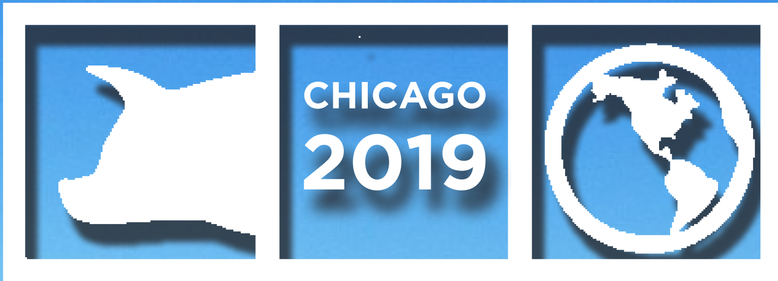
PROGRAM AND PROCEEDINGS FOR THE



North American PRRS Symposium

CHICAGO MARRIOTT MAGNIFICENT MILE

November 2-3, 2019

www.vet.k-state.edu/na-prrs/

EXECUTIVE DIRECTORS

Bob Rowland and Ying Fang

KANSAS STATE UNIVERSITY | College of Veterinary Medicine

The 2019 North American PRRS Symposium

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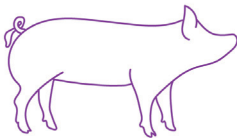

Chicago 2019

Welcome to 2019 NA PRRS Symposium
Emerging and Foreign Animal Diseases of Swine

Dear Friends,

The North American PRRS Symposium was initiated in November 2003 as the "International Workshop on PRRS". The conference continues to focus on the latest knowledge and tools for the control of PRRS, one of the most costly viral diseases to ever face a global swine industry. The latest threat to producers is the continued spread of African Swine Fever Virus (ASFV) throughout Southeast Asia and Europe.

This year we feature a special reception and poster session as a format to discuss and develop collaborative relationships related to exploring new research initiatives for PRRS, ASF and other emerging diseases. Also of note is the integration of the symposium with NC229 and the Conference of Researcher Workers in Animal Diseases (CRWAD), as the means to deliver a much larger and more comprehensive meeting experience. Join us in the celebration as CRWAD conducts its 100th annual meeting.

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

Enjoy the beautiful city of Chicago!

Raymond (Bob) Rowland and Ying Fang
Executive Directors of the 2019 NAPRRS Symposium

2019 North American PRRS Symposium Organizing Committees

2019 NA PRRSS Planning Committee

Executive Directors, NA PRRSS: Dr. Raymond R.R. Rowland
Kansas State University

Dr. Ying Fang
University of Illinois

Executive Director, CRWAD: Dr. David Benfield
The Ohio State University

Industry Liaison: Dr. Lisa Becton
National Pork Board

NA PRRSS Publicist: Joe Montgomery
Kansas State University

NA PRRSS Internal Coordinator and Proceedings Editor: Francine Rowland
Kansas State University

NA PRRSS Onsite Coordinator: Jennifer Stalley
Midwest Solutions

NA PRRSS and NC229 Joint Scientific Committee

Co-Chairs, Joint Scientific Committee: Dr. Sheela Ramamoorthy
North Dakota State University

Dr. Ying Fang
University of Illinois

Committee Members:

Dr. Kay Faaberg: *USDA Agricultural Research Service*

Dr. Montserrat Torremorell: *University of Minnesota*

Dr. Hiep Vu: *University of Nebraska*

Dr. Leyi Wang: *University of Illinois*

Dr. Dongwan Yoo: *University of Illinois*

Dr. Yanjin Zhang: *University of Maryland*

NA PRRSS Travel Fellowship Selection Committee

Dr. Joan Lunney: *USDA, ARS-BARC*

Dr. Dongwan Yoo: *University of Illinois*

Dr. Megan Niederwerder: *Kansas State University*

2019 Michael P. Murtaugh Memorial Lecture in Immunology
Sponsored by Boehringer Ingelheim Animal Health

”The Adaptive Immune Response to PRRSV”

Dr. Tobias Kaeser
North Carolina State University

The animal health and veterinary virology communities suffered a tremendous loss with the passing of Dr. Michael P. Murtaugh in 2018. Dr. Murtaugh was internationally recognized as a leader in the battle against swine diseases, including porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus-associated disease (PCVAD). Mike was one of the original PRRS researchers. He was the first to report on the genetics of PRRSV, which has laid the groundwork for investigations into antigenic variation, virus gene evolution, and vaccine development. He is generally considered “The PRRS Immunologist”.

Mike was best known for his leadership in the PRRS community. Mike is the founder of the conference we now know as the North American PRRS Symposium. It began in November 2003 as the “International Workshop on PRRS”, held in conjunction with CRWAD. He was director of the first PRRS USDA Coordinated Agricultural Project, or PRRS CAP-1. His vision for collaboration in research as a means to address producer needs is the same standard of practice applied today. Mike’s ideas of the 1990’s continue to ripple through the scientific community.

2019 North American PRRS Symposium Travel Fellowships/Scholarships

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

Through the generosity of the NA PRRS Symposium sponsors, we are able to provide almost $25,000 in travel support.

This year the Symposium welcomes a generous donation from **Elanco Animal Health**.

The David A. Benfield Student Travel Fellowship

Dr. David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished career 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 in the PRRS field. Currently, he is an associate vice president at The Ohio State University. Dr. Benfield’s generous donation initiated the Symposium 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.

The Bob Morrison Travel Scholarship

sponsored by Boehringer Ingelheim Animal Health

The Dr. Bob Morrison Travel Scholarship, sponsored by Boehringer Ingelheim Animal Health is given in memory of Dr. Bob Morrison, who was a professor in the Department of Veterinary Population Medicine at the University of Minnesota. His leadership 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, was the creator of the Swine Health Monitoring Program, an industry-wide effort to help the industry prevent the next “PRRSV”.

2019 Travel Fellowship/Scholarship Recipients

Elanco Animal Health Travel Fellowships

Ryan Jeon	Iowa State University
Jineui Kim	University of Illinois
Kyu-Sang Lim	Iowa State University
Xingyu Yan	Kansas State University

The David A. Benfield Student Travel Fellowships

Lilla Dénes	University of Veterinary Medicine Budapest
Christian La Londe	University of Montreal
Hepzibar Clilverd Maymo	Universitat Autònoma de Barcelona (UAB, Barcelona, Spain)
Anton Yuzhakov	Federal State Budget Scientific Institution "Federal Scientific Centre VIEV" (FSC VIEV)

The Bob Morrison Travel Scholarships

Sponsored by Boehringer Ingelheim Animal Health

Yulu Chen	Iowa State University
"Mark" Jian Cheng	Iowa State University
Felipe Mathias Weber Hickmann	Iowa State University
Yanli Li	Universitat Autònoma de Barcelona, Spain
Carolina Malgarin	University of Saskatchewan, Western College of Veterinary Medicine
Salik Nazki	Chonbuk National University, Iksan, Republic of Korea
Sergey Raev	Federal State Budget Scientific Institution "Federal Scientific Centre VIEV" (FSC VIEV)
Sofia Riccio	The Pirbright Institute & Institute of Infection and Global Health (IGH), University of Liverpool
Leticia Maria Pereira Sanglard	Iowa State University
Shaoyuan Tan	University of Minnesota
Heather L. Walker	The Ohio State University
Fangfeng Yuan	Kansas State University

2019 North American PRRS Symposium Final Program
November 2-3, 2019

FRIDAY, NOVEMBER 1, 2019	
	Registration, 1:00 PM - 5:00 PM – 5th Floor Registration Desk
SATURDAY, November 2, 2019	
	Registration, 7:00 AM - 5:00 PM – 5th Floor Registration Desk
8:00 AM-12:00 PM	***Session 1 – Chicago Ballroom A,B,C,D*** ***Recent Advances in the Control of Swine Diseases***
	Moderators: Ying Fang, University of Illinois; and Bob Rowland, Kansas State University
8:00 AM	**2019 Michael P. Murtaugh Memorial Lecture in Immunology** *Tobias Kaeser-North Carolina State University* "**The adaptive immune response to PRRSV"** *Sponsored by Boehringer-Ingelheim*
8:30 AM	*Chris Tuggle-Iowa State University*, "**The structure and function of the pig genome: Application to PRRSV research"**
9:00 AM	*Douglas Gladue-USDA Plum Island*, **"Deletion of a novel gene in African swine fever virus produces complete attenuation and protection against current outbreak strain ASFV-Georgia"**
9:30 AM	*Ana Reis- Pirbright Institute,* **"African swine fever virus: understanding virus-host interactions to aid vaccine development"**
10:00 AM	**Break**
10:30 AM-12:00 PM	***Special Session on the Control of PRRS in the Field***
	"**Shifting the PRRS research focus to grow/finish phase of production – What are we learning and implications for making progress on PRRS control**" *Sponsored by Boehringer-Ingelheim*
10:30 AM	**Moderator and Introduction**, *Montse Torremorell-University of Minnesota* " **Why should we focus our attention to PRRSV in growing pigs?"**
10:40 AM	*Jose Angulo-University of Minnesota*, **"Incidence of PRRS wild-type virus in growing pigs and risk factors for infection"**
11:00 AM	*Mariana Kikuti-University of Minnesota*, **"Evolution of PRRS viruses in growing pigs"**
11:20 AM	*Cesar Moura and Daniel Linhares-Iowa State University*, **"Controlling PRRS in growing pigs and dynamics of virus infection"**
11:40 AM	*Edison Magalhães and Daniel Linhares-Iowa State University*, **"Drivers of wean-to-finish mortality and the impact of disease status"**

12:00 PM	Lunch
1:15 PM-5:00 PM	Session 2 – Chicago Ballroom A,B,C,D *Invited talks and presentations from selected abstracts- Organized by NC-229*
	Moderators: Scott Kenny, Ohio State University; and Sheela Ramamoorthy, North Dakota State University
1:15 PM	**Dr. Bob Rowland, Kansas State University** **Opening Remarks and Recognition of Dr. David A. Benfield and Dr. Peter Johnson.**
1:30 PM	*Fangfeng Yuan*, "**Development and characterization of monoclonal antibodies against African Swine Fever virus"**
1:45 PM	*Salik Nazki*, "**Local immune responses play a vital role in PRRSV clearance from infected pigs"**
2:00 PM	*Kyu-Sang Lim*, **"Single-cell RNA-sequencing to identify transcriptional signatures in a PRRSV persistent cell line"**
2:15 PM	*Levon Abrahamyan*, "**Global profiling of nidovirus–host protein interactions by comparative proteomics (PEDV and PRRSV)"**
2:30 PM	*Ryan Jeon*, "**Effect of genotype at a GBP-5 marker on resilience to a polymicrobial natural disease challenge in pigs"**
2:45 PM	*Adthakorn Madapong*, "**Efficacy of type 1 PRRSV MLV against HP-PRRSV or co-challenge with PRRSV1 when vaccinated via IM or ID in pigs"**
3:00 PM	**Break**
3:30 PM	*Hepzibar Clilverd*, "**Characterization of PRRSV-1 transmission routes in an endemic farm identifies conserved phylogenetic clusters"**
3:45 PM	*Alex Pasternak*, "**The Fetal immune response following PRRSV2 challenge of third-trimester gestating gilts**
4:00 PM	*Carolina Malgarin*, "**PRRSV-infected fetuses show evidence of hypoxia and apoptosis"**
4:15 PM	*Lilla Dénes*, "**Detection of atypical porcine pestivirus (APPV) by in situ hybridization and electron microscopy in the brain tissues of CT affected piglets"**
4:30-5:00 PM	NC229 Business Meeting
5:30 PM-7:30 PM	*Special Reception and Poster Session* *4th Floor Avenue Ballroom* *(NA PRRS Symposium or All Access Pass Registered Participants Only)*
SUNDAY, November 3, 2019	
8:00 AM-12:00 PM	*Session 3 - CRWAD Sessions on Swine Diseases organized by CRWAD – 5th Floor, Chicago Ballroom E*

8:00 AM	*Nicholas Streauslin*, **“Repeatability of open reading frame 5 sequencing for PRRSV at different concentrations”**
8:15 AM	*Declan Schroeder*, **“Development of a bespoke PRRSV genomic toolkit”**
8:30 AM	*Christian Lalonde*, **“Whole genome sequencing of PRRSV lead to a better classification of clinical cases”**
8:45 AM	*Alex Pastenak,* **“Maternal and fetal thyroid hormone disruption following late gestation PRRSV2 challenge”**
9:00 AM	*Jenelle Dunkelberger,* **“Genomic regions associated with host response to infection with a highly pathogenic PRRSV strain”**
9:15 AM	*Jared Young,* **“Isolation and immortalization of PRRSV GP5 specific porcine B-cells”**
9:30 AM	**Break**
10:00 AM	*Sandra Rodriguez-las*, **“Study of pig behavior later in life associated with maternal immune activation”**
10:15 AM	*Y Li*, **“Generation of pigdendritic cells from Flt3 ligand-dependent bone marrow cultures and the infection by PRRSV1”**
10:30 AM	*Elisa Crisci*, **“Genomic characterization of PRRSV-1 Infection In pulmonary Innate Immune cells”**
10:45 AM	*Sofia Riccio*, **“Identification of host proteins that interact with non-structural proteins-lα and -lβ of prrsv-1”**
11:00 AM	*Jayesh Chaudhari*, **“Characterization of the swine immune responses to a synthetic live-attenuated PRRSV vaccine”**
11:15 AM	*Chang-Gi Jeong*, **“Evaluation of live attenuated chimeric PRRSV vaccine against Korean type 2 field strains in a reproductive model”**
12:00 PM	***End of NA-PRRSS/NC-229 Conference***

2019 North American PRRS Symposium
Selected Featured Speaker Abstracts

Number.	Speaker Name	Presentation Title	Institution or Organization
Speaker 01	Tobias Kaeser	The Adaptive Immune Response to PRRSV	North Carolina State University
Speaker 02	Chris Tuggle	The Structure and Function of the Pig Genome: Application to PRRSV research	Iowa State University
Speaker 03	Douglas Gladue	Deletion of a novel gene in African swine fever virus produces complete attenuation and protection against current outbreak strain ASFV-Georgia	USDA Plum Island
Speaker 04	Ana Reis	African swine fever: understanding virus-host interactions to aid vaccine development	Pirbright Institute
Speaker 05	Montse Torremorell	Why should we focus our attention to PRRSV in growing pigs?	College of Veterinary Medicine University of Minnesota

Speaker 01

The adaptive immune response to PRRSV

A.R. Kick, A.F. Amaral, L.M. Cortes, E. Crisci, G.W. Almond, **T. Käser***

North Carolina State University, Department of Population Health and Pathobiology, Raleigh, NC

The adaptive immune response contains two parts – a humoral and a cellular immune response. Both parts play an integral role in the fight and protection against pathogens such as PRRSV. Despite its importance, many parts of this adaptive immune response are still unknown – especially of the cellular adaptive immune response. To overcome this lack of understanding, the cellular immune response to both PRRSV vaccination and infection with two concurrent type-2 PRRSV strains was studied using polychromatic flow cytometry (pFCM). This method facilitated the combined analysis of the proliferation or IFN-γ production with the differentiation and homing patterns of three T-cell subsets – cytotoxic T lymphocytes, T-helper cells, and TCR-γδ T cells. This presentation will provide on the one side an overview of the advantages of pFCM; and it will summarize our findings on the humoral and cellular adaptive immune response with focus on the T-cell immune response to PRRSV.

