NORTH AMERICAN PRRS SYMPOSIUM 2017 CHICAGO

NSIF National Swine Improvement Federation

NA PRRS/NSIF Joint Meeting
PROGRAM
December 1-3, 2017
InterContinental Magnificent Mile
Chicago, Illinois

The 2017 North American PRRS Symposium wishes to thank the following sponsors for their generous support:

Boehringer Ingelheim

pork checkoff

zoetis™

Kansas State University College of Veterinary Medicine

Tetracore®

Newport Laboratories

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

BRI
Biosecurity Research Institute

MEDIAN Life Science

Aptimmune

Kansas State University | Institute for Commercialization

Kansas State Veterinary Diagnostic Laboratory

Dr. Dave Benfield (Travel Fellowship)
Dr. Bob Morrison Memorial (Travel Fellowship)

2017 NA PRRS Symposium – NSIF Conference Executive Committee

Executive Director:	Raymond R.R. Rowland *Kansas State University*
President NSIF:	Christopher Hostetler *National Pork Board*
Executive Director, CRWAD:	David Benfield *The* Ohio State University
Committee Members:	
Lisa Becton *National Pork Board*	Jay Calvert *Zoetis, Inc.*
Jack Dekkers *Iowa State University*	Megan Kilgore
Justin Fix *Smithfield Premium Genetics*	Reid Philips *Boehringer-Ingelheim Vetmedica, Inc.*
Proceedings Editor/Staff:	Francine Rowland *Kansas State University*
Conference Coordinator:	Megan Kilgore

North American PRRS Symposium Program and Scientific Committee

Jack Dekkers *Iowa State University*	Laura Miller *USDA ARS NADC*
Diego Diel *South Dakota State University*	Sheela Ramamoorthy *North Dakota State University*
Jennelle Dunkelberger *Topigs-Norsvin*	Raymond Rowland *Kansas State University*
Paul Lawrence *Newport Laboratories*	Nick Serao *Iowa State University*
Luis Gimenez-Lirola *Iowa State University*	

North American PRRS Symposium Travel Fellowship Selection Committee

Joan Lunney *USDA* – ARS - BARC	Montse Torremorell *University of Minnesota*

NSIF Committee

Chair:

Justin Fix
Smithfield Premium Genetics

Committee Members:

Mark Knauer
North Carolina State University

Amanda Minton
The Maschoffs

Steve Bretey
Allflex USA / Destron Fearing

Scott Price
Ivey's Spring Creek Farm, Inc.

Clint Schwab
The Maschhoffs

Kent Gray
Smithfield Premium Genetics

The 2017 NA PRRS Symposium is Dedicated to the Memory of Dr. Bob Morrison

The Dr. Bob Morrison Memorial Fellowship

The Dr. Bob Morrison Memorial Fellowship is given in memory of Dr. Bob Morrison, who was a professor in the Department of Veterinary Population Medicine at the University of Minnesota. He 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 fellowship, organized by Dr. Montse Torremorell and Dr. Joan Lunney, will provide travel support for future scientists who wish to follow in his footsteps.

The 2017 NA PRRS conference is dedicated to his memory. Bob will be missed.

2017 Bob Morrison Fellowship Awardees	
Yanhua Li, Kansas State University	Pengcheng Shang, Kansas State University
Kabita Pandey, South Dakota State University	Ana Stoian, Kansas State University
Austin Putz, Iowa State University	Qingzhan Zhang, University of Illinois

David A. Benfield Student Travel Fellowships

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. It is his generous donation that 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.

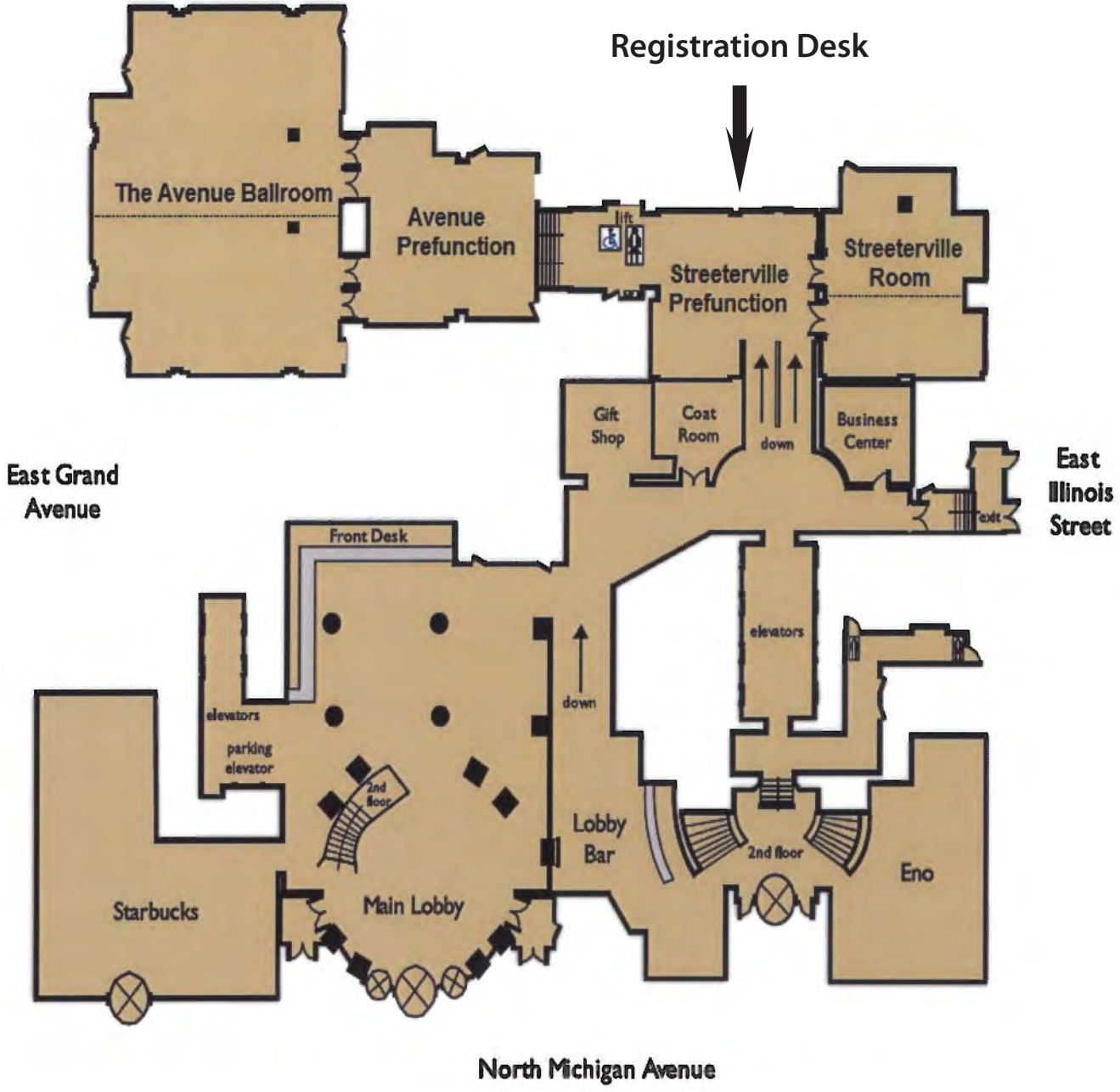
Joan Lunney, who is actively involved in mentoring younger scientists, is pleased to support the David A. Benfield Student Travel Fellowships for attendance at PRRS Symposia.

2017 Student Travel Fellowship Recipients	
Qian (Jessie) Dong	Iowa State University
Kinga Biernacka	Warsaw University of Life Sciences, Poland
Laura A. Constance	Kansas State University
Hanzhong Ke	University of Illinois at Urbana-Champaign
Yashavanth Shaan Lakshmanappa	OARDC, The Ohio State University
Shehnaz Lokhandwala	Kansas State University
Dagmara Magdalena Miłek	Warsaw University of Life Sciences, Poland
Carolina Maciel Malgarin	University of Saskatchewan, Canada
Vlad Petrovan	Kansas State University
Luca N. Popescu	Kansas State University
Aleksandra Woźniak	Warsaw University of Life Sciences, Poland
Liping Yang	University of Maryland

CE Credit Information

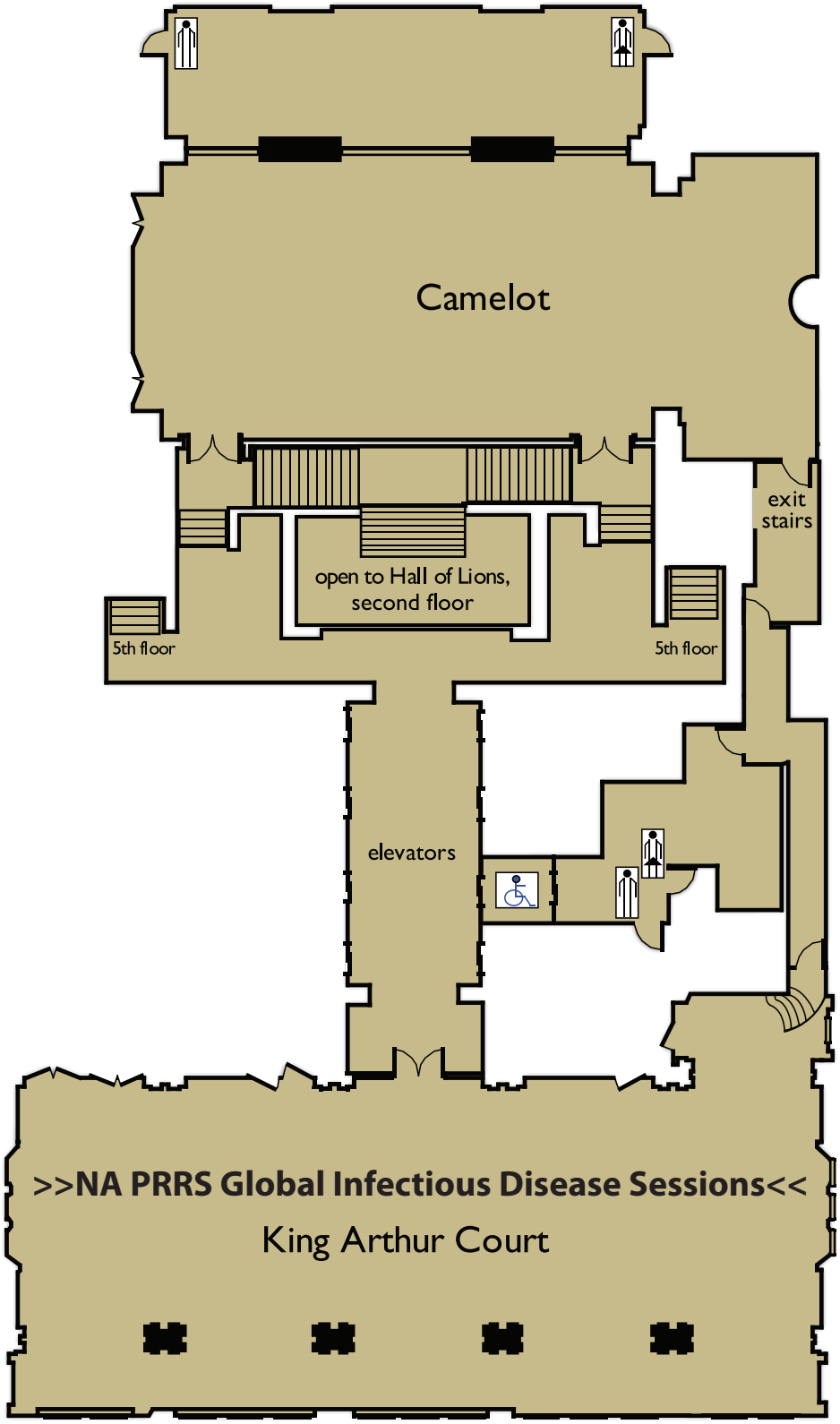
The 2017 North American PRRS Symposium and NSIF Conference programs have been approved for 19.5 hours of continuing education (CE) credit as accepted by the Kansas Board of Veterinary Examiners. Kansas Board of Veterinary Examiners approves continuing education hours based on the guidelines set forth by the AAVSB RACE approval program for veterinarians and veterinary technicians. Signed CE confirmation forms are included in the attendee registration packet.

Lobby Level

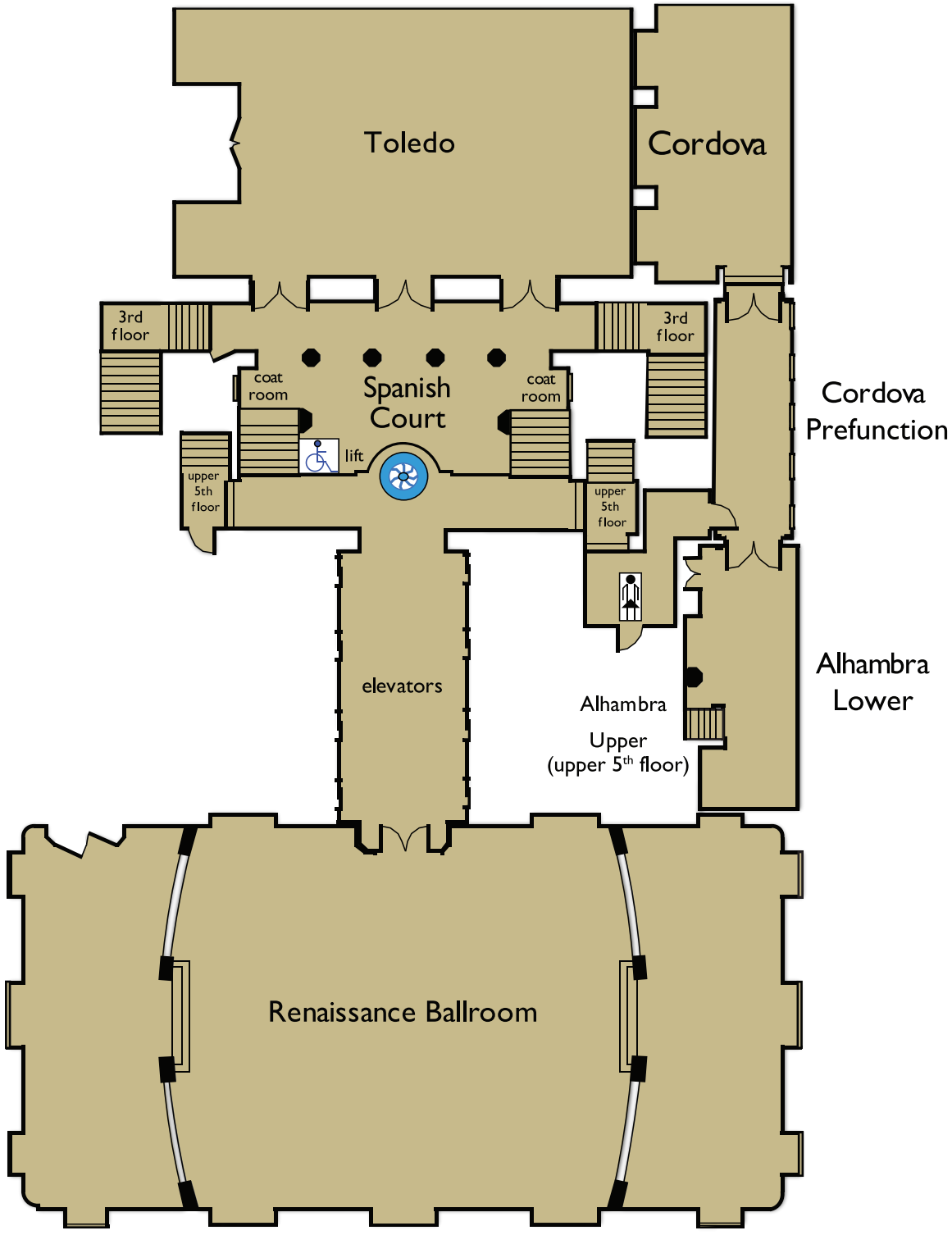


COCKTAIL RECEPTION & NA PRRS POSTER SESSION
Sponsored by Zoetis, Inc
The Avenue Ballroom and Streeterville Room

3RD Floor


Camelot
exit stairs
open to Hall of Lions, second floor
5th floor
5th floor
elevators
>>NA PRRS Global Infectious Disease Sessions<<
King Arthur Court

5TH Floor



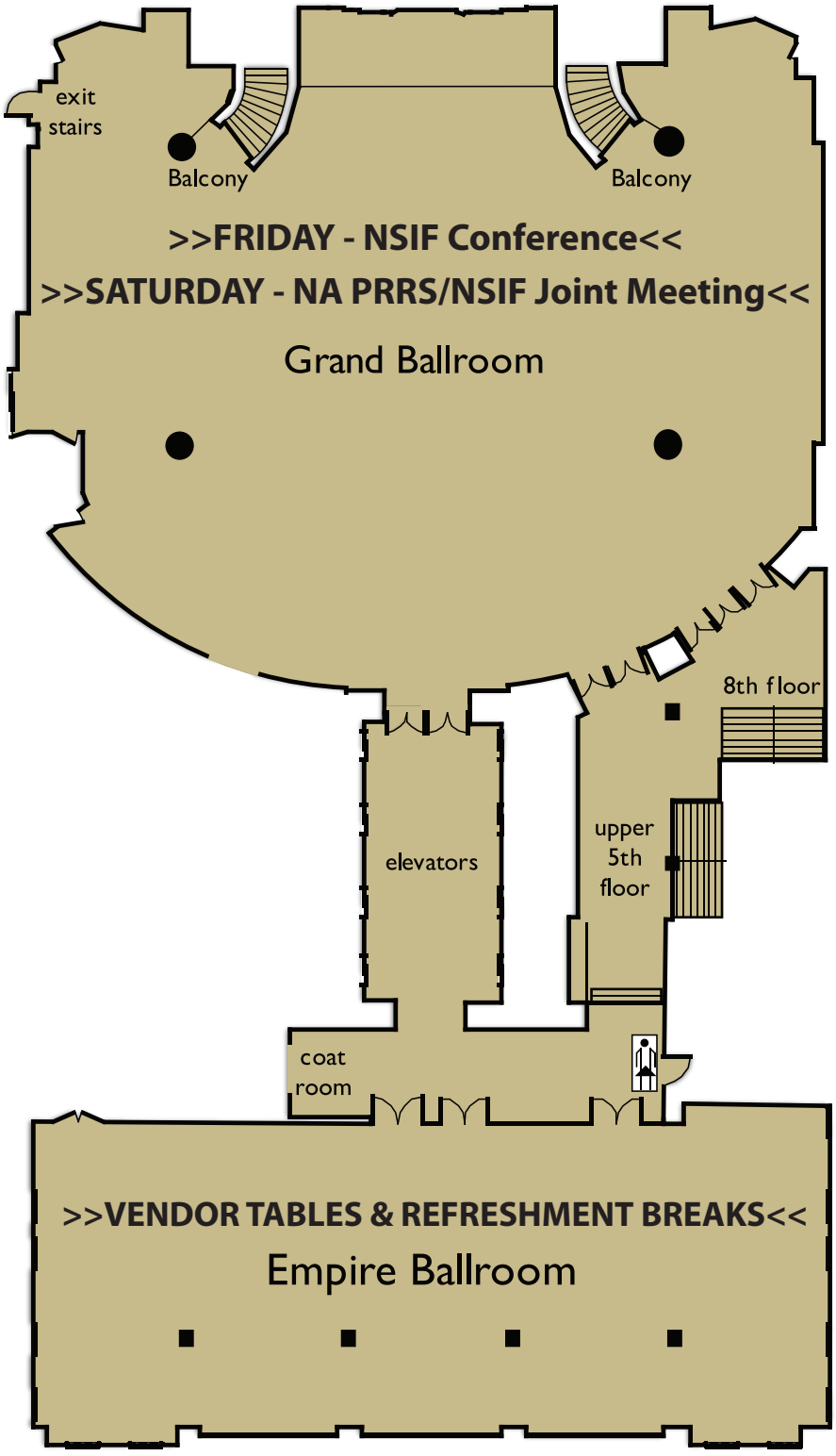
Renaissance Ballroom will host the following events:

>>FRIDAY LUNCH - NSIF Annual Meeting & Awards Presentation<<

>>SATURDAY LUNCH - NA PRRS/NSIF Joint Luncheon<<

>>SUNDAY BREAKFAST - NA PRRS Abstract Presentations<<

7TH Floor


exit
stairs
Balcony
Balcony
>>FRIDAY - NSIF Conference<<
>>SATURDAY - NA PRRS/NSIF Joint Meeting<<
Grand Ballroom
8th floor
elevators
upper
5th
floor
coat
room
>>VENDOR TABLES & REFRESHMENT BREAKS<<
Empire Ballroom

North American PRRS Symposium and National Swine Improvement Federation Conference

2017 NA PRRS-NSIF Joint Meeting Program

Thursday, November 30

4:00pm-6:00pm **Registration Desk Open**

Streeterville Prefunction Room, 1st Floor, InterContinental Hotel

Friday, December 1

7:00am-2:00pm **Registration Desk Open**

Streeterville Prefunction Room, 1st Floor, InterContinental Hotel

NSIF Conference - InterContinental Hotel, Grand Ballroom, 7th Floor

8:00am **Welcome**
Justin Fix, Conference Chair

8:15am **Beyond the Farm**
What is possible with big data? The human example.
Francia Mara – IRI
Pork in the Food Service Industry.
Stephen Gerike – National Pork Board
What's the future market pig look like, the buyer perspective?
Chuck Allison - Smithfield Foods

10:30am **Break**

10:45am **Competencies for the Future**
Moderator: Mark Knauer, North Carolina State University
University Perspective: Developing Human Capital for the U.S. Swine Genetics Field
Maynard Hogberg – Iowa State University
Industry Perspective: Developing Human Capital for the U.S. Swine Genetics Field
Tom Rathje – DNA Genetics
Roundtable Discussion to Follow

12:00pm NSIF Annual Meeting and Awards Presentation – Renaissance Ballroom, 5th Floor

The Friday afternoon NSIF Conference session will run concurrently with the NA PRRS Symposium session

1:30pm Graduate Student Award Presentation

2:00pm Technologies – The Next Step
Sexed Semen Technology
Richard Leach – Fast Genetics
Population Management Using Genomics (ROH)
Jeremy Howard – University of Nebraska

3:30 pm Break

3:45 pm Allflex USA/Destron Fearing Reception (NSIF Attendees)

5:00pm Conclusion of NSIF Program

5:30pm NSIF/NA PRRS Keynote Speaker – Rennaissance Ballroom, 7th Floor
Can Biotechnology Bring Home the Bacon?
Dr. Alison Van Eenennaam – University of California, Davis

North American PRRS Symposium
InterContinental Hotel, King Arthur Court, 3rd Floor

Global Infectious Disease

The Friday afternoon NA PRRS Sympsoium session will run concurrently with the NSIF Conference session

Moderators:
Paul Sundberg, SHIC; Ying Fang and Bob Rowland, Kansas State University

1:00pm Setting priorities for disease threats and strategies
Paul Sundberg – SHIC

1:20pm Perspectives on the development of global efforts for disease control
Alex Morrow – STAR-IDAZ IRC

1:45pm **Things your epidemiologist never told you about surveillance**
Jeff Zimmerman – Vet Diagnostic and Production Animal Medicine, Veterinary Diagnostic Laboratory, Iowa State University

2:05pm **Here we go again? Emergence of a novel swine enteric alphacoronavirus (SeACov) in Southern China**
Yao-Wei Huang – Zhejiang University

2:30pm **Break**

3:00pm **Novel approaches for assessing the risks of importing viruses from other countries through feed and feed ingredients**
Scott Dee – Pipestone; Diego Diel – South Dakota State University; Megan Niederwerder – Kansas State University

4:00pm **Emerging swine disease diagnostics and characterization: connecting basic research to real-world applications**
Ying Fang – Kansas State University

4:20pm **Comparison of historic and contemporary strains of Senecavirus A**
[Abstract #8]
Alexandra Buckley – USDA ARS VPRU

4:40pm **MinION Next-Generation Sequencing-based Routine Identification and Strain Typing of Porcine Reproductive and Respiratory Syndrome Virus**
[Abstract #52]
Kevin Linhares – Virginia Tech

5:00pm Conclusion of Global Infectious Disease Program

5:30pm **NSIF/NA PRRS Keynote Speaker – Rennaissance Ballroom, 7th Floor**
Can Biotechnology Bring Home the Bacon?
Dr. Alison Van Eenennaam – University of California, Davis

Saturday, December 2

7:00am-10:00am Registration Desk Open

Streeterville Prefunction Room, 1st Floor, InterContinental Hotel

NA PRRS/NSIF Joint Session – InterContinental Hotel, Grand Ballroom, 7th Floor

PRRS in the Field

[*special program sponsored by Boehringer Ingelheim Vetmedica, Inc*]

Moderator: Dr. Montse Torremorell, University of Minnesota

8:00am **Welcome**
Dr. Jens Kjaer – Boehringer Ingelheim Vetmedica, Inc

8:05am **PRRS Field Applicable Research Update**
University of Minnesota – Montse Torremorell

- Research update/summary
- What are we doing/learning?
- Field relevance & applications
- What's next?

9:00am **Iowa State University – Daniel Linhares**

- Research update/summary
- What are we doing/learning?
- Field relevance & applications
- What's next?

10:00am **Break – Empire Ballroom**

10:30 **University of Illinois – Jim Lowe and Ben Blair**
Are cull sow movements impacting disease transmission?

11:00am **BI-R&D – Mike Roof**
Current status – "State of the Union" – PRRS vaccine research

11:30am **Panel Discussion with Speakers**
Moderated by Montse Torremorell

12:00pm **LUNCH – Renaissance Ballroom, 5th Floor**

Translational Research: The Genetics of Disease Resistance Updates on Current Progress and a Vision for the Future

Moderators: Jack Dekkers – Iowa State University and Bob Rowland – Kansas State University

1:00pm **Introduction of the Program**
Jack Dekkers and Bob Rowland

1:10pm **PRRS overview and description of disease phenotypes and models for genetic research**
Bob Rowland – Kansas State University

1:40pm **Probing mechanisms of PRRS resistance**
Joan Lunney – USDA ARS BARC

2:10pm **New PRRS disease phenotypes as vaccine and genetic improvement targets**
Andrea Wilson – Roslin Institute

2:40pm **Break – Empire Ballroom**

3:10pm **Resilience and PRRS in a natural disease challenge model**
Graham Plastow – University of Alberta

3:40pm **Viral genetics and application to vaccine development**
Jay Calvert – Zoetis, Inc

4:10pm **Porcine reproductive and respiratory syndrome genetic resistance: an online class at destination of swine experts and professionals**
Perle Boyer – University of Minnesota

4:30pm **Using genetic selection and genomics to combat infectious disease**
Jack Dekkers – Iowa State University

5:00pm **Discussion**

6:00pm Poster Session and Reception

Streeterville Ballroom/Avenue Ballroom, 1st Floor
Sponsored by Zoetis, Inc.

Sunday, December 3

7:15 am BREAKFAST – Renaissance Ballroom, 5th Floor

NA PRRS Symposium – InterContinental Hotel, Renaissance Ballroom, 5th Floor

Presentations Selected from Abstracts

Moderators: Diego Diel – South Dakota State University and Laura Miller – USDA ARS NADC

8:00am Abstract #46
"Evaluation of the number of samples required to accurately quantify PRRS viral load at the maternal-fetal interface"
Carolina Malgarin – Western College of Veterinary Medicine, University of Saskatchewan

8:15am Abstract #33
"Concurrent but consecutive vaccination of modified live type 1 and type 2 PRRSV provides better protection in nursery pigs"
Yashavanth Shaan Lakshmanappa – Department of Preventive Medicine, The Ohio State University

8:30am Abstract #34
"Adenovirus-vectored novel African Swine Fever Virus multi-antigen cocktail elicit strong but non-protective immune responses in commercial pigs"
Shehnaz Lokhandwala – Dept. of Diagnostic Medicine/Pathobiology, Kansas State University

8:45am Abstract #23
"Fecal microbiota transplantation improves outcome in nursery pigs following co-infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2d"
Megan Niederwerder – Dept. of Diagnostic Medicine/Pathobiology, Kansas State University

9:00am **Abstract #24**
"Double-stranded viral RNA persists in vitro and in vivo during prolonged infection of PRRSV"
Pencheng Shang – Dept. of Diagnostic Medicine/Pathobiology, Kansas State University

9:15am **Abstract #14**
"Dynamic of adaptive immune responses following Senecavirus A infection in pigs"
Mayara Maggioli – Animal Disease Research & Diagnostic Laboratory, South Dakota State University

9:30am **Abstract #6**
"Inhibition of type III interferons in the intestinal epithelial cells by porcine epidemic diarrhea virus and innate immune evasion"
Qingzhan Zhang – Department of Pathobiology, University of Illinois

9:45am **Abstract #65**
"CD163 PSTII domain is required for PRRSV-2 infection:
Ana Stoian – Dept. of Diagnostic Medicine/Pathobiology, Kansas State University

10:00am **Break**

10:30am **Abstract #60**
"A dual ribosomal frameshifting mechanism transactivated by an arterivirus protein and host cellular factors"
Yanhua Li – Dept. of Diagnostic Medicine/Pathobiology, Kansas State University

10:45am **Abstract #55**
"Glycoprotein 3 of porcine reproductive and respiratory syndrome virus exhibits an unusual hairpin-like membrane topology"
Minze Zhang – Institute of Virology, Free University Berlin

11:00am **Abstract #5**
"Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid Protein Activates NF-kappa B through Binding to PIAS1"
Hanzhong Ke – Department of Pathobiology, University of Illinois

11:15am **Abstract #31**
"MYH9 Protein C-Terminal Domain Blocks PRRSV infection"
En-Min Zhou – College of Veterinary Medicine, Northwest A&F University, China

11:30am **Abstract #16**
"PRRSV hijacks karyopherin alpha6 to facilitate the viral replication"
Liping Yang – VA-MD College of Veterinary Medicine, University of Maryland

11:45am **Abstract #18**
"Relationships of polymorphisms in the regulator of G-protein signaling 16 gene with porcine circovirus type 2 viremia in naturally infected Yorkshire pigs"
Kyu-Sang Lim – Iowa State University

12:00pm **Closing remarks**

No.	Name	Institution	Abstract Title	Authors/Institutions/Organizations
Speakers				
S1	Boyer, Perle	University of Minnesota	Principles and applications of genetics and genomics to improve animal health: An online class at destination of swine experts and professionals	Perle Boyer[1], Montserrat Torremorell[1], Jack Dekkers[2] *[1]University of Minnesota, College of Veterinary Medicine, St. Paul, MN; [2]Iowa State University, College of Agriculture and Life Sciences, Ames, IA*
S2	Calvert, Jay	Zoetis	Viral genetics and application to vaccine development	Jay Calvert *Zoetis, Veterinary Medicine R&D, Kalamazoo, MI, USA*
S3	Dee, Scott Diel, Diego Niederwerder, Megan	Pipestone, South Dakota State University and Kansas State University	Survival of viral pathogens in animal feed ingredients under transboundary shipping models	Scott A. Dee[1*], Fernando V. Bauermann[2], Megan C. Niederwerder[5,6], Aaron Singrey[2], Travis Clement[2], Marcelo de Lima[2,3] Craig Long[2], Gilbert Patterson[4], Maureen A. Sheahan[5], Ana M. M. Stoian[5], Vlad Petrovan[5], Cassandra K. Jones[7], Jon De Jong[1], Ju Ji[8], Gordon D. Spronk[1], Jane Christopher-Hennings[2], Jeff J. Zimmerman[9], Raymond R.R. Rowland[5], Eric Nelson[2], Paul Sundberg[10], Diego G. Diel[2.] *[1]Pipestone Applied Research, Pipestone Veterinary Services, Pipestone, MN, USA. [2]Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA. [3]Faculdade de Veterinaria, Universidade Federal de Pelotas, RS, Brazil. [4]Center for Animal Health in Appalachia, Lincoln Memorial University, Harrogate, TN, USA. [5]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA. [6]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA. 7Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS, USA. [8]Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA, USA. [9]Veterinary Diagnostic Population Animal Medicine, Iowa State University, Ames, IA, USA.[10]Swine Health Information Center, Ames, IA, USA.*
S4	Dekkers, Jack C. M.	Iowa State University	Using genetic selection and genomics to combat infectious disease	Jack C. M. Dekkers *Department of Animal Science, Iowa State University*
S5	Fang, Ying	Kansas State University	Emerging swine disease diagnostics and characterization: connecting basic research to real-world applications	Ying Fang, *Kansas State University*