While our data on the humoral immune response confirm the delayed neutralizing antibody response for a modified live vaccine, neutralizing antibodies upon infection with current North Carolina strains NC174 and NC134 followed the timeline of the IgG response. Regarding the cellular adaptive immune response, our data establish an important role for T-cell subsets in the homo- and heterologous immune response to PRRSV: Cytotoxic T lymphocytes seem to play a crucial role in the lung and BAL; and Th cells seem to have a central role in combating PRRSV in blood as well as in the lymphoid and lung tissues. In comparison to these TCR-αβ T cells, TCR-γδ T cell showed a reverse homing pattern: While naïve TCR-αβ T cells home to lymphoid tissue via the expression of the chemokine receptor CCR7, naïve TCR-γδ T cells lack CCR7 expression and seem to home to non-lymphoid tissue. Upon activation of TCR-γδ T cells by PRRSV, proliferating cells upregulated CCR7; in contrast, IFN-γ producing TCR-γδ T cells lacked this CCR7 upregulation. These data also indicate a dual function for TCR-γδ T cells in PRRSV; but the specific role of TCR-γδ T cells in the different tissues will need to be addressed in future studies.

This novel system provides a powerful tool to decipher the cellular immune response to PRRSV. Thereby, it will provide not only valuable information to improve the design and evaluation of vaccines against PRRSV; but it will also facilitate the prediction of cross-protection of vaccines against various circulating PRRSV strains.

Speaker 02

The Structure and Function of the Pig Genome: Application to PRRSV research

Christopher K Tuggle and Jack C M Dekkers

This talk will describe the molecular and population genetic approaches to understanding immune responses to PRRSV infection, as well recent advances in improving the underlying genome assembly for the pig and annotation of the genome itself.

Analysis of genetic variation in the porcine genome and association of such variation to differences in host response to infection by PRRS virus is a central approach toward identifying host genetic resistance to this virus. One application of genome-wide tools to identify genetic variants associated with differences in host response to PRRSV infection was the use of SNP chips in Genome-Wide Association Studies (GWAS). Using such a GWAS for analysis of data from large-scale experimental infection studies of nursery pigs identified a genomic region and an associated genetic marker (WUR) that controls a significant portion of genetic differences in host control of PRRSV replication (as measured by viral titer in blood) as well as growth immediately post-infection. The causative locus was identified to be a splice variant in the GBP5 gene. This genetic marker is now being used for selection in several breeding programs. Although host response to PRRSV infection is heritable, further analysis has not found other major genes but smaller effect size associations have been uncovered, and the genes in the regions near the associated SNPs have been shown to be enriched for NK cell function using genome annotation information. This is intriguing since NK cells are an important cell type in combating viral infections. In other work, CD163 knock-out pigs have been found to be completely resistant to PRRSV infection. Although such completely resistant pigs have not been found in nature, recently, several SNPs in the CD163 gene were found to be associated with viremia in blood following PRRSV infection.

Additional approaches to find the genes important for resistance to PRRSV infection have used transcriptomic and proteomic surveys of immune tissues and blood, either prior to or during PRRSV infection of animals or cells in culture. Several pathways and cellular functions are associated with the immune response to PRRSV in blood transcriptomic response to infection.

Both GWAS and transcriptomic analyses are hampered by the lack of knowledge of the biological function of identified genes. Recently, the USDA has funded projects to increase our understanding of the functional components of the newly improved pig genome assembly, as part of a global consortium called Functional Annotation of Animal Genomes, or FAANG. Such functional knowledge can help us filter the most important variants to improve genetic resistance to PRRSV infection, as well as identify regulators and their target genes to clarify immune response networks. These functional annotation projects have a number of approaches, but focus on determining the RNAs expressed in mainly adult tissues, as well as the epigenetic marks that identify functional regulatory elements in these tissues. A focus more recently has been on immune tissues and cell types, including macrophages responding to LPS and poly-IC to mimic responses to bacterial and viral infections.

The discovery of genes and regulatory elements controlling the pig's response to PRRSV infection will provide new opportunities to interpret GWAS studies as described above, as well as identify genes that could be of interest to modify with the intent to improve disease resilience in pigs.

Speaker 03

Deletion of a novel gene in African swine fever virus produces complete attenuation and protection against current outbreak strain ASFV-Georgia

Manuel V. Borca[1], Elizabeth Ramirez Medina[1,2], Ediane Silva[1,3], Elizabeth Vuono[1,4], Ayushi Rai[1,5], Sarah Pruitt[1,5], Lauren G. Holinka[1], Lauro Velazquez Salinas[1,3], James Zhu[1], and **Douglas P. Gladue[1*]**

[1] Agricultural Research Service, U.S. Department of Agriculture, Plum Island Animal Disease Center, Greenport, NY 11944, USA.[2] Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT 06269, USA.[3] Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506.[4] Department of Pathobiology and Population Medicine, Mississippi State University, P.O. Box 6100, MS 39762.[5] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN 37830, 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 disease is devastating the swine industry in Central Europe and East Asia, with current outbreaks caused by circulating strains of ASFV derived from the 2007 Georgia isolate (ASFV-G), a genotype II ASFV. In the absence of any available vaccines, African Swine Fever (ASF) outbreak containment relies on control and culling of infected animals. Limited cross protection studies suggest that in order to ensure a vaccine is effective it must be derived from the current outbreak strain or at the very least from an isolate with the same genotype. Here we report the discovery that deletion of a previously uncharacterized gene, from the highly virulent ASFV-G produces complete virus attenuation in swine. Animals inoculated intramuscularly with the virus lacking this gene, ASFV-GΔ, in a dose range of 10^2 to 10^6 HAD_{50} remained clinically normal during the 28 day observational period. All ASFV-G-ΔXXX-infected animals had low viremia titers, showed no virus shedding, developed a strong virus-specific antibody response and, importantly, they were protected when challenged with the virulent parental strain ASFV-G. ASFV-G-ΔXXX is one of the few experimental vaccine candidate virus strains reported to be able to induce protection against the ASFV Georgia isolate, and the first vaccine capable of inducing sterile immunity against the current ASFV strain responsible for recent outbreaks.

Speaker 04

African swine fever: understanding virus-host interactions to aid vaccine development

Ana Reis

The Pirbright Institute, United Kingdom

African swine fever virus (ASFV) causes a haemorrhagic fever in domestic pigs and wild boar with lethality rates approaching 100%. The current epidemics of ASFV in Europe and Asian countries has highlighted the urgent need for an effective vaccine against this devastating disease. Live attenuated viruses provide the fastest route to achieve such a vaccine. Deletion or modification of genes involved in the evasion of the host defences, but not required for virus replication in cells, can be used to rationally attenuate ASFV. Several of these genes encode inhibitors of the type I interferon response, the main host antiviral pathway. ASFV also codes for proteins that inhibit host cell apoptosis, prolonging host cell survival to increase infectious virus production. The EP402R gene codes for a CD2 like protein that mediates the attachment of red blood cells to both infected cells and virions, hence playing an important role in viremia levels and virus persistence in blood. We tested different ASFV single or multiple deletion mutants in vivo and the outcomes of these experiments will be discussed. Our results show that a delicate balance between virus replication and induction of immunity is key to produce a safe and efficacious vaccine. This will probably require a specific combination of gene deletions, which may be vary according to the ASFV genopyte.

Speaker 05

Why should we focus our attention to PRRSV in growing pigs?

Montse Torremorell, DVM, PhD

College of Veterinary Medicine University of Minnesota, St. Paul, MN 55108

Porcine reproductive and respiratory syndrome (PRRS) continues to be the most important disease affecting the U.S swine industry. PRRS virus (PRRSV) causes significant economic losses to U.S swine producers with most of the cost attributed to losses in the growing period. The latest estimate by Holtkamp et al (2017) attributed 62% of the $581 million USD losses to growing pigs. Despite the significant losses in the growing pigs, most of the investments in PRRSV control and prevention strategies happen in the sow herd. This is in part due to the recognition that control in growing pigs starts with weaning a negative pig and thus the need to focus on protecting the sow herd. However, the sole focus of PRRS control in the sow herd may be holding us back in our efforts to control and eradicate PRRSV from regions. Results from the Morrison Swine Health Monitoring Project indicate that PRRSV incidence has not really decreased significantly since the project started 10 years ago. PRRSV incidence has ranged between 20 and 40% and it has been only in the years that the industry has been concerned about the spread of emerging viruses that the incidence has been in the lower range. Both 2013/14 and 2018/19 seasons had lower incidence and coincidentally they corresponded to the emergence of PEDV in the US and ASF in China, respectively, which no doubt heightened the industry awareness to disease prevention.

Evidence is also growing of the risk that finishing pigs represent to PRRSV infections into sow farms. An association between PRRSV prevalence in growing pigs and PRRSV detection in sow farms has been documented by Angulo and Yeske (2018). Temporality of PRRSV infection in growing pigs and sow farms has been observed and there is a general understanding that the risk of infections in sow farms increases as infection rates in growing pigs increase. Thus realistically we need to advance control of PRRS in growing pigs if we want to significantly advance control in sow farms. However, we have limited information on what is happening to PRRSV in growing pigs. We lack information on estimates of wild type PRRSV introductions, risk factors that trigger PRRSV infections, biosecurity measures effective at preventing and containing PRRSV in growing pigs and control measures able to maximize productivity while limiting viral spread. Thus the challenge and opportunity for the swine industry is to put attention to PRRSV control and prevention in growing pigs while keeping production costs at check. By focusing in improving disease prevention in growing pigs we will not only improve PRRSV control but we will have a more resilient industry capable to prevent the spread of other diseases since growing pigs are the current Aquila’s tendon of the US swine industry.

2019 North American PRRS Symposium Special Reception and Poster Session
November 2, 2019 – Chicago Marriott, 4th Floor - Avenue Ballroom

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 01	Chang-Gi Jeong	Evaluation of live attenuated chimeric PRRSV vaccine against Korean type 2 field strains in a reproductive model	Chonbuk National University
PRRS 02	Roman Pogranichniy	Ukraine: Seven years with African Swine Fever virus (ASF) - experience and recommendations	Kansas State University
PRRS 03	Carolina Malgarin	PRRSV-infected fetuses show evidence of hypoxia and apoptosis	Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan
PRRS 04	Pauline Guidoni	Placental tight junction integrity in PRRSV-2 infected fetuses	University of Saskatchewan
PRRS 05	Heather Walker	Tonsil scrapings for porcine reproductive and respiratory syndrome detection in growing pig populations	Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University
PRRS 06	Felipe Hickmann	Host-genomic scan for total antibody response during a PRRSV outbreak in purebred sows	Department of Animal Science Iowa State University
PRRS 07	Felipe Hickmann	Accuracy of genomic prediction for total antibody response in purebred sows during a PRRS outbreak	Department of Animal Science Iowa State University
PRRS 08	Leticia P Sanglard	Association between vaginal microbiome and antibody response to PRRS vaccination in commercial gilts	Department of Animal Science Iowa State University
PRRS 09	Leticia P Sanglard	Vaginal microbiome of PRRS-vaccinated gilts differs between animals with high and low farrowing performance	Department of Animal Science Iowa State University

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 10	Jian Cheng	Genetic parameters of disease resilience traits in wean-to-finish pigs from a natural disease challenge model	Iowa State University
PRRS 11	Jenelle Dunkelberger	Genomic regions associated with host response to infection with a highly pathogenic PRRSV strain	Topigs Norsvin USA
PRRS 12	Yulu Chen	Protein levels in the blood of young healthy pigs as indicators of disease resilience	Department of Animal Science Iowa State University
PRRS 13	Yanli Li	Generation of pig dendritic cells from Flt3 ligand-dependent bone marrow cultures and the infection by PRRSV1	Universitat Autònoma de Barcelona (UAB)
PRRS 14	Alex Pasternak	The Fetal immune response following PRRSV2 challenge of third-trimester gestating gilts	Department of Animal Science Purdue University
PRRS 15	Alex Pasternak	Thyroid hormone disruption in feeder pigs following experimental and natural PRRSV2 and polymicrobial infections	Department of Animal Science Purdue University
PRRS 16	Alex Pasternak	Maternal and fetal thyroid hormone disruption following late gestation PRRSV2 challenge	Department of Animal Science Purdue University
PRRS 17	Junsheng Dong	Development of an ELISA for the detection of swine antibodies in response to influenza B, C, and D viruses	Kansas State Veterinary Diagnostic Laboratory, Kansas State University
PRRS 18	Dmitiy Masiuk	Diagnosis of virulent strain of PRRS on sow farm in Ukraine	Dnipro State Agrarian and Economic University
PRRS 19	Jineui Kim	Expression attenuation of proinflammatory cytokines by NF-κB activation and type I IFN suppression-negative PRRSV	College of Veterinary Medicine, University of Illinois

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 20	Kathleen Walter	Immunosuppressive effects of PRRS virus are dampened in alveolar macrophages from arachidonate-fed piglets	North Carolina State University
PRRS 21	Salik Nazki	Local immune responses play a vital role in PRRSV clearance from infected pigs	College of Veterinary Medicine, Chonbuk National University
PRRS 22	Elisa Crisci	Genomic characterization of PRRSV-1 infection in pulmonary innate immune cells	North Carolina State University
PRRS 23	Levon Abrahamyan	Global profiling of nidovirus–host protein interactions by comparative proteomics (PEDV and PRRSV)	Faculty of Veterinary Medicine, University of Montreal
PRRS 24	Levon Abrahamyan	Polycations enhance the infectivity of two porcine nidoviruses (PRRSV and PEDV) of veterinary importance	Faculty of Veterinary Medicine, University of Montreal
PRRS 25	Alexandra Henao-Diaz	Detection of PRRSV-specific antibody in swine fecal samples	Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University
PRRS 26	Alexandra Henao-Diaz	Licensed commercial kit vs standardized overnight protocol. What works better for PRRSV OF ELISA testing?	Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University
PRRS 27	Alexandra Henao-Diaz	Influence of technician on PRRSV OF ELISA test results	Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University
PRRS 28	Ryan Jeon	Effect of genotype at a GBP-5 marker on resilience to a polypathogenic natural disease challenge in pigs	Department of Animal Science Iowa State University
PRRS 29	Sheela Ramamoorthy	Oral vaccine delivery for porcine epidemic diarrhea virus	North Dakota State University

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 30	Brett O'Brien	A comparison of vaccine setback and viremia following Prime Pac® PRRS RR and Ingelvac® PRRS MLV vaccination	Merck Animal Health
PRRS 31	Fangfeng Yuan	Developing a Parainfluenza Virus 5 (PIV5)-based PRRSV vaccine	University of Illinois at Urbana-Champaign
PRRS 32	Adthakorn Madapong	Efficacy of type 1 PRRSV MLV against HP-PRRSV or co-challenge with PRRSV1 when vaccinated via IM or ID in pigs	Chulalongkorn University
PRRS 33	Sim-In Lee	Intradermal inactivated vaccine against PCV2 and Mycoplasma hyopneumoniae (Mhp) induces protective immunity in pigs	Chonbuk National University
PRRS 34	Marie-Eve Koziol	Adjuvant for Porcine Epidemic Diarrhea and Transmissible Gastroenteritis Bivalent Inactivated Vaccines	Seppic
PRRS 35	Renukaradhya Gourapura	Corn based nanoparticle inactivated swine influenza virus vaccine augments mucosal immune response in pigs	The Ohio State University
PRRS 36	Victoria Primavera	Effect of incorporation of the FMDV 2B viroporin into a plasmid based virus-like particle production platform	Leidos
PRRS 37	Michael Puckette	Development and evaluation of a mammalian cell-culture-produced FMDV virus like particle vaccine in swine	U.S. Department of Homeland Security Science and Technology Directorate
PRRS 38	Federico Zuckermann	Comparative efficacy of inactivated and modified-live PRRS virus vaccines against a heterologous virus challenge	College of Veterinary Medicine, University of Illinois
PRRS 39	Sergei Raev	Porcine dermatitis and nephropathy syndrome in Russia: a case-report	Federal State Budget Scientific Institution Federal Scientific Centre VIEV

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PRRS 40	Anton Yuzhakov	Complete genome of a “Russian group” isolate of subtype 1 PRRSV-1 is different from the “classic” PRRSV-1 strains	Federal State Budget Scientific Institution Federal Scientific Centre VIEV
PRRS 41	Sofia Riccio	Identification of host proteins that interact with non-structural proteins-1α and -1β of PRRSV-1	University of Liverpool
PRRS 42	Declan Schroeder	Development of a bespoke PRRSV genomic toolkit	College of Veterinary Medicine, University of Minnesota
PRRS 43	Guehwan Jang	Assessment of the protective efficacy of a cold-adapted attenuated genotype 2b PEDV	Kyungpook National University
PRRS 44	Jonghyun Park	Genotypic and phenotypic characteristics of novel emerging lineage 1 PRRSV nsp2 deletion variants in South Korea	Kyungpook National University
PRRS 45	Hepzibar Clilverd	Characterization of PRRSV1 transmission routes in an endemic farm identifies conserved phylogenetic clusters	Department of Animal Health and Anatomy, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB)
PRRS 46	Young-Min Lee	Host factors involved in porcine reproductive and respiratory syndrome virus entry	Department of Animal, Dairy, and Veterinary Sciences, College of Agriculture and Applied Sciences, Utah State University
PRRS 47	Stacie Crowder	Medium chain fatty acids to reduce Porcine Reproductive Respiratory Syndrome Virus replication in MARC-145 cells	Department of Animal Science Purdue University
PRRS 48	Xingyu Yan	In vivo characterization of an emerging PRRSV variant with novel -2/-1 PRF signal in nsp2 region	Department of Pathobiology, University of Illinois Champaign-Urbana

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 49	Kyu-Sang Lim	Single-cell RNA-sequencing to identify transcriptional signatures in a PRRSV persistent cell line	Iowa State University
PRRS 50	Fangfeng Yuan	Development and characterization of monoclonal antibodies against African Swine Fever virus	Kansas State University
PRRS 51	Salman Butt	Long and short reads sequencing of total RNA for detection of pathogens in chicken respiratory clinical swabs	Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, US National Poultry Research Center, USDA-ARS
PRRS 52	Vladimir Celer	Epitope mapping of the ASFV p30 protein	University of Veterinary and Pharmaceutical Sciences Brno
PRRS 53	Bob Rowland	Preventing PRRS through modifications in the virus receptor CD163	Kansas State University
PRRS 54	Lilla Dénes	Detection of atypical porcine pestivirus (APPV) by RNAscope method and electron microscopy	University of Veterinary Medicine Budapest
PRRS 55	Gyula Balka	Design and development of photonic biosensors for swine viral diseases detection	University of Veterinary Medicine Budapest
PRRS 56	Scott Kenney	Replication of zoonotic genotype 3 hepatitis E virus (HEV) is enhanced by the ORF4 protein of human genotype 1 HEV	Food Animal Health Research Program, Department of Veterinary Preventative Medicine, The Ohio State University
PRRS 57	David Meekins	Improved methods for African Swine Fever Virus whole genome sequencing and genotyping	Kansas State University
PRRS 58	Natasha Gaudreault	Comprehensive transcriptomic analysis of African Swine Fever Virus infected pigs	Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University

Poster No.	Presenting Author	Title	Institution/Organization
PRRS 59	Tavis Anderson	An overview of research at the National Animal Disease Center on endemic and emerging viral diseases of swine	National Animal Disease Center
PRRS 60	Rolf Rauh	Development of a novel multiplex real-time PCR for the detection and differentiation of SADS-CoV, an emerging swine coronavirus	Tetracore
PRRS 61	Anoyatbekova Afshona Muzafarbekovna	First report of Pestivirus D (Border Disease Virus) identification in small ruminants in Tajikistan	Federal State Budget Institution “Federal Scientific Center VIEV”
PRRS 62	Victoria Vasilyevna Stafford	Immunohistochemical Diagnostics of Porcine Reproductive and Respiratory Syndrome	Federal State Budget Institution “Federal Scientific Center VIEV”
PRRS 63	Jeffrey Zimmerman	NC-229 Multistate Committee: Iowa State University Annual Report (2018 - 2019)	Iowa State University
PRRS 64	Kevin Lahmers	Virginia NC229: PRRS virus and other emerging viral diseases of swine	Virginia-Maryland College of Veterinary Medicine
PRRS 65	Steven Lawson	Detection and control of PRRS virus and emerging viral diseases of swine (NC-229 South Dakota)	South Dakota State University
PRRS 66	Hiep Vu	NC-229 Multistate Research Project: Nebraska Station Update	Department of Animal Science, Nebraska Center for Virology, University of Nebraska-Lincoln
PRRS 67	Bob Rowland	NC-229 Multistate Research Project: Kansas State Progress Report	Kansas State University

PRRS 01

Evaluation of live attenuated chimeric PRRSV vaccine against Korean type 2 field strains in a reproductive model

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Objective

PRRS vaccinology faces a major challenge of inadequate or no cross-protective immunity conferred by currently available vaccines. To broaden the cross-protective range of vaccine candidates, a chimeric virus was constructed and evaluated for its safety and cross-protective efficacy in pregnant sows. CB1 chimeric PRRSV was constructed using pFL12 backbone by replacing ORFs 3-6 with ORFs of two Korean field strains of most prevalent lineages of PRRSV-2 in Korea (K07-2273: Korean lineage C and K08-1054: lineage 5) and inserting attenuated sequences in ORF1a.

Methods

Six PRRSV-free pregnant sows at 60 days of gestation were divided into 3 groups of 2 sows each. Two groups were vaccinated with CB1 while the third was kept as non-vaccinated control. The vaccinated groups were challenged with K07-2273 and K08-1054, respectively, at 30 days post-vaccination (dpv) whereas the two unvaccinated pigs were challenged with K07-2273 and K08-1054, respectively at 90 days of gestation. Blood samples were collected at 0, 7, 14, 21 (dpv) and 0, 7, 14, 24 day post challenge (dpc). Live born piglets were evaluated for vertical transmission of virus until 28 days after birth.

Results

As compared to the non-vaccinated sows, the CB1-vaccinated sows presented lower viral loads in sera after challenge. All of the CB1-vaccinated sows were seropositive at 14 dpv, which was maintained up to 24 dpc, whereas the non-vaccinated sows remained seronegative prior to virus challenge. Moreover, decreased fetal death rate was perceived in the vaccinated sows and the neonates from the vaccinated sows weighed significantly higher than that of non-vaccinated pigs.

Conclusions

This study advocates that the cross-protective ability of CB1 verified against K08-1054 and K07-2273 strains may perhaps be facilitated not only due to the presence of structural proteins from these field strains but also by an immunogenic pFL-12 backbone. Therefore, CB1 opens possibilities for broadly effective vaccines for reproductive diseases against various PRRSV strains.

Financial Support

Rural development admin republic of Korea

PRRS 02

Ukraine: Seven years with African Swine Fever virus (ASF) - experience and recommendations

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Objective
This article describes the importance of ASF disease in Ukraine and chronological events with ASF in Ukraine from the beginning, as well as the monitoring of ASF initiated by AFO and supported by Ukrainian veterinary services.

Methods
Data from Ukrainian State Diagnostic laboratory on confirmed ASF cases in Ukraine were used in this study.

Results
2007-2012 - threat monitoring (eastern Europe): Georgia (2007) → Armenia, Azerbaijan, Iran and the Russian Federation. Active monitoring (threat of entry - increased control by the competent authorities).

2012 - Elimination of the first case of ASF in Ukraine (taking measures to prevent the spread of the disease, revision of the regulatory framework). In parallel, deregulation and change in state supervision (control) in the field of economic activity, reforming the state veterinary service → loss of effectiveness of control of market operators and traceability (control of movement of animals and products).

2014 - Confrontation in the country (military operations in the east), the entry of ASF - 16 cases (4 - domestic, 12 - wild), in three regions of Ukraine bordered by the Russian Federation and Belarus 11 districts in Ukraine.

2015 - 40 cases of ASF (34 - domestic, 5 - wild, 1 - infected object) in central Ukraine and in the south (29 districts in 9 oblasts).

2016 - 91 cases of ASF (84 - domestic, 7 - wild) diffuse spread of disease across the country (16 oblasts), except for several western and southeastern oblasts, mostly among domestic pigs.

2017 - 163 cases of ASF (119 - domestic, 38 - wild and 6 infected objects), spread of the disease with coverage of all (24) oblasts.

2018 - 145 cases (93 - domestic, 39 - wild, 13 - infected objects), tendency to decline (20 oblasts).

2019 - 44 cases (28 - domestic, 10 - wild, 6 - infected objects), reduction of the number of outbreak (currently only 13) areas.

Conclusions
New cases of ASF continue to appear in the swine population but not as often. There are many reasons why ASF is still present in Ukraine and the possibility of eliminating this disease exists.

PRRS 03

PRRSV-infected fetuses show evidence of hypoxia and apoptosis

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Objective

Pregnant sows infected with PRRSV can present fetal death and abortions. As dead fetuses display minimal or no gross lesions, the mechanisms of fetal death are not well understood. We aimed to investigate differences in gene expression related to apoptosis and hypoxia in PRRSV-infected and non-infected fetuses to elucidate the possible causes of fetal death following PRRSV infection.

Methods

Gene expression was assessed by RT-qPCR in brain and heart samples collected from fetuses on gestation day 97, 12 days after maternal inoculation. The genes of interest were selected based on their involvement in hypoxia (HIF1a, IDO1, VEGF) and apoptosis (CASP3, CASP7, CASP8, CASP9, RIPK1, RIPK3). Brain (n=48) and heart (n=56) were selected from 4 phenotypic groups: fetuses from non-inoculated gilts (CON); fetuses from inoculated gilts that escaped infection (UNINF); viable high viral load fetuses (HVL); and HVL meconium-stained fetuses (MEC). Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method and univariate non-parametric analysis (Kruskal Wallis followed by Dunn) was performed to determine group differences within genes.

Results

The fetal brain of the PRRSV infected animals (HVL and MEC) showed significant (P<0.05) increased gene expression of HIF1a, IDO1, CASP7, and CASP8, and a trend (P=0.5) towards increased RIPK3, compared to other groups. CASP3 was decreased in UNINF fetuses when compared to CTRL and MEC but not significantly altered among CTRL, HVL, and MEC. CASP9, RIPK1, RIPK3, and VEGF did not significantly differ among the groups. Fetal heart showed a significant (P<0.05) increase in expression of HIF1a, IDO1, and CASP8, on infected groups (HVL and MEC). CASP3, CASP9, RIPK1, and RIPK3 were only increased in HVL fetuses. VEGFa was only increased in MEC fetuses. CASP7 was the only gene to have decreased expression in HVL and MEC groups when compared to CTRL and UNINF.

Conclusions

In conclusion, there is significant evidence that hypoxia and apoptosis occur in the brain and heart of PRRSV-infected fetuses. These are important results, given the cruciality of both organs in animal survival.

Financial Support

Genome Prairie

PRRS 04

Placental tight junction integrity in PRRSV-2 infected fetuses

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Objective

Existing PRRS control strategies are not completely effective and alternative approaches are needed to prevent the disease. Although intrauterine growth restricted (IUGR) fetuses appear more resilient to transplacental PRRSV infection than normal fetuses, the exact mechanisms are not fully understood. The objective of this research is to assess tight junction proteins (TJ) within the porcine placenta and search for potential alterations in integrity that affect the movement of nutrients and PRRSV across the placental barrier.