S6	Huang, Yao-Wei	Zhejiang University	Emergency of a novel swine enteric alphacoronavirus (SeACoV) in southern China	Yao-Wei Huang[1]*, Yongfei Pan[2], Bin Wang[1], Pan Qin[1], Yong-Le Yang[1], Yanhua Song[2] *[1]Department of Veterinary Medicine, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China; [2]Hog Production Division, Guangdong Wen's Foodstuffs Group Co, Ltd, Xinxing, Guangdong, China.*
S7	Lunney, Joan K.	USDA-ARS, BARC	Probing mechanisms of PRRS resistance	Joan K. Lunney *USDA-ARS, BARC, APDL, Building1040, Beltsville 20705, MD, USA*
S8	Morrow, Alex	STAR-IDAZ IRC	Perspectives on the development of global efforts for disease control	Alex Morrow *STAR-IDAZ IRC*
S9	Plastow, Graham	University of Alberta	Resilience and PRRS in a natural disease challenge model	G. S. Plastow *Dept. of Agricultural, Food & Nutritional Science, University of Alberta*
S10	Rowland, Bob	Kansas State University	PRRS overview and description of disease phenotypes and models for genetic research	Raymond (Bob) Rowland *Department of Diagnostic Medicine and Pathobiology, Kansas State University*
S11	Wilson, Andrea	The Roslin Institute	New PRRS phenotypes as vaccine and genetic improvement targets	Andrea Doeschl-Wilson *The Roslin Institute, University of Edinburgh, Scotland UK*
Epidemiology, biosecurity, virus control in the field				
1	Perri, Amanda	University of Guelph	A case-control study investigating the early months of the porcine epidemic diarrhea (PED) outbreak in Canadian swine herds	A.M. Perri *1, Z. Poljak1, C. Dewey1, J.C.S. Harding2, T.L. O'Sullivan1. *Department of Population Medicine, University of Guelph1, Canada; Large Animal Clinical Sciences, University of Saskatchewan2, Canada.*
2	Clack, Herek	University of Michigan	Airborne inactivation of porcine reproductive & respiratory syndrome virus (PRRSv) by a packed bed dielectric barrier discharge non-thermal plasma.	Tian Xia1, My Yang2, Ian Marabella3, Darrick Zarling3, Bernard Olson3, Montserrat Torremorell2, Herek L. Clack1* *1Dept. of Civil & Environmental Engineering, University of Michigan, Ann Arbor, MI, 2Dept. of Mechanical Engineering, University of Minnesota, Twin Cities, Minneapolis, MN, 3Dept. of Veterinary Population Medicine, University of Minnesota, Twin Cities, St. Paul, MN.*
3	Bernal, Guillermo	MSD Mexico (Merck)	Field evaluation of a modified-live PRRS Type II vaccine in a positive herd in Mexico	G. Bernal1, R.Garcia1, A.F. de Grau2*, B.Thacker3, R. Jolie4 *1MSD México, México City México; 2 Intervet Canada Corporation, Kirkland, Quebec, Canada; 3Merck Animal Health, DeSoto, KS, USA; 4 Merck Animal Health, Madison, NJ , USA.*

Host response to infection, including host genetics and innate/adaptive immunity				
4	Jeon, Ji Hyun	Kyungpook National University	Importance of cellular cholesterol for the entry process of porcine nidoviruses	J.H. Jeon*, C. Lee *Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea*
5	Yoo, Dongwan	University of Illinois at Urbana-Champaign	Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid Protein Activates NF-kappa B through Binding to PIAS1	H. Ke*, S. Lee, D. Yoo *Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL. 61802.*
6	Yoo, Dongwon	University of Illinois at Urbana-Champaign	Inhibition of type III interferons in the intestinal epithelial cells by porcine epidemic diarrhea virus and innate immune evasion	Q. Zhang1*, H. Ke1, T. Fujita2, D. Yoo1 *1Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA 2Institute for Virus Research, Kyoto University, Kyoto, Japan*
7	Fang, Ying	Kansas State University	Multiplexed digital mRNA profiling of immune responses in pigs persistently infected with porcine reproductive and respiratory syndrome virus	Pengcheng Shang1, Rui Guo1, Celena A. Carrillo2, Crystal J. Jaing2, Ying Fang1 *1Kansas State University, Manhattan, Kansas, USA; 2Lawrence Livermore National Laboratory, Livermore, California, USA*
8	Buckley, Alexandra	USDA ARS VPRU	Comparison of historic and contemporary strains of Senecavirus A	Alexandra Buckley1*, Baoqing Guo2, Vikas Kulshreshtha1, Albert van Geelen1, Kyoung-Jin Yoon2, and Kelly Lager3 *1Oak Ridge Institute for Science and Education and National Animal Disease Center and U.S. Department of Agriculture, Ames, IA, USA 2College of Veterinary Medicine, Iowa State University, Ames, IA, USA 3National Animal Disease Center, U.S. Department of Agriculture, Ames, IA, USA*
9	Nan, Yuchen	Northwest A&F University	Glycosylation site of PRRSV GP5 is associated with broad neutralization of PRRSV infection	Y. Nan1*, G. Gu1, C. Wu1, Y. Zhang2, and E-M. Zhou1* *1College of Veterinary Medicine, Northwest A&F University, China; 2Molecular Virology Laboratory, VA-MD College of Veterinary Medicine and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA*
10	Fleming, Damarius	USDA-ARS-National Animal Disease Center	Effect of miRNA and tRNA gene expression on the homeostatic status of pigs infected with highly pathogenic PRRSV.	Damarius S. Fleming1,2 and Laura C. Miller2 *ORAU/ORISE Oak ridge, TN1, Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA-ARS2*

11	Miller, Laura	USDA-ARS-National Animal Disease Center	Transcriptome responses to respiratory virus infection of pigs within the tracheobronchial lymphnode following infection with PRRSV, PCV2 or IAV.	Laura C. Miller*1, Damarius S. Fleming 1,3, Gregory P. Harhay2, Marcus E. Kehrli Jr1, and Kelly M. Lager1 *1Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa 50010, USA; 2Animal Health Research Unit, US Meat Animal Research Center, USDA, Agricultural Research Service, Clay Center, Nebraska 68933, 3ORAU/ORISE Oak ridge, TN*
12	Miller, Laura	USDA-ARS-National Animal Disease Center	Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived cells to different stimuli	Laura C. Miller*1, Damarius S. Fleming1,6, Xiangdong Li2, Darrell O. Bayles3, Frank Blecha4 and Yongming Sang5 *1Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa 50010, USA; 2Pulike Biological Engineering, Lingbo Road, Luoyang Hi-New Tech Development Zone, Henan Luoyang Henan 471000, China; 3Infectious Bacterial Diseases Research Unit, National Animal Disease Center, USDA-ARS, 1920 Dayton Ave, Ames, IA 50010; 4Departments of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506; 5Department of Agricultural and Environmental Sciences, College of Agriculture, Human and Natural Sciences, Tennessee State University, 3500 John A. Merritt Boulevard, Nashville, TN 37209; 6ORAU/ORISE Oak ridge, TN.*
13	Van Geelen, Albert	USDA ARS VPRU	Live virus Immunization (LVI) with a recent 1-7-4 PRRSV isolate elicits broad protection against PRRSV challenge in finishing age swine.	Albert G. M. van Geelen2, Alexandra Buckley2, Vikas Kulshreshtha3, Susan Brockmeier1, Laura Miller1 Damarius Fleming2, Crystal Loving4, Kay Faaberg1 and Kelly lager1 *1: U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Virus and Prion Research Unit. 2: Oak Ridge Institute for Science and Education, 3: Iowa State College of Veterinary Medicine, 4: U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Food Safety and Enteric Pathogens*
14	Maggioli, Mayara F.	South Dakota State University	Dynamics of adaptive immune responses following Senecavirus A infection in pigs	M.F. Maggioli1*, S. Lawson1, M. De Lima2, L.R. Joshi1, T.C. Faccin3, F.V. Bauermann1, D.G. Diel1 *1Animal Disease Research & Diagnostic Laboratory, South Dakota State University, Brookings, SD, USA, 2Universidade Federal de Pelotas, Pelotas, Brazil, 3Universidade Federal de Santa Maria, Santa Maria, Brazil*
15	Zhang, Yanjin	University of Maryland	PRRSV inhibits interferon signaling by downregulating STAT2	L. Yang*, Z. Ma, S. Lin, and Y. Zhang *Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD*

16	Zhang, Yanjin	University of Maryland	PRRSV hijacks karyopherin alpha6 to facilitate the viral replication	L. Yang*, R. Wang, S. Yang, Z. Ma, S. Lin, and Y. Zhang *Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD*
17	Darbellay, Joseph	Vaccine and Infectious Disease Organization-International Vaccine Centre	Differential rates of viral replication and cell susceptibility to infection by PRRSV in multiple antigen presenting cell populations	J. Darbellay1*, J. Van Kessel1, V. Gerdts1 *1. Vaccine and Infectious Disease Organization-International Vaccine Centre, Saskatoon, Sk. Canada*
18	Hong, Ki-Chang	Korea University	Relationships of polymorphisms in the regulator of G-protein signaling 16 gene with porcine circo virus type 2 viremia in naturally infected Yorkshire pigs	K.S. Lim1*, S.H. Lee2, E.A. Lee2, J.M. Kim3, T. Chun2, K.C. Hong *1Iowa State University, Ames, IA, USA, 2Korea University, Seoul, Republic of Korea, 3National Institute of Animal Science, Wanju, Republic of Korea*
19	Constance, Laura	Kansas State University	Microbiome associations with weight gain in pigs after vaccination with a porcine reproductive and respiratory syndrome (PRRS) modified live virus (MLV) vaccine followed by challenge with PRRSV and porcine circovirus type 2 (PCV2b)	L.A. Constance1*, J.B. Thissen2, C.J. Jaing2, K.S. McLoughlin3 A.G. Cino-Ozuna1,4, R.R.R. Rowland1, M.C. Niederwerder1,4 *1Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; 2Physical & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; 3Computations Directorate, Lawrence Livermore National Laboratory, Livermore, CA 4Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS*
20	Dunkelberger, Jenelle	Topigs Norsvin USA	Genomic prediction of a PRRS-vaccinated training population to predict host response to PRRS virus-only or PRRS virus/PCV2b co-infection	JR Dunkelberger,1,2* NVL Serão,2 M Niederwerder,3 M Kerrigan,3 M Schroyen,2,4 CK Tuggle,2 J Lunney,5 RRR Rowland,3 and JCM Dekkers2 *1Topigs Norsvin USA, Burnsville, MN; 2Iowa State University, Ames, IA; 3Kansas State University, Manhattan, KS; 4University of Liège, Gembloux, Belgium; 5USDA, ARS, BARC, APDL, Beltsville, MD*
21	Ko, Haesu	Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA	Viral load and antibody titer of post-weaned pigs represent the host susceptibility to porcine circovirus type 2 (PCV2) infection	Haesu Ko1*, Kyung-Tai Lee1, Taehoon Chun2, Ki-Chang Hong2, and Jun-Mo Kim1 *1Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea, 2College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea*
22	Wang, Xiuquing	South Dakota State University	GTPase-activating protein - binding protein 1 (G3BP1) plays an antiviral role in Porcine Epidemic Diarrhea Virus replication	Kabita Pandey1*, Shuhong Zhong1, Eric Nelson2, Xiuqing Wang1 *Department of Biology and Microbiology1, Department of Veterinary and Biomedical Sciences, South Dakota State University2, Brookings, South Dakota 57007*

23	Niederwerder, Megan	Kansas State University	Fecal microbiota transplantation improves outcome in nursery pigs following co-infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2d	M.C. Niederwerder1,2,*, R.R.R. Rowland1, L.A. Constance1, M.L. Potter3, M.A. Sheahan1, R.A. Hesse1,2, and A.G. Cino-Ozuna1,2 *1Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, 2Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas*
24	Fang, Ying	Kansas State University	Double-stranded viral RNA persists in vitro and in vivo during prolonged infection of PRRSV	Rui Guo1, Pengcheng Shang1, Celena A. Carrillo2, Xingyu Yan1, Tao Wang1, Crystal J. Jaing2, Megan Niederwerder1, Raymond R. R. Rowland1, Ying Fang1 *1Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA 2Lawrence Livermore National Laboratory, Livermore, CA, USA*
25	Wang, Rong	Xi'an Jiaotong University	PRRSV-induced HMGB1 secretion via PKC-delta activation in triggering inflammatory response	R. Wang1*, Y. Zhang2, E. Liu1, L. Yang2 *1Laboratory Animal Center, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi, China 2Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD*
26	Dong, Qian	Iowa State University	Piglet Blood Transcriptome Response to Co-infection with PRRSV and PCV2, with or without prior Vaccination for PRRS	Qian Dong*1, Joan K Lunney2, Christopher K Tuggle1, James Reecy1, Raymond R R Rowland3, Jack C M Dekkers1 *1Department of Animal Science, Iowa State University, Ames, Iowa; 2USDA, ARS, BARC, APDL, Beltsville, Maryland; 3College of Veterinary Medicine, Kansas State University, Manhattan, Kansas*
27	Popescu, Luca	Kansas State University	GP5 of porcine reproductive and respiratory syndrome virus (PRRSV) as a target for homologous and broadly neutralizing antibodies	Luca N. Popescu1, Benjamin R. Trible1, Nanhua Chen2 and Raymond R. R. Rowland1* *1. Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS 66506 2. College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, 225009, P.R. China*
28	Rowland, Raymond	Kansas State University	Gene expression analysis of whole blood from pigs infected with low and high pathogenic African swine fever viruses.	Raymond R.R. Rowland4, Crystal Jaing1*, Jonathan Allen2, Andrea Certoma3, James B. Thissen1, John Bingham3, Brenton Rowe3, John R. White3, James W. Wynne3, Dayna Johnson3, David T. Williams3 *1Physical & Life Sciences Directorate, 2Computations Directorate, Lawrence Livermore National Laboratory, Livermore, CA, 3 CSIRO Australian Animal Health Laboratories, Geelong, Victoria, Australia, 4 Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS*

Vaccination strategies and therapeutics				
29	Lee, Sunhee	Kyungpook National University	Evaluation of the efficacy of an attenuated live vaccine based on a virulent type 2 porcine reproductive and respiratory syndrome virus strain in young pigs	S. Lee1*, S.-C. Lee2, H.-W. Choi2, Y.-H. Noh2, I.-J. Yoon2, C. Lee1 *1Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; 2Choongang Vaccine Laboratory, Daejeon, South Korea*
30	Philips, Reid	Boehringer Ingelheim Animal Health	Efficacy of Inngelvac PRRS X MLV against a heterologous PRRSV 1-3-4 RFLP Challenge	A. Patterson, B. Fergen, J. Hermann, G. Haiwick, R. Philips* *Boehringer Ingelheim Animal Health, Duluth, GA, USA*
31	Zhou, En-Min (Eric)	Northwest A&F University	MYH9 Protein C-Terminal Domain Blocks PRRSV infection	L. Li1, Y. Nan1, L. Zhang1, G. Hou1, B. Xue1, W. Sun1, G. Gu1, C. Wu1, Q. Zhao1, Y. Zhang2, G. Zhang3, Julian A. Hiscox4 and E-M. Zhou1* *1College of Veterinary Medicine, Northwest A&F University, China; 2Molecular Virology Laboratory, VA-MD College of Veterinary Medicine and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA; 4College of Animal Science and Veterinary Medicine, Henan Agricultural University, China; 5Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool L3 5RF, UK*
32	Parrillo, Matthew	University of Rhode Island	EpiCC: A new tool for selecting the optimal vaccine during an emerging outbreak	A.H. Gutiérrez2, L. Moise1, 2 , M. Parrillo1*, W.D. Martin2, A.S. De Groot1, 2 *1Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, 2EpiVax, Inc., Providence, RI*
33	Shaan Lakshmanappa, Yashavanth	The Ohio State University	Concurrent but consecutive vaccination of modified live type 1 and type 2 PRRSV provides better protection in nursery pigs	Yashavanth Shaan Lakshmanappa1*, Pengcheng Shang2*, Sankar Renu1, Santosh Dhakal1, Bradley Hogshead1, Pauline Bernardo1, Xinyu Yan2, Ying Fang2 and Renukaradhya J Gourapura1 *1Food Animal Health Research Program (FAHRP), OARDC, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH 44691, USA. 2Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.*
34	Lokhandwala, Shehnaz	Kansas State University	Adenovirus-vectored novel African Swine Fever Virus multi-antigen cocktail elicit strong but non-protective immune responses in commercial pigs	S. Lokhandwala1*, L. Popescu1, N. Sangewar1, C. Elijah1, V. Petrovan1, A. Stoian1, M. Olcha1, M. Kerrigan1, J. Bray2, S. Waghela2, R. Rowland1, W. Mwangi1. *1Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, 2Department of Veterinary Pathobiology, Texas A&M University, College Station, TX*

35	Zhang, Chenming (Mike)	Virginia Tech	Virus-like particle (VLP) based vaccine strategy for porcine reproductive and respiratory syndrome virus (PRRSV)	Yi Lu, Frank Gillam, Chenming Zhang* *Virginia Tech*
36	Puckette, Michael	DHS-S&T Plum Island Animal Disease Center	Demonstration of in vitro VLP production and in vivo immunogenicity utilizing a mutant FMDV 3C protease	Erica Martel1*; Justin Smith2; Ben Clark2; Melia Pisano2; Traci Turecek2; Juan M. Pacheco1; Jose Barrera2; John G. Neilan3; Max Rasmussen3; Michael Puckette3 *Plum Island Animal Disease Center (PIADC), Greenport, NY. [1]ORISE PIADC Research Participation Program, [2]Leidos Inc., [3]U.S. Department of Homeland Security Science & Technology*
Detection, diagnostics and surveillance				
37	Gimenez-Lirola, Luis	Iowa State University	Complete Antigenic Profile of Porcine Epidemic Diarrhea Virus	LG Giménez-Lirola1*, J Zhang1, JA Carrillo-Avila2, Q Chen1, R Magtoto1, K Poonsuk1, DH Baum1, PE Piñeyro1, J Zimmerman1 *1Iowa State University; 2Granada University (Spain)*
38	Lee, Changhee	Kyungpook National University	Molecular characterization of porcine deltacoronaviruses in South Korea, 2014–2016	C. Lee1*, G. Jang1, S.-H. Kim2, K.-K. Lee2 *1Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; 2Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon, South Korea*
39	Rauh, Rolf	Tetracore	A novel multiplex field deployable molecular assay for vesicular diseases of pigs.	Rolf Rauh1, Emma L. A. Howson2, Veronica L. Fowler2, Diego G. Diel3, Jane Christopher-Hennings3, Travis Clement3, Wm. M. Nelson1 *1Tetracore, Inc., 9901 Belward Campus Drive, Suite 300, Rockville, MD 20850, USA 2The Pirbright Institue, Ash Road, Pirbright, Woking, GU24 0NF UK. 3Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, South Dakota*
40	Angulo, Jose	Zoetis	Measurement System Analysis (MSA) applied to Restriction Fragment Length Polymorphism (RFLP) of PRRS vaccine viruses and recent field strains	J. Angulo1*, J.G. Calvert2 *1. Zoetis, Parsippany NJ, 2. Zoetis, Kalamazoo, MI.*
41	Bai, Jianfa	Kansas State University	Development of a Luminex Multiplex Assay for the Detection and Differentiation of Type 2 PRRSV Field Strains and the Four US Vaccine Strains.	Yin Wang1, Wanglong Zheng1,3, Elizabeth Porlsen1, Xuming Liu1,2, Jianqiang Zhang4, Kyoung-Jin Yoon4, Lalitha Peddireddi1,2, Richard Hesse1,2, Ying Fang2, Gary Anderson1,2 and Jianfa Bai1,2 *1Kansas State Veterinary Diagnostic Laboratory, 2Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; 3Yangzhou University, Yangzhou, China. 4Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA.*

42	Bai, Jianfa	Kansas State University	A multiplex real-time RT-PCR assay for simultaneous detection and differentiation of Influenza D, C, B and A viruses in swine and cattle	Xuming Liu1,2*, Hewei Zhang1,3, Yin Wang1, Yanhua Li2, Yuekun Lang2, Elizabeth Poulsen1, Lance Noll1, Nanyan Lu1, Russell Ransburgh1, Wanglong Zheng1,4, Wenjun Ma2, Lalitha Peddireddi1,2, Ying Fang2, Gary Anderson1,2, Jianfa Bai1,2 **(* X. Liu: presenter**) *1Kansas State Veterinary Diagnostic Laboratory, 2Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA. . 3Chinese Academy of Agricultural Sciences, Beijing, China; 4Yangzhou University, Yangzhou, China. 4Yangzhou University, Yangzhou, China.*
43	Wozniak, Aleksandra	Warsaw University of Life Sciences	Detection of novel porcine circovirus type 3 (PCV3) in Poland	A. Woźniak1*, D. Miłek1, K. Biernacka1, T. Stadejek1 *1Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, Warsaw, Poland*
44	Milek, Dagmara	Warsaw University of Life Sciences	Detection of porcine parvoviruses 1, 2, 3, 4, 5 and 6 in serum, feces and oral fluid of pigs in Poland	D. Miłek1*, A. Woźniak1, K. Biernacka1, T. Stadejek1 *Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland*
45	Zimmerman, Jeffrey	Iowa State University	Improving the tactics for PRRSV surveillance using oral fluids	YA Henao-Díaz1*, L Giménez-Lirola1, K Poonsuk1, C Wang1, 2, J Ji2, TY Cheng1, RG Main1, J Zimmerman1 *1Department of Veterinary Diagnostic and Production Animal Medicine and 2Department of Statistics, Iowa State University, Ames, Iowa, USA. 50011*
46	Li, Yanhua	Kansas State University	Development of a real-time RT-PCR assay for detecting porcine parainfluenza virus 1 infection in pigs	Yanhua Li1*, Ying Fang1, Chase Stahl3, Jianfa Bai1,2, Xuming Liu1,2, Lalitha Peddireddi1,2, Gary Anderson1,2 *1Department of Diagnostic Medicine & Pathobiology, Kansas State University, KS, 2Kansas State Veterinary Diagnostic Laboratory, Kansas State University, KS, 3Fairmont Veterinary Clinic, Fairmont, MN*
47	Malgarin, Carolina	University of Saskatchewan	Evaluation of the number of samples required to accurately quantify PRRS viral load at the maternal-fetal interface	C. M. Malgarin1*, S. E. Detmer1, D. J. MacPhee1, J. C. S. Harding1 *1Western College of Veterinary Medicine, University of Saskatchewan, Canada*

48	Trujillo, Jessie	Kansas State University	Point of Need Molecular Based Detection of African Swine Fever Virus	J.D. Trujillo1, 2*, K. Urbaniak1, 2, T. Wang3, I. Morozov1, 2, J. A. Richt 1, 2 *1Dept. of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS. 2Center of Excellence for Emerging and Zoonotic Animal Diseases, College of Veterinary Medicine, Kansas State University, Manhattan, KS. 3GeneReach, USA, MA, USA*
49	Urbaniak, Kinga	Kansas State University	Development of a new protocol for quantitative real-time PCR (qPCR) for the detection of African swine fever virus in from formalin-fixed, paraffin-embedded tissues.	A. Meekins1, 2, J. A. Richt1, 2, A. S. Davis1, J. D. Trujillo1, 2. *1Dept. of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS. 2Center of Excellence for Emerging and Zoonotic Animal Diseases, College of Veterinary Medicine, Kansas State University, Manhattan, KS.*
50	Clement, Travis	South Dakota State University	Development of rapid diagnostic capability for encephalomyocarditis virus (EMCV)	T. Clement1*, F. Bauermann1, L. Joshi1, M. Maggioli1, D. Rausch1, T. Faccin1&2, M. Fernandes1, F. Rodrigues1&2, M. Martins1&2, D. Diel1. *1South Dakota State University, Veterinary and Biomedical Sciences Department, Brookings, SD. 2Federal University of Santa Maria, Brazil.*
51	Frossard, Jean-Pierre	Animal and Plant Health Agency	Seasonality of Porcine Reproductive and Respiratory Syndrome diagnoses in Great Britain	J.P. Frossard1*, C.A. Bidewell2, J. Thomson3, S. M. Williamson2 *1Animal and Plant Health Agency, Virology Department, Weybridge, U.K., 2Animal and Plant Health Agency, Surveillance Intelligence Unit, Bury St Edmunds, U.K., 3Scottish Agricultural College (Consulting), Veterinary Services, Bush Estate, Penicuik, Scotland, U.K.*
52	Rotolo, Marisa	Iowa State University	Changes in swine production mandate changes in swine surveillance	M Rotolo1*, C Wang1, M Haddad1, Y Sun1, M Hoogland2, R Main1, J Zimmerman1 *1Iowa State University, Ames, IA, 2Smithfield Foods, Algona, IA*
53	Lahmers, Kevin	Virginia Tech	MinION Next-Generation Sequencing-based Routine Identification and Strain Typing of Porcine Reproductive and Respiratory Syndrome Virus	K.K. Lahmers1*, J.B. Stanton2, S.M. Todd1, T. LeRoith1 *1. Department of Biomedical Sciences & Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 2. Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA*

54	Yuan, Fangfeng	Kansas State University	Development and validation of real time RT-PCR assay for the detection of Atypical Porcine Pestivirus	Fangfeng Yuan1, Xuming Liu1, Jianfa Bai1, Gary Anderson1, Ying Fang1,2, Bailey Arruda3, Paulo Arruda3, Lalitha Peddireddi1 *1Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 2Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506.3Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa.*
55	Biernacka, Kinga	Warsaw University of Life Sciences	Comparison of six commercial ELISAs for the detection of antibodies against porcine reproductive and respiratory syndrome virus.	Kinga Biernacka1*, Anna Tyszka2, Tomasz Stadejek1 *1Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences–SGGW, Nowoursynowska 159c, 02-776 Warsaw, Poland, 2Veterinary Diagnostic Laboratory, Vet Lab Group, Ostrodzka 46, 11-036 Gietrzwald, Poland*
Virus structure and gene function (viral genetics)				
56	Zhang, Minze	Institute of Virology, Free University Berlin, Robert von Ostertag-Straße 7-13, 14163 Berlin, Germany.	Glycoprotein 3 of porcine reproductive and respiratory virus exhibits an unusual hairpin-like membrane topology	Minze Zhang and Michael Veit *Institute of Virology, Free University Berlin, Robert von Ostertag-Straße 7-13, 14163 Berlin, Germany*
57	Zhou, Lei	China Agricultural University	Identification of a novel linear B-cell epitope in nonstructural protein 11 of porcine reproductive and respiratory syndrome virus that are conserved in both genotypes	Nan Jiang, Huan Jin, Yi Li, Xinna Ge, Jun Han, Xin Guo, Hanchun Yang, Lei Zhou* *Key Laboratory of Animal Epidemiology of Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, People's Republic of China*
58	Gladue, Douglas	Plum Island Animal Disease Center, ARS, USDA	Prediction and evaluation of novel genetic function for previously uncharacterized genes in African swine fever virus.	M.V. Borca1, L.H .Holinka1, E. Ramirez-Medina1,2, K. Berggren1,3, V. O'Donnell4, L.B. Carey5, J.A. Richt6, D. P. Gladue1* *1. Agricultural Research Service, Plum Island Animal Disease Center, Greenport, NY 11944, USA 2. Department of Pathobiology, University of Connecticut, Storrs, CT , 06268 USA 3. Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831 4. Animal and Plant Health Inspection Service, Plum Island Animal Disease Center, Greenport, NY 11944, USA 5. Universitat Pompeu Fabra, Barcelona, Spain 08003 6. Kansas State University Manhattan, KS 66502, USA*
59	Fang, Ying	Kansas State University	RNA stem-loop structures and conserved regions in ORF6 are important for the replication of porcine reproductive and respiratory syndrome virus	Pengcheng Shang1, Yanhua Li1,2, Ian Brierley2, Andrew E. Firth2 and Ying Fang1 *1Kansas State University, Manhattan, USA; 2Department of Pathology, University of Cambridge, Cambridge, UK*

60	Fang, Ying	Kansas State University	A naturally occurring cross order recombinant of enterovirus and torovirus	Pengcheng Shang1, Saurav Misra2, Ben Hause1 and Ying Fang1 *1Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506; 2Department of Biochemistry & Molecular Biophysics, Kansas State University, Manhattan, KS 66506*
61	Fang, Ying	Kansas State University	A dual ribosomal frameshifting mechanism transactivated by an arterivirus protein and host cellular factors	Yanhua Li1,2*, Andrew E. Firth2, Ian Brierley2, Eric Snijder3, Jens Kuhn4, Ying Fang1 *1Kansas State University, Manhattan, Kansas, USA; 2University of Cambridge, Cambridge CB2 1QP, United Kingdom;3Molecular Virology Laboratory, Department of Medical Microbiology, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands; 4Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, USA.*
62	Petrovan, Vlad	Kansas State University	Antigenic and structural properties of African swine fever virus (ASFV) p30	V. Petrovan1*, M. V. Murgia1, Y. Fang1, R. R. R. Rowland1 *1Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan*
Swine genetics/genomics topics (NSIF-Related abstracts that don't involve infectious disease)				
63	Putz, Austin	Iowa State University	Quantifying Resilience Utilizing Feed Intake Data in A Natural Challenge Model for Disease Resilience in Wean-to-Finish Pigs	A. M. Putz1*, M. K. Dyck2, PigGen Canada3, J. C. S. Harding4, F. Fortin5, G. S. Plastow6, and J. C. M. Dekkers1 *1 Department of Animal Science, Iowa State University, Ames, IA 50011, USA 2 Department of Agricultural, Life, and Environmental Sciences, University of Alberta, AB, Canada 3 PigGen Canada, Guelph, ON, Canada 4 Large Animal Clinical Sciences, University of Saskatchewan, Canada 5 Centre de développement du porc du Québec inc. (CDPQ), Quebec, Canada 6 Livestock Gentec Centre, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada*
64	Mauch, Emily	Iowa State University	Genetic divergence and selection signatures in two selection experiments for residual feed intake	E.D. Mauch1*, B. Servin2, H. Gilbert2, and J.C.M. Dekkers1 *1 Department of Animal Science, Iowa State University, Ames, IA, USA 2 INRA, GenPhySE, Castanet-Tolosan, France*

65	Ferring, Cassandra	Iowa State University	Genetic Analysis of Reproductive Traits in Commercial Sows in Health Challenged Herds	C.L. Ferring1*, B.E. Mote2, P. Willson3, J.C.S Harding4, G.S. Plastow5, J.C.M. Dekkers1, and N.V.L Serão1, and PigGen Canada6 *1. Department of Animal Science, Iowa State University, Ames, IA 2. Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 3. Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, SK, Canada 4. Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, SK, Canada 5. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada 6. PigGen Canada, Guelph, ON, Canada*
Other (Virus-receptor interactions)				
66	Stoian, Ana	Kansas State University	CD163 PSTII domain is required for PRRSV-2 infection	A.M.M. Stoian*, R.R.R. Rowland *Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS*
67	Stoian, Ana	Kansas State University	Peptide sequence domains in SRCR5 of CD163 that contribute to recognition by PRRSV-2	A.M.M. Stoian*, R.R.R. Rowland *Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS*
68	Suleman, Muhammad	University of Saskatchewan	Evaluation of porcine trophectoderm cell line susceptibility to porcine reproductive and respiratory syndrome virus-2 infection and its possible role in viral transmission across maternal-fetal interface	M. Suleman1*, C.M. Malgarin1, S.E. Detmer1, D.J. MacPhee1, J.C.S. Harding1 *1Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.*

Principles and applications of genetics and genomics to improve animal health: An online class at destination of swine experts and professionals

Perle Boyer[1], Montserrat Torremorell[1], Jack Dekkers[2]
[1]University of Minnesota, College of Veterinary Medicine, St. Paul, MN; [2]Iowa State University, College of Agriculture and Life Sciences, Ames, IA

There has been a significant shift in genetic selection programs to include genetic strategies to improve animal health. These strategies range from single marker selection of pigs resistant to F-18 and K-88 E.coli to whole genome approaches against multiple traits and diseases. Efforts to genetically select animals that show resistance against PRRSV infections are undergoing and show promising results. However, genetics is a fast-evolving field that can be overwhelming. Genetic selection and genomic programs are complex and require a comprehensive understanding of the methods and strategies employed by breeding stock companies to deliver genetic improvement. Unfortunately, veterinarians are seldom exposed to genetics during their training years. The University of Minnesota in collaboration with Iowa State University is developing an online course centered on educating animal health professionals on genetic selection programs, and the application of genetic improvement strategies to enhance animal health.

This on-line course is part of the project "***Genetically Improving Resistance of Pigs to PRRS Virus Infections*****"** by Dekkers and collaborators .

Goal: To train veterinarians, geneticists and industry stakeholders on basic concepts of genetic selection and genomics and how to apply them to improve animal health

Format: Class materials are designed by a team of experts on genetics, health and education. Materials will include audio files, essential reading, presentations by reference people in the field.

By the end of the course, a student will be able to:

-Synthesize the epidemiologic and biological characteristics of an infectious disease.
-Explain the bases of a genetic improvement program and how a program works.
-Compare and contrast the various genetic selection and genomic tools and approaches and how they can be used to improve health.