Methods

Twenty-four paraffin embedded samples of maternal-fetal interface (MFI) from 6 non-infected (CON) and 18 PRRSV-2 infected fetuses (divided equally by group; IUGR, non-IUGR, meconium stained (MEC)) were randomly selected. Five-micrometer thick tissue sections were immunostained for claudin1 (CLDN1), claudin4 (CLDN4) and zonula occludins (ZO1). Microscopic staining intensity was scored in 10 regions of MFI using a semi-subjective scoring system (absent, mild, moderate, abundant), Image J software, and a series of custom macros.

Results

There were no group differences in CLDN4 in any region and no group differences in any TJ protein in areola and glandular epithelium regions. IUGR fetuses had a lower expression of CLDN1 than CON in maternal endothelial cells, and lower expression of ZO1 in maternal and fetal villus crypts as well as maternal and fetal endothelial cells. MEC fetuses had a lower expression of CLDN1 than CON in fetal villus crypt and tip as well as maternal and fetal endothelial cells. ZO1 expression in MEC was also lower in maternal and fetal villus crypts and fetal endothelial cells compared to CON. The only difference between non-IUGR and CON fetuses was in CLDN1 with lower expression in the fetal villus crypt and maternal endothelial cells.

Conclusions

There were no differences in TJ protein expression among the PRRSV-2 infected groups, only between control and infected fetuses. The results of this study will enhance our understanding of the biology of transplacental PRRSV-2 infection and may enable strategies to prevent or impede fetal infection.

Financial Support

Genome Prairie

PRRS 05

Tonsil scrapings for porcine reproductive and respiratory syndrome detection in growing pig populations

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Objective

Porcine reproductive and respiratory syndrome (PRRS) is the most costly swine disease, affecting pigs of all ages. Research on testing methods for growing pig populations is limited[1], and the use of tonsil scrapings (TS) for PRRS virus (PRRSV) detection has not been thoroughly investigated under field conditions. The objectives of this study were to describe the use of TS and oral fluids (OF) to detect PRRSV in growing pig herds; and investigate whether farm vaccination status was associated with PRRSV detection in TS.

Methods

Two PRRS positive farms were enrolled. The first was a 3,500-head wean-to-finish facility in which unvaccinated pigs were weaned from a newly positive sow farm. The second was a 2,800-head finisher in which pigs were vaccinated at processing. Eight individual TS and 8 pen-level OF samples were collected monthly from each farm as previously described[1,2] using fixed spatial sampling[3] for 3 months. Samples were tested using RT-PCR, and a Ct value < 37 was used to declare PRRSV positivity[4]. The association between farm vaccination status and TS PRRSV detection was tested using Pearson's chi-squared test in STATA 13.

Results

From 42 samples, 64.3% and 4.8% of TS and OF samples tested positive, respectively. Only two OF samples tested positive in April, one on each farm. The prevalence of positive TS decreased for both farms over the 3-month period. All sampled areas for the non-vaccinated and vaccinated farms tested positive at least once on TS. Overall prevalence of PRRSV in the unvaccinated and vaccinated herds was 66.7% and 61.1% respectively, and there was no association between farm vaccination status and a positive TS PCR (P = 0.71).

Conclusions

There are currently no established protocols for PRRSV sampling in growing pig populations; even though this represents a large proportion of swine herds. Our results showed tonsil scrapings may be a more sensitive sampling method for PRRSV detection in growing pigs. Sequencing results are pending.

[1]Prickett et al. 2008; [2]Hess et al. 2018; [3]Rotolo et al. 2017; [4]Gerber et al. 2013.

Financial Support

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PRRS 06

Host-genomic scan for total antibody response during a PRRSV outbreak in purebred sows

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Objective

Total antibody response, measured as sample-to-positive (S/P) ratio, has been proposed as an indicator trait to improve reproductive performance in pigs infected with the PRRS virus (PRRSV). Previous studies reported that S/P is controlled by two major host-genomic regions on chromosome (chr) 7 in Landrace sows during a PRRSV outbreak, however, these results have not been validated in other outbreak herds nor in other breeds. The objective of this work was to perform host-genomic analyses for S/P in Landrace and Duroc sows during a PRRS outbreak.

Methods

Serum samples were taken from 1228 purebred sows (538 Landrace and 690 Duroc) after a PRRSV outbreak for subsequent PRRS ELISA analysis and high-density single nucleotide polymorphism (SNP) genotyping (29871 SNPs). Heritability and genome-wide association studies (GWAS) were performed for S/P for each breed separately. All analyses were performed in ASReml and GenSel.

Results

The heritability estimates (± standard error) of S/P ratio during the PRRS outbreak were moderate, with 0.28±0.07 for Landrace and of 0.33±0.06 for Duroc. For Duroc, the GWAS identified a major quantitative trait locus (QTL) on chr 7 [24-26 megabases (Mb)] explaining 13.6% of the genetic variance (GV), and another one on chr 8 (13-25 Mb) explaining 3.7% GV. For Landrace, a QTL on chr 7 (23-25 Mb) explaining 31.1% GV was identified. For both breeds, the QTL identified on chr 7 harbors the major histocompatibility complex (MHC), the most important genomic region controlling the immune response in mammals.

Conclusions

These results validate previous results that S/P in PRRSV-infected sows during a PRRS outbreak is heritable and has an additive genetic component that can be explored by genomic selection, as well as the MHC region as the major genomic region controlling this trait. The QTL on chr 7 (130 Mb), reported in previous studies, was not identified in this population. On the other hand, a novel QTL on chr 8 was identified for Duroc. Additional research is needed to validate the QTL on chr8 found in Duroc sows.

PRRS 07

Accuracy of genomic prediction for total antibody response in purebred sows during a PRRS outbreak

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Objective

Previous studies have shown that genetic markers could be used to predict total antibody response to PRRS, measured as sample-to-positive (S/P) ratio, in commercial sows during acclimation or infected with the PRRS virus. The objective of this work was to assess the genomic prediction accuracies of S/P in commercial sows of different breeds during a PRRS outbreak.

Methods

Blood samples from 1228 sows (538 Landrace and 690 Duroc) after a PRRS outbreak were used for PRRS ELISA analysis and high-density single nucleotide polymorphism (SNP) genotyping (29871 SNPs). The training dataset was used to estimate SNP effects on S/P ratio in three different SNP sets: using all SNPs across the genome (SNPAll), SNPs within the MHC region (SNPMHC), and SNPs outside the MHC (SNPRest). Analyses were performed in three different scenarios: within breed, between-breed, and multi-breed. For within- and multi-breed, 5- and 10-fold cross-validations were used, respectively. Validation was performed for each breed for the multi-breed scenario. For between-breed, all the data for each breed were used for training and validation.

Results

For within-breed, accuracies of genomic prediction (AGP; ± standard deviation) were 0.73±0.06 for Landrace and 0.60±0.08 for Duroc for SNPAll. For SNPMHC, these were 0.60±0.05 (Landrace) and 0.50±0.09 (Duroc), whereas for SNPRest these were 0.41±0.10 (Landrace) and 0.45±0.07 (Duroc). For the between-breed analysis, AGP for SNPAll, SNPMHC, and SNPRest were -0.01, -0.32, and 0.10 for Landrace, respectively, and 0.09, 0.03, and 0.11 for Duroc, respectively. For the multi-breed analysis, AGP for SNPAll, SNPMHC, and SNPRest were 0.67±0.03, 0.55±0.06, and 0.43±0.05 when validating on Landrace, respectively, and 0.62±0.07, 0.49±0.10, and 0.49±0.08, when validating on Duroc, respectively.

Conclusions

Results show that genomic selection for S/P is moderate-high within-breed and when combining data from different breeds. On the other hand, when predicting S/P on a different breed, the negative results indicate that makers and quantitative loci are in opposite phases in these breeds.

PRRS 08

Association between vaginal microbiome and antibody response to PRRS vaccination in commercial gilts

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Objective

Previous reports have been shown that vaginal microbiome composition has been associated with health outcomes. This study estimated the impact of the vaginal microbial variance on the variation of sample-to-positive (S/P) ratio to PRRS vaccination and identified differences in the microbiome composition between animals with contrasting Ab responses.

Methods

Three-hundred and one commercial F1 gilts (Landrace x Large White) were vaccinated with a commercial modified live virus PRRS vaccine (day 0; D0). Vaginal swabs (n=576) were collected on D4 and D52 for subsequent 16S rRNA sequencing, and serum was collected on D52 for measurement of S/P using a commercial ELISA test. After quality control, 1,369 operational taxonomic units (OTUs) were identified and used for further analyses. Microbiability (m^2) was calculated as the proportion of the S/P variance explained by the OTUs using Bayesian Ridge Regression. In addition, gilts were split into two S/P groups: the top 10 (High) and bottom 10 (Low) S/P. A negative binomial model including the effects of S/P group, day of collection, and their interaction, was used to identify OTUs showing differential relative abundances.

Results

The estimated $m^2$for S/P was low on both days (0.07±0.07 and 0.01±0.01 for D4 and D52, respectively). Nine microbes exhibiting differential relative abundances were identified (q<0.05) between Low and High S/P groups. *Fusobacterium*was more abundant in the High group compared to the Low [$\log_2$fold-change (FC) = 2.7], while *Actinobacillus* ($\log_2$FC = -3.6), *Strepococcus* ($\log_2$FC = -2.7), *Campylobacter* ($\log_2$FC = -4.4), *Anaerococcus* ($\log_2$FC = -3.5), *Anaerococcus* ($\log_2$FC = -3.4), *Mollicutes* ($\log_2$FC = -3.3), *Peptostreptococcus* ($\log_2$FC = -2.9), and *Treponema* ($\log_2$FC = -2.2) were more abundant in the Low group.

Conclusions

Antibody response to PRRS MLV vaccination, measured as S/P ratio, was only slightly explained by the vaginal microbial variance. However, the vaginal microbiota differed between animals with low and high S/P ratio.

Financial Support

Smithfield Premium Genetics

PRRS 09

Vaginal microbiome of PRRS-vaccinated gilts differs between animals with high and low farrowing performance

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Objective

It has been shown that antibody response to PRRS is associated with reproductive performance. The existent relationship between microbiome and immune response suggests that microbiome may be an alternative indicator of reproductive performance. Therefore, this study characterized the vaginal microbiota of PRRS-vaccinated gilts with contrasting farrowing performance.

Methods

Two hundred and sixty-one commercial F1 gilts (Landrace x Large White) were vaccinated with a commercial MVL PRRS vaccine (day 0; D0). Vaginal swabs from all animals were collected on D4 and D52 for subsequent 16S rRNA sequencing. After quality control, 1,369 operational taxonomic units (OTUs) were used for further analyses. First-parity data were available, including total born alive (NBA) and number born dead (NBD). Gilts were split into two farrowing performance groups based on the NBA, NBD, and a combination of high farrowing performance for NBA and NBD (Index): the top 10 (High) and bottom 10 (Low) performances. A negative binomial model including group, day of collection, and their interaction was used to identify differentially abundant OTUs (DAMs). Canonical discriminant analyses (CDA) were performed for each day separately to classify the samples into two groups: High and Low farrowing performance. OTUs were selected for the CDA using stepwise selection ($P<0.10$). A leave-one-out cross-validation (LOOCV) was used to assess the predictive ability of the OTUs to classify samples into the two groups.

Results

Six DAMs ($q<0.05$) between farrowing groups were identified for NBD and Index, respectively. The number of OTUs selected on the CDA analyses ranged from 14 to 16 for all traits. The correct classification rates into the two groups obtained in the LOOCV were 0.95 for all traits in both days.

Conclusions

These results indicate that the vaginal microbiome composition of gilts following vaccination with a PRRS MLV vaccine differs between animals classified as High and Low performance. In addition, selected microbes collected after PRRS MLV vaccination may be used to identify animals with high parity one performance.

Financial Support

Iowa Pork Producers Association

PRRS 10

Genetic parameters of disease resilience traits in wean-to-finish pigs from a natural disease challenge model

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Objective

The objective of this study was to estimate genetic parameters of performance and resilience traits from a natural disease challenge model in wean-to-finish pigs.

Methods

Data were from 3,139 Yorkshire x Landrace wean-to-finish pigs that were genotyped for over 700,000 SNPs. The natural challenge was established by bringing naturally infected animals into a nursery and finish barn at CDPQ, targeting various viral and bacterial diseases, and maintained by entering batches of 60–75 healthy nursery pigs every 3 weeks in a continuous flow system. Traits analyzed included average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), residual feed intake (RFI), carcass weight (CWT), dressing percentage (DRS), lean yield (LYLD), carcass back fat (CBF), carcass loin depth (CLD), mortality, number of health treatments per day (TRT), day-to-day variation in feed intake and in duration at the feeder ($RMSE_{FI}$ and $RMSE_{DUR}$), and the proportion of off-feed days based on 5% quantile regression (QR_{FI} and QR_{DUR}). For finishing ADG, ADFI, FCR, $RMSE_{FI}$, $RMSE_{DUR}$, $RMSE_{FI}$, and $RMSE_{DUR}$, only pigs that survived to slaughter were included in analysis. Analysis was by a mixed linear model with genomic relationships.

Results

Estimates of heritability were 0.20 and 0.31 for ADG in the challenge nursery and finishing barn, respectively, 0.06 and 0.03 for mortality, and 0.10 and 0.00 for TRT. In the finishing barn, estimates of heritability were 0.36 for ADFI, 0.29 for FCR, 0.47 for RFI, 0.10 for $RMSE_{FI}$, 0.27 for $RMSE_{DUR}$, 0.10 for QR_{FI}, 0.24 for QR_{DUR}, 0.22 for CWT, 0.17 for DRS, 0.46 for LYLD, 0.49 for CBF, and 0.31 for CLD.

Conclusions

Estimates of heritability were within the range reported in literature for most growth and carcass traits. Heritabilities for mortality and TRT were much higher in the challenge nursery than in the finisher; the opposite was true for ADG. Estimates of genetic correlations of performance and resilience traits with mortality and number of treatments will be estimated. Funded by Genome Canada, Genome Alberta, USDA-NIFA, and PigGen Canada.