Viral genetics and vaccines

Jay G. Calvert
Zoetis, Veterinary Medicine R&D, Kalamazoo, MI, USA.

The performance of a commercial modified live viral vaccine depends on many factors. Some of these factors are beyond the control of the vaccine designer, including the health, genetics, and nutrition of the host animal, environmental conditions, immunological history of the herd, and co-infections with pathogens and/or other vaccines. In contrast, the vaccine designer has the ability to determine the genetic characteristics of the vaccine virus in two ways: (1) Careful selection of the starting virus isolate (starting genetics), and (2) choosing the method and the endpoint of attenuation (final genetics). This presentation will focus on the theoretical and practical aspects of these two processes, from the point of view of a professional vaccine designer employed by a major animal health company.

The selection of the most relevant field strain to be the parent of a new vaccine can be difficult process, and must be made at the very beginning of the project. It can easily take 5-7 years to move from strain selection to USDA license, especially for a virus like PRRSV that causes both respiratory and reproductive diseases. Choosing the most recent "hot" strain is a risky strategy, since these new variants appear and disappear frequently, and do not necessarily leave behind an economically important clade of descendants after 5-7 years. In addition, one must consider the pattern of strain evolution for the virus in question. In the case of PRRSV, there is an ever increasing diversity of new strains that are not closely related to each other. The ancestral strains are not necessarily replaced by the new strains, but can persist and give rise to yet more variation. Since maximizing cross-protection against diverse field strains is critically important for a PRRS vaccine, selection of an ancestral strain can maximize the degree of genetic similarity between the vaccine and all current field strains, and thereby maximize the number of immunological epitopes shared by the vaccine and challenge viruses. Usually the inherent virulence and immune dysregulation characteristics of the starting virus are not major factors, since these properties can be altered during the attenuation process.

For conventional modified live vaccines, the attenuation process involves isolating the field virus and adapting it to an appropriate cell line. Once adaptation to the new cell line has occurred, many serial passages are performed, usually in parallel. Periodically the virus is tested for safety in pigs, and for efficacy against one or more challenge viruses. The attenuation process is generally concluded once the candidate vaccine has become safe enough to pass the required USDA safety tests, since additional attenuation risks loss of efficacy (over attenuation). During attenuation, two distinct types of genetic changes occur in the genome of the virus: (1) Strong selection for rapidly occurring mutations that allow the virus to replicate well on the new cell line, which is generally quite different from the primary porcine alveolar macrophage in the natural host, and (2) Gradual accumulation of mutations in viral genes that are not needed for replication in cell culture, including those genes that cause immune dysfunction in the pig. Since one cannot predict which random mutations will occur in what order, each parallel attenuation attempt will give a different result.

Survival of viral pathogens in animal feed ingredients under transboundary shipping models

Scott A. Dee[1*], Fernando V. Bauermann[2], Megan C. Niederwerder[5,6], Aaron Singrey[2], Travis Clement[2], Marcelo de Lima[2,3] Craig Long[2], Gilbert Patterson[4], Maureen A. Sheahan[5], Ana M. M. Stoian[5], Vlad Petrovan[5], Cassandra K. Jones[7], Jon De Jong[1], Ju Ji[8], Gordon D. Spronk[1], Jane Christopher-Hennings[2], Jeff J. Zimmerman[9], Raymond R.R. Rowland[5], Eric Nelson[2], Paul Sundberg[10], Diego G. Diel[2.]

[1]Pipestone Applied Research, Pipestone Veterinary Services, Pipestone, MN, USA. [2]Animal Disease Research and Diagnostic Laboratory, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA. [3]Faculdade de Veterinaria, Universidade Federal de Pelotas, RS, Brazil. [4]Center for Animal Health in Appalachia, Lincoln Memorial University, Harrogate, TN, USA. [5]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA. [6]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS, USA. 7Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS, USA. [8]Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA, USA. [9]Veterinary Diagnostic Population Animal Medicine, Iowa State University, Ames, IA, USA.[10]Swine Health Information Center, Ames, IA, USA.

The goal of this study was to evaluate survival of important viral pathogens of livestock in animal feed ingredients imported daily into the United States under simulated transboundary conditions. Eleven viruses were selected based on global significance and impact to the livestock industry, including Foot and Mouth Disease Virus (FMDV), Classical Swine Fever Virus (CSFV), African Swine Fever Virus (ASFV), Influenza A Virus of Swine (IAV-S), Pseudorabies virus (PRV), Nipah Virus (NiV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Vesicular Disease Virus (SVDV), Vesicular Stomatitis Virus (VSV), Porcine Circovirus Type 2 (PCV2) and Vesicular Exanthema of Swine Virus (VESV). Surrogate viruses with similar genetic and physical properties were used for 6 viruses. Selected surrogates belonged to the same virus families as the target pathogen, and included Senecavirus A (SVA) for FMDV, Bovine Viral Diarrhea Virus (BVDV) for CSFV, Bovine Herpesvirus Type 1 (BHV-1) for PRV, Canine Distemper Virus (CDV) for NiV, Porcine Sapelovirus (PSV) for SVDV and Feline Calicivirus (FCV) for VESV. For the remaining target viruses, the actual pathogens were used. Virus survival was evaluated using Trans-Pacific or Trans-Atlantic transboundary models involving representative feed ingredients, transport times and environmental conditions, with samples tested by PCR, VI and/or swine bioassay. SVA (representing FMDV), FCV (representing VESV), BHV-1 (representing PRV), PRRSV, PSV (representing SVDV), ASFV and PCV2 maintained infectivity during transport, while BVDV (representing CSFV), VSV, CDV (representing NiV) and IAV-S did not. Notably, more viruses survived in conventional soybean meal, lysine hydrochloride, choline chloride, vitamin D and pork sausage casings. These results demonstrate survival of certain viruses in specific feed ingredients (“high-risk combinations”) under conditions simulating transport between continents and provides further evidence that contaminated feed ingredients may represent a risk for transport of pathogens at domestic and global levels.

Using genetic selection and genomics to combat infectious disease

Jack C. M. Dekkers
Department of Animal Science, Iowa State University

Over the past decades, genetic improvement has been a very important contributor to improved productivity in the swine industry. Recent development in the use of genomic information is further enhanced genetic selection for productivity. Although host response to disease has been shown to be under partial genetic control of the host, genetic improvement of host response to infectious disease or resilience has been hampered by the pyramidal structure of swine breeding programs, with high biosecurity in the nucleus population where selection occurs, along with the limited availability of traits or tools that can be used to identify and select pigs with better genetics for host response to disease. The availability of genomics, however, provides opportunities for such selection, provided appropriate phenotypic and/or genomic information is available. The purpose of this presentation is to present the current state-of-the-art in strategies for genetic improvement of host response to disease and opportunities for the future.

Emerging swine disease diagnostics and characterization: connecting basic research to real-world applications

Ying Fang, Kansas State University

Emerging swine infectious diseases have increased significantly during the past decade. Timely control and prevention of new disease outbreak require integration of a wide range of expertise and application of modern technologies. Molecular tools, including next generation sequencing and pathogen microarray, have played a key role in discovery of emerging pathogens. Recently, with a collaborative effort among researchers, diagnosticians, and field practitioners, we have identified and characterized a panel of emerging viral pathogens, including atypical porcine pestivirus, porcine circovirus, porcine parainfluenza virus, Seneca Valley virus (SVV), and recombinant enterovirus/torovirus (EVG-ToV). Key diagnostic reagents (monoclonal antibodies, antigens, etc.) have been generated and applied in field use for detecting this panel of pathogens. With the support from Swine Health Information Center, diagnostic assays have been developed (are under developing) for these emerging pathogens. We further applied basic research tools to facilitate in depth characterization of these viral pathogens; particularly, the application of reverse genetics system for SVV and recombinant EGV-ToV accelerated the structure-function analysis of viral RNA and protein sequences. This system also facilitates studies into host immune responses and viral immune evasion and pathogenesis. In addition, molecular mechanisms underlying the emergence of new pathogens have been explored. This is spotlighted by the study of a novel case of cross order genetic recombination between enterovirus and torovirus. These studies represent our collaborative effort to apply contemporary knowledge and technologies for emerging infectious disease control and prevention.

Emergence of a novel swine enteric alphacoronavirus (SeACoV) in southern China.

Yao-Wei Huang[1]*, Yongfei Pan[2], Bin Wang[1], Pan Qin[1], Yong-Le Yang[1], Yanhua Song[2]
[1]Department of Veterinary Medicine, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China; [2]Hog Production Division, Guangdong Wen's Foodstuffs Group Co, Ltd, Xinxing, Guangdong, China.

Outbreaks of diarrhea in newborn piglets without detection of transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV), have been recorded in a pig farm in southern China since February 2017. Isolation and propagation of the pathogen in cell culture resulted in discovery of a novel swine enteric alphacoronavirus (tentatively named SeACoV) related to the bat coronavirus HKU2 identified in the same region a decade ago. Specific fluorescence signal was detected in cells infected with SeACoV by using a positive sow serum collected in the same farm, but not by using TGEV-, PEDV- or PDCoV-specific antibody. Electron microscopy observation demonstrated that the virus particle with surface projections was 100 to 120 nm in diameter. Complete genomic sequencing and analyses of the SeACoV prototype GD-01/2017 strain indicated that the extreme amino-terminal domain of the SeACoV spike (S) glycoprotein associated with the enteric tropism had an extremely high variability, harboring 75-amino-acid (aa) substitutions and a 2-aa insertion, compared to that of HKU2, which is likely responsible for the extended host range or interspecies transmission. The isolated GD-01/2017 virus was infectious in pigs when inoculated orally into 3-day-old newborn piglets, with induction of clinical signs of diarrhea similar to PED and detection of fecal virus shedding, confirming that it is a novel swine enteric alphacoronavirus representing the fifth porcine coronavirus. Further detailed characterization of this emerging virus is in progress.

2017 North American PRRS Symposium in Chicago, IL. Dec.1-3, 2018

Probing mechanisms of PRRS resistance

Joan K. Lunney
USDA-ARS, BARC, APDL, Building1040, Beltsville 20705, MD, USA

Our goal is to assess the role of host genetics in determining efficacy of porcine reproductive and respiratory syndrome (PRRS) vaccine responses and of resistance/susceptibility to PRRS virus (PRRSV) infection or coinfection with PRRSV and porcine circovirus (PCV2). This talk will focus on our efforts to identify mechanisms of anti-PRRS responses in the young pig. Our early research, as part of the PRRS Host Genetics Consortium, involved experimental infection trials of large numbers of commercial nursery pigs. Based on deep phenotyping and genomics a major gene, GBP5 on swine chromosome 4 (SSC4), was identified as the putative causative mutation associated with decreased viral load and improved weight gain. A genetic marker (WUR) near GBP5 can be used by industry for selection of PRRS resistant pigs (with just 1 copy of the "B" allele conferring resistance). Our later trials affirmed the positive effect of this WUR allele on pig responses to a second type 2 PRRSV strain and to PRRS vaccination and co-infection with PRRSV and PCV2b. We pursued gene (3'RNA-seq and NanoString assays) and protein expression analyses to explore the blood transcriptome and identify mechanisms and biomarkers involved in controlling anti-PRRS responses. For these studies we used the nursery pig model in which 50% of 200 pigs received a PRRS vaccination (Vx); after 28 days all were challenged with PRRSV and PCV2 coinfection. Blood RNA was probed to identify differentially expressed (DE) genes associated with anti-viral response phenotypes. Samples from 7 sib sets (AA or AB for the WUR SSC4 marker; with/without Vx; 28 pigs total) were used for RNA preparation from Tempus tube-preserved blood samples collected following vaccination (4, 7, 11, 14, 21 days) and challenge (0, 4, 7, 11, 14, 21, 28 days). Differential gene expression (DE gene) was determined using both 3'RNA-seq and a swine immune-focused, 230 gene NanoString codeset (developed at BARC based on biomarkers previously predicted to alter PRRS resistance and susceptibility). Data will be presented on DE gene expression, and particularly on immune related genes, that may contribute to controlling PRRS vaccination and PRRSV and PCV2 coinfection challenge responses. Further studies were aimed at identifying genes and biomarkers that regulate effective vaccine responses and anti-viral phenotypes. This NanoString codeset was used to probe mechanisms of PRRSV persistence in tonsil and to assess PRRSV resistance versus susceptibility of fetuses within litters after congenital PRRSV infection. These studies should reveal new markers to use for improved PRRS resistance. Overall genetic selection can be a valuable tool for the swine industry to combat PRRS. These studies were completed by members of the PRRS Host Genetics Consortium with recent funding (USDA ARS, USDA NIFA grant # 2013-68004-20362; National Pork Board grant #14-224 and Genome Canada).

Perspectives on the development of global efforts for disease control.

Animal diseases can cause serious social, economic and environmental damage, impact on animal welfare and in some cases directly threaten human health. An increasing number of the major disease problems or threats faced by the livestock industry are of a global nature. Global challenges need global solutions and these can only be achieved in the required timeframe through a common and coordinated research effort. To achieve this an international forum of R&D programme owners/managers and international organisations was established to share information, improve collaboration on research activities and work towards common research agendas and coordinated research funding on the major animal diseases affecting livestock production and/or human health. Specifically STAR-IDAZ International Research Consortium aims to coordinate research at international level to contribute to new and improved animal health strategies for at least 30 priority diseases/infections/issues. Twenty-two Partners from 14 countries including one international research organisation (ILRI), one charity, the European Commission and three industry bodies have signed the Letter of Intent to participate. The target deliverables include candidate vaccines, diagnostics, therapeutics, procedures and key scientific information/tools to support risk analysis and disease control. The preliminary list of target diseases include Porcine Reproductive and Respiratory Syndrome, Influenza, Foot and Mouth Disease, African Swine Fever, Corona viruses and the bacterial component of Porcine Respiratory Disease Complex. A number of these conditions have been identified elsewhere as diseases where the development of new or improved vaccines could reduce antimicrobial use in pig production.

Resilience and PRRS in a natural disease challenge model

G. S. Plastow

Dept. of Agricultural, Food & Nutritional Science, University of Alberta

Disease resilience has been defined as the ability of animals to continue to perform in the face of infection challenges. Genetic selection for disease resilient pigs cannot be achieved in the high health nucleus units at the top of the swine breeding pyramid. In this case our aim was to establish a challenge model for growing pigs that reflected the multi-agent nature of disease at the commercial level. Resilient pigs would be those that when infected would be able to mount an efficient immune response, recover relatively quickly and continue to grow efficiently to market weight. One of the major aims of the project is to identify predictors for resilience. Ideal predictors would be those that can be measured in young animals in high health facilities such as genetic nucleus herds. Samples are collected for a large array of different assays prior to introducing pigs to the model. The challenge was developed at a test station in Canada by introducing common pathogens via “seeder” pigs from different low health status barns along with naïve pigs. Once established a new batch of naïve pigs is introduced every three weeks. The challenge includes three strains of type 2 PRRSV and swine influenza, as well as bacterial agents such as *Mycoplasma hyopneumoniae, Haemophilus parasuis,* and *Streptococcus suis.* Growth, feed intake and treatments are recorded and heritable variation has been observed for performance as well as morbidity and mortality. More than 2,000 pigs have been introduced to the challenge model to date.

The challenge model was established by a team including Michael Dyck (University of Alberta), Frédéric Fortin (Centre de développement du porc du Québec inc.), Jack Dekkers (Iowa State University), John Harding (University of Saskatchewan) and PigGen Canada (https://piggencanada.org/).

PRRS overview and description of disease phenotypes and models for genetic research

Raymond (Bob) Rowland, Department of Diagnostic Medicine and Pathobiology,
Kansas State University

Previously known and newly identified members of the family, *Arteriviridae*, were recently organized into five new genera. In this new scheme, lactate dehydrogenase-elevating virus (LDV) and PRRSV are grouped together as the only members of the genus, *Rodarterivirus*. PRRS viruses of European and North American origin are designated as species, PRRSV-1 and PRRSV-2, respectively. PRRSV possesses a complicated surface structure which is the source of many of its unique pathogenic properties. The virus envelope is dominated by a heterodimer composed of GP5 and the matrix (M) protein. Minor envelope proteins include a heterotrimer of GP2, GP3, and GP4, along with two small proteins, E and ORF5a protein. The GP2,3,4 heterotrimer interacts with the CD163 receptor on the surface of macrophages. In 2007, the National Pork Board (NPB) formed the PRRS Host Genetics Consortium (PHGC). The goal of the PHGC is to identify genomic markers that lessen the impact of PRRS on the commercial pig industry. In the first PRRS model developed by the PHGC, groups of 200 three- to four-week-old nursery pigs are infected with a well-characterized PRRSV isolate. The piglets are followed for 42 days after infection; a period covering the acute phase of infection and early onset of persistence. Piglets and parents are genotyped for >60,000 single-nucleotide polymorphisms (SNPs) using the Porcine SNP60 Bead Chip. PRRSV-related disease traits, or phenotypes, are measured. These include virus load, weight gain, anti-nucleocapsid (N) antibody, virus neutralizing activity, morbidity, and mortality. Deeper phenotyping approaches, such as RNA-Seq, are being applied to the infection models. Based on these results, we have identified several regions of the swine genome that influence phenotypic responses, including *Sus scrofa* chromosome (SSC) 4 (weight gain and virus load) and SSC 7 (antibody response). The SSC 4 marker, WUR10000125 (WUR), locates to a 1.0 Mb region occupied by members of the guanylate binding protein (GBP) family of interferon-inducible genes. Each GBP possess a globular head with guanine binding and GTPase activities, followed by a leucine-rich tail. GBP5, which possess an additional isoprenylation motif on the C-terminal end, is the protein affected by the WUR mutation. GBP5 anchored to the Golgi is predicted to affect PRRSV infection at several levels. A second infection model is the translation of the PHGC nursery model to the field. Pigs are followed from the nursery all the way to finish in pig herds with endemic PRRS. A third model, designed to reproduce PRRSV-associated disease, incorporates co-infection of pigs with PRRSV and PCV2. Additional phenotypic measurements include morbidity and mortality. The experimental or natural infection of almost 5,000 pigs has resulted in an improved understanding of the dynamics of PRRSV infection at the population level. The identification of genomic markers linked with specific disease traits creates the opportunity to select for pigs with improved responses to PRRSV infection.

Acknowledgements: Significant portions of this work were supported by USDA NIFA "Genetically Improving Resistance of Pigs to PRRS Virus Infection", Award No. 2013-68004-20362; and several NPB awards supporting the PHGC.

New PRRS disease phenotypes as vaccine and genetic improvement targets

Andrea Doeschl-Wilson, The Roslin Institute, University of Edinburgh, Scotland UK

Disease resistance, which may be defined as the ability of a host to counteract pathogen entry or within host replication, has been described as the number 1 target trait for genetic improvement. However, for diseases such as PRRS, to which no natural complete resistance seems to exist, two other host traits influence the health and performance status of pig herds exposed to the PRRS virus: These are *tolerance*, the ability of a pig to maintain high performance levels despite infection, and *infectivity*, the ability of a pig, once infected, to transmit the infection to other pigs. To date, the mechanisms and genetic regulation of tolerance and infectivity are largely unknown. Determining the genetic (co)regulation of these traits is crucial in order to prevent that genetic selection for resistance does not accidentally generate tolerant PRRSV superspreaders.

The PRRS Host Genetics Consortium (PHGC) dataset, in which ~1600 genotyped commercial cross-bred growing pigs were infected in 8 separate batches with a virulent PRRSV type-2 isolate, was used to explore tolerance and infectivity characteristics of pigs with known genetic difference in resistance to PRRSV. Using random regression models, we show that pigs that were previously identified to vary genetically in their resistance to PRRSV, also exhibit genetic variation in tolerance. Moreover, the beneficial allele of single nucleotide polymorphism (SNP) WUR10000125 (WUR) on chromosome 4 not only seems to confer large effects on resistance, but also positive effects on tolerance. Moreover, statistical analyses of the viremia profiles of these PHGC pigs revealed substantial differences in individual viremia profile characteristics with potential effects on PRRSV transmission. Whereas viremia profiles were uniphasic for most pigs (i.e. viremia levels steadily declined towards undetectable levels after the peak virus load was reached at around 4-10 days post infection), about 20% of pigs exhibited a viremia rebound within 4-6 weeks of infection. The presence of such rebounders in a pig herd are of concern given their potential impact on disease transmission and the efficacy of control strategies. Rebound was found to be not heritable, suggesting that rebound is not under host genetic control. To investigate this further, we developed a mathematical model of the within-host immune response to PRRS virus infection and fitted it to the PHGC dataset. The model results imply that viremia rebound can indeed occur as a result of difference in the immune competence of pigs alone and offers, for the first time, insights into potential causative immune mechanisms generating rebound. In particular, the model results suggest that vaccines or genetic control strategies promoting strong neutralizing and cytolytic responses, ideally associated with low apoptotic activity and cell permissiveness, would successfully prevent rebound. Future studies that validate these theoretical findings and provide a deeper understanding of the genetics and mechanisms underlying PRRS transmission dynamics are warranted.

The work was carried out as a collaboration between the PHGC with the Roslin Institute (Doeschl-Wilson group), Iowa State University (Dekkers group), Wageningen University (Mulder group) as the lead academic partners for exploring tolerance genetics, and INRIA (Touzeau group) for the mechanistic modelling.

A case-control study investigating the early months of the porcine epidemic diarrhea (PED) outbreak in Canadian swine herds

A.M. Perri *[1], Z. Poljak[1], C. Dewey[1], J.C.S. Harding[2], T.L. O'Sullivan[1].
Department of Population Medicine, University of Guelph[1], Canada; Large Animal Clinical Sciences, University of Saskatchewan[2], Canada.

Porcine epidemic diarrhea (PED) was first reported in Canada in January 2014 on a southwestern Ontario farm. Preliminary epidemiological investigations of the initial Ontario PED cases identified contaminated feed originating from a single feed company (FC) as the probable source of infection. It was subsequently determined that the spray-dried porcine plasma (SDPP) from FC was found to reproduce the infection under experimental conditions, however the complete feed containing the contaminated SDPP did not. This finding lead to FC voluntarily recalling their nursery feed products containing SDPP in February 2014. The objective of this study was to use a case-control epidemiological approach to evaluate the role of feed in the early phase of the PED outbreak in Canada, after controlling for potential confounders.

The period of interest for this study was from January 22nd to March 1st 2014. A total of 22 Canadian swine herds (n=9 case herds; n=13 control herds) agreed to participate in the case-control study. A case was defined as any Canadian swine herd, identified within the study period, with typical clinical signs (acute onset and severe neonatal watery diarrhea with high mortality) and a confirmed positive diagnostic test (RT-PCR) result for PED virus. Control herds were randomly selected and matched to case herds based on province, herd size, herd type, and time (initial day of PED clinical signs for the matched case herd and 30 days prior to this date). A questionnaire was administered to each producer for the case and control herds. A mixed multivariable logistic regression model, with the matched herds accounted for as a random effect, was constructed to assess associations between the number of pig/people movements onto and off farms, the number of feed deliveries received, whether a herd received any feed from FC, the quantity of potentially contaminated (PC) feed received from FC, and herd biosecurity measures with the odds of a PED outbreak.

More case herds received feed from FC (n=8/9) than control herds (n=3/13). The mean amount of PC feed received from FC was higher ($P<0.05$) for case herds (98.89 tonnes) compared to control herds (6.35 tonnes). The odds of a PED outbreak was 38 times greater for herds that received PC feed from FC ($P=0.007$) than herds that did not receive PC feed from FC. In contrast, herd biosecurity measures and the frequency of movements onto and off of sites through people, other livestock and animals, and different fomites were not associated ($P>0.05$) with a herd-level outbreaks of PED during the study period.

In conclusion, this study supports the role of PC feed from FC as a significant risk factor for PED viral transmission during the early phase of the Canadian outbreak, after adjusting for other possible risk factors.

Acknowledgements: Thank you to all participating producers, veterinarians and collaborators. Funding was provided by OMAFRA, Ontario Pork, and NSERC- CRD.

Airborne inactivation of porcine reproductive & respiratory syndrome virus (PRRSv) by a packed bed dielectric barrier discharge non-thermal plasma.

Tian Xia[1], My Yang[2], Ian Marabella[3], Darrick Zarling[3], Bernard Olson[3], Montserrat Torremorell[2], Herek L. Clack[1*]

[1]Dept. of Civil & Environmental Engineering, University of Michigan, Ann Arbor, MI, [2]Dept. of Mechanical Engineering, University of Minnesota, Twin Cities, Minneapolis, MN, [3]Dept. of Veterinary Population Medicine, University of Minnesota, Twin Cities, St. Paul, MN.

Porcine reproductive and respiratory syndrome virus (PRRSv) has been detected in air more than 9 km downwind of infected swine. Applying HEPA filtration to ventilation air supplied to hog barns involves structural retrofits to buildings that can be costly, in addition to the periodic replacement of used filters. Non-thermal plasmas (NTPs) are electrical discharges comprised of reactive radicals and excited species that inactivate viruses and bacteria. Our previous experiments using a packed bed non-thermal plasma reactor demonstrated effective inactivation of bacteriophage MS2 as a function of applied voltage and power, ranging from less than one-log inactivation at < 20 kV and a few watts to greater than two-log inactivation at 30 kV. The present study examined the effectiveness of the same reactor in inactivating aerosolized PRRSv. A PRRSv solution containing ~10^5 $TCID_{50}$/ml was aerosolized at a rate of 3 ml/min by an air-jet nebulizer and introduced into air flows of 5 or 12 cfm followed by NTP exposure in the reactor. Twin impingers upstream and downstream of the reactor collected samples of the virus-laden air flow. Subsequent $TCID_{50}$ assay and quantitative polymerase chain reaction (qPCR) analyses of the collected samples determined the pre- and post-treatment abundance of infective PRRSv (in $TCID_{50}$/ml) as compared with the abundance of the viral genome (qPCR), whether infective or rendered inactive by NTP exposure. An optical particle sizer measured upstream and downstream aerosol size distributions, giving estimates of aerosol filtration by the reactor. The results showed that PRRSv was inactivated to a similar degree as MS2 at the same conditions, with the 1.3-log inactivation of PRRSv achieved at 20 kV and 12 cfm air flow rate. Differential pressure across the reactor was minimal compared to HEPA filters and a consumer-grade ozone filter reduced residual ozone concentrations down to levels commensurate with the ambient laboratory environment. The results demonstrate the potential of properly optimized NTPs for preventing infiltration of PRRSv into hog barns with ventilation air.

Field evaluation of a modified-live PRRS Type II vaccine in a positive herd in Mexico

G. Bernal[1], R.Garcia[1], A.F. de Grau[2*], B.Thacker[3], R. Jolie[4]
[1]MSD México, México City México; [2] Intervet Canada Corporation, Kirkland, Quebec, Canada; [3]Merck Animal Health, DeSoto, KS, USA; 4 Merck Animal Health, Madison, NJ , USA.

Introduction

For more than two decades, porcine reproductive and respiratory syndrome virus (PRRS) has caused severe economic losses to the Mexican pork industry. The disease is widespread and serological surveys demonstrated that farms have been infected since 1992. Herd-to-herd biosecurity and PRRS specific control programs vary among swine operations in Mexico. Efforts to eradicate the disease through regional control and depopulation of herds have yielded mixed results and acute outbreaks are still common. Furthermore, viral dissemination between farms is high, resulting in the emergence of unique strains due to recombination and evolution. The preferred approach for PRRS control in this region has been to maintain a uniform level of immunity through a combination of vaccination and live virus inoculation. However, these practices have been unable to uniformly stop PRRS circulation and disease outbreaks still occur.

Objective

To evaluate the efficacy in young pigs of a recently approved PRRS vaccine (Prime Pac® PRRS, MSD Animal Health, Boxmeer, Netherlands) in a highly PRRS challenged, single-strain affected operation located in central Mexico by comparison with the farm's previous vaccination program. The specific goal for the Prime Pac PRRS vaccination was to reduce mortality and increase productivity parameters of nursery pigs raised in this operation.

Material and Methods

The study was conducted in a nursery operation that received the weekly production from a 1,600 sow farm. The study involved 10 weekly groups and a total of 8,000 pigs; 5 groups and 4,000 pigs that were not vaccinated and 5 subsequent groups and 4,000 pigs that received Prime PAC PRRS intramuscularly at 14 days of age. The pigs were housed in three nursery sites for 6-7 weeks. Production parameters evaluated in the nursery phase included mortality, daily weight gain and feed conversion.

Results

Mortality rate following vaccination with Prime Pac PRRS declined from 8.3% to 2.5% in nursery, a 69.9% reduction. Daily gain increased by 7.3% (from 439 to 471 grams/day) while feed conversion improved by 0.5% (from 1.71 to 1.70 feed/gain).

Conclusion

Vaccination is an important tool for controlling PRRS in endemically infected herds. In this operation, Prime Pac® PRRS significantly improved farm profitability based on reduced mortality, increased daily weight gain and improved feed conversion compared with not vaccinated piglets.

Importance of cellular cholesterol for the entry process of porcine nidoviruses

J.H. Jeon*, C. Lee

Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) represent emerging and re-emerging porcine nidoviruses that continue to threaten pork-producing countries around world, leading to huge financial losses to the global swine industry. Cholesterol, a major constituent of lipid rafts, maintains the tight packaging of sphingolipids, and several proteins are partitioned into these microdomains. Cholesterol depletion destroys this structural order, leading to disorganization of lipid raft microdomains and dissociation of bound proteins. Therefore, plasma membrane cholesterol plays important roles in the infection processes of various non-enveloped and enveloped viruses. Although cholesterol is known to affect the replication of a broad range of viruses *in vitro*, its significance and role in porcine nidovirus infection remains to be elucidated. In the present study, therefore, we investigated the requirement for cellular or/and viral cholesterol and its mechanism of action in porcine nidovirus infection. Independent depletion of cholesterol from the plasma membrane of target cells by methyl-β-cyclodextrin (MβCD) significantly impaired PRRSV and PEDV infection in a dose-dependent manner. These inhibitory effects on viral replication were partially reversible by replenishment with exogenous cholesterol. In contrast, porcine nidoviruses were shown to be resistant to pharmacological reduction of cholesterol content in the viral envelope. These data indicated that cholesterol-enriched microdomains are essential for PRRSV and PEDV in the cellular membrane, but not in the viral membrane. The antiviral activity of MβCD on porcine nidovirus infection was found to be predominantly exerted when used as a treatment pre-infection or prior to the viral entry process. Further experiments revealed that pharmacological depletion of cellular cholesterol primarily interferes with virus binding and penetration and subsequently influences post-entry steps of the PRRSV and PEDV replication cycle, including viral RNA and protein biosynthesis and progeny virus production. In addition, pharmacological sequestration of cellular cholesterol suppressed the replication of a newly emerged porcine deltacoronavirus (PDCoV), suggesting its essential function common to nidovirus infection. Altogether, our results suggest that cholesterol in the cellular membrane is critical for porcine nidovirus entry, and that disruption of the cholesterol-dependent entry process may be an excellent therapeutic option for nidovirus infection in human or veterinary subjects.

Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid Protein Activates NF-kappa B through Binding to PIAS1

H. Ke*, S. Lee, D. Yoo.
Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL. 61802.

Porcine reproductive and respiratory syndrome virus (PRRSV) triggers the onset of inflammation during infection, and pro-inflammatory cytokines including interleukin (IL)-1 beta, IL-6, IL-8, and TNF-alpha have been shown to be upregulated in virus-infected porcine alveolar macrophages (PAMs), suggesting the activation of NF-kappa B by PRRSV. We show in the present study that in cells infected with PRRSV or cells expressing PRRSV nucleocapsid (N) protein, the RelA (p65) subunit of NF-kappa B was increasingly phosphorylated and translocated to the nucleus to result in the activation of NF-kappa B. By yeast two hybrid screening using N as a bait, the protein inhibitor of activated STAT1 (PIAS1) was identified from PAMs as a molecular partner of N. PIAS1 binds to RelA and prevents NF-kappa B activation by interfering RelA-DNA binding in the nucleus and thus is a negative regulator of NF-kappa B. The N binding to PIAS1 was confirmed by co-immunoprecipitation and colocalization studies. To map the binding domains of the PIAS1 and PRRSV N proteins, deletions and truncations were made, and the binding domain of PIAS1 was mapped to the N-terminal fragment. This domain was shown to be the sole domain that binds to RelA to prevent it from binding to kappa B sites, demonstrating the correlation between the N-PIAS1 interaction and the NF-kappa B activation. For N, the region between 37 and 72 amino acids was found to interact with PIAS1, and this region overlapped with the nuclear localization signal of N. And this region was also shown to activate the NF-kappa B signaling in the NF-kappa B promoter-based reporter assay, confirming the correlation between N-PIAS1 binding and NF-kappa B activation. By competition assay, we show that, compared to RelA, the binding of PIAS1 to N is preferred, and this interaction was validated in PRRSV-infected cells. Taken together, PRRSV N binds PIAS1 to release NF-kappa B from PIAS1, and as a consequence, NF-kappa B becomes activated. This is a novel strategy of PRRSV for NF-kappa B signaling activation.

Inhibition of type III interferons in the intestinal epithelial cells by porcine epidemic diarrhea virus and innate immune evasion

Q. Zhang1*, H. Ke1, T. Fujita2, D. Yoo1
[1]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[2]Institute for Virus Research, Kyoto University, Kyoto, Japan

Porcine epidemic diarrhea (PED) is a highly contagious acute enteric disease characterized by vomiting, watery diarrhea, and severe dehydration accompanied by high mortality in suckling piglets. PEDV emerged in the US in 2013 and became endemic, posing significant economic concerns. PEDV infects epithelial cells of the small intestine. Accumulating evidence suggests that type III interferon (IFN-lambda) plays a key role to maintain the innate antiviral state in the mucosal epithelial cell surface in the gut, and in turn, enteric viruses may have evolved to evade the IFN-lambda responses during infection. To study the innate immune evasion of PEDV from the type III IFN response, we first developed a pig intestinal epithelial cell line susceptible for PEDV infection and named it IPEC-DQ. IPEC-DQ cells efficiently supported PEDV replication, and potently and selectively induced the production of type III IFNs. Of four isotypes of type III IFNs, IFN-lambda-1, -lambda-3, and -labmda-4 were expressed upon stimulation with double-stranded RNA analog. Interestingly, IFN-lambda-2 was deficient in various porcine cells, indicating that pigs may be deficient for IFN-lambda-2 expression. In PEDV-infected cells, the production of type III IFNs was inhibited, suggesting the PEDV-mediated suppression of type III IFNs in these cells. The recombinant IFN-lambda-1 and IFN-lambda-3 restricted the PEDV replication over time in a dose-dependent manner, indicating a potent antiviral activity of the type III IFNs. IFN regulatory factor 1 (IRF1) is a key regulator for type III IFNs, and we further show that PEDV blocked the IFN-lambda promoter activation by interfering both IRF1 and NF-κB. PEDV did not interfere the expression of IRF1, but rather inhibited its nuclear translocation. The decrease in the number of peroxisomes was evident in PEDV-infected cells. Our study for the first time demonstrates that PEDV evades the type III IFN response in the intestinal epithelial cells. Our finding may facilitate to design a novel approach to control not only PEDV but also other enteric viral infections.

Multiplexed digital mRNA profiling of immune responses in pigs persistently infected with porcine reproductive and respiratory syndrome virus

Pengcheng Shang[1], Rui Guo[1], Celena A. Carrillo[2], Crystal J. Jaing[2], Ying Fang[1]

[1]Kansas State University, Manhattan, Kansas, USA; [2]Lawrence Livermore National Laboratory, Livermore, California, USA

The capacity to establish and maintain an asymptomatic persistent infection is one of the hallmarks of PRRSV infection. Tracking of the global changes in the host gene expression profile during viral infection contributes to the better understanding of viral pathogenesis and identification of potential diagnostic / therapeutic targets. To better understand the host-pathogen interaction during PRRSV persistent stage, we developed a swine gene specific multiplexed immune gene mRNA profiling assay based on the nanostring nCounter RNA-array technology, a high-throughput digital gene expression system. The nCounter swine immune gene panel includes 189 widely studied innate/adaptive immune-related genes plus 3 internal controls. The assay was used to analyze swine immune gene expression in tracheobronchial lymph nodes from PRRSV-infected pigs during acute and persistent infection stages. Results showed that 55 immune genes were significantly upregulated in acutely infected pigs compared to mock or persistently infected pigs. Persistently infected pigs had gene expression pattern more close to that of mock infected pigs. Further gene ontology analysis showed that most of genes in acutely infected pigs were enriched in innate immune pathways and cytokine responses. Taken together, this study established a swine immune gene mRNA profiling assay and demonstrated the feasibility of utilizing nCounter RNA-array technology in rapid analysis of host immune gene expressions in infected animals.

Comparison of historic and contemporary strains of Senecavirus A

Alexandra Buckley[1]*, Baoqing Guo[2], Vikas Kulshreshtha[1], Albert van Geelen[1], Kyoung-Jin Yoon[2], and Kelly Lager[3]

[1]Oak Ridge Institute for Science and Education and National Animal Disease Center and U.S. Department of Agriculture, Ames, IA, USA
[2]College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[3]National Animal Disease Center, U.S. Department of Agriculture, Ames, IA, USA

Objective: After Koch's postulates were fulfilled and SVA was proven to be a causative agent for vesicular disease, speculation remained surrounding the sudden increase in SVA infections and why attempts to reproduce disease had failed in the past. One hypothesis was that contemporary strains were more pathogenic than historical strains. Our objective was to study disease progression of historical and contemporary SVA isolates in growing pigs.

Materials and Methods: Commercial swine aged 16-20 weeks old (n=54) were split into 6 challenge groups (n=8) and 1 control group (n=6). Three historical isolates (2001, 2011, 2012) and three contemporary isolates (2015) with inoculum titers ranging from $10^{5.1}$ – $10^{6.8}$ TCID50/mL were given intranasally. Animals were regularly bled, rectal swabbed, and oral swabbed. Animals were also observed daily for any clinical signs of vesicular disease. The sampling period ranged from 0 days post inoculation (dpi) to 14 dpi. Serum and swabs were tested by real-time PCR for SVA nucleic acid detection. Serum was also tested for neutralizing antibody response to the challenge virus by virus neutralization assay and cross neutralizing antibodies to other challenge isolates.

Results: All isolates used in the study were able to induce clinical disease with the development of vesicles either on the coronary bands or the snout. The number of pigs presenting with clinical signs in each challenge group ranged from 5/8-8/8. Just under half of the animals that developed vesicles had more than one lesion present by the end of the study. All animals in each challenge group replicated virus and had a least one PCR positive rectal swab for SVA. There were slight differences in onset and duration of shedding among the six different isolates, but overall most pigs were positive for SVA in oral and/or rectal swabs by 4 dpi and were still shedding virus at 14 dpi. Neutralizing antibody titers to both homologous and heterologous strains used in this study are pending. Sequencing results and comparison between the 6 isolates is also pending.

Conclusions: This study demonstrated that vesicular disease can be experimentally reproduced in growing pigs with both historic and contemporary isolates of SVA. In addition, the results suggested there were not large differences in clinical presentation between strains, which was contrary to our hypothesis. Further research will be needed to help determine the cause of the sudden increase in vesicular disease due to SVA infection in the United States swine population.

Glycosylation site of PRRSV GP5 is associated with broad neutralization of PRRSV infection

Y. Nan[1*], G. Gu[1], C. Wu[1], Y. Zhang[2], and E-M. Zhou[1*]

[1]College of Veterinary Medicine, Northwest A&F University, China; [2]Molecular Virology Laboratory, VA-MD College of Veterinary Medicine and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA;

Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-stranded, enveloped RNA virus which belongs to the genus Arterivirus, family *Arteriviridae* and order *Nidovirales* . Currently, all PRRSV isolates were divided into *PRRSV-1* and *PRRSV-2* strains which share approximately 60% nucleotide sequence identity and have serotype difference. Although heterologous nature of PRRSV isolates lead to great obstacle for developing effective vaccine, pig sera samples contain broad neutralizing antibodies for PRRSV were reported. Up to date, there is no report of identification for a conserved epitope in PRRSV which is related with broad neutralization of PRRSV infection.

In this study, we occasionally obtained a clone of monoclonal antibody (Mab) developed against PRRSV-GP5 with ability to recognizing Marc145 cell infected with variety PRRSV strains. Although antibody isotype analysis suggesting this Mab is IgM, further analysis suggesting that this Mab recognizing all PRRSV-2 strains tested in our labs, which including classical strains(VR2332, CH-1a, NVSL97, VR2385), HP-PRRSV strains(HuN4, JXA1, SD16), MN-184 and NADC-30 like strain and is able to capture purified PRRSV virion. More importantly, viral neutralization assay suggested that this Mab is able neutralizing infection of all PRRSV strains tested, which indicating conserved epitope is existing among heterologous PRRSV isolates. Additionally, detail analysis demonstrates this Mab recognizes a confirmation epitope located in GP-5 requiring glycosylation. Treatment with purified PRRSV virions with PNGase F abolish recognition of this Mab. Compared with polyclonal serum to GP-5 which recognizing all GP-5 from cytoplasm in PRRSV infected cells, this Mab only recognize GP5 located in trans Golgi, the location of glycosylation occurred and it recognition can be blocked by brefeldin A (inhibitor for Golgi transportation).

In conclusion, our data suggest there are novel epitope required glycosylation in PRRSV-GP5 which is conserved among all PRRSV-2 strains and is associated with broad neutralization of PRRSV infection.

Effect of miRNA and tRNA gene expression on the homeostatic status of pigs infected with highly pathogenic PRRSV.

Damarius S. Fleming[1,2] and Laura C. Miller[2]
ORAU/ORISE Oak ridge, TN[1], Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA-ARS[2]

It has been established that reduced susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV) has a genomic component. This component, however, is a multi-faceted composition of coding and non-coding genetic elements that function as regulators of immune function. Our study focuses on the small non-coding (sncRNA) side of this response in pigs because of emergence of various sncRNAs shown to play important roles in the human viral immunity. Among these sncRNAs are the microRNA (miRNA) and transfer RNA (tRNA) molecules. Our study looks at changes in expression of these sncRNAs to produce information on how gene function in the pig can become dysregulated and subsequently respond to the virus. The objective of the study is to identify differences in miRNA and tRNA gene expression between healthy and highly pathogenic PRRSV challenged pigs.

The study was conducted using total RNA extracted from pig whole blood taken from a total of 24 pigs split into either control (sham inoculation) or infected pigs at 1, 3, and 8 days post infection. Sequencing of the samples produced 100bp single end libraries for transcriptomic analysis of sncRNA gene expression.

The results indicated statistically significant changes in sncRNA expression were dependent on time and treatment, in which, miRNA expression was variable while tRNA expression declined steadily post-infection. The results of this study highlights changes in sncRNA expression that have the potential to unlock new targets for understanding the effect of PRRSV on pig homeostasis.

Transcriptome responses to respiratory virus infection of pigs within the tracheobronchial lymphnode following infection with PRRSV, PCV2 or IAV.

Laura C. Miller*[1], Damarius S. Fleming [1,3], Gregory P. Harhay[2], Marcus E. Kehrli Jr[1], and Kelly M. Lager[1]

[1]Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa 50010, USA; [2]Animal Health Research Unit, US Meat Animal Research Center, USDA, Agricultural Research Service, Clay Center, Nebraska 68933, [3]ORAU/ORISE Oak ridge, TN

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major respiratory pathogen of swine that has become extremely costly to the swine industry worldwide, often causing losses in production and animal life due to their ease of spread. However, the intracellular changes that occur in pigs following viral respiratory infections are still scantily understood for PRRSV, as well as, other viral respiratory infections. The aim of this study was to acquire a better understanding of PRRS disease by comparing gene expression changes that occur in tracheobronchial lymph nodes of pigs infected with either porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), or swine influenza virus (IAV) infections.

The study identified and compared gene expression changes in the TBLN of 16 pigs following infection by PRRSV, PCV2, SIV, or sham inoculation. Total RNA was pooled for each group and time-point (1, 3, 6, and 14 DPI) to make 16 libraries, for analysis by Digital Gene Expression Tag Profiling (DGETP). The data underwent standard filtering to generate a list of sequence tag raw counts that were then analyzed using multidimensional and differential expression statistical tests.

The results showed that PRRSV, IAV and PCV-2 viral infections followed a clinical course in the pigs typical of experimental infection of young pigs with these viruses. Gene expression results echoed this course, as well as, uncovered genes related to shared and unique host immune responses to the 3 viruses.

By testing and observing the host response to other respiratory viruses, our study has elucidated similarities and differences that can assist in development of vaccine and therapeutics that shorten or prevent a chronic PRRSV infection.

Comparative analysis of signature genes in PRRSV-infected porcine monocyte-derived cells to different stimuli.

Laura C. Miller*[1], Damarius S. Fleming[1,6], Xiangdong Li[2], Darrell O. Bayles[3], Frank Blecha[4] and Yongming Sang[5]

[1]Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa 50010, USA; [2]Pulike Biological Engineering, Lingbo Road, Luoyang Hi-New Tech Development Zone, Henan Luoyang Henan 471000, China; [3]Infectious Bacterial Diseases Research Unit, National Animal Disease Center, USDA-ARS, 1920 Dayton Ave, Ames, IA 50010; [4]Departments of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506; [5]Department of Agricultural and Environmental Sciences, College of Agriculture, Human and Natural Sciences, Tennessee State University, 3500 John A. Merritt Boulevard, Nashville, TN 37209; [6]ORAU/ORISE Oak ridge, TN.

Monocyte-derived DCs (mDCs) are major target cells in porcine reproductive and respiratory syndrome virus (PRRSV) pathogenesis; however, the plasticity of mDCs in response to activation stimuli and PRRSV infection remains unstudied. In this study, we polarized mDCs, and applied genome-wide transcriptomic analysis and predicted protein-protein interaction networks to compare signature genes involved in mDCs activation and response to PRRSV infection. Porcine mDCs were polarized with mediators for 30 hours, then mock-infected, infected with PRRSV strain VR2332, or a highly pathogenic PRRSV strain (rJXwn06), for 5 h. Total RNA was extracted and used to construct sequencing libraries for RNA-Seq. Comparisons were made between each polarized and unpolarized group (i.e. mediator vs. PBS), and between PRRSV-infected and uninfected cells stimulated with the same mediator. Differentially expressed genes (DEG) from the comparisons were used for prediction of interaction networks affected by the viruses and mediators. The results showed that PRRSV infection inhibited M1-prone immune activity, downregulated genes, predicted network interactions related to cellular integrity, and inflammatory signaling in favor of M2 activity. Additionally, the number of DEG and predicted network interactions stimulated in HP-PRRSV infected mDCs was superior to the VR-2332 infected mDCs and conformed with HP-PRRSV pathogenicity.

Live virus Immunization (LVI) with a recent 1-7-4 PRRSV isolate elicits broad protection against PRRSV challenge in finishing age swine.

Albert G. M. van Geelen[2], Alexandra Buckley[2], Vikas Kulshreshtha[3], Susan Brockmeier[1], Laura Miller[1] Damarius Fleming[2], Crystal Loving[4], Kay Faaberg[1] and Kelly lager[1].
1: U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Virus and Prion Research Unit. 2: Oak Ridge Institute for Science and Education, 3: Iowa State College of Veterinary Medicine, 4: U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Food Safety and Enteric Pathogens.

PRRSV infection is the most economically important disease affecting domestic swine herds in the United States and in many countries. Commercially available vaccines are often based on older viral strains and offer limited efficacy against heterologous challenge. Live virus immunization (LVI), a form of autologous vaccine, is based on immunizing healthy swine with serum from a diseased pig infected with a recent local isolate affecting that farm or area. Since the emergence of PRRS, this practice has been frequently utilized with reported success in immunizing replacement gilts, suggesting it does confer better protection than commercial vaccines. However, recently there have been field reports that LVI is not as efficacious as it once was, implying there may have been a change in how PRRSV interacts with the pig.
Materials and Methods: To evaluate the efficacy of LVI with contemporary 1-7-4 strains, 60 finishing age swine were used in this study and divided into 7 groups. Three groups were challenged with 2ml serum I.M. from an NADC34 infected pig, a current 1-7-4 strain. Each of these LVI groups were again challenged after six weeks intranasally with 5 x 10E4 TCID50 of either NADC34 (homologous challenge), NADC36, a close relative (98.4% homologous whole genome), or SDSU73, a known moderately pathogenic 1-4-4 strain that is 83.2% homologous with NADC34. Each of these groups were compared with an equivalent but naïve group and evaluated for pyrexia, ADG, viral load, lung lesions and virus neutralization titer.
Results: LVI with NADC34 protected all three LVI groups against homologous or heterologous challenge by lowering viral load by almost three logs, by eliminating negative effects on ADG of all three viruses and by eliciting broadly neutralizing calculated antibody titers of greater than 100 at 50% inhibition in 5 out of 8 pigs in the homologous challenge group, 5 out 8 in the NADC36 group, and 7 out of 9 in the SDSU73 group.
Conclusions: LVI with NADC34 induces equivalent neutralization and protection per group from challenge with the NADC34, NADC36 or heterologous virus SDSU73. When sera of individual pigs were examined by western blotting, individually distinct reactivity patterns against viral proteins were observed between pigs in a group, suggesting individually distinct anti-PRRSV antibody responses. Future work aims to elucidate if a predictive value can be gleaned from these patterns for efficacy of protection against future challenge.

Dynamics of adaptive immune responses following Senecavirus A infection in pigs

M.F. Maggioli1*, S. Lawson1, M. De Lima2, L.R. Joshi1, T.C. Faccin3, F.V. Bauermann1, D.G. Diel1;

1Animal Disease Research & Diagnostic Laboratory, South Dakota State University, Brookings, SD, USA, 2Universidade Federal de Pelotas, Pelotas, Brazil, 3Universidade Federal de Santa Maria, Santa Maria, Brazil.

Senecavirus A (SVA) is an emerging picornavirus that causes vesicular disease (VD), which is clinically indistinguishable from foot-and-mouth disease (FMD) in pigs. Many aspects of SVA infection biology and the host immune responses to infection remain unknown. In the present study, finishing pigs were infected with a contemporary SVA strain (SD15-26), and the ensuing humoral and T cell responses were evaluated during acute infection (14 days) or after disease resolution (at day 35 pi). We assessed levels of neutralizing antibodies, and the levels of IgM and IgG directed against the external capsid proteins VP1, VP2 and VP3. Additionally, cellular immune responses to SVA were assessed by flow cytometric analysis. For this, IFN-γ expression and proliferative responses by T cells were evaluated upon recall stimulation with uv-inactivated virus (uvSVA) or with individual capsid proteins (VP1, VP2 and VP3). Infection elicited neutralizing antibody as early as 5 days pi, which coincided and was strongly correlated with VP2- and VP3-specific IgM responses. Levels of anti-VP1, -VP2 and -VP3 IgG antibodies increased around days 10 (for VP-3) or 14 pi (for VP1 and VP2). Specific T cell responses to SVA were first detected at day 3 pi, and were highly significant starting at day 7 pi (as measured by both IFN-γ expression and proliferation). T cell responses to all three external capsid proteins were detected, with VP2 and VP3 dominating those responses. Both CD4 and CD8 T cell responses were detected, with CD4 T cells being the predominant T cell subset responding to SVA infection. In summary, SVA elicits robust B and T cell activation early upon infection, with high neutralization titers associated with IgM activity. T and B cell responses are directed against all three external capsid proteins.

PRRSV inhibits interferon signaling by downregulating STAT2

L. Yang*, Z. Ma, S. Lin, and Y. Zhang

Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD

Type I interferons (IFNs) play a crucial role in the antiviral response of the innate immunity. Porcine reproductive and respiratory syndrome virus (PRRSV) antagonizes the antiviral response by inhibiting IFN synthesis and downstream signaling. 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 type I IFN-activated JAK/STAT signaling. The objective of this study was to investigate the PRRSV effect on STAT2 signaling. Here, we discovered that PRRSV downregulates STAT2 to inhibit the IFN-activated signaling. Both type 1 and type 2 PRRSV strains decreased STAT2 protein levels, whereas they had minimum 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. Together, these results demonstrate that PRRSV antagonizes type I IFN signaling by accelerating STAT2 degradation, in addition to the other mechanisms previously identified. This study provides further insight into the PRRSV interference with the type I IFN signaling and the consequent perturbation of the host immune response.

PRRSV hijacks karyopherin alpha6 to facilitate the viral replication

L. Yang*, R. Wang, S. Yang, Z. Ma, S. Lin, and Y. Zhang

Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD

PRRSV is a positive-sense single-stranded RNA (+ssRNA) virus. A common feature for +ssRNA viruses is that they induce intracellular membrane rearrangements to form replication complexes (RCs) in the cytoplasm of infected cells to increase replication efficiency and protect the viral genome from host anti-viral response. The virus RNA synthesis occurring inside the RCs needs both viral and host proteins. However, it remains unknown how the RCs interact with the surrounding environment, especially the transportation of RNA and proteins into and out of the RCs. The objective of this study was to determine the mechanism of PRRSV interaction with host transport factors. In this study, we discovered karyopherin alpha6 (KPNA6, also known as importin-alpha7) is exploited by PRRSV, through translocation in the cells to enhance viral replication. The karyopherin proteins, involved in nucleocytoplasmic trafficking, are critical for macromolecule movement and subcellular localization in cells. Upon PRRSV infection, KPNA6 protein level is elevated due to viral interference with the ubiquitin-proteasomal degradation, which results in extension of KPNA6 half-life. The PRRSV nsp12 protein induced KPNA6 elevation. RNA interference (RNAi)-mediated KPNA6 silencing inhibited PRRSV replication. Notably, in PRRSV-infected cells, KPNA6 is translocated to the viral replication complexes (RCs) at the perinuclear region. Specifically, PRRSV nonstructural protein 2 (nsp2) interacts with KPNA6 and mediated its relocation. These results indicate that PRRSV hijacks and translocates it to the viral RCs to enhance the viral replication. This finding provides insights into PRRSV pathogenesis and virus–host interactions.

Differential rates of viral replication and cell susceptibility to infection by PRRSV in multiple antigen presenting cell populations

J. Darbellay1*, J. Van Kessel1, V. Gerdts1

1. Vaccine and Infectious Disease Organization-International Vaccine Centre, Saskatoon, Sk. Canada

The tropism of PRRSV has been shown to be restricted to cells that express CD163. The majority of these cells are of myeloid origin and are responsible for the stimulation of both naïve and memory T cell responses. Thus, we chose to investigate the rates of viral replication within different antigen presenting cell (APC) populations using flow-cytometry and rt-qPCR. Additionally, we used flow-cytometry to compare levels of cell surface molecule expression associated with antigen presentation in non-infected and infected APCs. Using monocyte-derived Mϕs, monocyte derived DCs (MoDCs), and Flt3L-derived bone-marrow DCs (BMDCs), we first compared the susceptibility of the cells *in vitro* to infection with PRRSV. Intracellular detection of PRRSV N was comparable and prominent 24 hours post infection in MoDCs and Mϕs (non-stimulated, M1, and M2). Furthermore, using rt-qPCR we found that viral copies in the supernatant increased at similar rates in MoDCs and Mϕs. On the other hand, we found that although CD163+ BMDCs are susceptible to infection, in comparison to the myeloid derived APCs, the rate of viral replication is much lower in CD163+ BMDCs. We continued by analyzing various cell surface molecules associated with antigen presentation. We observed that levels of MHCI and MHCII expression on the surface of infected Mϕs are both down-regulated. However, our previous results have shown that the down-regulation of MHCI and MHCII do not prevent Mϕs from stimulating a recall response in PRRSV exposed animals. In comparison, both CD163+ BMDCs and MoDCs showed an up-regulation of MHCI and MHCII molecules when infected with PRRSV. Our results indicate that the susceptibility and rate of replication of PRRSV in BMDCs in comparison to MoDCs and Mϕs is different, which suggests that BMDCs are responsible for the induction of cell mediated immunity, whereas the myeloid cell populations play a prominent role during the resolution of infection in addition to stimulating memory T cell responses upon reinfection.

Relationships of polymorphisms in the *regulator of G-protein signaling 16* gene with porcine circo virus type 2 viremia in naturally infected Yorkshire pigs.

K.S. Lim[1]*, S.H. Lee[2], E.A. Lee[2], J.M. Kim[3], T. Chun[2], K.C. Hong[2].

[1]Iowa State University, Ames, IA, USA, [2]Korea University, Seoul, Republic of Korea, [3]National Institute of Animal Science, Wanju, Republic of Korea.

Porcine circo virus associated disease (PCVAD) is ubiquitous in the swine industry worldwide. The main etiology is porcine circo virus type 2 (PCV2), which is a small size circular DNA virus. For that reason, vaccination against PCV2 is generally applied in Korea. However, not all pigs that are infected with PCV2 have clinical symptoms related with PCVAD, so we could hypothesize that some pigs are resilient against PCV2 infection. Here, we identified single nucleotide polymorphisms (SNPs) in the porcine *regulator of G-protein signaling 16* (*RGS16*) gene, which is known to directly interact with PCV2 ORF3, and analyzed their relationships with viremia in weaning Yorkshire pigs. Of in total 22 SNPs, 10 SNPs were in the 5'upstream region and 12 SNPs were in intron regions. We chose a farm that had a naturally occurring PCV2 infection. A total of 142 pigs that were seronegative for PCV2 at 4 weeks of age were genotyped, and their PCV2 DNA viremia at 10 weeks of age was measured in serum samples. As a result, SNP2 and SNP8 in the 5'upstream region and SNP11 in intron 1 were associated with viremia at 10 weeks of age ($P < 0.05$). The effect of these SNPs was confirmed by haplotype analysis ($P < 0.05$). Taken together, these preliminary results support the relationship between *RGS16* and host response against PCV2 infection and can be used as a basis of further studies of resistance or resilience to PCV2 in naturally infected populations.

Microbiome associations with weight gain in pigs after vaccination with a porcine reproductive and respiratory syndrome (PRRS) modified live virus (MLV) vaccine followed by challenge with PRRSV and porcine circovirus type 2 (PCV2b)

L.A. Constance[1*], J.B. Thissen[2], C.J. Jaing[2], K.S. McLoughlin[3] A.G. Cino-Ozuna[1,4], R.R.R. Rowland[1], M.C. Niederwerder[1,4]

[1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; [2]Physical & Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; [3]Computations Directorate, Lawrence Livermore National Laboratory, Livermore, CA [4]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2b) are two of the most significant pathogens affecting the swine industry. Worldwide, co-infections are common, resulting in respiratory disease and decreased growth rate. PRRS modified live virus (MLV) vaccines are widely used to reduce PRRS-associated loses; however, vaccination does not provide sterilizing immunity or prevent weight gain variation after PRRSV challenge. Previous research has shown that the gastrointestinal microbiome is associated with outcome following co-infection with PRRSV and PCV2b. The objective of this study was to determine if similar associations were present between microbiome profiles and outcome of pigs vaccinated with a PRRS MLV and subsequently challenged with PRRSV and PCV2b. Twenty-eight days post-vaccination (dpv) and at the time of PRRSV/PCV2b challenge, fecal samples were collected from an experimental population of 50 nursery pigs. Additionally, fecal samples were collected at three and six weeks post-challenge. At 42 days post-infection (dpi), 20 pigs were retrospectively identified as having high or low growth rates after co-infection. Average daily gain post-challenge was 0.94 ± 0.06 kg and 0.71 ± 0.11 kg in high and low growers ($p < 0.001$; unpaired t-test). PRRSV and PCV2 replication were similar throughout the study and no significant differences were detected in the severity of lymphoid depletion between the two groups. Using the Lawrence Livermore Microbial Detection Array (LLMDA), the microbiomes of the two growth rate groups were determined both pre and post-challenge. Preliminary microbiome analyses have shown that overall increased species diversity as well as increased members of the *Streptococcaceae* and *Veillonellaceae* families may be beneficial for growth rate following vaccination and subsequent PRRSV/PCV2 co-infection. Additionally, increased numbers of species within the phylum Firmicutes may be favorable for higher growth. Microbiome differences were most prevalent at 0 dpi (28 dpv) when compared to 3 and 6 weeks post-infection. Overall, this data suggests that the microbiome may play a role in predisposing outcome following PRRS vaccination and co-challenge; however, additional testing and further analysis using 16S ribosomal RNA sequencing will be employed to confirm preliminary results.

This work was supported by the USDA NIFA Award #2013-68004-20362, the State of Kansas National Bio and Agro-Defense Facility Fund, and the Science and Technology Directorate of the United States Department of Homeland Security under contract number D15PC00276.

Genomic prediction of a PRRS-vaccinated training population to predict host response to PRRS virus-only or PRRS virus/PCV2b co-infection

JR Dunkelberger,[1,2*] NVL Serão,[2] M Niederwerder,[3] M Kerrigan,[3] M Schroyen,[2,4] CK Tuggle,[2] J Lunney,[5] RRR Rowland,[3] and JCM Dekkers[2]

[1]Topigs Norsvin USA, Burnsville, MN; [2]Iowa State University, Ames, IA; [3]Kansas State University, Manhattan, KS; [4]University of Liège, Gembloux, Belgium; [5]USDA, ARS, BARC, APDL, Beltsville, MD

The ability to identify pigs with increased natural resistance to PRRS would be a valuable tool for the swine industry. Genetic selection occurs in high-health herds, and thus disease phenotypes are not available. One possibility is to use PRRS vaccination response for one set of individuals to predict genetic susceptibility to infection for another set of individuals using genotypes from single nucleotide polymorphism (SNP) panels, i.e. based on genomic prediction. To evaluate the feasibility of this approach, the objectives of this study were to evaluate the ability of a PRRS-vaccinated training population to predict host response to PRRS virus (PRRSV)-only infection or PRRSV/PCV2b co-infection using genomic prediction.

Pigs in the co-infected population originated from three groups of 200 commercial Large White x Landrace nursery pigs from PRRS Host Genetics Consortium (PHGC) trials. Pigs were randomly sorted into two rooms; pigs in one room were vaccinated with a modified live virus PRRS vaccine, while pigs in the other room were not (Non-Vx). Post vaccination, repeated serum samples and body weights were collected to quantify vaccination viral load (VL) and weight gain (WG), respectively. Four weeks later, all pigs were co-infected (Co-X) with field strains of PRRSV and PCV2b and followed for 42 days. For the PRRSV-only infected population, consisting of pigs from 9 earlier PHGC trials, WG and PRRS VL were calculated from 0 to 42 and 0 to 21 days, respectively. Genomic prediction analyses were then conducted to evaluate the predictive ability of vaccination VL/WG for PRRS VL/WG using the Non-Vx group post Co-X and the PRRSV-only infected pigs as validation populations using the following SNP subsets: 1.) SNPs across the genome (Whole Genome) and 2.) SNP WUR10000125 (WUR), previously associated with PRRS (WUR Only). Prediction accuracies were computed as the correlation between the prediction and phenotype in the validation population, divided by the square root of heritability.

In general, better predictive ability was obtained when pigs from the same (SS), rather than different (DS), genetic sources were used for training/validation. Prediction accuracies for PRRS VL following PRRSV-only infection for WUR (Whole Genome) were 0.38 (0.39) and 0.35 (0.34) when using SS or DS, respectively. For PRRS VL post Co-X, prediction accuracies were 0.43 (0.49) and 0.40 (0.47), respectively. For WG following PRRSV-only infection, prediction accuracies for WUR (Whole Genome) were 0.45 (0.45) and 0.21 (0.21), respectively, and -0.05 (-0.06) and -0.02 (-0.02) for WG post Co-X using pigs from SS and DS, respectively.

In conclusion, prediction accuracies were similar when training/validating on the Whole Genome versus the WUR Only SNP subset. Thus, selecting on WUR genotype is sufficient or superior to whole genome prediction of PRRS vaccination response for host response to infection with a field isolate of PRRSV. However, prior research shows that SNPs other than WUR do have predictive ability when using a PRRSV-infected training population. This research was supported by USDA-NIFA grants 2012-38420-19286 and 2013-68004-20362.

Viral load and antibody titer of post-weaned pigs represent the host susceptibility to porcine circovirus type 2 (PCV2) infection

Haesu Ko[1*], Kyung-Tai Lee[1], Taehoon Chun[2], Ki-Chang Hong[2], and Jun-Mo Kim[1]

[1]Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju 55365, Republic of Korea, [2]College of Life Sciences and Biotechnology, Korea University, Seoul, 02841, Republic of Korea

Correspondence to KCH (kchong@korea.ac.kr) and JMK (junmosss@korea.kr).