Financial Support

PigGen Canada

PRRS 11

Genomic regions associated with host response to infection with a highly pathogenic PRRSV strain

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Objective

Results from a previous study provide evidence of substantial genetic variation in host response to deliberate PRRSV challenge followed by natural challenge with numerous secondary pathogens. Using this same data set, the objective of this follow-up study was to characterize this genetic variation by identifying the genomic regions associated with host response to infection.

Methods

Commercial crossbred pigs (*n*=1,446) were used for this study. Pigs were farrowed at a commercial sow farm and transported to a research facility at weaning where they were vaccinated for PRRS, then inoculated with the 1-7-4 PRRSV isolate four weeks later. Individual phenotypes were recorded during the challenge phase, including mortality and clinical score at 13 and 42 days post-infection, CS13 and CS42, respectively. Pigs were genotyped using the Illumina 50K single nucleotide polymorphism (SNP) chip. Genome-wide association studies were performed by fitting each SNP as a fixed effect regressed on breeding value for mortality, CS13, or CS42. SNPs with a $-\log_{10}$ P-value > 6 were considered significant and a quantitative trait locus (QTL) database was used to identify previously-reported QTL within a 2-Megabse (Mb) window spanning +/- 1 Mb on either side of significant SNPs.

Results

Significant SNPs explaining up to 6% of the genetic variance were detected on: 1) *Sus scrofa* chromosome (SSC)2 for mortality; 2) SSC9 and SSC14 for CS42; and 3) SSC14 for both CS42 and mortality. Previously-reported QTL for the following traits were detected within these regions: 1.) mycoplasma hypopneumoniae antibody titer; 2.) haptoglobin concentration, Immunoglobulin G, salmonella count, and interferon-gamma to interleukin-10 ratio; and 3.) white blood cell number, platelet count, and plateletcrit.

Conclusions

In conclusion, several significant, biologically relevant genomic regions were associated with mortality and CS42 post-infection. These regions may harbor genetic markers that could be used to select pigs for improved host response to multifactorial PRRSV-challenge for pigs reared in commercial environments.

PRRS 12

Protein levels in the blood of young healthy pigs as indicators of disease resilience

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Objective

Disease resilience is the ability to maintain performance under pathogen exposure. Selection for disease resilience is difficult because nucleus breeding populations must be kept in a bio-secure environment. Biomarkers that can be measured at an early age in high-health conditions could overcome this limitation. Proteins play crucial roles in most biological processes and the blood proteome has been used to identify biomarkers for some human diseases. The objective was to explore the blood proteome of young healthy pigs for potential biomarkers for disease resilience.

Methods

Seven batches of 60-75 healthy weaned Yorkshire x Landrace Barrows (405 total) were entered into a quarantine nursery and blood samples were collected after acclimation, at ~27 days of age. One week later, the pigs were moved to the nearby natural challenge facility at CDPQ, which was established by seeding the barn with pigs that were naturally infected with multiple pathogens and maintained using a continuous flow system. Quantitative protein expression levels were quantified in the blood samples using a Tandem Mass Tag (TMT)-based LC-MS/MS method. Associations of the level of the 50 proteins with the lowest number of missing values across pigs, with performance and disease resilience, were evaluated using mixed linear models.

Results

Several proteins showed suggestively significant associations ($p<0.05$) with disease resilience traits, including growth rate, day-to-day variation in feed intake, and the number of health treatments in the natural challenge facility.

Conclusions

In conclusion, protein expression in the blood of young healthy pigs may be associated with subsequent disease resilience and shows potential as a source of biomarkers for disease resilience.

Financial Support

PigGen Canada

PRRS 13

Generation of pig dendritic cells from Flt3 ligand-dependent bone marrow cultures and the infection by PRRSV1.

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Objective

The present work aims to develop pig conventional DC (cDC) using a Flt3L-dependent bone marrow (BM) culture system and assess their susceptibility to PRRSV1.

Methods

BM were cultured with human Flt3L (20 ng/ml) for 14 days with half of the medium changed every 3 days. The resulting cultures (Flt3L-DC) were characterized by flow cytometry (FC) with $CD14^{-}MHCII^{hi}CADM1^{hi}CD172a^{-/lo}$ gated as potential cDC1, and $CD14^{-}MHCII^{hi}CADM1^{hi}CD172a^{+}$ as cDC2. Each cell type was sorted, or further stained with anti-CD1/CD11R3/CD163/DEC205/CD11R1; or confirmed by relative gene expression (Flt3, XCR1 and FcεRIα); or inoculated with a PRRSV1 isolate at MOI of 0.1. The production was optimized by adding stem cell factor (SCF) or by a two-step procedure that firstly incubated with Flt3L and SCF for 6 days to expand progenitors, then introduced GM-CSF or IL-4 maintaining for 12 days.

Results

The resulting culture by Flt3L contained a subset with the phenotype of cDC2 and $CD1^{+}$, $CD11R3^{+}$, $CD163^{-}$, $DEC205^{lo}$, and $CD11R1^{lo}$ was identified. These cells expressed high levels of Flt3 and FcεRIα mRNA, consistent with the cDC2 residing in pigs. However, although a subset with the phenotype of cDC1 was found, they did not highly express XCR1 or Flt3, accordingly were not canonical cDC1. Addition of SCF to cultures increased the yield of cells by 25%. The two-step protocol produced a $DEC205^{+}MHCII^{lo}CADM1^{-}$ subset. Functional characterization of these cells is still under study.

No infection in cDC2 was detected by 48 hpi (titration or FC). But when the whole population was used, the productive infection (by titration) with 10.0±3.3% of PRRSV-positive cells (by FC) was detected. PRRSV-labeling was present in both $CD163^{+}$ and $CD163^{-}$ cells (both within $CD14^{-}$ subset). The pre-incubation and culture of Flt3L-DC with an anti-CD163 polyclonal antibody (100 µg/ml) that blocked PRRSV infection in alveolar macrophages (MOI 0.5 by 10 hpi) resulted in a reduction of replication but not a complete blocking, indicating that $CD163^{-}$ cells might be infected.

Conclusions

Flt3L mainly produced cDC2 which were not susceptible to PRRSV1.

PRRS 14

The Fetal immune response following PRRSV2 challenge of third-trimester gestating gilts

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Objective
Even moderately virulent strains of PRRSV2 cause disruptions in fetal development that can lead to substantial fetal mortality. Here we report the fetal immune response during late gestation maternal infection in relation to infection status and meconium staining as a marker of reduced fetal viability.

Methods
Pregnant Landrace x Yorkshire gilts were experimentally challenged with PRRSV2 (strain NVSL 97-7895) at gestation day 85 and euthanized at 21 days post infection. Fetuses exhibiting pulsations in the umbilical cord were categorized as live prior to euthanasia and further subdivided into viable (VIA; fetuses with normal skin colouring) and meconium stained (MEC; fetuses with inspissated yellow-brown material on skin). Fetuses were then further subdivided by serum and thymus viral load to identify uninfected (UNIF n=30), high viral load viable (HV-VIA n=54) or high viral load meconium stained (HV-MEC n=26) or non-infected controls (CON n=18). Expression of 8 cytokines (TNFα, IFNα, IFNβ, IFNγ, CCL2 CCL5 CXCL10 and IL10) were evaluated by qPCR in the fetal thymus and spleen. The levels of TNFα, IFNα, IFNβ, IFNγ and CCL2 where then measured in fetal serum by ELISA.

Results
Upregulation of cytokine gene expression in thymus and spleen was largely restricted to high viral load (HV-VIA, HV-MEC) fetuses, with the exception of IFNα which was upregulated only in UNIF relative to CON. Among HV fetuses, significant differences were found between gene expression and abundance of type 1, but not type 2, interferons. Expression of CCL5 was found to be significantly elevated in both thymus and spleen of HV-MEC relative to HV-VIA fetuses. Additional minor but significant differences between HV-MEC and HV-VIA fetuses were found in splenic CCL10 and IFNβ, and thymic TNFα and IL10.

Conclusions
The fetal immune response following maternal PRRSV2 infection is largely compartmentalized to infected fetuses. Few differences in the immune response between VIA and MEC fetuses were found suggesting cytokine responses are not a primary factor resulting in meconium staining or reduced viability.

Financial Support
Genome Prairie

PRRS 15

Thyroid hormone disruption in feeder pigs following experimental and natural PRRSV2 and polymicrobial infections

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Objective

Thyroid hormones are powerful regulators of growth, development and basal metabolic rate. In other species this system is susceptible to disruption in the face of severe illness in a process termed non-thyroidal illness syndrome (NTIS). Here we evaluate the susceptibility of feeder pigs to NTIS after exposure to natural polymicrobial pathogen challenge and following controlled challenge with PRRSV2.

Methods

Natural challenge: Serum samples were obtained from 3 batches of nursery-aged pigs (n=208) at 0, 14 and 42 days post exposure (DPE) to an array of pathogens, including PRRSV, in a natural disease challenge barn (NDCB) operated by Centre de développement du porc du Québec and PigGen Canada. *PRRSV challenge*: Serum was obtained at 0, 4, 7 and 11 days post inoculation (DPI) from nursery pigs challenged with either PRRSV2 NVSL 97-7895 (NVSL, N=102) or KS-2006-72109 (KS06, N=83) by the PRRS Host Genome Consortium (PHGC). Animals from 6 PHGC trials were selected in a two by two design based on z-score for high (HW; >0.75) and low (LW; <-0.75) average daily gain (0-42 DPI) and high (HV) and low (LV) viral load (serum AUC 0-21 DPI). Levels of total T3 and T4 were determined by radioimmunoassay.

Results

Natural challenge: Animals in the NDCB show a significant reduction in both T3 and T4 at 14 DPE, with levels of T3 partially rebounding by 48 DPE, while T4 levels remain depressed. *PRRSV challenge*: PHGC animals show a similar decrease in both hormones at 4, 7 and 11 DPI. Levels of both hormones were significantly lower at >4 DPI in animals challenged with NVSL vs KS06. The degree of disruption in thyroid hormones was significantly lower for HW than for LW PRRSV-challenged animals regardless of viral load.

Conclusions

Feeder pigs exhibit a disruption in the thyroid hormone system consistent with NTIS when exposed to a natural disease challenge including PRRSV and other major porcine pathogens. The same effect was observed following PRRSV2-only challenge, with the degree of disruption associated with resilience (i.e. growth rate following challenge), rather than resistance to infection (i.e. viremia).

Financial Support

NSERC-CRD

PRRS 16

Maternal and fetal thyroid hormone disruption following late gestation PRRSV2 challenge

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Objective

Thyroid hormones play a critical role during pregnancy, regulating fetal development and maturation. This system can be disrupted for an as yet unknown purpose, in response to severe illness in a process termed non-thyroidal illness syndrome (NTIS). Here we evaluate the susceptibility of pregnant gilts and their fetuses to NTIS following third-trimester experimental challenge with PRRSV2.

Methods

Serum from pregnant Landrace x Yorkshire gilts (N=57) experimentally challenged with PRRSV2 (strain NVSL 97-7895) at gestation day 85 was evaluated for total T3 and T4 by radioimmunoassay (RIA) at 0, 2, 6, 19 and 21 days post infection (DPI) along with equivalent time controls from uninfected gilts (N=18). Serum total T3 and T4 were also assessed in fetuses collected at 11 or 21 DPI and classified based on viral load in serum and thymus as either uninfected (UNIF n=28 & 201), high viral load viable (HV-VIA n=19 & 171) or high viral load meconium stained (HV-MEC n=26 & 93) or controls (CON n=30 & 56).

Results

Infected gilts showed a significant decrease in both T3 and T4 at 2 and 6 DPI, with evidence of rebound at 19 DPI in a pattern consistent with previously established viremia. At 21 DPI, T3 and T4 were significantly decreased relative to CON in all fetal groups derived from infected gilts, however, levels in both HV-VIA and HV-MEC were also significantly reduced relative to UNIF fetuses. At 11 DPI UNIF fetuses show no significant decrease relative to CON fetuses while levels in HV-VIA and HV-MEC were significantly depressed. Interestingly, at 21 DPI meconium staining, a marker of reduced viability, was associated with significantly elevated T3 relative to HV-VIA group, with a difference found to be trending toward significance at 11 DPI.

Conclusions

During reproductive PRRSV2 infection, both gilts and fetuses show a significant disruption in thyroid hormones consistent with severe NTIS. Amongst highly infected fetuses T3 suppression was more severe in the resilient HV-VIA fetuses relative to their susceptible (HV-MEC) counterparts, suggesting NTIS may play a protective role in the fetus.

Financial Support

Genome Prairie

Development of an ELISA for the detection of swine antibodies in response to influenza B, C, and D viruses

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Objective
Influenza is a highly contagious viral respiratory disease caused by influenza A, B, C, and D viruses (IAV, IBV, ICV, and IDV). The objective of this study was to develop an enzyme-linked immunosorbent assay (ELISA) for the detection of swine antibody responses against IBV, ICV, and IDV infections.

Methods
Serum samples and oral fluids collected from experimentally inoculated piglets and from clinically confirmed field swine samples were used to generate IBV, ICV, and IDV positive and negative antibody standards. Optimized full-length NP genes of IBV (coding 560aa NP), ICV (565aa), and IDV (552aa) were synthesized and cloned into expression vector pET-28a(+) and transformed into the *E. coli* protein expression strain BL21(DE3). The expressed recombinant NP protein sizes are approximate 66.69 KD, 68.85KD and 66.32KD for IBV, ICV and IDV, respectively. Indirect ELISA procedure and conditions were optimized by using purified NP proteins, standard positive/negative antibody samples, and goat anti-swine IgG labeled with horseradish peroxidase (HRP) as secondary antibody.

Results
The OD_{405} cutoff values were 0.103, 0.293, and 0.073, for IBV, ICV, and IDV positive (>) or negative (<), respectively, determined by ELISA tests on swine serum samples. The OD_{405} detected from positive IBV, ICV, and IDV serum samples are up to 2.643, 0.632 and 1.091, respectively. Our data indicated the background signals in negative seral samples detected by the ELISA were lower than the cutoff values. Specific IBV, ICV, and IDV ELISA results also showed there were no cross-reaction signals detected from the serum samples positive for the non-target influenza A, B, C, and D viruses. The signal intensities generated on oral fluids were very weak in this study.