Piglets from the commercial farms have been prevalently infected to porcine circovirus type 2 (PCV2), a circular DNA virus classified in the *Circoviridae* family, in the genus *Circovirus*. PCV2-infected piglets typically manifest clinical symptoms such as weight loss, respiratory distress, reproductive failure, diarrhea, wasting, and jaundice, leading to significant economic burdens in swine farming. However, the PCV2 infections to piglets could be usually measured using postmortem evaluation methods. So, it is required to evaluate PCV2 infections in live pigs without conducting an autopsy so that the tested pigs can be used to study host susceptibilities. In this study, blood parameters such as PCV2 viral load and antibody titer were used to classify host susceptibilities of post-weaned pigs having naturally PCV2 infections. We took measurements for PCV2 DNA viral load (log copies/ml), Anti-PCV2 antibodies (SNc ratio), and leukocyte count (10^3/μl) in blood samples collected from the pigs at 10 weeks of age (69.25 ± 2.17 days). Then we categorized two classifications based on the PCV2 viral load (high, > 10^5 copies/ml; intermediate, 10^5 to 10^3 copies/ml; and low, < 10^3 copies/ml) and the SNc ratio (positive, ≤ 0.50; negative, > 0.50), respectively. It showed no significant effect between the two classifications, as well as on the leukocyte counts. Next, we grouped PCV2-susceptible and PCV2-resistant pigs by combining the two classifications. The susceptible group showed a high PCV2 viral load (> 5.0 log PCV2 copies/ml) with negative PCV2 antibody level, whereas the resistant group showed a low PCV2 viral load (< 3.0 log PCV2 copies/ml) with positive PCV2 antibody level. The susceptible group showed a significantly higher viral load ($p<0.001$) and PCV2 SNc ratio ($p<0.001$) than did the resistant group. Furthermore, the total leukocyte counts were considerably lower in the susceptible group ($p=0.018$). In conclusion, the combination of the two classifications resulted in the suggestion that our criteria using the PCV2 viral load and antibody titer may have potential for evaluating susceptibility against PCV2 infection.

GTPase-activating protein -binding protein 1 (G3BP1) plays an antiviral role in Porcine Epidemic Diarrhea Virus replication

Kabita Pandey1*, Shuhong Zhong1, Eric Nelson2, Xiuqing Wang1
Department of Biology and Microbiology[1], Department of Veterinary and Biomedical Sciences, South Dakota State University[2], Brookings, South Dakota 57007
Corresponding author: Xiuqing.wang@sdstate.edu

Porcine Epidemic Diarrhea Virus (PEDV) belongs to the *Coronaviridae*, and causes severe diarrhea and dehydration in nursing piglets. This emerging disease leads to significant economic loss in the swine industry worldwide. Currently, limited information is known regarding the role of innate immunity in PEDV infection. Stress granules (SGs) are sites of mRNA storage that are formed in response to various conditions of stress, including viral infections. Ras-GTPase-activating protein (SH3 domain) binding protein 1 (G3BP1) is a key stress granule-resident protein that nucleates stress granule assembly. Some virus infections cause SGs and the formation of SGs is considered as an indication of an antiviral innate response that limits translation of the viral genes. The primary objective of the study was to examine the role of G3BP1 and SGs in PEDV replication. We observed that infection of Vero cells with PEDV-CO induced formation of stress granules at 24 hours after infection. Overexpression of G3BP1 in Vero cells lowered virus replication by approximately 100-fold compared to the vector control. Knockdown G3BP1 by silencing RNA enhanced the viral replication by 100-fold compared to silencing control RNA. We also found that. knockdown of G3BP1 significantly promoted the viral genome replication and mRNA expression of viral genes. An increase in the mRNA of the proinflammatory cytokines such as Interleukin-1Beta and Tumor Necrosis Factor – Alpha was also observed in PEDV-infected G3BP1 knockdown cells compared to PEDV-infected control cells. Taken together, our results demonstrate that PEDV induces SGs formation and G3BP1 plays an antiviral role in virus replication.

.

Fecal microbiota transplantation improves outcome in nursery pigs following co-infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2d

M.C. Niederwerder[1,2,*], R.R.R. Rowland[1], L.A. Constance[1], M.L. Potter[3], M.A. Sheahan[1], R.A. Hesse[1,2], and A.G. Cino-Ozuna[1,2]

[1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas
[2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas
[3]Abilene Animal Hospital, Abilene, Kansas

Co-infections involving porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2d) are common on a global scale and contribute to significant losses in swine production due to poor weight gain and respiratory disease in growing pigs. The diversity and composition of the gut microbiome, or collection of microorganisms living in the gastrointestinal tract, has been shown to impact outcome following PRRSV/PCV2 co-infection in nursery pigs. Previous work by our group has demonstrated a consistent association between increased microbiome diversity and improved outcome characteristics following co-infection, including reduced virus replication, improved weight gain, and decreased clinical disease. In the current study, we sought to determine the effects of modulating the microbiome through fecal microbiota transplantation (FMT) on outcome following co-infection. Sibling pairs from a high health commercial source were divided into two groups at weaning and housed separately. The FMT group (n = 10) was administered the transplant for 7 consecutive days prior to co-infection and the control group (n = 10) was administered a mock-transplant with sterile saline over the same time period. All pigs were co-infected with PRRSV and PCV2d and followed for 42 days post-infection (dpi). Clinical signs of porcine circovirus associated disease (PCVAD), such as muscle wasting, dyspnea, tachypnea, pallor, lethargy, rough hair coat and nasal discharge, peaked between 18 and 30 dpi. Morbidity and mortality due to PCVAD was significantly higher in the control group compared to the transplanted group; 70% mortality compared to 20% mortality was seen in the control and transplanted groups, respectively ($p = 0.0447$, Mantel-Cox test). The majority of control pigs lost weight (70%) whereas only 30% of FMT pigs had evidence of weight loss at any time during the study. On 21 and 28 dpi, increased PCV2d replication and increased PRRSV replication, respectively, were detected in the serum of control pigs. Seroconversion for PRRSV and PCV2 was detected with the FMT pigs having higher antibody titers for both viruses. Upon examination at necropsy, the FMT group had reduced severity of lesions characteristic of PRRSV/PCV2 co-infection. Overall, this study provides evidence that microbiome modulation through FMT prior to co-infection with PRRSV and PCV2d improves outcome and suggests that a microbiome therapeutic may be an alternative method for disease control.

This work was supported by the Kansas State University College of Veterinary Medicine Success for Young Investigators Grant Program, start-up funds provided by the Kansas State University College of Veterinary Medicine, and the State of Kansas National Bio and Agro-Defense Facility Fund.

Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV

Rui Guo[1], Pengcheng Shang[1], Celena A. Carrillo[2], Xingyu Yan[1], Tao Wang[1], Crystal J. Jaing[2], Megan Niederwerder[1], Raymond R. R. Rowland[1], Ying Fang[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA

[2]Lawrence Livermore National Laboratory, Livermore, CA, USA

Porcine reproductive and respiratory syndrome virus (PRRSV) infection can be divided into at least two distinct stages: acute infection and persistence. Initial acute infection leads to the rapid cytopathic replication of the virus in host cells, resulting in the release of large amount of PRRSV into the blood circulation. The infection then progresses into asymptomatic persistent stage, in which the virus is no longer detected in blood and viral replication is primarily localized in lymphoid organs, including tonsil and lymph nodes. Currently, little is known about the mechanism of PRRSV persistence. In this study, a cellular model of persistent infection was established using PRRSV-infected MARC-145 cells passaging 109 times in vitro. Strand-specific quantitative RT-PCR analysis revealed that plus- and minus-strand viral RNAs were present at nearly equivalent levels; and immunofluorescence microscopy and RNAase I treatment analysis showed that double-stranded RNA (dsRNA) conformation existed in persistently infected cells. In contrast, plus- and minus-strand viral RNAs were present at about 46:1 ratio in acute infected cells. Consistent with the data generated from *in vitro* cell culture system, there was about 3.3-fold reduced ratio of plus versus minus-strand viral RNAs in lymphoid tissues from PRRSV-infected pigs at 52 days post infection (dpi), in comparison to that of PRRSV-infected pigs at 10 dpi. The results were further confirmed using lymphoid tissues collected from pigs at 70 dpi, in which reduced ratios of plus versus minus-strand viral RNAs were consistently detected. Immunohistochemistry analysis showed that viral dsRNAs were detected aggregating inside the germinal centers of tonsil and lymph nodes from PRRSV persistence pigs, while most of the dsRNAs were detected in marginal zones of lymphoid tissues in acute PRRSV-infected pigs. Our results suggest that the PRRSV dsRNA could function as a mediator for viral persistence. The viral dsRNA persistence in germinal centers of lymphoid tissues may reveal a novel mechanism for PRRSV to escape antiviral immune responses.

PRRSV-induced HMGB1 secretion via PKC-delta activation in triggering inflammatory response

R. Wang[1]*, Y. Zhang[2], E. Liu[1], L. Yang[2]

[1]Laboratory Animal Center, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi, China
[2]Molecular Virology Laboratory, VA-MD College of Veterinary Medicine, University of Maryland, College Park, MD

Porcine reproductive and respiratory syndrome (PRRS) causes significant economic losses to the swine industry across the world. Severe pulmonary inflammatory injuries, considered as its main pathology, are seen in infected pigs. It was known that secreted high mobility group box 1 protein (HMGB1) acted as a cytokine mediator of inflammation and enhanced inflammatory response. However, the mechanism of PRRSV-induced HMGB1 secretion is not known. In this study, we found that HMGB1 secretion and PKC-delta activation were induced by different PRRSV strains in both MARC-145 cells and primary pulmonary alveolar macrophages (PAM), whereas UV-inactivated PRRSV failed to do so. HMGB1 secretion was positively correlated with PKC-delta activation in PRRSV-infected MARC-145 cells in a dose and time-dependent manner. PKC-delta inhibitor and PKC-delta siRNA significantly suppressed PRRSV-induced HMGB1 translocation and secretion without hampering virus replication. This indicates that PKC-delta plays an important role in the PRRSV-mediated HMGB1 secretion. In addition, in PRRSV-infected cells, PKC-delta siRNA silencing also led to the downregulation of inflammatory cytokines, such as IL-1beta and IL-6. Moreover, overexpression of PRRSV proteins in HEK293 cells showed that ORF2b and ORF5a products led to HMGB1 secretion and PKC-delta activation in a dose-dependent manner. These results indicate that PRRSV induces HMGB1 secretion via PKC-delta activation and that PRRSV ORF2b and ORF5a products are responsible for the induction. This finding contributes to our understanding on the inflammatory response and pathogenesis of PRRSV infection.

Piglet Blood Transcriptome Response to Co-infection with PRRSV and PCV2, with or without prior Vaccination for PRRS

Qian Dong[*1], Joan K Lunney[2], Christopher K Tuggle[1], James Reecy[1], Raymond R R Rowland[3], Jack C M Dekkers[1]

[1]Department of Animal Science, Iowa State University, Ames, Iowa; [2]USDA, ARS, BARC, APDL, Beltsville, Maryland; [3]College of Veterinary Medicine, Kansas State University, Manhattan, Kansas

Co-infections of pigs with PRRSV and PCV2 is a common model for PRRS and PCVAD in the field, with PRRSV potentiating replication of PCV2. The objectives of this research were 1) to identify the blood transcriptome response of commercial nursery pigs to vaccination for PRRS and after co-infection with PRRSV and PCV2, and 2) to estimate the effect on blood gene expression of pig WUR genotype that marks a major gene (GBP5) associated with susceptibility to PRRS. PRRS Host Genetics Consortium trial 16 used about 200 Large White/Landrace cross barrows from one genetic supplier. Shortly after weaning, pigs were randomly assigned to one of 2 rooms and pigs in one room were vaccinated using a PRRS modified lived vaccine. On day 28 post vaccination (dpv) pigs in both rooms were challenged with field isolates of PRRSV and PCV2b and followed for 28 days post infection (dpi). In total, 190 blood samples collected from 14 piglets from each room at 10 time points including 4, 7, 11, 14 dpv [for vaccinated pigs (Vac) only] and 0, 4, 7, 11, 14, 28 dpi were selected for transcriptome analysis using 3' RNAseq (QuantSeq). The 28 piglets were selected from seven litters that had one piglet for each of 4 Vac by WUR genotype combination: Vac-AA, Vac-AB, nonVac-AA, nonVac-AB. The QuantSeq raw data were processed through a pipeline similar to Bluebee using version 11.1 of the pig genome from Ensembl. For each time point, differential expression analysis was done using QuasiSeq with a generalized linear model that included vaccination status, WUR genotype, Vac x WUR interaction, RIN score, and lane. For 4, 7, 11, and 14 dpv between two WUR genotypes, 25, 100, 0, 9 differentially expressed genes (DEGs) were identified, respectively ($q \leq 0.2$). For 0, 4, 7, 11, 14, and 28 dpi, no DEGs were found between the two WUR genotypes and 8, 0, 31, 3, 3, 0 DEGs were identified between vaccination status ($q \leq 0.2$). One and 24 DEGs were significant for the Vac x WUR interaction on 0 and 7 dpi, respectively ($q \leq 0.2$). The maximum number of DEGs between WUR genotypes was on 7 dpv, and that between vaccination status, and for the Vac x WUR interaction was on 7 dpi after co-infection. These timepoints coincided with the peak viral levels. Using Ingenuity Pathway Analysis (IPA), the DEGs identified between WUR genotypes on 7 dpv may play an role in infectious diseases, inflammatory response, cell-to-cell signaling and interaction, reproductive and cardiovascular system development and function. The DEGs identified for vaccination status on 7 dpi may participate in inflammatory response, metabolic disease, infectious diseases and endocrine system disorders. The DEGs identified for the Vac x WUR interaction on 7 dpi may play a role in connective tissue disorders, inflammatory disease, organismal injury and abnormalities, and tissue morphology. Taken together, this study suggests that the expression of genes involved in the immune response were associated with a pig's vaccination status, WUR genotype and their interaction. This project was funded by Genome Canada, USDA-ARS, USDA-NIFA grant 2013-68004-20362. We would also like to acknowledge contributions from members of the PRRS Host Genetics Consortium.

GP5 of porcine reproductive and respiratory syndrome virus (PRRSV) as a target for homologous and broadly neutralizing antibodies

Luca N. Popescu[1], Benjamin R. Trible[1], Nanhua Chen[2] and Raymond R. R. Rowland[1]*

1. Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS 66506
2. College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, 225009, P.R. China

Virus neutralization (VN) responses during infection with porcine reproductive and respiratory syndrome virus (PRRSV) range from narrowly focused antibodies with only homologous neutralizing activity against the virus used for infection, to antibodies that can neutralize both Type 1 and Type 2 viruses, which we call broadly neutralizing antibody (bnAb). Even though neutralizing epitopes are likely distributed among several structural glycoproteins, this paper focuses on the ectodomain region of GP5 as a model system for investigating the role for neutralizing and non-neutralizing antibodies in protection and disease. Epitope B within GP5 possesses several features of a broadly neutralizing epitope, including similarities to a linear broadly neutralizing epitope described for E2 of hepatitis C virus (HCV). In the proposed model, the conserved epitope in GP5 is blocked not only by homologous neutralizing and non-neutralizing antibodies, against the flanking hypervariable domains, but also by conformational alterations throughout GP5. Continuous escape from homologous neutralization provides a mechanism for persistence. The proposed mechanism for immune evasion is not unique to PRRSV, but can be found in other persistent viruses, such as HCV. Virus replication properties that favor the production of broadly neutralizing antibodies provide insights towards the development of a new generation of broadly protective PRRS vaccines.

Gene expression analysis of whole blood from pigs infected with low and high pathogenic African swine fever viruses.

Raymond R.R. Rowland[4], Crystal Jaing[1*], Jonathan Allen[2], Andrea Certoma[3], James B. Thissen[1], John Bingham[3], Brenton Rowe[3], John R. White[3], James W. Wynne[3], Dayna Johnson[3], David T. Williams[3]

[1]Physical & Life Sciences Directorate, [2]Computations Directorate, Lawrence Livermore National Laboratory, Livermore, CA, [3] CSIRO Australian Animal Health Laboratories, Geelong, Victoria, Australia, [4] Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS

ASFV is a macrophage-tropic virus responsible for a transboundary disease that threatens swine production world-wide. Since there are no vaccines available to control ASF after an outbreak, obtaining an understanding of the virus-host interaction is important for developing new intervention strategies. In this study, RNA-Seq was used to characterize differentially expressed genes (DEGs) in pigs infected with a low pathogenic ASFV isolate, OUR T88/3 (OURT), or the highly pathogenic Georgia 2007/1 (GRG). After infection, pigs infected with OURT showed no or few clinical signs. In contrast, infection with GRG resulted in clinical signs consistent with acute ASF. Because of morbidity, all GRG pigs were euthanized between 7 and 10 days after infection. Whole blood RNA was collected at day 0 and at euthanasia day (ED) or 7 to 10 days for the GRG pigs, and on days 3, 7, 14 and 28 days after infection with OURT. Since ASFV replicates in circulating macrophages, RNA-Seq detected the expression ASFV genes from the whole blood of the GRG pigs. ASFV gene reads were not detected in any of the OURT pigs, consistent with the absence of ASFV nucleic acid. The number of reads for the GRG genes constituted about 0.1% of the overall reads. RNA-Seq detected the expression of 109 named ASFV genes and multi-gene families (MGFs). The highest mean level of expression was observed for MGF 360-15R, followed by *A276R*, an early gene involved in blocking the induction of IFN-beta. A third highly expressed ASFV gene was *DP71L*, which codes for a protein involved in the dephosphorylation of P-eIF2a. The twenty most highly upregulated host genes in the GRG pigs were associated with; 1) increased numbers of circulating macrophages (increased expression of *CD163* and *SIGLEC-1*), 2) the cellular response of macrophages to virus or ASFV infection, and 3) NK cell activation. Upregulated genes in the OURT pigs included genes associated with macrophage function and NK activation. Even though GRG and OURT possess different pathogenic properties, there was significant DEG overlap. For example 513 DEGs were detected for the OURT samples versus 367 for GRG. Overlap consisted of 85 DEGs. Application of human and pig gene atlases showed that most DEGs were associated with immune function. Together, the data confirm previous studies describing the response of macrophages and lymphocytes to ASFV infection, as well as some unique gene pathways upregulated in response to infection.

Evaluation of the efficacy of an attenuated live vaccine based on a virulent type 2 porcine reproductive and respiratory syndrome virus strain in young pigs

S. Lee[1]*, S.-C. Lee[2], H.-W. Choi[2], Y.-H. Noh[2], I.-J. Yoon[2], C. Lee[1]

[1]Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; [2]Choongang Vaccine Laboratory, Daejeon, South Korea

Porcine reproductive and respiratory syndrome virus (PRRSV) is a globally ubiquitous swine viral pathogen that causes significant financial losses worldwide. The type 2 PRRSV lineage 1 that includes virulent MN184 strain and its relative strains has severely affected the pork industry in South Korea since the early 2010s. We previously obtained a cell-adapted PRRSV strain, CA-2-MP120, by sequentially passaging the virulent MN184-related strain CA-2 on MARC-145 cells for 100 passages and on cultured porcine alveolar macrophage (PAM) cells for additional 20 passages, and reported an attenuated phenotype of CA-2-MP120 compared to its parental virus. In the present study, the efficacy of the cell-attenuated PRRSV CA-2-MP120 strain as a modified live vaccine was assessed in the natural host. Three-week-old PRRSV-free pigs were inoculated intramuscularly with CA-2-MP120 ($10^{6.0}$ 50% tissue culture infective dose [$TCID_{50}$]) and then challenged intramuscularly with the 20^{th} passage virus of CA-2 strain (CA-2-P20, $10^{6.3}$ $TCID_{50}$/2 ml) at 57 days post-immunization (dpi). Clinical symptoms, humoral immune response, viral growth, and different tissue lesions were monitored. All animals were euthanized at 14 days post-challenge (dpc) for post-mortem examination. None of piglets showed any obvious changes in daily weight gain, body temperature, or clinical signs of disease at any point during the experiment compared to the control group. Antiviral antibody detection by ELISA revealed that seroconversion occurred in all vaccinated pigs by 11 dpi and that the mean S/P ratio of the antibody test peaked at 21 dpi, which were maintained until the end of study. Although all pigs in the virus-challenged unvaccinated control group seroconverted by 7 dpc, their mean S/P ratios were significantly lower than those of the virus-challenged vaccinated group. All vaccinated animals developed viremia by 3 dpi which persisted until 54 dpi, while unvaccinated control groups remained viremia-negative during the 54-day post-immunization period but were viremic at 4 dpc (61 dpi). Interestingly, no viremia was detected in the vaccinated pigs during the 14-day post-challenge period. Furthermore, no pigs in the virus-challenged vaccinated group shed virus nasally, orally or rectally throughout the entire experiment. Compared to the challenge control, vaccinated pigs showed much milder pathological lung and lymph node lesions. These results indicated that CA-2-MP120 can provide effective protection against the virulent wild-type PRRSV strain. Altogether, our data suggest that the attenuated CA-2-MP120 strain is a promising candidate for developing a safe and efficacious live PRRSV vaccine.

Efficacy of Ingelvac PRRS® MLV against a heterologous PRRSV 1-3-4 RFLP challenge

A. Patterson, B. Fergen, J. Hermann, G. Haiwick, R. Philips*
Boehringer Ingelheim Animal Health, Duluth, GA, USA

Introduction

Ingelvac PRRS® MLV has demonstrated heterologous protection against current and relevant PRRSv challenge isolates. However, the efficacy of Ingelvac PRRS® MLV against the current virulent PRRSV RFLP 1-3-4 isolate has not been reported to date. The objective of this study was to evaluate the efficacy of two commercially available PRRSV vaccines in a three-week-old pig respiratory challenge model, using a heterologous PRRSV RFLP 1-3-4 field strain that was isolated in 2016.

Materials and Methods

At approximately three weeks of age (D0), 102 PRRSV naïve piglets pigs were randomized into groups, and intramuscularly vaccinated with 2 ml of either a placebo (n=36), Ingelvac PRRS® MLV (n=33) or Fostera® PRRS (n=33). Pigs were housed in rooms by group during the vaccination period. At D28 of the study, all pigs were comingled and challenged with 1.0 mL intramuscularly and 2.0 mL intranasally (1 mL per nostril) of diluted serum containing 7.97 log gc/mL of PRRSV 1-3-4. Serum samples were collected periodically from D0 through D42. Serum samples were tested by RT-PCR for the presence of viremia and by ELISA for the presence of anti-PRRSV antibody. Viremia testing for PRRSV was reported as "positive or negative" and quantified in units of genomic copies/ml serum. Pigs were weighed at D0, day of challenge (D28), and termination of the study (D42) to assess average daily weight gain (ADWG). On D42, all pigs were necropsied and lungs were scored for the presence of macroscopic lesions. Data were analyzed using generalized and linear mixed statistical models.

Results and Conclusion

Table 1. Day 42 lung lesions (median %), mortality (%) and post-challenge ADWG (lbs) in vaccinated and non-vaccinated pigs

Treatment	Lung Lesions	ADWG in lbs.	Percent Mortality
Ingelvac PRRS® MLV	14.9[a]	0.317[a]	15.2[a]
Fostera® PRRS	27.4[a]	0.052[c]	21.2[a]
Placebo	58.3[b]	-0.250[b]	61.1[b]

*Different letters indicate significant differences at $P \leq 0.05$.

Ingelvac PRRS® MLV has 85.5% similarity to the PRRSV 1-3-4 challenge strain based on ORF5 sequence. The challenge isolate selected resulted in severely impacted ADWG (-0.25 lbs), lung lesions (58.3%) and mortality (61.1%) in the challenge control group. The results confirmed anecdotal evidence of the severity of disease associated with this PRRSV 1-3-4 isolate. Despite this virulent challenge, Ingelvac PRRS® MLV demonstrated heterologous protection by significantly reducing lung lesions and mortality, and significantly improving ADWG compared to the challenge controls. Compared to challenge control pigs, Ingelvac PRRS® MLV vaccinated pigs had significantly lower levels of post-challenge viremia at D29, D31, D33, D35 and D42. In addition, pigs vaccinated with Ingelvac PRRS® MLV had significantly higher ADWG compared to the Fostera® PRRS vaccinated group. This study demonstrates the ability of Ingelvac PRRS® MLV to protect against a relevant and contemporary PRRS challenge.

MYH9 Protein C-Terminal Domain Blocks PRRSV infection

L. Li[1], Y. Nan[1], L. Zhang[1], G. Hou[1], B. Xue[1], W. Sun[1], G. Gu[1], C. Wu[1], Q. Zhao[1], Y. Zhang[2], G. Zhang[3], Julian A. Hiscox[4] and E-M. Zhou[1*]

[1]College of Veterinary Medicine, Northwest A&F University, China; [2]Molecular Virology Laboratory, VA-MD College of Veterinary Medicine and Maryland Pathogen Research Institute, University of Maryland, College Park, MD, USA; [4]College of Animal Science and Veterinary Medicine, Henan Agricultural University, China; [5]Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool L3 5RF, UK

PRRSV has negatively impacted the swine industry worldwide since 1987. PRRSV vaccines have not been effective, in part, because PRRSV is constantly evolving and re-emerging as new variants, due to its high mutation rate that exceeds rates of all known RNA viruses (on the order of 10^{-2} mutations/site/year). Current PRRSV modified live vaccines only confer protection to homologous virus strains, with limited or no protection against heterologous strains. Thus, a high frequency of atypical PRRS outbreaks are observed in vaccinated herds, spurring efforts to find alternative strategies to vaccines for PRRSV prevention and control.

Numerous studies demonstrate that PRRSV infection is mediated by various cellular receptors or factors such as heparin sulfate (HS), vimentin, CD151, CD163, sialoadhesin, DC-SIGN, and MYH9. However, only CD163 and MYH9 have been shown to be indispensable for PRRSV infection. Recently, by probing PRRSV permissive cells with an anti-idiotypic monoclonal antibody (Mab2-5G2), we identified MYH9, encoded by the gene *MYH9*, as the novel cellular host factor that binds to PRRSV GP5. Subsequently, additional investigations demonstrated that MYH9 serves as a host factor for PRRSV and plays an indispensable role in PRRSV internalization by host cells.

In recent years, data from other groups have demonstrated that MYH9 functions as a cellular receptor for several viruses, including herpes simplex virus-1, severe fever with thrombocytopenia syndrome virus, and Epstein-Barr virus, which suggest a novel role of MYH9 in virus infections. Meanwhile, our research has recently demonstrated that the C-terminal portion of MYH9 (designated PRA) interacts with Mab2-5G2 and GP5.

In this study, we investigated whether soluble PRA could serve as a novel blocking agent of PRRSV infection. Our data showed that preincubation of PRRSV with PRA inhibited virus infection of susceptible cells (MARC-145, PK-15^{CD163}, CRL2843^{CD163}, and PAMs) in a dose-dependent manner. Notably, PRA also exhibited broad-spectrum ability to inhibit infection with diverse genotype 2 PRRSV strains. Analysis of the interaction between PRA and PRRSV-GP5 revealed that PRA is able to capture PRRSV virions. While PRA does not block attachment of PRRSV virions to PAMs, it inhibits PRRSV internalization by preventing membrane redistribution of MYH9.

In conclusion, our data suggest that MYH9 protein C-terminal domain could serve as a novel broad-spectrum inhibitor of infection by heterogeneous genotype 2 PRRSV strains.

EpiCC: A new tool for selecting the optimal vaccine during an emerging outbreak

A.H. Gutiérrez[2], L. Moise[1, 2] , M. Parrillo[1*], W.D. Martin[2], A.S. De Groot[1, 2]
[1]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, [2]EpiVax, Inc., Providence, RI

We have developed a novel immunoinformatics tool for comparing potential vaccine strains to outbreak strains using HLA- or SLA- epitope for humans and pigs. Here we applied this tool to existing Porcine reproductive and respiratory syndrome virus (PRRSV) vaccines and circulating strains of PRRSV in US swine herds. EpiCC can be used to compare T cell epitope content between vaccine strains and outbreak strains. It evaluates T cell epitope cross-conservation to develop an epitope-based relatedness or EpiCC score.

Methods: We used PigMatrix to identify T cell epitopes in the complete proteomes of 70 non-redundant PRRSv strains and existing PRRSV vaccines (GP5 Inglevac MLV, GP5 Inglevac ATP, GP5 Fostera). Epitopes predicted to bind to common class I and class II SLA alleles were identified. The epitopes of vaccine and outbreak strains were compared and an EpiCC score was calculated.

Results: We observed that epitope content and conservation varied between US-based pig herd outbreak strains. A threshold of protection was defined using previous EpiCC studies; using this threshold the vaccine efficacy against the selected strains was estimated. We found that none of the vaccines is predicted to protect against all PRRSV strains, however GP5 Inglevac MLV would protect against 7 PRRSV strains, GP5 Inglevac ATP would protect against 4 PRRSV strains and GP5 Fostera would protect against 15 PRRSV strains.

Conclusions: EpiCC gives vaccine researches an additional tool for developing vaccine strains and could also help pork producers pick the appropriate vaccine in a PRRSv outbreak.

Concurrent but consecutive vaccination of modified live type 1 and type 2 PRRSV provides better protection in nursery pigs

Yashavanth Shaan Lakshmanappa[1]*, Pengcheng Shang[2]*, Sankar Renu[1], Santosh Dhakal[1], Bradley Hogshead[1], Pauline Bernardo[1], Xinyu Yan[2], Ying Fang[2] and Renukaradhya J Gourapura[1]

[1]Food Animal Health Research Program (FAHRP), OARDC, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH 44691, USA. [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA.

Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant economic loss to the swine industry worldwide. The current available vaccines do not provide sufficient heterologous protection. In this study, the level of protection against both PRRSV genotypes following concurrent vaccination was evaluated. Conventional 4-5 weeks old PRRSV free pigs (n=12 per group) were vaccinated with modified live virus (MLV) strains of both type 1 and type 2 PRRSV. The type 1 and type 2 MLVs were administered either in combination on the same day (group 1) or 3 days apart (group 2, type 1 MLV followed by type 2 MLV). At day 42, half of the pigs per group (n=6) were challenged with homologous type 1 or type 2 PRRSV. The pig experiment was terminated at 10 days post challenge. Quantitative RT-PCR (qRT-PCR) result showed that type 1 PRRSV RNA was detectable from day 3-42 in group 2 pigs, while only low level of type 1 PRRSV RNA was detected from day 28-42 in group 1 pigs. The type 2 PRRSV RNA levels were comparable and detected from day 7-42 in both groups of pigs. After challenge, the mean viral load of type 1 PRRSV is lower in group 2 pigs than that of group 1 pigs, while no replicating type 2 virus was detected in both groups of pigs. In TBLN that restimulated with the respective challenge virus, the test for recalled lymphocytes response showed enhanced IFN-gamma secreting T-helper/memory and cytotoxic T lymphocytes in both pig groups. In stimulated PBMCs, only T-helper/memory cells were IFN-gamma positive in type II virus challenged animals. In conclusion, vaccination of pigs with both PRRSV genotypes at 3 days apart (type 1 MLV followed by type 2 MLV) provides better protection and clearance of viral infection than those pigs vaccinated simultaneously with both type 1 and type 2 MLVs.

Adenovirus-vectored novel African Swine Fever Virus multi-antigen cocktail elicit strong but non-protective immune responses in commercial pigs

S. Lokhandwala[1]*, L. Popescu[1], N. Sangewar[1], C. Elijah[1], V. Petrovan[1], A. Stoian[1], M. Olcha[1], M. Kerrigan[1], J. Bray[2], S. Waghela[2], R. Rowland[1], W. Mwangi[1].

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, [2]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

African Swine Fever Virus (ASFV) is a high-consequence transboundary animal pathogen that places a huge economic burden on affected countries. The pathogen causes a hemorrhagic disease in swine with a case fatality rate close to 100%. Lack of any treatment or vaccine for the disease make it critical that safe and efficacious vaccines be developed to safeguard the swine industry. Previously, we evaluated the immunogenicity of seven adenovirus-vectored novel ASFV antigens, namely A151R, B119L, B602L, EP402RΔPRR, B438L, K205R and A104R by immunizing commercial swine with a cocktail of recombinant adenoviruses formulated in an adjuvant. The cocktail primed strong ASFV antigen-specific IgG responses as well as ASFV-specific IFN-gamma responses that were recalled upon boosting. To evaluate protective efficacy of the antigen cocktail, we replicated the experiment above and subsequently challenged the pigs with 10^4 HAD_{50} of ASFV-Georgia 2007/1 isolate. The cocktail induced very strong ASFV antigen-specific IgG responses against each antigen in all vaccinees as previously observed. These responses underwent rapid recall upon boost four weeks post-priming. However, upon challenge, the pigs in the treatment group had higher mean clinical scores, mean body temperatures, and decreased WBC counts as compared to the controls. Notably, the mean body temperatures of the pigs in the treatment group was significantly ($p<0.05$) higher than the controls on day four post-challenge. In addition, six of the pigs in the treatment group and only three of the control pigs had to be euthanized on day five post-challenge for animal welfare reasons. Overall, the data suggests that the ASFV-antigen specific antibodies induced in the pigs enhanced ASF virus uptake by macrophages following challenge. The outcome also suggests that the IgG responses induced by these antigens are non-protective and that development of protective ASFV subunit vaccine will require an immunization strategy that will elicit strong cytotoxic T lymphocyte response while limiting humoral response.

Virus-like particle (VLP) based vaccine strategy for porcine reproductive and respiratory syndrome virus (PRRSV)

Yi Lu, Frank Gillam, Chenming Zhang*

Virginia Tech

Porcine reproductive and respiratory syndrome virus (PRRSV) has devastated the swine industry for decades, but no effective vaccines are commercially available. Virus-like particle (VLP) based vaccine presents a safe and potentially effective strategy over modified live virus (MLV) based vaccine against PRRSV. Hepatitis B core antigen (HBcAg) is known for its ability to enticing strong immunological responses. Our overall goal is to develop a VLP based universal vaccine for PRRSV using HBcAg as a carrier for various virus immunogenic epitopes. The objectives of this particular work are to investigate whether or not chimeric HBcAg carrying different epitopes can be successfully expressed in *E. coli*, readily purified, and self-assemble into virus-like particles. Our results show that all chimeric proteins were successfully expressed in *Escherichia coli.* The proteins were purified using ion exchange (IEX) chromatography followed by immobilized metal ion affinity chromatography (IMAC) to homogeneity. Particle assembly was confirmed through both dynamic light scattering (DLS) and transmission electron microscopy (TEM). The animal trials will be conducted in the future to confirm the immunogenicity and virus neutralizing activity of the vaccines.

Demonstration of in vitro VLP production and in vivo immunogenicity utilizing a mutant FMDV 3C protease

Erica Martel1*; Justin Smith2; Ben Clark2; Melia Pisano2; Traci Turecek2; Juan M. Pacheco1; Jose Barrera2; John G. Neilan3; Max Rasmussen3; Michael Puckette3

Plum Island Animal Disease Center (PIADC), Greenport, NY. [1]ORISE PIADC Research Participation Program, [2]Leidos Inc., [3]U.S. Department of Homeland Security Science & Technology

Foot-and-mouth disease virus (FMDV) is a worldwide threat to livestock causing major economic loss. Current FMDV subunit vaccines offer safe manufacture but utilize wild-type FMDV 3C protease (3Cpro) for processing of the P1 polypeptide to yield virus-like particles (VLPs). Wild-type 3Cpro cytotoxicity may limit production yields or expression platform options. Here we identify 3Cpro mutants that enhance transgene output relative to wild-type 3Cpro, and show abundant VLP production in bacterial and mammalian cells. Utilizing the 3C(L127P) mutant, Guinea pigs and cattle were administered purified FMDV VLPs produced in either E. coli or HEK293 cells resulting in high-titer virus neutralizing antibodies. Moreover, in vivo expression of VLPs using the 3C(L127P) mutant in a well-characterized Ad5Blue vector system protected cattle from FMDV challenge 14 days after a single immunization. Equivalent swine studies are in progress. These results demonstrate a potentially significant advance for improved manufacture of next-generation molecular subunit FMDV vaccines.

Funding: DHS S&T funded Leidos, Inc. through award HSHQDC-14-F-00035. ORISE is managed by ORAU under DOE contract number DE-AC05-06OR23100.

Complete antigenic profile of porcine epidemic diarrhea virus

LG Giménez-Lirola[1*], J Zhang[1], JA Carrillo-Avila[2], Q Chen[1], R Magtoto[1], K Poonsuk[1], DH Baum[1], PE Piñeyro[1], J Zimmerman[1]
[1]Iowa State University; [2]Granada University (Spain)

The pig immune system has the ability to recognize specific porcine epidemic diarrhea virus (PEDV) proteins and to respond by producing specific antibodies. However, in the field, pigs are exposed to different coronaviruses, e.g., transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), and porcine deltacoronavirus (PDCoV), which are known to share genetic and antigenic traits. The aim of this study was to identify highly sensitive and specific PEDV antigen targets for the antibody-based differential diagnosis of coronavirus-related enteric disease.

In this study, 72 7-week-old pigs were randomized to six groups; each group consisted of 12 pigs in one room, with 6 pens per room and 2 pigs per pen. Each group of pigs was inoculated with PEDV non-S INDEL, TGEV Miller, TGEV Purdue, PRCV, PDCoV, or culture medium (uninoculated control group). Serum samples (n = 792) were collected from all groups on day post-infection (DPI) –7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42. Virus shedding within groups and absence of cross-contamination between groups was confirmed by rRT-PCR through the collection period (49 days). Five polypeptides derived from PEDV structural proteins spike (S1), nucleocapsid (N), membrane (M), and envelope (E) were recombinantly generated using a prokaryotic (E, N, and M) or eukaryotic (S1) expression system, and purified by affinity chromatography. The antibody response to the different recombinant polypeptides generated was evaluated using a multiplex fluorescent microbead-based immunoassay (FMIA, Luminex®). The antibody response to whole-virus (WV) particles was also evaluated and compared to the response against individual PEDV structural proteins.

Antibody assay cut-offs were selected to provide 100% diagnostic specificity for each target (S1 non S-INDEL, S1 S-INDEL, N, M, E, and WV). The earliest IgG antibody response was detected at day 7–10 post-infection, mainly directed against S1. With the exception of non-reactive protein E, we observed similar antibody ontogeny and pattern of seroconversion for S1 (non-S INDEL, S INDEL), N, M, and WV antigens. Recombinant S1 provided the best diagnostic sensitivity, regardless of PEDV strain, with no cross-reactivity detected against TGEV, PRCV, or PDCoV pig antisera. The WV particles showed some cross-reactivity against TGEV Miller and TGEV Purdue antisera, while N protein presented some cross-reactivity against TGEV Miller. The M protein was highly cross-reactive against TGEV and PRCV antisera.

This study demonstrated that variations in the antibody response against different PEDV structural proteins have important implications in the diagnosis of PEDV infection. We also successfully identified targets of interest (e.g., S1) for the diagnosis of PEDV, providing a truly molecular immunological view of antigenic distribution and a complete antibody cross-reactivity profile between PEDV and other porcine enteric coronaviruses.

Molecular characterization of porcine deltacoronaviruses in South Korea, 2014–2016

C. Lee[1]*, G. Jang[1], S.-H. Kim[2], K.-K. Lee[2]

[1]Animal Virology Laboratory, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; [2]Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon, South Korea

Porcine deltavoronavirus (PDCoV), a member of the genus *Deltacoronavirus* in the family *Coronaviridae* of the order *Nidovirales*, is a newly emerged swine enteric coronavirus causing severe clinical diarrhea and intestinal pathological damage in piglets. In the present study, we aimed to further investigate the prevalence and full-length genome sequence analysis of PDCoV from clinical cases associated with diarrhea from Korean swine farms. Here, a nested reverse transcription (RT)-PCR approach for the detection of PDCoV was developed to identify and characterize etiologic agent(s) associated with diarrheal diseases in piglets in South Korea. A PCR-based method was applied to investigate the presence of PDCoV in 683 diarrheic samples collected from 449 commercial pig farms in South Korea from January 2014 to December 2016. The molecular-based survey indicated a relatively high prevalence of PDCoV (19.03%) in South Korea. Among those, the monoinfection of PDCoV (9.66%) and co-infection of PDCoV (6.30%) with porcine epidemic diarrhea (PEDV) were predominant in diarrheal samples. The full-length genomes or the complete spike (S) genes of the most recent strains identified in 2016 (KNU16-07, KNU16-08, and KNU16-11) were sequenced and analyzed to characterize PDCoV currently prevalent in South Korea. We found a single insertion-deletion signature and dozens of genetic changes in the S genes of the KNU16 isolates. Phylogenetic analysis based on the entire genome and spike protein sequences of these strains indicated that they are most closely related to other Korean isolates and all previous and recent Korean strains are grouped within the US PDCoV clade. However, Korean PDCoV strains formed different branches within the same cluster, implying that the virus continues to evolve and adapt to its natural host in the field. Our data will advance the understanding of the molecular epidemiology and evolutionary characteristics of PDCoV circulating in South Korea.

A novel multiplex field deployable molecular assay for vesicular diseases of pigs.

Rolf Rauh[1], Emma L. A. Howson[2], Veronica L. Fowler[2], Diego G. Diel[3], Jane Christopher-Hennings[3], Travis Clement[3], Wm. M. Nelson[1]
[1]Tetracore, Inc., 9901 Belward Campus Drive, Suite 300, Rockville, MD 20850, USA
[2]The Pirbright Institue, Ash Road, Pirbright, Woking, GU24 0NF UK.
[3]Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, South Dakota

INTRODUCTION *

There are several vesicular diseases of pigs which are clinically indistinguishable that can only be differentiated by laboratory tests, namely: Foot-and-Mouth Disease (FMD); swine vesicular disease (SVD); vesicular stomatitis (VS); and Senecavirus A (SVA).

MATERIALS AND METHODS *

For FMD testing two independent real-time RT-PCR assays targeting different regions (5'UTR and 3D) of the FMDV genome are routinely used to test sample material submitted to the World Reference Laboratory for FMD (WRLFMD). An assay targeting 3D region of FMD viral genome was developed by Tetracore (Callahan et al) and was recently revised based on the new sequences.

For the development of the SVA assay Tetracore collaborated with South Dakota State University (SDSU) and the Pirbright Institute. SDSU assessed whether the genetic differences between current isolates and the isolates obtained prior to 2015 would affect the detection efficacy of the SVA diagnostic test in use at the South Dakota Animal Disease Research and Diagnostic laboratory (ADRDL). For this, the real-time PCR assay currently in use at the ADRDL was tested against several US SVA isolates obtained in 2015 and 2016 and against nine SVA isolates obtained between 1988 and 2002. The SVD assay is currently in development with the Pirbright Institute.

RESULTS *

In a field study in Africa the lyophilized FMD reagents and the T-COR8™, a portable rRT-PCR platform, were compared against the gold-standard laboratory-based rRT-PCR and alternative molecular technologies RT-LAMP and RT-RPA. In this ongoing study, a robust sample preparation method for serum, esophageal-pharyngeal fluid and epithelial suspensions were developed to negate the need for RNA extraction prior to rRT-PCR. The final rRT-PCR protocol and associated lyophilized reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infections. The ability of the T-COR8 FMD rRT-PCR test to utilize simple sample preparation, amplification and detection methods offers promise for rapid in situ FMD diagnosis and demonstrates an important transition for FMDV-specific molecular assays into formats suitable for field diagnostic use. The SVA assay which targets a conserved region of the SVA genome efficiently detected all SVA isolates. Results from the SDSU study provided an improved understanding of genetic diversity of contemporary SVA isolates recently associated with vesicular diseases in the US and in Brazil.

DISCUSSION AND CONCLUSIONS *

The availability of diagnostic assays allowing for rapid and specific (differential) detection and identification of FMD, SVA and SVD in swine naive populations is mandatory. Testing of suspect cases in field rapidly utilizing T- COR 8 field portable system will help considerably to contain the disease in the event of a future outbreak and to understand the rapid disease progression.

Measurement System Analysis (MSA) applied to Restriction Fragment Length Polymorphism (RFLP) of PRRS vaccine viruses and recent field strains

J. Angulo1*, J.G. Calvert2
1. Zoetis, Parsippany NJ, 2. Zoetis, Kalamazoo, MI.

Differential molecular diagnosis of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) began in the Mid-1990's to help veterinarians monitor PRRS control programs. The most commonly available tool for PRRSv classification at that time involved RT-PCR amplification of ORF5 followed by Restriction Fragment Length Polymorphism (RFLP). Nowadays ORF5 sequencing has become the standard method for determining the degree of genetic relatedness between PRRS viruses, and RFLP patterns are automatically generated from the sequence by most US diagnostic laboratories. The categorization of vaccine and field viruses by RFLP pattern provides a common shorthand language for practitioners to refer to different clusters of related viruses in their production systems. However, the relationship between RFLP pattern and genetic relatedness (by ORF5 sequence) is far from perfect. The same RFLP pattern can emerge in different lineages that are only distantly related. Measurement System Analysis (MSA) is a mathematical method of determining how much the variation within the measurement process contributes to overall process variability. It uses statistical tools to determine the amount of variation contributed by the measurement system and it is well used in quality improvement programs to identify measurement errors called as Precision and Accuracy of any single measurement. The objective of this study was to use the MSA method to determine how well RFLP patterns predict actual genetic similarity. For the purposes of this analysis, genetic similarity is defined as ≥98% nucleotide identity, which corresponds to no more than 12 nucleotide changes within the 600-nucleotide ORF5 gene. A "Type 1 gage study" was run for each of the six most prevalent PRRSv RFLP patterns to measure bias and repeatability. The RFLP patterns (1-3-2 (n=63), 2-5-2 (n=33), 1-7-4 (n=29), 1-4-4 (n=14), 1-3-4 (n=12), and 1-26-2 (n=10)) were analyzed from a database of 159 ORF5 sequences collected from 2014-2016 in the USA via the Zoetis STOMP Plus program. The average bias for each RFLP pattern was calculated against Genbank references for Fostera® PRRS (KP300938), Ingelvac PRRS® MLV, or the index sequences in the STOMP Plus database for PRRSv field strains. Bias is the mean percent genetic distance above (positive number) or below (negative number) the 2% sequence variation allowed around the index sequence for that RFLP pattern. Therefore a bias of 0.00% is divergent by exactly 2% (12 nucleotides) from the index sequence for that RFLP pattern. The study showed that the average bias was relatively high for RFLP patterns 1-4-4 (4.22%), 1-3-4 (8.73%) and 1-26-2 (3.53%), indicating that these RFLP patterns are shared by recent field viruses that are only distantly related to the index sequence. In contrast, average bias was much lower for RFLP patterns 1-3-2 (-0.153%), 2-5-2 (-0.272%) and 1-7-4 (-0.193%). For both vaccine viruses and 1-7-4 viruses, the majority of recent field isolates with these patterns are within 2% genetic distance from the index sequence. In a separate analysis of the same database, all sequences with ≥98% nucleotide identity to a given index sequence were sorted by RFLP pattern. The percentages of closely related sequences with the same RFLP pattern as the reference were as follows: 1-3-2: 85.0%, 2-5-2: 96.7%, 2-6-2: 3.3%, 1-7-4: 76.5%, 1-21-4: 14.7% 1-7-3: 8.8%, 1-4-4: 66.7%, 1-4-3: 22.2%, and 1-2-3: 11.1%. These analyses confirm the inherent limitations of RFLP for PRRSv classification. The complete ORF5 sequence should be used for more accurate determination of genetic relatedness between PRRSv strains, and any interpretation should also include herd history (vaccination and/or infection dynamics).

Development of a Luminex Multiplex Assay for the Detection and Differentiation of Type 2 PRRSV Field Strains and the Four US Vaccine Strains.

Yin Wang[1], Wanglong Zheng[1,3], Elizabeth Porlsen[1], Xuming Liu[1,2], Jianqiang Zhang[4], Kyoung-Jin Yoon[4], Lalitha Peddireddi[1,2], Richard Hesse[1,2], Ying Fang[2], Gary Anderson[1,2] and Jianfa Bai[1,2]
[1]Kansas State Veterinary Diagnostic Laboratory, [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; [3]Yangzhou University, Yangzhou, China. [4]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA.

Since its emergence in North America in the late 1980s, type 2 PRRSV becomes one of the most problematic pathogen in swine production systems, which causes significant economic losses in the US. The viral genome has undergone constant changes and new variants have evolved upon time, and that makes molecular diagnosis very challenging. There are four type 2 PRRS vaccines, Ingelvac MLV, Ingelvac ATP, Fostera and Prime Pac, have been used in the US. Differentiating vaccine strains from the field strains is important to guide and improve vaccine applications. Due to that the vaccine strains are very similar to some of the field strains, it is often difficult to differentiate them from the field strains. Currently the most used method of vaccine differentiation is by ORF5 sequencing, which is expensive and time consuming. The Luminex xTAG assay is a bead-based nucleic acid detection that hypothetically can analyze more than 100 different nucleotide sequences in a single reaction. In this study, a Luminex-based multiplex assay was developed to detect the vast majority of type 2 PRRSV field strains, at the same time to differentiate the four vaccine strains that have been used in the US. A collection of 694 full or near-full genomes of North American type 2 PRRSV strains were analyzed. Two pairs of primers targeting the M and N genes were designed for detection. The coverage for each set is 85.4% and 91.2%, respectively, with a combined coverage of 98.1%, by an *in silico* analysis. Four pairs of primers targeting in NSP2 genes of vaccine strains were designed for differentiation. Testing on a number of field strains and the four vaccine strains indicated that the assay detected all PRRS strains and identified each of the four vaccine strains correctly. The analytical sensitivity was performed by 10-fold serial dilutions of three vaccine strains (MLV, ATP and Fostera) and a cloned genomic piece of Prime Pac. To evaluate the limit of detections (LODs), real-time (r) reverse-transcription (RT) PCR assays (rRT-PCR) were also used for comparison. The LODs of the Luminex assay were equivalent to Ct 35.8 by rRT-PCR for MLV, Ct 33.2 for ATP, Ct 31.2 for Fostera, and Ct 36.1 for Prime Pac, which are similar to that generated by rRT-PCRs.

Abstract for 2017 NAPRRS

A multiplex real-time RT-PCR assay for simultaneous detection and differentiation of Influenza D, C, B and A viruses in swine and cattle

Xuming Liu[1,2*], Hewei Zhang[1,3], Yin Wang[1], Yanhua Li[2], Yuekun Lang[2], Elizabeth Poulsen[1], Lance Noll[1], Nanyan Lu[1], Russell Ransburgh[1], Wanglong Zheng[1,4], Wenjun Ma[2], Lalitha Peddireddi[1,2], Ying Fang[2], Gary Anderson[1,2], Jianfa Bai[1,2] (* X. Liu: presenter)

[1]Kansas State Veterinary Diagnostic Laboratory, [2]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA. . [3]Chinese Academy of Agricultural Sciences, Beijing, China; [4]Yangzhou University, Yangzhou, China. [4]Yangzhou University, Yangzhou, China.

Influenza is a highly contagious viral respiratory disease caused by different influenza viruses including influenza A, B, C, and D viruses (IAV, IBV, ICV, and IDV). Timely detection and differentiation of influenza viruses is important for prevention and intervention. IAV in human and animals has been well studied and various mature diagnostic assays are available. In this study, TaqMan real-time RT-PCR assays were developed and validated for detection of IBV, ICV, and IDV in singular and triplex formats. The current USDA and CDC IAV real-time RT-PCR assays were incorporated, and a multiplex real-time PCR panel assay (IDV, ICV, IBV, IAV, and an internal control targeting the host 18S rRNA gene) has been developed for rapid detection and differentiation of the four influenza viruses. Specific real-time PCR primers and probes were designed to target the most conserved gene regions upon the analyses of all available and full- or near-full segment sequences of IBV, ICV, and IDV. Cloning primers flanking the real-time PCR target regions were designed, and positive control plasmids harboring the real-time PCR target regions were constructed. Both the plasmid DNA samples and the *in vitro* transcribed RNA samples from the respective plasmids were used to determine the analytical sensitivity/limit of detection (LOD). The assay coverage rate (perfect matches of all real-time PCR primers and probe) of IBV qPCR is 95.8% over 5,261 Matrix sequences, ICV qPCR is 99.4% over 157 Matrix sequences, and IDV qPCR is 100% over 23 PB1 sequences. The singular and multiplex RT-qPCR protocols were optimized and validated for the identification of IBV, ICV, IDV, and IAV, with PCR efficiencies (E) 90%~110% and correlation coefficients (R^2) >0.99. The multiplex assay was also highly specific in detecting the target influenza virus without cross-reactivity among influenza viruses and other common pathogens in swine and cattle. In conclusion, the high-coverage, high-throughput, low-cost, multiplex real-time RT-PCR assay established in this study will be efficiently and conveniently used for simultaneous detection and differentiation of IAV, IBV, ICV, and IDV.

Key Words: Influenza virus, qPCR, multiplex assay

Detection of novel porcine circovirus type 3 (PCV3) in Poland

A. Woźniak1*, D. Miłek1, K. Biernacka1, T. Stadejek1

[1]Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, Warsaw, Poland

Porcine circovirus type 3 (PCV3) is a novel pathogen discovered in 2016 in USA and in 2017 in China and Poland. PCV3 is suspected to be a causative agent of porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, respiratory disease and multisystemic inflammation.

The objective of this study was to investigate the prevalence and molecular diversity of PCV3 in serum samples collected from14 pigs farms from different provinces of Poland representing different sow herd size and health status.

Materials and methods

Serum samples (n = 1050) were collected from randomly selected 8-10 pigs at the age of 3-20 weeks from 14 conventional pig farms in Poland in 2013-2017. From 12 farms, 10-20 samples from randomly selected sows were also obtained. The samples were pooled by 4-6 before DNA extraction and tested with in house real time PCR for PCV3. Samples with Ct values $\leq$37 were considered positive. ORF2 fragment from one serum pool per farm was sequenced.

Results

PCV3 DNA was detected in 12 out of 14 tested farms. The prevalence on the positive farms ranged from 5.9% to 65% serum pools. Overall, PCV3 was detected in 46 out of 184 (24.5%) serum pools from pigs and in 9 out of 31 serum pools from sows (29.0%). The virus was most common in weaned pigs and finishers (26.1% and 28.0% of serum pools, respectively). Only one serum pool from 3-week-old piglets was PCV3 positive (5.0%). The analysis of partial ORF2 sequences from 9 farms showed that the nucleotide identity ranged from about 95% to >99%. Interestingly, some were >99% identical to previously published sequences from China and USA. No correlation between the presence of PCV3 in serum and health status of pigs was found.

Discussion and Conclusion

The results indicate that PCV3 is highly prevalent in Poland and probably in Europe. 3-week-old piglets are mostly free from infection, what suggests protective role of passive immunity. PCV3 is the most common in finishers. Apparently, the course of PCV3 infection in the analyzed farms was subclinical. Surprisingly, analysis of partial ORF2 sequences shows high genetic diversity of this virus. Further studies about PCV3 are needed to expand global knowledge of the role and evolution of this newly emerged virus.

Detection of porcine parvoviruses 1, 2, 3, 4, 5 and 6 in serum, feces and oral fluid of pigs in Poland

D. Miłek1*, A. Woźniak1, K. Biernacka1, T. Stadejek1

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

Introduction

The aim of the study was to compare the presence of porcine parvoviruses PPV1-PPV6 DNA in serum, feces and oral fluid samples of pigs in 4 Polish production farms: BA, GR, PA, PR.

Material and Methods

The serum, feces and oral fluid samples were collected once (cross-sectional) from pigs of 3 to 17 weeks of age. From each sampled age group 10 serum and 10 fecal samples were collected and tested after pooling by 5. Oral fluid samples were collected one per pen and tested individually. Only in 3-week-old pigs oral fluid samples were obtained individually using cotton swabs and tested after pooling by 5. Total DNA was extracted with QIAamp DNA Mini Kit or QIAamp cador Pathogen Mini Kit (Qiagen) according to the manufacturer's instructions. Three duplex in house real-time PCR assays were performed in 40 cycles (PPV1 and PPV2, PPV3 and PPV6, PPV4 and PPV5).

Results

PPV2, PPV3, PPV4, PPV5 and PPV6 were detected in most of the farms and PPV1 only in GR farm. In all farms from two to six PPVs were co-circulating. Overall, in oral fluid PPV1, PPV2, PPV3, PPV4, PPV5 and PPV6 were detected in 5.4%, 70.3%, 27.0%, 21.6%, 29.7%, 27.0% samples, respectively. In serum PPV1, PPV2, PPV3, PPV4, PPV5 and PPV6 were detected in 1.6%, 67.2%, 21.9%, 9.4%, 15.6%, 18.8% of pools, respectively. In feces PPV1, PPV2, PPV3, PPV4 and PPV6 were detected in 3.1%, 29.7%, 10.9%, 10.9%, 15.6%, 9.4% of pools, respectively. The viruses were present only in weaned and fattening pigs. Majority of positive samples reacted with low to moderate Ct values (Ct>30). Interestingly, exceptionally low Ct values in serum pools were detected for PPV2 (Ct from 13.6 to 17.6) in 5-9 weeks old pigs, for PPV3 (Ct from 12.0 to 15.2) in 11 and 15 weeks old pigs, and for PPV6 (Ct from 10.7 to 17.3) in 13 and 15 weeks old pigs.

Discussion and Conclusion

Infections with all novel parvoviruses are common in Polish pigs. PPV2 appeared to be the most prevalent of PPV species. The infection was most common in weaners and fatteners where PPV2 through PPV6 were detected in all types of samples with high frequency. No PPVs detection in 3-week-old pigs indicates that passive immunity may be protective. The lowest detection frequency was in fecal samples and the highest in oral fluids. Thus, for PPV1-6 surveillance by real-time PCR oral fluid testing can be recommended. Exceptionally low Ct values in serum of PPV2, PPV3 and PPV6 infected pigs indicate high levels of viremia and raise the question on the importance of this viruses for pig health. Further studies are warranted to assess the role of novel PPVs infections for pig health.

Improving the tactics for PRRSV surveillance using oral fluids

YA Henao-Díaz[1*], L Giménez-Lirola[1], K Poonsuk[1], C Wang[1, 2], J Ji[2], TY Cheng[1], RG Main[1], J Zimmerman[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine and [2]Department of Statistics, Iowa State University, Ames, Iowa, USA. 50011.

INTRODUCTION

In 1982, Calvin Schwabe wrote, "*The preventive veterinary medical practice of the future* (would) *largely be a form of on-going on-farm research, based upon surveillance and emphasizing the fact that diagnosis as such closely resembles a research process.*" Given the size and complexity of today's pork industry and the speed with which pathogens move, the need for such an approach is more urgent than ever. Schwabe's vision has never been realized because routine surveillance using individual animal samples (serum) is too difficult and expensive. In contrast, oral fluid (OF) samples are easily collected, welfare-friendly, and an excellent surveillance sample. However, OF often contain feed, feces, and other environmental contaminants that may affect pipetting accuracy and/or test performance. Sample quality is an issue that must be resolved. Extensive processing (filtration or centrifugation) of OF samples is not compatible with high-throughput laboratories. However, treatment of samples with "coagulants" (chemicals used to clarify liquid matrices) is an approach that has not been previously explored. Therefore, the objective of this study was to evaluate the effect of chitosan-based clarification of oral fluids on the detection of PRRSV antibody.

METHODS

OF samples of known status were generated by vaccinating pigs (n = 17) with a PRRSV MLV vaccine. Individual pig samples were collected from day post vaccination -7 to 42 and subdivided into 4 aliquots. Each aliquot was treated with one coagulant formulation (A, B, C) with the 4th aliquot serving as an untreated control (NC). All samples were tested by PRRSV OF ELISA immediately after treatment (day post-treatment DPT 0). Samples were then held at 4°C and re-tested on DPTs 2, 4, 6, and 14.

RESULTS

Among all DPTs, no difference was detected in the proportion of positive PRRSV antibody samples among treatments (Cochran's Q, $p > 0.05$). A repeated measures multiple comparison analysis with Tukey adjustment found no significant difference in ELISA S/P responses between treatments by storage time.

CONCLUSIONS

"Coagulants" clarify by destabilizing the charges on particles suspended in liquid, thereby allowing them to clump and fall out of solution. Clarification of oral fluid samples using chitosan-based formulations did not affect the PRRSV oral fluid antibody ELISA either immediately or over time (DPT). The results suggested that chitosan (or other coagulants) could improve oral fluid handling characteristics without affecting antibody-testing results. This direction holds potential to place the oral fluid specimen and PRRSV surveillance into a next level.

Development of a real-time RT-PCR assay for detecting porcine parainfluenza virus 1 infection in pigs

Yanhua Li[1*], Ying Fang[1], Chase Stahl[3], Jianfa Bai[1,2], Xuming Liu[1,2], Lalitha Peddireddi[1,2], Gary Anderson[1,2]

[1]Department of Diagnostic Medicine & Pathobiology, Kansas State University, KS

[2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University, KS

[3]Fairmont Veterinary Clinic, Fairmont, MN

Porcine parainfluenza virus 1 (PPIV1) was first reported in the pig nasopharyngeal samples in Hong Kong in 2013. Recently, PPIV1 was determined to be widespread in US commercial swine herds. However, no validated diagnostic assay is available for PPIV1 detection. In this report, a one-step real-time quantitative RT-PCR assay (qRT-PCR) targeting the viral hemagglutinin-neuraminidase (HN) gene of PPIV1 was developed and validated. No cross-reactivity was observed with nucleic acid prepared from common swine pathogens, including PRRSV, PCV2, IAV. The limit of detection was determined to be 10 copies per 20 µl reaction using *in vitro* transcribed HN RNA. The performance of this assay was further validated with 120 pig nasal swabs (60 known positive and 60 known negative for PPIV1) and 20 oral fluid (8 known positive and 12 known negative for PPIV1). The qRT-PCR results were consistent with RT-PCR and DNA sequencing of HN gene. This assay was further used to screen the diagnostic samples collected from 10 different farms. Among 310 nasal swab samples that we have tested, 202 samples from 8 farms were PPIV1 positive. Overall, this qRT-PCR assay demonstrates sufficient sensitivity and accuracy for detecting PPIV1 RNA. It will be a useful tool for the rapid diagnosis of PPIV1 infection and in aid of PPIV1 epidemiological surveillance.

Evaluation of the number of samples required to accurately quantify PRRS viral load at the maternal-fetal interface

C. M. Malgarin[1]*, S. E. Detmer[1], D. J. MacPhee[1], J. C. S. Harding[1]
[1]Western College of Veterinary Medicine, University of Saskatchewan, Canada

A previous study looking for PRRS viral particles by immunofluorescence microscopy showed that a focal distribution of the virus can occur in the maternal-fetal interface (MFI; endometrium and attached fetal placenta). It was observed that from 6 analyzed subsamples from the same MFI section, virus was detected in only one, leading to the conclusion that testing a single subsample might not be the most accurate approach. The objectives of this study were to assess the number of subsamples required to accurately quantify viral load (VL) across the entire endometrium (END) and placenta (PLC) supporting each fetus; to determine if viral load is consistent across all of the subsampling locations; and to determine if pooling subsamples before or after extraction affects accuracy of VL quantification. Five pieces (subsamples) of MFI were collected from fetuses of gilts inoculated with PRRSV-2 at gestation day 85 and terminated 2 to 14 dpi. Piece 1 was adjacent the umbilical stump, and pieces 2-5 were positioned towards the ovarian tip, uterine body, mesometrial and anti-mesometrial borders. Virus from manually separated END and PLC from each of 24 fetuses was extracted using commercial kits, and the pieces were pooled as follows: a) the five pieces were extracted individually, b) a pool of five tissue homogenates was made prior to extraction, and c) a pool of RNA was made after extraction of individual pieces. PRRSV RNA was quantified (target copies/mg) by RT-qPCR. The gold standard was considered the average of the five individually extracted and quantified samples. Lin's concordance coefficients (r_c) were used to compare the level of agreement in viral RNA concentration between individual pieces and pools to the gold standard; higher values reflect stronger association between the pool and gold standard. Table 1 shows the mean VL, r_c and coefficient of variation (CV) for five individual pieces, hypothetical pools of 2, 3 and 4 pieces calculated by averaging specific pieces, as well as for the homogenate pool and RNA pool. There was no significant difference in VL among the five different locations of sampling. We conclude that 4 pieces strategically located around each fetus would be the closest result to the gold standard, but at least three strategically located subsamples from each fetus are recommended to obtain an accurate measurement of the PRRSV RNA concentration in the PLC and END. The results show that there is no advantage in pooling RNA from individually extracted subsamples and no disadvantage in pooling homogenates of tissues.

Table 1: Mean and mean coefficient of variation for single subsamples, pools of 2, 3 and 4, homogenate pool and RNA pool; regression coefficient (r^2) measuring association between the groups, the pools and the gold standard.

	ENDOMETRIUM							PLACENTA						
	Single sample	**Pools of 2**	**Pools of 3**	**Pools of 4**	**Pool of 5***	**Tissue pool**	**RNA pool**	**Single sample**	**Pools of 2**	**Pools of 3**	**Pools of 4**	**Pool of 5***	**Tissue pool**	**RNA pool**
VL mean	4.49	4.88	5.06	5.14	5.19	5.20	4.91	2.50	3.03	3.35	3.61	4.03	3.46	3.33
CV mean**	0.51	0.40	0.34	0.31	0.29	0.36	0.42	0.97	0.87	0.81	0.77	0.74	0.74	0.75
r_c mean	0.671	0.835	0.927	0.967	1	0.881	0.805	0.520	0.680	0.772	0.863	1	0.622	0.674

*Pool of 5 = mean of 5 individual samples (gold standard)
** CV mean = coefficient of variation

This work is supported by Genome Canada, Genome Prairie (Saskatchewan Ministry of Agriculture), and Genome Alberta.

ABSTRACT TITLE
Point of Need Molecular Based Detection of African Swine Fever Virus

AUTHOR(S)
J.D. Trujillo[1, 2*], K. Urbaniak[1, 2], T. Wang[3], I. Morozov[1, 2], J. A. Richt [1, 2]

[1]Dept. of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS.
[2]Center of Excellence for Emerging and Zoonotic Animal Diseases, College of Veterinary Medicine, Kansas State University, Manhattan, KS.
[3]GeneReach, USA, MA, USA.