Conclusions
In conclusion, the ELISA developed in this study using recombinant NP as antigen can detect and differentiate swine antibodies in serum in response to influenza B, C and D viruses. This ELISA offers a useful tool for screening and follow-up diagnostics that is an integral part of epidemiological surveys and outbreak investigations.

Financial Support
Swine Health Information Center

PRRS 18

Diagnosis of virulent strain of PRRS on sow farm in Ukraine

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Objective
The studies were performed on a pig farm of the farrowing-fattening cycle with 5,000 sows. The objective of the research was to study pathogenesis of novel virulent PRRS-associated infections with unique case scenario. Analysis of anamnestic and clinical data indicates problems with reproduction in sows and respiratory pathology in pigs of the growing and fattening groups.

Methods
For the diagnosis of respiratory infections, serum from pigs of the fattening group were tested using ELISA test systems for the presence of antibodies to *PRRSV, SIV, ADV* (ID Vet, France). The biological material was examined by qPCR (Exopol, Spain; Biosellal, France).

Results
As a result, 100% of the tested serum samples contained antibodies to *PRRSV* and *SIV*. Oral fluid from pigs of the growing group was also tested using qPCR, in which were detected NA *PRRSV* ($2{,}54\times10^6$ g.e.), HPS ($1{,}58\times10^6$ g.e.), *M. hyorhinis* ($1{,}27\times10^6$ g.e.) were identified, APP 2 serotypes ($7{,}21\times10^5$ g.e.) and *P. multocida* ($5{,}64\times10^5$ g.e.).

The further investigation of *PRRSV* infections from sows with paired blood sera were selected 1 week before and 2 weeks after farrowing in which seroconversion of antibodies to *PRRSV* were detected. The SP in 3 out of 5 sows increased 3-4 times. In the chest fluid from stillborn pigs was found *PRRSV* virus of $3{,}86\times10^7$ g.e. In the testes of piglets 3-5 days old was found RNA virus *PRRSV* in the amount of 10^3-10^4 g.e.

The phylogenetic analysis showed that the *PRRSV* strains under study, isolated from the test material from piglets and sows, had 83.8% homology to reference European PRRS strain Lelystad (M96262.2), 83.7% to vaccinal strain Pyrsvac (DQ345726.1), 84% to vaccinal strain Unistrain (DQ345725.1), 83.5% to vaccinal strains Porcillis-dv (KJ127878.1).

Conclusions
Therefore, pathological changes in sows' reproductive and respiratory tracts and in piglets are caused by a unique *PRRSV*, by which piglets are infected through the placenta. At 5 weeks of age in pigs there is activation of *PRRSV* infection, which is complicated by the influence of *SIV*, HPS, *M. hyorhinis*, APP and *P. multocida* in this herd.

Expression attenuation of proinflammatory cytokines by NF-κB activation and type I IFN suppression-negative PRRSV

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Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses the innate immunity, namely type I interferons (IFNs-α/β). PRRSV also activates NF-κB signaling and thus the secondary infection may trigger enhanced expression of proinflammatory cytokines which may result in severe porcine respiratory disease complex (PRDC). Among the PRRSV proteins, nsp1β was identified as the potent IFN antagonist, and L126 was determined as the active residue for IFN suppression. For NF-κB activation, the viral nucleocapsid (N) protein was found the effector protein, and the nuclear localization signal (NLS) in N overlapped the NF-κB activation domain. In the present study, both IFN suppression and NF-κB activation functions were eliminated from PRRSV by reverse genetics, and double-mutant PRRSVs were examined in cells for their immunological phenotype.

Methods

Extralong inverse PCR was conducted using full-length infectious clones to delete L126 in nsp1β and NLS in N from PRRSV. A series of mutant PRRSVs were then rescued by reverse genetics. Mutations were confirmed by sequencing from 'passage 4' viruses. RT-qPCR was used to determine mRNA expressions for 14 swine-specific cytokines and chemokines in PAMs infected with individual mutant PRRSV at different times postinfection. Statistical analyses were performed using Student t-tests and statistical significance was expressed as $P < 0.05$.

Results

Among the 14 cytokines and chemokines, the expression of GM-CSF was lower at 12 h postinfection in PAMs infected by NLS mutant. The expressions of IL-1α, MCP-1, IP-10, TNF-α, IL-1β, IL-6, IL-8, and RANTES were also significantly lower ($P < 0.05$ to 0.001) in PAMs infected with NLS-mutant PRRSV compared to wild-type PRRSV. The down regulated genes were mostly driven by NF-κB.

Conclusions

Our study demonstrates that NLS-mutant PRRSV represents NF-κB activation-negative and attenuates the expression of proinflammatory cytokines compared to wild-type PRRSV. This finding supports the hypothesis that the mutant PRRSV may relieve the clinical severity of PRDC caused by coinfection of PRRSV and other swine pathogens.

Financial Support

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PRRS 20

Immunosuppressive effects of PRRS virus are dampened in alveolar macrophages from arachidonate-fed piglets

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Objective

Porcine Reproductive and Respiratory Syndrome Virus (PRRS) contributes to 33% of reported disease cases in preweaned pigs despite preventive practices. Long-chain polyunsaturated fatty acids (LC-PUFA), arachidonic acid (ARA; n-6) and eicosapentaenoic acid (EPA; n-3), elicit immunomodulatory effects. Arachidonic acid exhibits increased eicosanoid synthesis with binary effects, leading to enhanced antiviral and inflammatory cytokine production during the early stages of infection and subsequent inflammatory resolution. This study evaluated whether PUFA enrichment could dampen the immunosuppressive effects that PRRS elicits on antiviral cytokine transcription, cell infectivity and viability.

Methods

Porcine alveolar macrophages (PAM) enriched with LC n-6 and n-3 from dietary supplementation were infected with PRRS (MOI 0.5) for 48 hours. Cytokine and viral mRNA abundance was quantified by qRT-PCR. Protein quantification was analyzed by ELISA. Viral infectivity and viability were assessed by flow cytometry.

Results

A 20-fold increase in COX2 mRNA at 6h post infection (pi) correlated with a 22% increase in prostaglandin E_2 concentration in LC n-6 PAM compared to control (CON) and an 84% increase compared to LC n-3 for 24hpi ($P < 0.0001$). By 12hpi, interferon (INF)-α mRNA increased 73% in LC n-6 PAM, while viral nucleocapsid transcripts (N) were 67% lower relative to LC n-3 ($P < 0.0001$). By 24 and 48hpi, N was 66% lower in LC n-6 and n-3 PAM compared to CON. At 6 and 24hpi, lipoxygenase 5 (ALOX5) and interleukin-6 (IL-6) mRNA were 2.5-fold higher in LC n-6 PAM relative to CON ($P < 0.0001$). Tumor necrosis factor (TNF)-α mRNA was 2-fold higher in LC n-6 PAM compared to CON ($P < 0.0001$), but declined in both LC n-6 and n-3 by 12hpi and remained lower than that of CON by 48hpi. The amount of PRRS positive PAM decreased 50% in PUFA-enriched PAM compared to CON, however viability was unaffected.

Conclusions

These results suggest that dietary LC n-6 PUFA can dampen some immunosuppressive effects of PRRS in PAM by altering antiviral and inflammatory responses, thus decreasing viral replication within 24h.

Financial Support

North Carolina State University Laboratory of Developmental Nutrition

PRRS 21

Local immune responses play a vital role in PRRSV clearance from infected pigs

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Objective

Despite extensive investigations, the host immune factors responsible for the clearance of PRRSV from pigs are poorly understood. Previous attempts have mainly focused on certain aspects of immune responses, offering a limited picture of the host defense system. In this study, investigations on local vs systemic and innate vs adaptive immune responses during the course of infection were performed in order to better understand the host defense against PRRSV.

Methods

Among 4 weeks old, 100 pigs, 75 pigs were infected with PRRSV-JA142 strain and 25 pigs were kept uninfected until the end of study. Then, the infected and negative control pigs were euthanized at 3, 10, 21, 28 and 35-days post-challenge (dpc) to collect blood, lung, bronchoalveolar lavage (BAL) and bronchial lymph nodes (BLN). The pathogenicity of virus was assessed based on body temperature, weight gain and microscopic lung lesion scores. Humoral responses were measured as anti-PRRSV IgG and serum virus-neutralizing (SVN) antibodies whereas, cellular immune responses were evaluated in peripheral blood, lung, BAL and BLN samples of all pigs.

Results

The infected pigs displayed the highest viral loads in the serum and lungs between 3 and 10 dpc resulting in mild to moderate interstitial pneumonia, which resolved with the clearance of virus by 28 dpc. PRRSV induced weak and delayed SVN antibody responses, and altered the dynamics of the respiratory dendritic cell/ macrophage network in challenged pigs. At the peak viremic stage, reduced populations of alveolar macrophages (AMs) were perceived in infected pigs. Interestingly, monocyte derived-macrophages and dendritic cells (DC), and conventional DC frequencies increased, which coincided with the early induction of local T cell responses. However, a delayed systemic T cell response was measured in peripheral blood mononuclear cells (PBMCs) with significant induction at the post-viremic stage.

Conclusions

The results highlight the importance of local immune responses in restraining PRRSV primarily by early initiation of protective T cell responses at the virus replication sites in the host.

Financial Support

Ministry of food agriculture forestry and fisheries in the Republic of Korea

PRRS 22

Genomic characterization of PRRSV-1 infection in pulmonary innate immune cells

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Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) has an extensive impact on pig production and, due to its recombination properties, presents a vast genetic diversity worldwide. PRRSV is divided in two species, type 1 (European origin, PRRSV-1) and type 2 (North American origin, PRRSV-2) and within PRRSV-1 specie, PRRSV-1.3 strains such as Lena are more pathogenic and trigger a higher Th1 response than PRRSV-1.1 such as Lelystad or Flanders 13 (FL13).

To date, the molecular interactions of PRRSV with primary lung mononuclear phagocytes (MNP) subtypes such as conventional dendritic cells type 1 (cDC1), cDC2, monocyte-derived DCs (moDC) and parenchymal macrophages (AM-like/PIMs) have not been thoroughly investigated.

Methods

Here, we describe the transcriptome profiles of *in vitro* FL13- and Lena-infected parenchymal MNP and of *in vivo* FL13-infected parenchymal MNP subpopulations obtained using RNAseq.

Results

In vitro, we found respectively 4,500 and 23 differentially expressed genes (DEGs) in Lena-infected MNP and FL13-infected MNP compared to mock-infected cells confirming the potent modulation induced by Lena. Considering the low number of DEGs obtained with conventional statistics in FL13 condition, we decide to use a machine learning approach to unravel other relevant genes in FL13-infected cells. Roughly, 500 additional genes were predicted and enriched in 19 IPA canonical pathways; in particular, two of them, related to the oxidative phosphorylation and mitochondrial dysfunction, were not shared with the 202 pathways found in Lena-infected cells. Transcriptomic data from *in vivo* sorted cells (alveolar macrophages, AM-like/PIMs, cDC1, cDC2, moDC) delineated cell specific clusters and confirmed the low number of DEGs during FL13 infection.

Conclusions

These data indicate that, whereas Lena strongly triggers the innate lung immune system, FL13 keeps antiviral and inflammatory AM/DC functions silent. Some transcriptomic clues might relate FL13' stealth to virus-induced mitochondrial dysfunctions. Validations of key DEGs are currently in progress.

Financial Support

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PRRS 23

Global profiling of nidovirus–host protein interactions by comparative proteomics (PEDV and PRRSV)

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Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses and considered as the primary emerging livestock pathogens worldwide. The long-term objective of our project is to gain a better understanding of the molecular mechanisms of interactions between animal nidoviruses and their hosts in order to develop new antiviral strategies. We hypothesized that the tight interactions between host and viral proteins define the fate of nidoviral infection and pathogenesis.

Methods

Virus-host interactions are highly dynamic, leading to important changes in the intracellular levels of host proteins. Virus-induced modulation of the intracellular environment creates conditions that are more favorable for infection and spread. Consequently, proteomic characterization of the nidovirus-infected cells in a time-resolved manner will provide dynamic and global mapping of virus-host interactions. To address this objective, we are using a quantitative comparative proteomic approach. More specifically, we analyzed proteomic patterns in host cells during viral infection, and we characterized protein composition of the virions and extracellular microvesicles (EMV) produced by PEDV or PRRSV infected cells.

Results

We found that PRRSV and PEDV infections affected the abundance levels of numerous host proteins associated with EMV. Our data showed that indeed the nidovirus infection resulted in significant alterations in the host cell proteome and that both viruses induced specific changes, unique to their molecular pathogenesis. E.g.,the abundance of proteins involved in immune responses was changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and cytoskeletal system were affected in abundance. Moreover, PEDV significantly modulated biological pathways such as entry into the host cells, type I IFN signaling, defense response to viral infection, etc.

Conclusions

Dynamic proteomics greatly facilitates our understanding of the molecular details of virus-host interactions.

Financial Support

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PRRS 24

Polycations enhance the infectivity of two porcine nidoviruses (PRRSV and PEDV) of veterinary importance

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Objective
Our research project is focused on emerging swine pathogens of unprecedented economic significance, such as the porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV). We hypothesized that the composition of PRRSV and PEDV virions and proteomic profiles of virally infected cells should reflect the complex interplay between the virus and host factors, which shape the course of infection. Host-virus interactions are highly dynamic; thus, a certain optimization is needed in order to investigate the spatio-temporal regulation of viral infection.

Methods
To address our hypothesis, we synchronized and increased virus entry into the cells and studied the proteomic patterns of infected cells in a time-resolved mode. Positively charged molecules (polycations) and spinoculation were successfully used for lentiviral gene transfer, and it was shown that both approaches increased the infectivity of retroviruses. Therefore, we evaluated the effects of two polycations (polybrene and DEAE-dextran) on the PEDV and PRRSV infectivity, viral production and cytotoxicity on Vero and MARC-145 cell cultures.

Results
We demonstrated that polycations greatly enhanced the efficiency of nidovirus entry and infection. Thus, polycations can be used for the optimization of PRRSV and PEDV infection, improved detection, and vaccine production. Currently, we are evaluating the effect of spinoculation and combined treatment (polycations and spinoculation) on PRRSV and PEDV entry. Importantly, the effect of the polycations and spinoculation on the PRRSV and PEDV infection has never been studied before.