ABSTRACT
African swine fever (ASF) is a highly contagious, commonly fatal disease of swine caused by a large, macrophage-tropic, double stranded DNA virus, ASF virus (ASFv). Natural hosts for ASFV include wild suids and arthropod vectors (Ornithodoros genus). ASFV is endemic in Africa and Sardinia. The recent spread to Transcaucasia, the Russian Federation and Eastern Europe warrant serious concern of further spread into Europe. Rapid detection and response to ASF outbreaks is paramount to mitigate economic and animal losses.

For rapid on-site detection, the qPCR assay for ASFv p72 gene was adapted for insulated isothermal PCR (iiPCR) on the portable device, POCKIT (GeneReach USA). LOD_{100} for this assay was determined on the portable device and compared to the NAHLN (USDA) reference assay on the laboratory thermocycler utilizing non-infectious ASFv controls and purified ASFv DNA from the following ASF viruses: Arm07, E70, Ken05 and OURT 88/3. LOD_{100} for the reference assay performed on the laboratory thermocycler (BioRad CFX) using Quanta qPCR Fast mix II was between 1.2 and 12 ASFv DNA copies. The iiPCR assays on POCKIT yielded similar sensitivities with LOD_{100} between 1-50 ASFv DNA copies. The deployable device demonstrated excellent agreement and reproducibility when compared to the USDA reference assay performed on the laboratory thermocycler. Testing of negative serum samples (n=25) yielded no false positives and a clinical specificity of 100%. Studies using formalin inactivated tissues (n=36 tested in duplicate) attained from experimentally infected pigs resulted in equivalent sensitivity for the deployable device and the reference assay (100% sensitivity). Samples tested contained from 1 to 10^6 copies of ASFv DNA and comprise 6 diagnostically relevant tissue types. Testing of whole blood, fresh tissues and oral fluids are underway.

These data demonstrate the future utility of iiPCR assays on the deployable device, POCKIT device for accurate and sensitive detection of high impact pathogens under field conditions.

Development of a new protocol for quantitative real-time PCR (qPCR) for the detection of African swine fever virus in from formalin-fixed, paraffin-embedded tissues. K. Urbaniak[1, 2]*, D. A. Meekins[1, 2], J. A. Richt[1, 2], A. S. Davis[1], J. D. Trujillo[1, 2]. [1]Dept. of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS. [2]Center of Excellence for Emerging and Zoonotic Animal Diseases, College of Veterinary Medicine, Kansas State University, Manhattan, KS.

African swine fever virus (ASFV) is endemic in Africa and Sardinia and causes a highly contagious, fatal disease in pigs. Recent spread to Transcaucasia, Northern Asia and Eastern Europe causes concern for continued outbreaks. Formalin-fixed, paraffin-embedded tissues (FFPET) are non-infectious specimens with application in surveillance and diagnostics. However, recovery methods for ASFV DNA from FFPET are poorly developed. Recently, we developed a successful protocol for rapid recovery of ASFV DNA from FFPET. Here, we evaluate the FFPET protocol for quantitate qPCR detection of ASFV DNA and compare it to fresh, frozen tissue (FFT).

Tissues from pigs (n=15) experimentally infected with ASFV Arm07, E70 and Ken05 were tested, specifically heart, liver, spleen, tonsil, and superficial cervical lymph nodes (LN) as well as kidney, lung, and renal and gastrohepatic LN. Deparaffinization of FFPET, ATL-proteinase K digestion and DNA de-crosslinking were performed in a single tube. FFT lysates derived from 20% tissue homogenate were processed using ATL-proteinase K digestion. Lysates were processed using automated magnetic bead extractions optimized for DNA recovery. Quantitative qPCR for the detection of the ASFV p72 and actin DNA were performed using Quanta Fast Mix II. The gene copy number (CN) was calculated via plasmid standard curves. The results were presented as ASFV CN per 1000 CN of actin.

Overall sensitivity for detection of ASFV in FFPET was 100% as compared to FFT. Two pigs had ASFV CN outside the quantitative range of qPCR and were not included in inter-protocol equivalency analysis. Eighty-seven percent of the 100 tissue samples from 13 pigs resulted in equivalent detection ASFV CN between FFPET and FFT. The remaining 13% processed as FFPET had 1-2 logs less ASFV detected as compared to FFT. For the two pigs with the low ASFV CN, FFPET was more sensitive for one pig (Arm07), while for the second (Ken05) FFT was more sensitive.

To our knowledge, this is the first study to quantify ASFV CN in FFPET. Its high sensitivity and comparability to FFT confirms FFPET as an alternative specimen for ASFV qPCR diagnostics, vital to advancing ASF eradication, control, and surveillance efforts globally.

Development of rapid diagnostic capability for encephalomyocarditis virus (EMCV)

T. Clement1*, F. Bauermann1, L. Joshi1, M. Maggioli1, D. Rausch1, T. Faccin1&2, M. Fernandes1, F. Rodrigues1&2, M. Martins1&2, D. Diel1.

[1]South Dakota State University, Veterinary and Biomedical Sciences Department, Brookings, SD. [2]Federal University of Santa Maria, Brazil.

Encephalomyocarditis virus (EMCV) is a positive sense single-stranded RNA virus that is capable of infecting several animal species including swine and humans. Rodents are thought to be the natural reservoirs of EMCV, spreading the virus to other susceptible animal species through contaminated feces or carcasses. EMCV causes acute myocarditis and mortality in young pigs or reproductive failure in sows. Mortality rates ranging from 20-100% have been reported in neonatal piglets. The genome has a unique coding region flanked by two untranslated regions (UTR). The 5' UTR is between 800-1200 nucleotides (nt) long and is believed to be highly conserved among different EMCV strains. The objective of this study is to develop and validate a probe-based real time PCR assay for rapid detection of encephalomyocarditis virus (EMCV). Given the high degree of conservation, the 5'UTR region of the EMCV genome was selected as the target sequence for the development of the real time assay. In order to obtain the animal samples for validation of the assay, 5 week old piglets were experimentally infected and divided into three groups consisting of a control group (n=10) mock infected with DMEM, EMCV ATCC-VR 1479 (mouse heart source) infected group (n=10), and EMCV 1988 Hawaii field strain (piglet source) infected group (n=10). Oral, nasal, and rectal swabs were collected from the piglets on days 0, 3, 7, 10, 14, and 22 post inoculation (pi). Blood serum was also collected from the piglets. Heart, liver, spleen, tonsil, lymph node, intestine, and brain were collected from all animals at necropsy for PCR testing. The group inoculated with EMCV strain Hawaii presented detectable EMCV RNA in the heart of eight out of the ten piglets. EMCV was not detected in any of the piglet hearts of the ATCC-VR 1479 piglets nor in the control mock group. Analytical performance of the assay was tested for repeatability, analytical specificity and sensitivity, and diagnostic specificity and sensitivity. Inclusive analytical specificity (100%) was analyzed by detecting five different EMCV strains which had 82.6% - 89.7% similarity amongst themselves when whole genome sequencing was completed on each strain. Exclusive specificity (100%) was analyzed by testing clinical samples that were known to be positive for PRRSV, PCV2, PEDV, PDCoV, SVA, and porcine enterovirus 1, 3 and 7 in triplicate. Heart was the best tissue sample for the detection of EMCV. This new assay has been well validated against multiple strains of EMCV and could be used with confidence by the VDL's.

Seasonality of Porcine Reproductive and Respiratory Syndrome diagnoses in Great Britain

J.P. Frossard[1]*, C.A. Bidewell[2], J. Thomson[3], S. M. Williamson[2].

[1]Animal and Plant Health Agency, Virology Department, Weybridge, U.K., [2]Animal and Plant Health Agency, Surveillance Intelligence Unit, Bury St Edmunds, U.K., [3]Scottish Agricultural College (Consulting), Veterinary Services, Bush Estate, Penicuik, Scotland, U.K.

PRRS has been shown to be seasonal in the U.S.A., Canada and also in Vietnam, with the number of new cases or outbreaks diagnosed showing a consistent pattern of increasing during specific months of the year, and decreasing in others. This study aimed to determine whether such a seasonal pattern also exists for PRRS in Great Britain (GB), where only PRRSV-1 is present and PRRSV-2 has not been detected.

Diagnostic data from the Animal and Plant Health Agency and from Scottish Agricultural College Veterinary Services dating from 2005 to 2017 were analysed. The quarterly rates of PRRS diagnoses for diagnostic submissions were determined. Concurrent diagnoses for submissions where PRRS was diagnosed were also examined to determine their seasonality and to identify any temporal association with PRRS.

The diagnostic rate for PRRS shows a seasonal trend in GB for the period 2005-2017, with peaks of disease between November and May, and a reduction in the diagnostic rate during the third quarter of most years. The percentage of diagnoses of PRRS from total diagnosable submissions ranged from 2.8% to 11.2% (mean 7.6%) for the first quarters of the year (January to March), from 1.6% to 11.9% (mean 6.5%) for the second quarters, from 0.5% to 10.7% (mean 4.9%) in the third quarters, and from 1.2% to 12.7% (mean 7.1%) in the fourth quarters. Every year there was an increase in the rate of diagnoses of new PRRS cases from the third to the fourth quarter, with this increase continuing through Q1 and Q2. The most common concurrent diagnoses established were pneumonia due to *Pasteurella multocida*, disease due to *Streptococcus sui*s serotypes, and salmonellosis due to *S. Typhimurium* and monophasic ST-like variants. These three most common concurrent infections and 42 other concurrent diagnoses did not show seasonality trends in association with PRRS.

One hypothesis to explain this seasonal pattern is that colder, damper and darker conditions during the winter months enable better survival and spread of the PRRS virus on fomites and in the environment, while also making cleaning and disinfection procedures of pig accommodation, vehicles and equipment harder to perform effectively. It is also possible that environmental temperature and ventilation issues during colder months predispose to more severe respiratory disease outbreaks due to PRRS which are more likely to prompt laboratory investigations. A fuller understanding of the causes of the seasonality of PRRS will aid in the implementation of more effective control measures for the disease.

Changes in swine production mandate changes in swine surveillance

M Rotolo1*, C Wang1, M Haddad1, Y Sun1, M Hoogland2, R Main1, J Zimmerman1
[1]Iowa State University, Ames, IA, [2]Smithfield Foods, Algona, IA

Since the 1980's, swine surveillance has been based on the assumption that subjects are independent of each other ("hypergeometric distribution"). Under this assumption, it can be shown that 30 samples from a population are sufficient to achieve a 95% probability of detection, if 10% of the population is infected. In swine surveillance, "30" has become the standard. However, farms have changed dramatically since the 1980's, which raises the question: does this assumption hold in contemporary production systems?

Methods and Results

A recent study made it possible to evaluate the spatiotemporal patterns associated with the spread of PRRSV. Oral fluids were collected from every occupied pen (108 pens; ~25 pigs per pen) in 3 commercial wean-to-finish barns on one finishing site for 8 weeks for a total of 972 OF samples. Samples were completely randomized and then tested for PRRSV by RT-rtPCR. Thereafter, the data were analyzed for spatial autocorrelation using threshold distance as the spatial weight matrix. Moran's I, a quantitative measure of spatial autocorrelation (calculated using GeoDa 1.10) showed positive global spatial autocorrelation in the distribution of PRRSV within barns: the subjects were not independent. LISA (Local Indicators of Spatial Association) analysis identified PRRSV clusters within barns and showed that the spatiotemporal pattern of clusters differed among barns.

Discussion

The RT-rtPCR data showed that PRRSV moved from pen-to-pen, with the result that positive pens clustered together (positive spatial autocorrelation). Tobler was the first to codify spatial autocorrelation in his First Law of Geography: "everything is related to everything else, but near things are more related than distant things." This simple, intuitive concept has huge implications for the way we conduct disease surveillance because it violates the assumption of hypergeometric distribution. Spatial autocorrelation appeared as the swine industry evolved in size and structure over the previous 20 years. That is, as the industry shifted from small, outdoor herds to larger, highly structured populations housed in confinement. Our surveillance methods have not kept pace with changes in the industry. In particular, the presence of spatial autocorrelation signals the need to reevaluate and explore new surveillance methodologies that account for positive spatial autocorrelation.

MinION Next-Generation Sequencing-based Routine Identification and Strain Typing of Porcine Reproductive and Respiratory Syndrome Virus

K.K. Lahmers1*, J.B. Stanton2, S.M. Todd1, T. LeRoith1

1. Department of Biomedical Sciences & Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA
2. Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA

Detection and characterization of pathogens by their nucleic acid is a universally accepted approach. This is typically accomplished by polymerase chain reactions; however, this requires knowledge of suspected pathogens and is limited in pathogen genotyping. Next-generation sequencing allows for an unbiased identification of organisms and provides information that can be used for genotyping. The nanopore-based MinION was used to detect and correctly genotype multiple Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infections in porcine fluids using a custom library for the, open-source software, Centrifuge, which includes prokaryotic and viral RefSeq genomes as well as additional sequences from targeted porcine viruses (e.g., 700+ PRRSV genomes). This low cost, commercially-available sequencer provides long reads in realtime, which allows for spanning of repetitive regions and coverage of full viral genomes in a single read. In addition to identifying PRRS virus, co-infections with other viruses and bacteria were identified, demonstrating the unbiased nature of this assay. Analysis of >10 Gbases of data can be completed using Centrifuge on a laptop within 90 seconds of full sequencing runs. In summary, nanopore-based sequencing is a financially and technically achievable diagnostic tool, which allows for rapid and effective identification and genotyping of PRRSV with the added benefit of identifying co-infections.

Keywords: PRRSV, Next generation sequencing, nanopore, metagenomic analysis, genotyping

Development and validation of real time RT-PCR assay for the detection of Atypical Porcine Pestivirus

Fangfeng Yuan[1], Xuming Liu[1], Jianfa Bai[1], Gary Anderson[1], Ying Fang[1,2], Bailey Arruda[3], Paulo Arruda[3], Lalitha Peddireddi[1]

[1]Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506
[2]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506.
[3]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Atypical Porcine Pestivirus (APPV) was reported as the etiologic agent for type A-II congenital tremor in newborn piglets. A sensitive and reliable PCR-based diagnostic test is critical for accurate detection of APPV. We developed a quantitative real-time RT-PCR (qRT-PCR) assay for reliable detection of all APPV strains. The assay design also included a swine 18S rRNA internal control to monitor PCR inhibitions. A positive control plasmid containing APPV-target region was constructed and a serial of 10-fold dilutions of *in vitro* transcripts obtained from the plasmid was used to determine limit of detection (LOD). Individual 18S rRNA and APPV qRT-PCR assays were optimized separately and then combined into a duplex assay. The individual and duplex assays had correlation coefficients of 0.997 and PCR amplification efficiencies of 91-92%. Comparison of detection limit and analytical sensitivity between assays indicated no inhibition of PCR sensitivity, when both assays are combined. The detection limit for APPV target, based on analytical sensitivity, is ~12 copies per reaction, which corresponds to a Ct of ~38 for both individual and duplex reactions. Assay specificity was verified using nucleic acids (NA) of other closely related pestiviruses and the NA from clinical samples positive for other common swine pathogens. No cross reactivity was observed. Data from six independent runs, including 5 replicates of three clinical samples with three Ct ranges, were utilized to assess inter-assay repeatability and intra-assay reproducibility. This analysis demonstrated intra-and inter-assay coefficients of variation of 0.57% and 1.46%, respectively, with a PCR efficiency of 102.13%. Screening of 758 porcine clinical samples from Kansas State Veterinary Diagnostic Lab identified 110 APPV-positive (Ct $\leq$38) samples, suggesting 14.52 % prevalence of APPV in the US swine herds. Among the sample types tested, oral fluid had lower average Ct compared to others. Detection of APPV positives cases from post weaning and grower pig populations suggests APPV persistent infections. Further studies are needed to support this speculation.

Comparison of six commercial ELISAs for the detection of antibodies against porcine reproductive and respiratory syndrome virus.

Kinga Biernacka[1*], Anna Tyszka[2], Tomasz Stadejek[1]
[1]Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences–SGGW, Nowoursynowska 159c, 02-776 Warsaw, Poland
[2]Veterinary Diagnostic Laboratory, Vet Lab Group, Ostrodzka 46, 11-036 Gietrzwald, Poland

Objectives:
The aim of this study was to compare sensitivity and specificity of six ELISAs commercially available in Europe.

Methods:
A total of 392 serum samples from individual pigs were included in the study. For the analysis of the specificity 139 sow sera from 5 PRRSV-naïve farms were used. For the sensitivity assessment, 253 sera from five PRRSV- positive farms were used. The following ELISA kits were compared: CIVTEST SUIS E/S (Hipra, Spain), IDEXX PRRS X3 Ab Test (IDEXX, USA), INgezim PRRS 2.0 (Ingenasa, Spain), VetExpert PRRS Ab ELISA 4.0 (Bionote, Korea), Pigtype PRRSV Ab (Qiagen) and PrioCHECK PRRSV Antibody ELISA (Thermo Fisher, USA). ELISA's were performed strictly following the producers recommendations.

Results:
The specificity assessed based on the analysis of 139 sow serum samples from PRRSV negative farms was estimated for Hipra and IDEXX for 100%, followed by Bionote and Qiagen with 98.6% specificity, Ingenasa with 96.6% and Thermo Fisher with 94.2% specificity. The proportion of positive results obtained from the analysis of serum samples from PRRSV positive farms ranged from 67.2% (with Thermo Fisher's PrioCHECK PRRSV Antibody ELISA) to 52.6% (with Hipra's CIVTEST SUIS E/S). The sensitivity was measured considering the samples positive in IDEXX ELISA as truly positive (100% sensitivity). The sensitivity of Thermo Fisher was estimated for 96.3%, Qiagen for 95.1%, Ingenasa for 91.6%, Bionote for 85.9% and Hipra for 80.2%.

Conclusions:
IDEXX PRRS X3 Ab Test remains to be a reference for serological diagnosis globally. However, relatively large number of alternatives are available in different countries, often at very competitive prices. Our comparison indicates that the ELISAs alternative to IDEXX suffer from either compromised sensitivity or specificity, when tested on real life diagnostic samples. Based on this study, with a limited number of samples from 10 Polish pig farms, Qiagen's Pigtype PRRSV Ab was showed to be only slightly less specific and sensitive than IDEXX ELISA, and can be also recommended for PRRSV serological surveillance and monitoring.

Acknowledgment:
The study was supported by NCN grant 2013/11/B/NZ7/04950 and KNOW Consortium "Healthy Animal - Safe Food" , Ministry of Science and Higher Education decision 05-1/KNOW2/2015.

Glycoprotein 3 of porcine reproductive and respiratory virus exhibits an unusual hairpin-like membrane topology

Minze Zhang and Michael Veit

Institute of Virology, Free University Berlin, Robert von Ostertag-Straße 7-13, 14163 Berlin, Germany.

The glycoprotein Gp3 consists of an N-terminal signal peptide, a 180 amino acids long and highly glycosylated domain, a hydrophobic conserved region (20 aa) and a variable unglycosylated C-terminal domain (50-60 aa). Gp3 is supposed to form a complex with Gp2 and Gp4 in virus particles, but secretion of the protein from infected cells has also been reported.
Here we analyzed the membrane topology of Gp3 from type-1 and -2 PRRSV strains. First, we found that the N-terminal signal peptide of Gp3 (and also from lactate dehydrogenase-elevating virus) is cleaved despite the presence of a carbohydrate in its vicinity. This is in contrast to Gp3 of equine arteritis virus where a carbohydrate attached at a similar position prevents processing.
Second, we confirmed that a fraction of wild-type Gp3 is secreted from transfected cells; Gp3 from type-1 strains (Lelystad, Lena) to a greater extent than Gp3 from type-2 strains (VR 2332, IAF-Klop, XH-GD). In contrast to intracellular Gp3, secreted Gp3 contains complex-type carbohydrates, indicating that it passed through the secretory pathway. Since intracellular and secreted Gp3 have identical SDS-PAGE mobility after deglycosylation, the secreted form is not derived from proteolytic cleavage.
Next we used a fluorescence protease protection assay to show that the C terminus of Gp3, fused to GFP, is resistant against proteolytic digestion in permeabilized cells. Furthermore, glycosylation sites inserted into the C-terminal part of Gp3 are used. Both experiments indicate that the C-terminal part of Gp3 is translocated into the lumen of the endoplasmic reticulum.
Deletion of the hydrophobic region, but not of the variable C-terminus greatly enhances secretion of Gp3. In addition, fusion of the hydrophobic region of Gp3 to GFP promotes complete membrane anchorage of this (otherwise soluble) protein. Bioinformatics suggests that the hydrophobic region might form an amphipathic helix. Accordingly, exchanging two or three amino acids in the hydrophobic face of the helix by alanine enhanced secretion of Gp3 to the same extent as deletion of the whole hydrophobic region. Exchanging the same amino acids in the context of the viral genome did not prevent release of virus particles (as shown by qPCR), but the particles are not infectious. This is consistent with the proposed role of Gp3 in virus entry.
In sum, Gp3 exhibits a very unusual hairpin-like membrane topology. The signal peptide is cleaved and the C-terminus is exposed to the lumen of the ER. Membrane attachment is caused by a short hydrophobic region, which might form an amphiphilic helix. This rather weak membrane anchoring might explain why a fraction of the protein is secreted. We speculate that secreted Gp3 might function as a “decoy”, which distracts antibodies away from virus particles.

Identification of a novel linear B-cell epitope in nonstructural protein 11 of porcine reproductive and respiratory syndrome virus that are conserved in both genotypes

Nan Jiang, Huan Jin, Yi Li, Xinna Ge, Jun Han, Xin Guo, Hanchun Yang, Lei Zhou*

Key Laboratory of Animal Epidemiology of Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, People's Republic of China

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important pathogens, that hinder the development of global pork industry. Two major identified genotypes of PRRSV, initially circling in the Europe (genotype 1) and North American (genotype 2), were found to surprisingly genetically divergent, which only share approximately ~60% nucleotide sequence identity, however these two genotypes present similar genomic organization, clinical symptoms, and temporal emergence. The PRRSV nonstructural protein 11 (nsp11), with the nidoviral uridylate-specific endoribonuclease (NendoU) domain, is essential for PRRSV genome replication and it also contributes to host innate immunity suppression from various pathway. However, the immunogenicity and immune structure of PRRSV nsp11 have not been well investigated yet. In this study, a monoclonal antibody (mAb) against PRRSV nsp11, named as 3F9, was screened out from the mouse immunized with recombinant His-nsp11$_{40\text{-}223aa}$ protein that expressed by truncated fragment covering 118- 669 nt of PRRSV nsp11. Subsequently, a series of partially overlapped fragments, covered the nsp11$_{40\text{-}223aa}$, were expressed to test the reactivity with mAb 3F9 by western blotting, and the ^{111}DCREY^{115} was found to be the core unit of the B-cell epitope recognized by mAb 3F9. Further investigation indicated that both genotype 1 (GZ11-G1) and genotype 2 (JXwn06) PRRSV can be recognized by mAb 3F9, due to the ^{111}DCREY^{115} is conserved in both genotype virus. Meanwhile, this epitope, localized at the surface of nsp11 in 3D structure, is confirmed to be able to react with pig sera collected form PRRSV infected pigs. These findings do not only contribute to the understanding of the immunogenicity and immune structure of PRRSV nsp11, it also provides an mAb tool for further investigate the function of nsp11, as well as indicating the diagnostic potential for this epitope.

Prediction and evaluation of novel genetic function for previously uncharacterized genes in African swine fever virus.

M.V. Borca[1], L.H .Holinka[1], E. Ramirez-Medina[1,2], K. Berggren[1,3], V. O'Donnell[4], L.B. Carey[5], J.A. Richt[6], D. P. Gladue[1]*

1. Agricultural Research Service, Plum Island Animal Disease Center, Greenport, NY 11944, USA
2. Department of Pathobiology, University of Connecticut, Storrs, CT , 06268 USA
3. Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37831
4. Animal and Plant Health Inspection Service, Plum Island Animal Disease Center, Greenport, NY 11944, USA
5. Universitat Pompeu Fabra, Barcelona, Spain 08003
6. Kansas State University Manhattan, KS 66502, USA

African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal disease of domestic pigs that has significant economic consequences for the swine industry. The viral genome encodes for more than 150 genes, and only a select few of these genes have been studied in some detail. Here we report the characterization of open reading frames encoding genes Ep152R and L83L, both predicted to be immune system regulators. EP152R contains a complement control module/SCR domain which similar to Vaccinia virus proteins involved in blocking the immune response during viral infection. L83L has been identified as an Il-1β binding protein. Here we describe for the first time the role of these two genes in African swine fever infection. Interestingly, analysis of host-protein interactions between EP152R with using a yeast two-hybrid screen, identified BAG6, a protein previously identified as being required for ASFV replication. Attempts to construct recombinant virus having a deleted EP152R gene, were consistently unsuccessful indicating that EP152R is an essential gene. However, we were successful in deleting L83L in the current highly virulent outbreak strain ASFV-G, allowing us to compare the pathogenesis of our mutant virus lacking L83L with that of parental ASFV-G.

RNA stem-loop structures and conserved regions in ORF6 are important for the replication of porcine reproductive and respiratory syndrome virus

Pengcheng Shang[1], Yanhua Li[1,2], Ian Brierley[2], Andrew E. Firth[2] and Ying Fang[1]

[1]Kansas State University, Manhattan, USA; [2]Department of Pathology, University of Cambridge, Cambridge, UK

In Nidoviruses, the high-order RNA structures in untranslated regions and transcription regulating sequences are crucial for virus replication. Bioinformatic analysis on all available full-length genome sequences of porcine reproductive and respiratory syndrome virus (PRRSV) in GenBank suggested the existence of two stem-loop (SL1 and SL2) RNA structures, an extended stem-loop (extSL) formed by a conserved region (CR) and downstream complementary sequence within ORF6 region of both genotypes. A panel of full-length cDNA clones that contains synonymous mutations at these regions was constructed to investigate the roles of SL1, SL2 and CR involved in viral replication. Two panels of full-length cDNA clones containing synonymous mutations at these regions were generated to investigate the roles of SL1, SL2 and extSL played in RNA transcription. In type I PRRSV, SL1 and extSL mutants showed 2-log lower virus titer than that of wild-type (WT) virus, indicating that SL1 and extSL are important for virus replication. Similar results were obtained in type 2 PRRSV; especially, mutations disrupting extSL significantly impaired the virus replication, generated nonviable viruses. The stem region of SL2 is crucial for virus replication in both genotypes, while virus replication was severely attenuated only in type 2 PRRSV when the loop region of SL2 (B-TRS 7.1) was mutated. Quantitative RT-PCR (qRT-PCR) analysis of minus strand RNAs on type 2 PRRSV further showed that compared with those of WT virus, lower levels of minus genomic RNAs were produced by SL1 and extSL mutants, while the relative ratios of minus sgRNA 7.1 were decreased in loop2 and extSL mutants. These data suggest that SL1, SL2 and CR are crucial for virus replication. The detailed mechanism of these RNA structures involved in virus replication is under active investigation.

A naturally occurring cross order recombinant of enterovirus and torovirus

Pengcheng Shang[1], Saurav Misra[2], Ben Hause[1] and Ying Fang[1]

[1]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506; [2]Department of Biochemistry & Molecular Biophysics, Kansas State University, Manhattan, KS 66506

Enteroviruses have been implicated in a wide range of diseases in human and animals. In this study, a novel enterovirus (species G; EVG KS16-08) was identified from a diagnostic sample using metagenomics and complete genome sequencing. The viral genome shared 87.5% amino acid identity and 73.5 % nucleotide identity with prototype EVG strain (PEV9 UKG/410/73). Remarkably, a 582 nucleotide insertion was determined in the 2C/3A junction region of the viral genome, which was flanked by 3Cpro cleavage sites at 5'- and 3'- ends. Sequence analysis revealed that this insertion region encodes a predicted protease that is mostly close to torovirus (ToV) papain-like protease (PLP) with 54-68% amino acid identity. Structure homology modeling predicts that this protease adopts a fold and catalytic site characteristic of a minimal PLP catalytic domain. The structure is similar to that of foot-and-mouth disease leader protease and to the core catalytic domains of coronavirus PLPs, all of which are de-ubiquitinating and deISGylating enzymes toward host cell substrates. More importantly, recombinant ToV-PLP protein derived from this novel enterovirus also showed strong deuibiquitination and deISGylation activity, and demonstrated the capability to suppress IFN-beta expression. Subsequently, ToV-PLP knockout recombinant virus was generated using reverse genetics. In comparison to that of wild type cloned virus, the mutant virus infected cells showed impaired growth property and increased expression level of innate immune genes. These results suggest that ToV-PLP functions as an innate immune antagonist, while the enterovirus may gain fitness with acquisition of ToV-PLP through the recombination event.

A dual ribosomal frameshifting mechanism transactivated by an arterivirus protein and host cellular factors

Yanhua Li[1,2*], Andrew E. Firth[2], Ian Brierley[2], Eric Snijder[3], Jens Kuhn[4], Ying Fang[1]

[1]Kansas State University, Manhattan, Kansas, USA;
[2]University of Cambridge, Cambridge CB2 1QP, United Kingdom;
[3]Molecular Virology Laboratory, Department of Medical Microbiology, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands;
[4]Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, USA.

Arteriviruses are a group of enveloped, positive-stranded RNA viruses that infect animals. They can cause persistent or asymptomatic infections, and also acute disease associated with a respiratory syndrome, abortion or lethal haemorrhagic fever. The family includes porcine reproductive and respiratory syndrome virus (PRRSV), equine arteritis virus (EAV), mouse lactate dehydrogenase-elevating virus (LDV), simian hemorrhagic fever virus (SHFV), and a number of more recently identified members, many of which are of simian origin. Recently, a novel case of -2/-1 programmed ribosomal frameshifting (-2/-1 PRF) was identified in PRRSV. The -2/-1 PRF leads to the translation of two additional viral proteins, nsp2TF (from -2 PRF) and nsp2N (from -1 PRF). Remarkably, this dual ribosomal frameshifting mechanism is transactivated by a viral protein nsp1beta and host factors PCBPs. Critical elements for -2/-1 PRF in PRRSV, including the slippery sequence (RGGUUUUU or RGGUCUCU) and 3' C-rich motif, were also identified in all nine species of simian arteriviruses. Interestingly, in four simian arteriviruses (MYBV-1, KRCV-1, KRCV-2, and SHEV), the slippery sequence (XXXUCUCU instead of XXXUUUUU) cannot facilitate -1 PRF to generate nsp2N. The nsp1beta of simian hemorrhagic fever virus was identified as a key factor that transactivates both -2 and -1 ribosomal frameshifting, and a universally conserved Arg^{114} in arteriviruses is essential to its function. The involvement of PCBPs in -2/-1 PRF in PRRSV and simian arteriviruses was also demonstrated using the *in vitro* translation system. Furthermore, PRRSV nsp1beta could stimulate -2/-1 PRF with the SHFV -2/-1 frameshifting sequences, while SHFV nsp1beta could stimulate -2/-1 PRF with the PRRSV -2/-1 frameshifting sequences. Taken together, these data suggest that -2/-1 PRF is an evolutionarily conserved mechanism employed in arteriviruses for the expression of additional viral proteins.

Antigenic and structural properties of African swine fever virus (ASFV) p30

V. Petrovan1*, M. V. Murgia1, Y. Fang1, R. R. R. Rowland1

[1]Department of Diagnostic Medicine & Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan

African swine fever (ASF) in the most important foreign animal disease threatening the US swine industry. The etiologic agent is African swine fever virus (ASFV), a large DNA enveloped virus and currently the only member of the *Asfarviridae* family. The viral DNA encodes for at least 54 structural proteins. Among the structural proteins is the 204 amino acid membrane protein, p30, which is expressed in abundance during early infection and highly immunogenic, thus providing important targets for serology and vaccination. SDS PAGE of recombinant polypeptide p30 fragments used for screening a panel of monoclonal antibodies (mAbs), identified a fragment covering amino acids 50-150 that migrated at a higher molecular weight than predicted. Computer analysis showed that a peptide sequence covering amino acids 91-137 is highly hydrophilic, enriched in glutamic acid residues, and possessing properties consistent with an intrinsically disordered protein region (IDPR). Based on the preliminary findings, we hypothesize that p30 could contain a region of intrinsic disorder.

A panel of mouse monoclonal antibodies (mAbs) was prepared against the recombinant p30 protein expressed in *E.coli*. One mAb, 47-3, recognized an epitope within the 42-amino acid region, 60-101. Consistent with a linear epitope, 47-3 reacted with p30 on a western blot (WB). In contrast, mAb 62-35, which recognized the C-terminal half of p30 failed to react with any of the 40 to 50 amino acid polypeptide fragments, suggesting the presence of a larger conformational epitope. However, 62-35 retained the ability to react with p30 in a western blot, a property associated with IDPR.

Taken together, mAbs against ASFV p30 provide important reagents for use in development of diagnostic assays and for understanding the unique immunological and structural properties of p30.