Conclusions
The calculation of titers of virus stocks and the estimation of viral load in clinical samples for many viruses often involve cytopathic effect (CPE) quantification in plaque-forming units (PFU) or similar approaches. Thus, optimization of the efficiency of viral infection is of high importance for a variety of research applications, diagnostics, and vaccine production.

Financial Support
Fonds de recherche du Québec – Nature et technologies (FRQNT)

PRRS 25

Detection of PRRSV-specific antibody in swine fecal samples

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Objective

Routine surveillance is mandatory to control and/or eliminate PRRSV, but breeding herds are rarely monitored because sampling adult animals is challenging in commercial production. Fecal samples (FS) are easy to collect and do not require animal handling. The presence of antibody in feces (coproantibody) has been reported in human, sheep, mice, primates, and other species. In swine, coproantibody against ASF, CSF, HEV, and PEDV have been documented. Therefore, this pilot study evaluated the detection of PRRSV-specific antibody in fecal samples using a commercial ELISA kit designed for oral fluids

Methods

Pigs (n=12) were vaccinated with a live virus vaccine (Ingelvac® PRRS MLV) and individually sampled from -5 to 42 days post-vaccination (DPV). A total of 112 serum, 512 oral fluids (OF), and 513 FS were tested using commercial PRRSV IgG ELISAs (IDEXX OF Ab test, IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc). Serum and OF samples were tested as directed by the ELISA manufacturer. FSs were diluted 1:1 with ELISA kit diluent containing 1000 ppm chitosan and then assayed on the PRRSV OF ELISA using a sample volume of 200µL

Results

Using a cutoff of S/P ≥ 0.4, the first positive serum ELISA and OF-ELISA samples were detected on 8 DPV. Using a cutoff of S/P ≥ 0.1, the first FS-ELISA-positive samples appeared on 10 DPV. A ROC analysis was conducted under the assumption that samples collected prior to DPV 7 were true negatives and samples collected after DPV 11 were true positives. The analysis estimated the diagnostic sensitivity and specificity of both the serum and OF-ELISAs at (99%, 99%), whereas the FS-ELISA was (81%, 99%)

Conclusions

This pilot study demonstrated that detectable levels of PRRSV coproantibody are present in feces and that their kinetics mirrors antibody in serum and oral fluids. The test is sufficiently diagnostically specific, but significant improvements in diagnostic sensitivity are necessary. With this improvement, the FS-ELISA would provide a practical and efficient approach to testing adult animals (sows or boars) in PRRSV surveillance programs

Financial Support

National Pork Board

PRRS 26

Commercial kit vs standard overnight protocol. What works better for PRRSV OF ELISA testing?

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Objective

ELISA detects PRRSV-specific antibodies beyond 6-8 months post infection revealing the PRRSV pre-exposed pigs and aviremic virus "carriers". Serum PRRSV ELISA was adapted to oral fluids (OF) by standardizing an overnight protocol and then commercial OF-ELISA kits were marketed. As the OF-ELISA testing demand increases also does the need of high throughput testing in veterinary diagnostic laboratories (VDL). Therefore, this study evaluated the diagnostic differences between the overnight standard PRRSV OF ELISA (ELISA ON) and the same-day-test IDEXX PRRSV OF Ab ELISA (ELISA SD) licensed in the US

Methods

Experimental known PRRSV status OFs (Panel 1, n=600) collected from 12 pigs over a period of 50 days post vaccination (DPV, -7 to 42), and field unknown PRRSV status OFs (Panel 2, n=600) submitted to ISU-VDL were tested on both ELISAs. ROC-AUC analysis evaluated the diagnostic performance of both ELISAs under different cut-offs, using the sample Panel 1. Wilcoxon and Cochran tests contrasted the results in both Panel 1 and Panel 2

Results

Panel 1: ELISA ON showed lower S/P (Wilcoxon, $p>0.05$) and lower positivity rate (Cochran, $p<0.001$) than ELISA SD, classifying 349/600 (58.2%) samples as positive, whereas ELISA SD classified 390/600 (65%). ELISA ON produced 1 false positive and 35 false negatives, and ELISA SD produced 4 and 2, respectively. ELISA SD showed better dxSe than ELISA ON (98.8% vs 90.1%), but ELISA ON showed slightly better dxSp (98.8% vs 99.4)

Panel 2: ELISAs S/P were significantly different (Wilcoxon, $p>0.05$). ELISA ON showed lower positivity rate than ELISA SD (Cochran, $p<0.05$), classifying 53/600 (8.8%) samples as positive, whereas ELISA SD classified 70/600 (11.7%)

Conclusions

The commercial IDEXX PRRSV OF Ab ELISA test offers earlier detection of PRRSV antibody positives because shows higher S/P values, and therefore higher number of false positive results could be alleged. However, this study demonstrated that the commercial kit offers a reliable diagnostic performance while is suitable to high throughput VDLs

Financial Support

National Pork Board

PRRS 27

Influence of technician on PRRSV OF ELISA test results

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Objective

Commercial PRRSV oral fluid (OF) ELISAs provide good diagnostic performance and testing swine OF specimens for PRRSV antibody is a convenient way to evaluate the PRRSV status of pigs. Like most serum ELISAs, PRRSV OF ELISA reactions are read as optical density (OD), ODs are converted to sample-to-positive (S/P) ratios using a formula that uses plate control ODs to standardize reactions across plates. The test result is then determined by the kit cut-off. This study evaluated the variation in PRRSV OF ELISA test results when the same set of samples was tested by two trained technicians on the same equipment

Methods

OFs (n=600) from pigs of known PRRSV status were tested on the IDEXX PRRS OF Ab Test by two trained technicians (T1 and T2). Quantitative and qualitative test results were compared by non-parametrical analyses

Results

T1 produced higher S/P than T2 (median S/P 1.99 vs 1.91; Wilcoxon, $p < 0.0001$). The proportion of positive results was similar between T1 and T2 393/600 (65.5%) vs 390/600(65%) (Cochran, $p = 0.18$). No difference was found between T1 or T2 results in terms of diagnostic performance (ROC-AUC Pairwise comparison, $p = 0.41$). However, 2/168 (1.2%) negative samples were misclassified as positive by both technicians, and 2/372(0.54%) and 4/372(1.07%) positive samples were negative by T1 and T2, respectively

An investigation into the cause of the quantitative differences in results revealed that loading the positive and negative plate controls immediately before or after the samples had been allocated to the plate affected control ODs and, therefore, all sample S/P calculations

Conclusions

Veterinary diagnostic laboratories implement a variety of standard operating procedures (SOP) for sample handling, equipment calibration, etc., to control for these factors. This study found that even “inoffensive” or very small differences in performing an ELISA test can introduce a measureable impact on results. Although the deviation from the SOP did not affect significantly the binary diagnostic results, it underlined the fact that test repeatability is dependent on attention to detail

Financial Support

National Pork Board

PRRS 28

Effect of genotype at a GBP-5 marker on resilience to a polypathogenic natural disease challenge in pigs

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Objective
The objective of this study was to determine whether a polymorphism linked to the GBP5 gene (WUR0000125, WUR), previously shown to be associated with host response to porcine reproductive and respiratory syndrome (PRRS) virus infection, is also associated with resilience to a polymicrobial natural disease challenge.

Methods
The study was conducted at CDPQ from late 2015 to early 2019. Using a continuous flow system, a new batch of 60-75 naive Yorkshire x Landrace nursery pigs was introduced every 3 weeks into a natural challenge facility that was seeded with multiple viral and bacterial pathogens, including PRRSV and swine influenza, effectively simulating a severe commercial environment that enables expression of disease resilience. Phenotypes recorded were growth rate, feed intake, backfat, loin depth, veterinary treatments, and mortality. Pigs were genotyped using a 600K SNP panel. Data from 3126 pigs was analyzed using a univariate linear mixed model that included WUR genotype, pen enrichment, entry age, and batch as fixed effects, and pen, sow, and animal genetics as random effects. For mortality, a logistic link function was used.

Results
Genotype frequencies were 0.86, 0.13, and 0.01 for AA, AG, and GG respectively. The G allele was favorable for most traits, with the contrast of AA vs [AG+GG] being significant for average daily gain in the challenge nursery (0.253 vs 0.269 kg/d, p=0.039), and for the number of treatments in the challenge nursery (1.30 vs 1.16, p=0.048), in the finisher (0.402 vs 0.343 p=0.103), and across both the nursery and finisher (p=0.0013). Mortality was 4.1, 1.7, and 4.3% higher for the AA genotype than for the [AG+GG] genotype in the challenge nursery, finisher, and across both the nursery and finisher, respectively, but not significantly different from 0% (p=0.59, 0.19, 0.18).

Conclusions
In conclusion, the G allele at the GBP5 marker is also associated with resilience to a polymicrobial disease challenge in swine.

Financial Support
USDA-NIFA

PRRS 29

Oral vaccine delivery for porcine epidemic diarrhea virus

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Objective

Porcine epidemic diarrhea virus (PEDV) of the coronaviride family, causes acute diarrhea, vomiting, dehydration, mortality rates ranging between 80-100% in neonatal pigs, leading to severe economic losses to the pork industry. Passive transfer of PEDV-specific IgA antibodies to suckling piglets via colostrum is critical for protection. In pregnant sows, orally administered, attenuated PEDV vaccines are more effective than inactivated vaccines. However, both live and killed PEDV vaccines require substantial improvement as outbreaks continue to occur in vaccinated herds and more virulent strains emerge periodically. The objective of this study is to determine whether improving oral delivery of the vaccine antigen will in turn improve vaccine efficacy.

Methods

We had previously reported on an orally-delivered, minimally replicative vaccine against PEDV which was highly effective and safe in weanling piglets. In this study, a proprietary, biodegradable, oral vaccine delivery system was developed to deliver this vaccine antigen to pregnant sows. Varying combinations of detergents, lipids and salts were assessed for their abiltiy to form vescicles and entrap vaccine antigen.

Results

At the currently optimized composition, about 70% of antigen was packaged by the delivery system and no significant cytotoxicity was detected in Vero cells.

Conclusions

Thus, the optimized vaccine delivery formulation was effective in forming inact vescles which could deliver antigen to cultured cells without causing cytotoxic effects. Following assessment of the delivery system's ability to withstand acid and enzymatic degradation, the safety and efficacy of the oral vaccine delivery and vaccine will be tested in a pregnant sow model.

Financial Support

National Institute for Food and Agriculture

PRRS 30

A comparison of vaccine setback and viremia following Prime Pac® PRRS RR and Ingelvac® PRRS MLV vaccination

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Objective

Two comparative post-vaccination serological and relative vaccine setback evaluations were conducted in pigs vaccinated with either Prime Pac® PRRS RR or Ingelvac® PRRS MLV.

Methods

PRRSV naïve pigs were weaned at 20-28 DOA, blocked by litter and weight, and randomly assigned to treatment groups (T01: Prime Pac PRRS RR, (Study 1 N=60, Study 2 N=120); T02: Ingelvac PRRS MLV, (Study 1 N=60, Study 2 N=120); T03: unvaccinated sentinels, (Study 1 N=15, Study 2 N/A)). Pigs received their assigned vaccine IM a few days post-weaning. In study 1, treatment groups were housed in separate rooms while in study 2, each treatment group was housed by pen (N=20/treatment group) in one barn. Pigs were bled (Study 1 – all pigs; Study 2 – 20 pigs/group) one day prior to vaccination and weekly post-vaccination for six weeks. Pigs were weighed at 0 and 28 dpv (Study 1) and at 0, 14, 28, and 42 dpv (Study 2). All serum was tested by PRRS qPCR and PRRS IDEXX ELISA. PRRSV sequencing was performed on a subset at every sampling. All data was analyzed with appropriate statistical analysis methods depending on data type.

Results

There was no cross-contamination of PRRSV strains between treatment groups during either study based on PRRSV sequencing results. Both vaccinated groups were 97% viremic by 14 dpv. Prime Pac PRRS RR vaccinated pigs had significantly lower viral loads at most time points post-vaccination ($P<0.01$) and exhibited a more transient viremia than pigs vaccinated with Ingelvac PRRS MLV in both evaluations. Both vaccinated groups showed >90% seroconversion rate by 21 dpv. However, T01 vaccinated pigs averaged lower S/P values than T02 at each time point ($P<0.01$). In Study 1, all three groups had an average starting weight of 15.1 lbs. but an average final weight of T01=30.1 lbs., T02 =27.7, and T03=29.4.

Conclusions

In Study 2, there was a trend of overall higher ADG (1.7 lb. advantage) in Prime Pac PRRS RR vaccinated pigs compared to Ingelvac PRRS MLV ($P=0.07$) and a significant difference in ADG (T01=0.90 lbs. and T02 = 0.84) during the first two weeks post-vaccination ($P<0.01$).

PRRS 31

Developing a Parainfluenza Virus 5 (PIV5)-based PRRSV vaccine

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Objective
Porcine reproductive and respiratory syndrome (PRRS) has caused tremendous economic losses to the swine industry since its appearance. A safe and effective vaccine is needed to prevent large-scale outbreaks. Parainfluenza virus 5 (PIV5), a paramyxovirus, is an attractive vaccine vector. It has been characterized as safe, stable, efficacious, and cost-effective to produce in large scales. PIV5 has been used as a vaccine vector for various viral pathogens, such as avian influenza viruses, rabies, respiratory syncytial virus and HIV. In this study, we tested the feasibility of using PIV5 as a vaccine vector for PRRSV vaccine development.

Methods
Towards this goal, we have generated a panel of recombinant PIV5 expressing PRRSV antigens that include all known 14 nonstructural proteins (nsp 1α/β, nsp2TF, nsp2-12) and 6 structural proteins (GP2a-5, M and N). Immunogenicity and efficacy of these PIV5-PRRSV vaccine candidates were tested in a nursery pig model. Pigs were immunized with a mixture of the recombinant viruses or mock-vaccinated for 28 days and challenged with PRRSV strain VR2332.

Results
The results showed that PIV5-PRRSV-immunized pigs generated robust cellular immune responses. While immunized piglets were still infected after PRRSV challenge, these piglets continued to gain weight, comparable to the group of negative control pigs (mock-vaccinated and uninfected).