Quantifying Resilience Utilizing Feed Intake Data in A Natural Challenge Model for Disease Resilience in Wean-to-Finish Pigs

A. M. Putz[1*], M. K. Dyck[2], PigGen Canada[3], J. C. S. Harding[4], F. Fortin[5], G. S. Plastow[6], and J. C. M. Dekkers[1]

[1] *Department of Animal Science, Iowa State University, Ames, IA 50011, USA*
[2] *Department of Agricultural, Life, and Environmental Sciences, University of Alberta, AB, Canada*
[3] *PigGen Canada, Guelph, ON, Canada*
[4] *Large Animal Clinical Sciences, University of Saskatchewan, Canada*
[5] *Centre de développement du porc du Québec inc. (CDPQ), Quebec, Canada*
[6] *Livestock Gentec Centre, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada*

The objective of this project was to quantify resilience using feed intake (FI) data during the grow-finish phase from a natural challenge study in Québec, Canada. Resilience is defined as the ability to maintain production under challenge conditions. Pigs that are affected by disease typically have a temporary reduction in feed intake. Therefore, maintaining a steady increase in feed intake per day over time could represent resilient phenotypes, while those that have large fluctuations in feed intake could be considered less resilient. This data represents 21 batches of Yorkshire x Landrace barrows from multiple suppliers that were entered into a continuous flow natural challenge barn for a total of 1341 animals. The barn provided exposure to a number of common viral and bacterial pathogens. Root mean square error (RMSE) from individual regressions of daily feed intake on age within animal was calculated as a measure of resilience. Phenotypically, the RMSE measure was favorably predictive of mortality (p-value = 0.009), number of treatments per 180 days (p-value < 0.001), finishing ADG (p-value < 0.001), feed conversion ratio (p-value < 0.001), and residual feed intake (p-value < 0.001). A similar measure of RMSE was calculated using duration at the feeder instead of feed intake and was found to also be phenotypically predictive of these same performance traits. Heritability estimates for daily FI RMSE and Duration RMSE were 0.22 (±0.07) and 0.25 (±0.08), respectively. Moving averages (3, 5, 7, 15, and 31 days) were utilized to reduce some of the noise from daily FI records. Heritability estimates declined as the moving average increased from 3 to 31 days (from 0.22 to 0.09 for FI RMSE and from 0.23 to 0.10 for Duration RMSE). This research demonstrates a heritable resilience measure can be extracted from feed intake data. Funding from Genome Alberta (ALGP2), Genome Canada, Swine Innovation Porc, CDPQ, and PigGen Canada are greatly acknowledged.

Genetic divergence and selection signatures in two selection experiments for residual feed intake

E.D. Mauch[1*], B. Servin[2], H. Gilbert[2], and J.C.M. Dekkers[1]

[1] Department of Animal Science, Iowa State University, Ames, IA, USA
[2] INRA, GenPhySE, Castanet-Tolosan, France

Residual feed intake (RFI) is a measure of feed efficiency that is defined as the difference between observed and predicted feed intake based on production and maintenance requirements. In the 2000's, two independent, yet similar, selection experiments began for RFI in purebred Yorkshire and Large White pigs at Iowa State University (ISU) and The French National Institute for Agricultural Research (INRA), each producing a Low RFI (more feed efficient) and a High RFI line (less feed efficient), and continued for 10 and 8 generations, respectively. The objectives of the present study were to (1) assess the genetic differentiation between and within the ISU and INRA populations and (2) identify selection signatures for RFI. Genotypes from the PorcineSNP60 and the GGP HD Porcine SNP panels were collected on 3,065 and 863 pigs from the ISU and INRA populations, respectively. For objective 1, a weighted principal component analysis (wPCA) was carried out using the R package *wcmdscale* with 39,176 SNPs that were in common between the SNP panels. Results of the wPCA showed that PC1 explained 20.6% of the genotypic variation and differentiated the ISU and INRA populations, while PC2 (7.7%) differentiated the ISU RFI lines and PC3 (5.9%) differentiated the INRA RFI lines. For objective 2, haplotype models were fit that account for population structure and linkage disequilibrium (Servin et al., 2014) using the hapflk v. 1.3 software to analyze SNP genotypes for signatures of selection in the ISU and INRA populations. Further quality control of SNP genotypes was performed, resulting in 33,203 and 31,737 SNPs retained for the ISU and INRA populations and 30,676 SNPs that were in common between populations. To identify genomic regions with changes in haplotype frequencies that were beyond those expected from drift, p-values were calculated empirically by fitting a Normal distribution with mean and variance estimates that were robust to outliers, both separately for each population, as well as combined. Genome build 11 was utilized for SNP location but a search for previously reported QTL (prQTL) in genomic regions with selection signatures was carried out on the PigQTLdb using genome build 10.2. At a false discovery rate (FDR) of less than 0.2, regions on *Sus scrofa* chromosomes (SCC) 2, 13, and 16 showed selection signature peaks for the independent or combined populations. On SSC 2, a region (103 to 106 Mb) was detected in the combined and ISU analyses, which contains a prQTL for feed intake. On SSC 13, from 189 to 191 Mb, a region was detected in the combined and INRA analyses, which contains a prQTL for feed conversion ratio (FCR). Two regions on SSC 16 (33 to 39 and 53 to 54 Mb) were detected only in the ISU analysis, which contain prQTL for feed intake and FCR. Results of this study indicate that identifying selection signatures in selection experiments is difficult in small populations that exhibit substantial genetic drift. By combining two independent populations, regions that appeared to be subject to selection in both selection experiments for RFI were identified, with higher significance in one population ($P < 0.002$) and moderate significance ($P < 0.17$) in the other population (i.e. SSC 2 and 13), along with regions that were unique to the ISU population (i.e. SSC 16). Funding provided by AFRI-NIFA grant #2011-68004-30336 (ISU) and the Chateaubriand Fellowship.

Genetic Analysis of Reproductive Traits in Commercial Sows in Health Challenged Herds

C.L. Ferring[1*], B.E. Mote[2], P. Willson[3], J.C.S Harding[4], G.S. Plastow[5], J.C.M. Dekkers[1], and N.V.L Serão[1], and PigGen Canada[6]

1. Department of Animal Science, Iowa State University, Ames, IA 2. Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 3. Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, SK, Canada 4. Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, SK, Canada 5. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada 6. PigGen Canada, Guelph, ON, Canada

Sow reproduction is a key component of profitability in the swine industry. A high producing sow that stays in the herd through her most productive parities produces more piglets and enhances profitability. Reproductive traits are difficult to select for due to low heritability and the age at which that traits are expressed. However, genomic information can be used to detect quantitative trait loci (QTL) for these traits, and thus may improve the accuracy of selection. Therefore, the purpose of this study was to estimate genetic parameters and identify QTL associated with reproductive traits in commercial crossbred sows.

Performance data from 1 to 4 parities and genotypes (40K SNPs) were available from 21 commercial farms on Large White x Landrace sows from 7 breeding companies (PigGen Canada). A total of 7,635 farrowing records from 2,604 sows were used for analyses. Traits analyzed included total number of piglets born (TB), number of piglets born alive (NBA), number of stillborn piglets (SB), number of mummified piglets (MUM), and number of piglets weaned (NW). Traits were analyzed by parity. In addition, for each trait, the sum of all parities was analyzed as a measurement of lifetime performance. Genetic parameters (heritability) were estimated using Bayes-C0. Genome-wide associations were performed in GenSel using Bayes-B (pi=0.995). The model used for both analyses included the fixed effect of contemporary group, which was defined as the group of gilts that entered a farm on the same date.

Heritability estimates for parities 1 to 4 ranged from 0.05 (NW) to 0.13 (TB), from 0.07 (NW) to 0.18 (TB), from 0.08 (MUM) to 0.15 (TB), and from 0.09 (MUM) to 0.18 (NW), respectively. For lifetime performance, estimates ranged from 0.10 (MUM) to 0.27 (NW). In parity 1, regions associated with TB, NBA, and SB explained 13.9% (chromosome [SSC]1 and SSC6), 10.8% (SSC2 and SSC10), and 20.9% (SSC13) of the genetic variance (GV), respectively. For parity 2, regions associated with SB, MUM, and NW explained 3.9% (SSC11), 3.8% (SSC4), and 10.7% (SSC15) of the GV. In parity 3, regions associated with NW accounted for 4.7% (SSC7) of the GV. In parity 4, regions associated with TB and NW explained 10.6% (SSC13) and 15.8% (SSC130 of the GV, respectively. For lifetime performance, regions associated with NBA, SB, and NW accounted for 14.6% (SSC1 and SSC12), 7.3% (SSC9), and 15.0% (SSC13) of the GV, respectively.

These results indicate that reproductive traits are lowly heritable, but lifetime performance is, overall, more heritable than traits defined by parity. The GWAS revealed QTL that correspond to corpus luteum number, teat number, age at puberty, TB, NBA, SB, and MUM, all traits that are related to reproduction. A novel QTL was also found in NBA for parity 1.

Financial support of PigGen Canada, Canadian Swine Health Board, Genome Alberta and Swine Innovation Porc is appreciated.

CD163 PSTII domain is required for PRRSV-2 infection

A.M.M. Stoian*, R.R.R. Rowland
Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine,
Kansas State University, Manhattan, KS

CD163, a receptor for PRRSV, is composed of nine scavenger receptor cysteine-rich (SRCR) domains and two proline-serine-threonine (PST) regions. Previous work by us showed that pigs lacking CD163 expression on macrophages are protected from infection (Wells *et al.*, 2017). Furthermore, SRCR5 is required for infection with both PRRSV genotypes (Van Gorp *et al.*, 2010; Burkard *et al.*, 2017). The purpose of this study was to identify other domains in CD163 that are necessary for infection with a PRRSV-2 isolate. The model system consisted of transfecting HEK cells with a plasmid that expressed CD163 as an EGFP fusion protein. After two days, the cells were infected with a PRRSV-RFP virus. A positive result was visualized as a cell expressing both green and red fluorescence. Several domain deletion constructs were tested. As expected, all constructs lacking SRCR5 were infected. The deletion of the PSTII domain also made transfected cells resistant to infection. The 16 amino acid PSTII is divided into two regions: the Exon 13 region composed of 12 amino acids and the Exon 14 portion consisting of the four amino acids, GRSS, which are on the external surface of the plasma membrane. The remainder of Exon 14 includes the transmembrane domain and a portion of the cytoplasmic tail. The CD163 PSTII partial and complete deletion constructs were amplified by incorporating primers that possessed *Sac*II restriction sites. The substitution of the GRSS peptide with AAAS had little effect on infection. However, the complete deletion of the 12 amino acids corresponding to Exon 13 prevented infection. Flow cytometry incorporating the porcine CD163 mAb, 2A10, confirmed that all constructs were expressed on the surface of transfected HEK cells. The importance of SRCR5 and PSTII domains in PRRSV infection indicates that the viral protein complex on the surface of the virion may form multiple contacts with CD163.
This work was supported by the National Pork Board project grant #16-181 and USDA NIFA award 2017-67015-26774.

References

Wells KD et al. 2017. Replacement of Porcine CD163 Scavenger Receptor Cysteine-Rich Domain 5 with a CD163-Like Homolog Confers Resistance of Pigs to Genotype 1 but Not Genotype 2 Porcine Reproductive and Respiratory Syndrome Virus. J Virol. In press.

Van Gorp H et al. 2010. Identification of the CD163 protein domains involved in infection of the porcine reproductive and respiratory syndrome virus. J Virol. 84:3101-3105.

Burkard C et al. 2017. Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathog 13(2): e1006206.

Peptide sequence domains in SRCR5 of CD163 that contribute to recognition by PRRSV-2

A.M.M. Stoian*, R.R.R. Rowland
Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine,
Kansas State University, Manhattan, KS

CD163, a receptor for PRRSV, is a scavenger protein composed of nine scavenger receptor cysteine-rich (SRCR) domains and two acid proline-serine-threonine (PST) regions. Our previous work showed that deletion of SRCR5 or PSTII is sufficient to prevent infection CD163 plasmid-transfected cells with a PRRSV-2 isolate. The overall goal of the current research is to identify the minimum changes in CD163 sufficient to make cells resistant to infection with PRRSV-2. The overall approach included the insertion of proline-arginine (PR) dipeptides along the 101 amino acid SRCR5 peptide sequence. The dipeptide insertions were accomplished by placing *Sac*II restriction sites about every 30 bp along the SRCR5 cDNA. When placed in the same reading frame, the *Sac*II sequence, CCG CGG, codes for a proline-arginine dipeptide. The results showed a wide range in infection rates from mutations that had little effect compared to others that almost completely blocked infection. Three PR insertions, located at positions 9, 55 and 100 of SRCR5, produced the greatest reduction in infection, with only a small percentage of CD163-EGFP showing infection after 48 hrs. The results for a space-filling model, based on x-ray crystallography of porcine SRCR5, showed that the three mutations are predicted to be located on a single face of the SRCR5 polypeptide. Even though the PR-9 and PR-100 mutations are located at the ends of the SRCR5 polypeptide sequence, the three dimensional structure predicts that both mutations locate in close proximity to each other, and likely affect the same binding region. The ribbon structure shows that the PR-9 insertion, located between Ile-8 and Pro-9, is predicted to create a short alpha helical structure in front of the β1 strand. One consequence is the re-orientation of proline-8 to the backside of the polypeptide face. The PR-55 insertion, located between Ala-54 and His-55, is predicted to create a kink within the loop structure between β4 and β5, which results in the disappearance of Ala-54 from the protein surface. And finally, the PR-100 mutation, inserted between Val-99 and Cys-100, is predicted to result in the early termination of the β7 strand. Together, the computer model data indicate that the PR insertion causes only localized changes in protein structure.

Acknowledgements. We thank Dr. Dongwan Yoo (University of Illinois, College of Veterinary Medicine, USA) who made available the original CD163 construct. This work was supported by the National Pork Board project #16-181 and USDA NIFA award # 2016-09462, 2017-2020.

Evaluation of porcine trophectoderm cell line susceptibility to porcine reproductive and respiratory syndrome virus-2 infection and its possible role in viral transmission across maternal-fetal interface

M. Suleman[1*], C.M. Malgarin[1], S.E. Detmer[1], D.J. MacPhee[1], J.C.S. Harding[1]

[1]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.

Abstract

The porcine maternal-fetal interface constitutes the major barrier against pathogens crossing from gilt to fetuses. Porcine reproductive and respiratory syndrome virus-2 (PRRSV-2) can not only breach this barrier, but also causes severe reproductive failure characterized by high fetal mortality leading to substantial economic losses to the swine industry. The porcine epitheliochorial placenta contains a single layer of strategically placed fetal trophectoderm cells that play physical, physiological and immunological roles necessary for allowing the optimal development of the fetus. The exact route of PRRSV transmission across the maternal-fetal interface (MFI) is not well defined. Thus, we evaluated a well characterized porcine trophectoderm cell line (PTr2) for its permissiveness to PRRSV-2 infection (NVSL 97-7895) and thus, its possible role in PRRSV transmission across the MFI. PTr2 cells were infected with PRRSV-2 at two different multiplicity of infections (MOI; 1 and 5) and PRRSV-2 was detected with immunofluorescence (IF), reverse-transcriptase quantitative PCR (RT-qPCR), flow cytometry (FCM) and electron microscopy (EM). Preliminary data was obtained after extracting viral RNA from infected PTr2 using commercial kits and a probe-based RT-qPCR targeting PRRSV ORF5 to assess viral load in samples at 0, 24 and 48 h post-infection. We detected PRRSV-2 load tended to decrease slightly between 24 and 48 h (in both MOI of 1 and 5) indicating no viral replication in PTr2 cells up to 48 h post-infection and a possible initial uptake of virus followed by release or neutralization of viral particles by PTr2. Further validation was done by IF of PRRSV-2 infected PTr2 cells and after 72 h incubation the cells were stained with PRRSV-2 specific monoclonal antibody SDOW17 (RTI®, USA). Images obtained using CellSens® software and an Olympus® IX83 microscope equipped with a high resolution Zyla camera showed the presence of PRRSV-2 positive staining within PTr2 cells. We used FCM to quantify the number of PTr2 cells infected by PRRSV, and after 72 h infection we found 4.7 ±0.09% and 12.9 ±0.27% cells infected at MOI of 1 and 5 respectively. Currently, samples are being processed for EM detection of PRRSV within PTr2 cells and also some additional experimentation to identify the mechanism of viral uptake by PTr2 cells. Recently published studies strongly suggest that a highly interesting cross-talk and intercellular communication system exists at the porcine MFI that is mediated by extracellular vesicles (EV). Further investigation is underway to explore if PRRSV is potentially exploiting EV for active transmission from dam to fetuses. This project was supported by Genome Canada, Genome Alberta and Genome Prairie (Saskatchewan Ministry of Agriculture). The PTr2 cell line was kindly provided by Dr. Chandrakant Tayade and Dr. Laurie A. Jaeger.

2017 Symposium Speakers

Keynote Speaker:

Dr. Alison Van Eenennaam is a Cooperative Extension Specialist in the field of Animal Genomics and Biotechnology in the Department of Animal Science at University of California, Davis. She received a Bachelor of Agricultural Science from the University of Melbourne in Australia, and both an MS in Animal Science, and a PhD in Genetics from UC Davis. Her publicly-funded research and outreach program focuses on the use of animal genomics and biotechnology in livestock production systems. Her current research projects include the development of genomic approaches to select for cattle that are less susceptible to disease, the development of genome editing approaches for livestock, and applied uses of DNA-based information on commercial beef cattle operations. She has given over 450 invited presentations to audiences globally, and uses a variety of media to inform general public audiences about science and technology. She frequently provides a credentialed voice on controversial topics including cloning and genetically engineered plants and animals. Dr. Van Eenennaam was the recipient of the 2014 Council for Agricultural Science and Technology (CAST) Borlaug Communication Award, and the 2016 Beef Improvement Association (BIF) Continuing Service Award.

Speakers:

Chuck Allison is the Director of Livestock Procurement and Pork Quality at Smithfield Foods. In his role, he has oversight for procuring 54,000 hd of market hogs a day for Smithfield's East Coast harvesting facilities and for the employees involved in handling the animals at each plant. In addition, he also serves as a liaison between the farms and plants, as well as works closely with Smithfield Premium Genetics to ensure our animals meet our customers' needs. Prior to this role, Chuck was heavily involved in Research and Development for both Fresh and Packaged pork products at Smithfield. Chuck received his MS and Ph.D. from Michigan State University and recently completed his MBA from The College of William and Mary.

Dr. Perle Boyer is an Assistant Professor in Veterinary Population Medicine (VPM) department at the College of Veterinary Medicine, University of Minnesota. Dr. Boyer received her DVM degree from the National Veterinary School of Toulouse, France and her Master of Specialized Veterinary Medicine from North Carolina State University. Dr. Boyer is coordinating the education efforts of the swine group at the College level, including DVM student lectures, continuing education and online learning. Her research efforts are divided between farm-applied projects in adequation with the needs of pork producers and developing new educational techniques in veterinary instruction. Member of the planning committee of the Allen D. Leman swine conference, Dr. Boyer is in charge of the DVM student session and in the faculty committee in charge of the Morrison Swine Innovator Prize.

Jay Calvert: After completing his Ph.D. in Genetics at Purdue University in 1988, **Dr. Jay Calvert** did post-doctoral research on fish viruses at the University of Guelph and on poultry viruses and vectors with the USDA-ARS in East Lansing Michigan. Since 1994 he has been employed by Zoetis and legacy companies (Pfizer Animal Health, SmithKline Beecham Animal Health), and stationed at Lincoln Nebraska, Groton Connecticut, and Kalamazoo Michigan. Dr. Calvert has contributed to a number of vaccine projects for livestock and companion animals, but since 1994 his emphasis has been primarily on swine viruses and especially the PRRS virus. He led the team of scientists that constructed the first infectious cDNA clone of a Type-2 PRRS virus in 1998, and a similar team in 2004 that identified the cellular protein CD163 as a primary receptor for PRRS viruses, being necessary and sufficient to convert common cell lines from PRRSV non-permissive to PRRSV permissive. Dr. Calvert oversaw the generation of novel CD163-expressing cell lines as well as the attenuation of the Fostera® PRRS vaccine (launched in 2012) and the Suvaxyn® PRRS MLV vaccine for Europe (2018). Dr. Calvert is currently exploring alternative vaccine technologies for PRRSV and other pathogens as a Research Director at Zoetis. In his free time he likes to wade rivers in search of trout and salmon, foster homeless dogs, and design/build/fly hobby rockets.

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 8.8M in research funds, has published 148 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.

Dr. Jack Dekkers grew up in the Netherlands and received B.Sc. and M.Sc. degrees from the Wageningen Agricultural University and a Ph.D. from the University of Wisconsin in Animal Breeding. From 1989 to 1997 he was on faculty at the University of Guelph, working closely with the Canadian industry on genetic improvement of dairy cattle. He moved to Iowa State University in 1997, where he currently is a C.F. Curtiss Distinguished Professor and Leader of the Animal Breeding and Genetics group. Current research focuses on the genetic basis of feed efficiency and health in pigs and on the integration of quantitative and molecular genetics, with applications to swine and poultry.

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).

Dr. Ying Fang is a professor at Kansas State University. During the past 18 years, Dr. Fang has been deeply involved in the study of molecular pathogenesis of porcine reproductive and respiratory syndrome virus. Current research program is expending to other emerging/foreign viral pathogens. Her research interests have been focused on understanding the basic molecular mechanisms of viral pathogenesis, and applying this knowledge to develop strategies for the diagnoses, prevention and treatment of viral disease. The laboratory has well-established technologies and pig model system for vaccine and diagnostic assay development. Fang's research has been supported by research grants from the US Department of Agriculture, National Institute of Health, National Pork Board and industry partners. In collaboration with other researchers, she published more than 60 papers in peer-reviewed prestigious journals, and has been invited to give presentations by various universities and professional conferences. Dr. Fang has received various research awards, including Zoetis Animal Health Award for Research Excellence in 2017, Distinguish Researcher Award in 2013, Intellectual Property Commercialization Award in 2010, Dean's Research Award in 2006, SIGMA XI Research Paper Award in 2004, and SIGMA XI Research Proposal Award in 1999.

Stephen Gerike, Assistant Vice President - Channel Marketing at the National Pork Board, creates and oversees the annual strategic marketing plan for the retail, foodservice and alternate food sales channel industries. This includes national trade advertising, the channel marketing websites, newsletters, editorial and industry networking and relations. Stephen developed the annual Pork Summit event for editors and Taste of Elegance winners, the annual Pork Crawl editor event, and is responsible for pork quality and culinary education for the American Culinary Federation, the Research Chef's Association, the International Corporate Chefs Association, the Global Culinary Innovators Association, and targeted retail and restaurant accounts. Stephen previously held the position of Director, foodservice marketing and national food service marketing manager with the National Pork Producers Council before joining the National Pork Board on July 1, 2001. He previously held the position of Senior Manager, Sysco Brand Marketing for Sysco Corporation in Houston, Texas. He also has sales and marketing experience with Compass Marketing, Sysco Foodservices of Baltimore and was the Executive Chef at the historic Mohonk Mountain House, Chef de Cuisine at the Annapolis Hotel, and The Treaty of Paris Restaurant within The Maryland Inn, part of the Historic Inns of Annapolis. He gained his culinary training through a formal apprenticeship in the Philadelphia, PA area. Stephen graduated from Rutgers University with a Bachelor of Arts degree and was a Certified Executive Chef within the American Culinary Federation. He resides in the town of Price on Maryland's Eastern Shore.

Dr. Maynard Hogberg is professor and chair emeritus of the Department of Animal Science at Iowa State University. A native of southwest Iowa, he received his B.S., M.S. and Ph.D. from Iowa State University. In 1976 he joined the faculty in animal husbandry at Michigan State University with a teaching, research and extension appointment in swine nutrition and management. Dr. Hogberg was appointed chair of Michigan State's Department of Animal Science in 1984. He led an effort that created a vision to strength animal agriculture in the state of Michigan, which resulted in substantial state funding for modernization of facilities and programmatic support of new faculty positions and operating funds. After serving on the faculty at Michigan State for 27 years, he was named chair of the Department of Animal Science at Iowa State University in 2003. He has strengthened the department to better service the research and teaching needs for the livestock industry in the state. Dr. Hogberg and his colleagues developed a vision for animal agriculture in Iowa that has become the guiding document to increase research funding for animal agriculture. He has been an active leader in several professional scientific organizations. He served as president of both the American Society of Animal Science, the Federation of Animal Science Societies and president-elect of the National Association for the Advancement of Animal Science. He served on the Symposium Planning Committee for the Food Animal Integrated Research (FAIR 2002) and on the writing committee for FAIR 2006, which prioritized research for animal agriculture in the United States. Dr. Hogberg has been recognized for numerous awards and honors, including Distinguished Service Awards from the Pedigreed Livestock Council, the National Pork Board and the National Swine Improvement Federation. He retired from Iowa State University in 2015

Dr. Jeremy Howard grew up north central Iowa and received a Bachelor's degree at Iowa State University then earned a Master's degree at the University of Nebraska-Lincoln under the direction of Dr. Matt Spangler. He attended graduate school at North Carolina State University and obtained a Ph.D. under the direction of Dr. Christian Maltecca, Department of Animal Science. Dr. Howard's work at NCSU included projects related to random regression models, variation in swine drug metabolism and the use of genomic information to manage a population. Currently a post-doctoral researcher at University of Nebraska-Lincoln, Dr. Howard is working on the application of genomic selection in a swine breeding population.

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. He was the recipient of"The Thousand Young Talents Plan" of China in 2014. Dr. Huang has authored or co-authored 56 publications in peer review journals such as mBio, Journal of Virology and Emerging Infectious Diseases. His current research is mainly focused on epidemiology, biology and vaccine development of swine enteric coronaviruses.

Dr. Richard Leach is Fast Genetics' US-based Senior Geneticist. Dr. Leach obtained a B.Sc. in Genetics from the University of Liverpool, followed by a Ph.D. in Genetics from the University of Edinburgh's Roslin Institute. Prior to Fast Genetics, Dr. Leach worked as geneticist for a large swine genetics company, focusing on customer-oriented R&D. Additionally, Dr. Leach previously worked as a quantitative geneticist researcher at the United States Department of Agriculture. Today, Dr. Leach contributes to Fast Genetics' genetic advancement through his management and involvement in a multitude of molecular and quantitative genetics projects. Dr. Leach also provides technical support to the US sales team and customers.

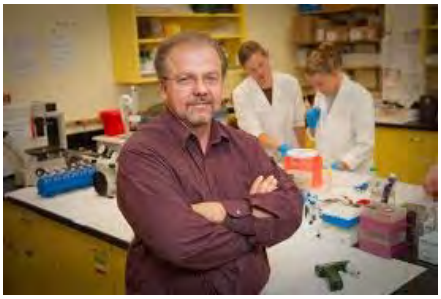
Dr. Joan Lunney is a Supervisory Research Scientist and an internationally recognized authority on pig immunology and genomics. Her lab is located at the USDA Agricultural Research Center (ARS), Beltsville Agricultural Research Center (BARC) in Beltsville, MD USA. Dr. Lunney obtained her B.S. in Chemistry from Chestnut Hill College, Philadelphia, PA, and her Ph.D. in Biochemistry from The Johns Hopkins University, Baltimore, MD. Her current research focuses on swine immunology, genomics, and resistance to diseases, particularly to porcine reproductive and respiratory syndrome (PRRS). She coleads the US PRRS Host Genomics Consortium (PHGC) which assesses the role of genetics in determining pig resistance and susceptibility to PRRS virus infection, pathology and associated growth effects. The PHGC effort has been expanded to functional genomics and proteomics as well as to a pregnant gilt model of PRRS virus infection in collaboration with Canadian scientists. She works with industry, particularly through the National Pork Board and PigGen Canada, Inc., and animal health and breeding companies, to expand her research efforts. She is a member of the Science and Industry Advisory Committee (SIAC) of Genome Canada. Dr. Lunney has led international workshops characterizing monoclonal antibodies (mAb) reactive against swine cell subset, or CD, antigens and immune proteins, the cytokines and chemokines. She coleads the US-UK Collaborative Swine Immune Toolkit effort aimed at developing new mAb, immune reagents and quantitative assays for assessment of pig health and vaccine responses and for use in biomedical models of human health and disease. Dr. Lunney is very actively involved in mentoring younger scientists, particularly women scientists. She was selected as a fellow of the American Association for the Advancement of Science (1998) and of the International Society for Animal Genetics (2017) and received the ARS Beltsville Area Scientist of the Year Award (2010). She has served on numerous grant panels, journal editorial boards and in leadership positions for animal genetic and veterinary immunology societies.

Dr. Alex Morrow, BA, MVB, PhD, MRCVS is veterinary surgeon with seventeen 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.

Dr. Megan Niederwerder is an Assistant Professor at the Kansas State University College of Veterinary Medicine in the Department of Diagnostic Medicine/Pathobiology and the Kansas State Veterinary Diagnostic Laboratory. She received her DVM from Kansas State University in 2009 and after 3 years as a practicing veterinarian, Dr. Niederwerder returned to Kansas State to complete a PhD in swine viral diseases. Dr. Niederwerder's research has been focused on understanding how the microbiome plays a role in outcome following viral infection and how feed plays a role in the introduction and transmission of viral diseases. She was selected as a finalist for the 2016 AVMA Young Investigator Award for her research. Dr. Niederwerder serves as the course coordinator and teaches veterinary virology to second year veterinary students as well as provides outreach service for the veterinary diagnostic laboratory.

Dr. Graham Plastow is a Professor of Livestock Genomics at the University of Alberta and CEO of Livestock Gentec. A pioneer of the application of genomics in animal breeding, he spent more than 20 years in the agri-food industry before returning to academia in 2007. He is interested in the application of genomics to increase value for all of the links within protein value chains (from the animal breeder to the consumer) with a focus on improving animal health .

Tom Rathje currently serves in the role of Chief Technical Officer at DNA Genetics LLC, Columbus, NE. A native of Iowa, he grew up in Clinton on a diversified cattle and cropping operation. Tom received his B.S. in Animal Science from Iowa State University (1989), and his M.S. (1991) and PhD. (1995) in Animal Breeding and Genetics from the University of Nebraska where he currently holds an adjunct professorship in the Department of Animal Science. Tom's M.S. Thesis focused on the effects of selection for testis size on sperm production in boars. His Ph.D. dissertation involved establishing one of the very first QTL mapping studies using selected populations. The study focused on identifying the association of QTL with variation in reproductive performance in swine. He has been with DNA Genetics since its founding in 1995 and has played a key role in the development of the company, being responsible for implementation of the breeding program within nucleus and customer production systems and working in management to develop and support the growth of the company. DNA Genetics has grown to become the second largest provider of swine genetics to the North American market and produces the most widely used terminal line in the swine industry, influencing over 30% of the pork produced. In this position he continues to pursue interests in genetic research including: application of genomic selection in pigs, disease susceptibility, sow longevity, identification of novel traits to improve production efficiencies and furthering the application of animal breeding principles to improve livestock populations at the commercial level. Tom has been a member of ASAS since 1990 and has served on the swine species committee from 2002-2004, serving as chair in 2004. He was the recipient of the Outstanding Young Agribusiness Award from the Midwest Section of ASAS in 2005. He has recently (2016) completed a term on the board of directors for the National Swine Improvement Federation serving as Vice-President and President. Tom has also served in other capacities being a past member of the USDA Swine Germplasm Preservation Committee, the National Pork Board Animal Science committee and currently serves on the NP101 Stakeholder Committee for US MARC. His personal interests include partnering with his wife, Kristi, to keep up with their three adult children, son-in-law and now two grandchildren. Tom and Kristi have a growing business breeding and marketing purebred Simmental cattle.



Raymond R. R. "Bob" Rowland is a Professor in the Department of Diagnostic Medicine and Pathobiology of Kansas State University's College of Veteinary 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.

Dr. Andrea Wilson: The Doeschl-Wilson group investigates how the genetics of individuals affects the spread of infectious disease, both within an animal and between animals. We are an interdisciplinary group of scientists aiming to effectively combine field and laboratory experiments with mathematical modelling and quantitative genetics theory, with the ultimate aim to improve livestock health and resilience.

Dr. Jeff Zimmerman has had a research/teaching appointment in the Iowa State University Veterinary Diagnostic Laboratory since 1990. His research focus is on epidemiology, disease ecology, and the development of cost-effective methods to monitor, quantify, and ameliorate the effect of pathogens on livestock health and productivity. He is the co-editor of Diseases of Swine (9th, 10th, 11th editions) and co-author of 120 refereed publications.

NOTES

NOTES

NOTES

NOTES

NOTES

NOTES

25th International Pig Veterinary Society Congress

2018 International PRRS Symposium

2018.06.10-06.14

Healthy pig Safe pork

25TH International PIG Veterinary Society Congress

2018 International PRRS Symposium