Conclusions
These data indicate that we have generated a PIV5-based PRRSV vaccine candidate that was effective in maintaining performance of production animals after PRRSV challenge.

Financial Support
USDA

Efficacy of type 1 PRRSV MLV against HP-PRRSV or co-challenge with PRRSV1 when vaccinated via IM or ID in pigs

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Objective

The objective of this study was to test the efficacy of type 1 PRRSV modified-live vaccine (UNISTRAIN® PRRS) when administrated intramuscularly or intradermally in pigs against either single challenge with PRRSV2 (Highly Pathogenic PRRSV, HP-PRRSV) or in co-challenge with PRRSV1 (Pan European) and PRRSV2 (HP-PRRSV).

Methods

Forty-two, PRRSV-free pigs at 3-week-old were randomly allocated into 7 groups with 6 pigs each, including IM/PRRSV2 (G1), ID/PRRSV2 (G2), NV/PRRSV2 (G3), IM/PRRSV1+2 (G4), ID/PRRSV1+2 (G5), NV/PRRSV1+2 (G6), and NV/Unch (G7), respectively. G1 and G4 were intramuscularly vaccinated with type 1 PRRSV (UNISTRAIN® PRRS). Meanwhile, G2 and G5 were intradermally vaccinated with UNISTRAIN® PRRS. G3, G6 and G7 were left non-vaccinated. At 35 days post-vaccination (DPV), G1, G2 and G3 were challenged with PRRSV2 and G4, G5 and G6 were co-challenged with PRRSV1 and PRRSV2. Pigs in G7 were left non-challenged. Blood sample and peripheral blood mononuclear cells (PBMC) were collected and assayed for immune response using ELISA and ELISPOT assays. Pigs were necropsied at 7- and 35 days post-challenge (DPC). PRRSV RNA, lung lesion and pathological examination were evaluated.

Results

Following vaccination, there was no difference in antibody response among vaccinated groups. ID-vaccinated pigs had shorter viremic phase and lower RNA level compared to IM-vaccinated pigs. ID-vaccinated pigs had significant lower IL-10 levels, and significant higher IFN-g-producing cells compared to IM-vaccinated pigs. Following challenge, ID-vaccinated pigs had significantly lower PRRSV RNA and lung lesion compared to IM-vaccinated pigs at 7 DPC but showed no difference at 35 DPC.

Conclusions

The results suggested that UNISTRAIN® PRRS administrated, either ID or IM, can provide protection against challenge with PRRSV2, either alone or in co-challenge with PRRSV1, as demonstrated by reducing lung lesion and viremia. ID route might represent an alternative to improve vaccine efficacy as it provided lower IL-10 and higher IFN-γ-PC.

Financial Support

Thailand Research Fund

PRRS 33

Intradermal inactivated vaccine against PCV2 and *Mycoplasma hyopneumoniae (Mhp)* induces protective immunity in pigs

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Objective

Nowadays, a renewed interest in intradermal vaccine delivery driven by the fact that the dermis and epidermis are rich in antigen-presenting cells is increasing. This study was designed to evaluate the efficacy of inactivated vaccines against PCV2 and *Mhp* in pigs.

Methods

A total of 15, 6 week-old, PCV2 and *Mhp* seronegative pigs were divided into 3 groups of 5 pigs each. Pigs in two groups were intradermally vaccinated with 0.2 ml of PCV2 and *Mhp* vaccines using a needle-free intradermal applicator while the pigs in the third group were kept as a non-vaccinated control. At 21 days post vaccination, pigs in one of the vaccinated groups and the non-vaccinated group were intranasally challenged with 2.0 ml of PCV2 and *Mhp* while the other vaccinated group was maintained as a vaccine control. Vaccine efficacy was assessed by observing weight gains, pathogen loads and pathological changes in pigs. In addition, humoral and cellular immune responses were evaluated in serum and whole blood, respectively.

Results

After challenge with PCV2 and *Mhp*, vaccinated pigs revealed significantly higher antibody responses and body weight gains; and lower clinical scores and PCV2 or *Mhp* loads in serum, nasal swabs or lungs as compared with non-vaccinated pigs. Intriguingly, the significant higher levels of CTLs, $CD8^{-}$ γδ T-cells, Th1 cells and Th17 cells at 14 days post challenge was displayed in the vaccinated pigs than non-vaccinated pigs.

Conclusions

This study demonstrated the efficient induction of protective immune responses against PCV2 and *Mhp* in pigs using a relatively small volume of vaccines via the intradermal route. PCV2 and *Mhp* needle-free vaccination was observed to be less irritating to pigs and induce competent defense against both of the pathogens, probably due to effective antigen delivery to antigen presenting cells in dermis.

Financial Support

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PRRS 34

Adjuvant for Porcine Epidemic Diarrhea and Transmissible Gastroenteritis Bivalent Inactivated Vaccines

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Objective

Porcine epidemic diarrhea (PED) and Transmissible Gastroenteritis (TGE) are highly contagious, intestinal infectious diseases. These two diseases are characterized by severe diarrhea, vomiting and dehydration. PEDV and TGEV infections occur in pigs of all ages, infections are most serious in piglets, with high morbidity and mortality. Adjuvants are required to improve vaccines efficacy. Montanide™ GEL 01 PR (GEL 01) is an innovative adjuvant based on a high molecular weight polyacrylic polymer. Here, we evaluated the immunopotentiating performance and safety of GEL 01 in a PED-TGEV bivalent inactivated vaccine (BIV).

Methods

Safety of vaccine based on GEL 01 used at 10% was compared to 3 types of vaccines and to a control group without antigen or adjuvant. 3 pregnant sows / grp were injected 6 weeks before delivery in the Houhai acupoint and 3 piglets of 3 days old were injected 1 mL IM. Pyrogenicity, general reactions, local reactions, effects of the vaccination on pregnancy and piglets health were assessed.

In a second trial, efficacy of the same vaccines was determined on 3 pregnant sows / grp and their piglets. 3 pregnant sows / grp were injected 4 mL IM each at 6 and 3 weeks before delivery in the Houhai acupoint. The PED and TGEV viruses seroneutralization titers in blood were measured before injection, at 6 and 3 weeks pre-delivery and at delivery. IgA titers of PEDV were measured in colostrum or in milk at farrowing, at 2 and 4 weeks post-farrowing.

The protection rate after sows vaccination through maternal milk was evaluated. 30 breastfed piglets were challenged orally 5 days after birth with 100 LD50 of either TGEV or PEDV, duration of hyperthermia, intestinal lesions & viremia were measured.

Results

Efficacy trial showed GEL 01 vaccine produced the highest TGEV & PEDV neutralizing antibody titers in sows among all groups. It induced the highest IgA PEDV titers in milk.

Conclusions

GEL 01 allows for safe, efficient and protective PED-TGEV IB vaccines for both sows and piglets. GEL 01 is the most adapted adjuvant for pregnant sows, and confers immunity to offspring through milk IgA.

PRRS 35

Corn based nanoparticle inactivated swine influenza virus vaccine augments mucosal immune response in pigs

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Objective

Swine influenza virus (SwIV) is a respiratory viral infection in pigs caused by the Influenza A virus (IAV). Unfortunately, available SwIV vaccines fail to provide robust cross-protective mucosal immunity required to control IAV infection and transmission in pigs. Nano-11 is a amphiphilic nanoparticle obtained from sweet corn-derived phytoglycogen. The nature of Nano-11 facilitates preparation of the nanoparticle vaccine by electrostatic forces created between positively charged Nano-11 and negatively charged killed IAV antigen (Ag) or peptides. Our previous intranasal vaccine studies using Nano-11 entrapped with killed SwIV H1N2 Ag (Nano-11+KAg) demonstrated the adjuvant effect of Nano-11; but there was unsatisfactory reduction of the challenge H1N1 virus titers in the pigs airway. To improve the efficacy of Nano-11+KAg vaccine, we co-adsorbed adjuvant Poly (I:C) (negative charge) in Nano-11 with KAg or conserved ten IAV peptides.

Methods

In this experiment pigs were intranasally vaccinated and then challenged with heterologous H1N1 virus.

Results

In summary, mucosal IgA antibody titer was increased in the airways of Nano-11+KAg+Poly(I:C) group

in comparation to commercial vaccine and Nano-11+peptides+Poly(I:C) group. An increased frequency of IFN-g+gdT cells against the vaccine virus in Nano-11+KAg+Poly (I:C) vaccine group was observed. Similarly, an increased frequency of IFN-g+gdT cells and cytotoxic T cells against vaccine and challenge viruses in Nano-11+peptides+Poly (I:C) group compared to control Poly (I:C) group. In contrast, commercial vaccine group induced substantially higher IgG and hemagglutination inhibition titers in comparison with all the other experimental groups.

Conclusions

Reduction of challenge virus load in Nano-11+KAg+Poly(I:C) group was not further improved over the earlier study performed without Poly (I:C). In conclusion, improvements in the Nano-11 flu vaccine formulation is required if we want to take advantage of this easy to prepare nanoparticle based vaccine formulation.

PRRS 36

Effect of incorporation of the FMDV 2B viroporin into a plasmid based virus-like particle production platform

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Objective

Insertion of the Foot-and-mouth disease virus (FMDV) 2B viroporin gene into the Foot-and-mouth disease Ad5 vaccine is reported to improve immunogenicity despite 2B expression being detrimental to cell survival. We recently developed a plasmid based FMDV virus-like particle vaccine platform, which utilizes only the P1 polyprotein and 3C protease. We investigated whether insertion of 2B into this platform would enhance transgene expression or alter processing of the P1 polypeptide using either the wild type FMDV 3C protease or the L127P 3C protease mutant.

Methods

We mirrored the 2B sequence of the FMDV Ad5 vaccine for insertion into VLP expression plasmids with either wild type or the L127P mutant of the FMDV 3C protease. Plasmids were transfected into mammalian cell culture, and we monitored luciferase expression, a correlate to overall transgene expression. We confirmed activity by measuring the processing of the FMDV P1 polypeptide using western blots. To examine if viroporin activity plays a role previously established, mutations designed to disrupt pore activity were tested.

Results

The effect of incorporating 2B on transgene expression differed depending if the wild type or L127P FMDV 3C protease was used. Incorporation of the wild type FMDV 3C protease, as used in the FMD Ad5 vaccine, resulted in a significant increase in transgene expression. Incorporation of the less toxic, L127P 3C protease mutant resulted in a significant decrease in transgene expression. When 2B pore activity was disrupted in L127P-containing constructs, there was a significant increase in transgene expression compared to functional 2B constructs, but levels were lower than those found in constructs lacking 2B.

Conclusions

We found that more immunogenic material was produced with the combination of 2B and wild type 3C in our transgene expression system. The lack of enhancement with 2B and the L127P mutant suggested that 2B may play a role in reducing the cellular burden of expressing wild type 3C.

Financial Support

U.S. Department of Homeland Security

PRRS 37

Development and evaluation of a mammalian cell-culture-produced FMDV virus like particle vaccine in swine

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Objective

To determine if novel Foot-and-mouth disease virus (FMDV) virus-like particles (VLPs) are effective at preventing clinical Foot-and-mouth disease (FMD) in swine challenged with FMDV O1 Manisa.

Methods

We utilized plasmids encoding the P1 polypeptide of FMDV O1 Manisa and the 3C(L127P) protease mutant to produce VLPs in HEK293-T cells. Extracted cytosolic fractions were concentrated using a centrifugal concentrator. The VLP antigens were administered to pigs as a full dose (3 mg), ¼ dose, and 1/16 dose using a prime vaccination and a boost at 28 days post-vaccination (dpv). An inactivated FMDV O1 Manisa antigen was the positive control, and pigs treated with PBS were the infected, non-vaccinated controls. Two groups of pigs received a single inoculation of the full dose of VLPs and the inactivated FMDV. All vaccines were formulated with Seppic Montanide 201 VG adjuvant. Swine were challenged at 14 days post-vaccination (dpv) or 42 dpv/14 days post-boost by contact with pigs infected with FMDV O1 Manisa. We measured FMDV neutralizing antibodies post-immunizations and prior to infection. After infection, we assessed the pigs for clinical FMD and measured viremia.

Results

Eighty percent of animals in the prime/boost groups were protected (n=5 pigs per group), while thirty-three percent of pigs (n=3) that received only a single dose of the VLPs were protected from clinical FMD. On the day of challenge, all of the pigs that received antigens in the prime/boost strategy were positive for neutralizing antibodies, while only the VLP single dose group was 100% positive. Viremia was prevented in 60-80% of the pigs that received the antigens in a prime/boost regimen, while only one of six pigs that received a single dose of vaccine was protected.

Conclusions

The mammalian cell-culture-produced VLPs were effective at preventing clinical FMD in pigs following challenge with FMDV O1 Manisa. We anticipate that this VLP platform will be readily adaptable to accommodate new constructs tailored to match field strains. We continue to develop methods to enhance protection from clinical FMD elicited by VLPs.

Financial Support

U.S. Department of Homeland Security

PRRS 38

Comparative efficacy of inactivated and modified-live PRRS virus vaccines against a heterologous virus challenge

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Objective

We compared the efficacy of a prototype inactivated PRRS virus vaccine with a commercial modified live virus (MLV) vaccine against a heterologous virus challenge in pigs.

Methods

All viruses used for this study belong to the North American PRRS virus genotype. Two groups of weanling pigs (23±2 days of age) were immunized intramuscularly with either a prototype inactivated vaccine mixed with WOW adjuvant or with a commercial MLV vaccine (Prevacent PRRS, Elanco). A third group received vaccine diluent mixed with the same WOW adjuvant. Pigs immunized with the inactivated virus or the mock vaccine received a booster shot 25 days later. At 69 days of age, all of pigs were challenged intranasally with a virulent lineage 5 PRRS virus strain 16244B, which is heterologous (≤96% homology) to either vaccine. The pigs were monitored for 12 days after the challenge for clinical signs of pneumonia, and blood samples collected to determine the extent of viremia and virus-specific immunity.

Results

Pigs vaccinated with either vaccine exhibited PRRS virus-specific interferon-gamma secreting T cells in their blood as well as serum antibodies specific for PRRS virus. Similarly, both groups exhibited significant levels of protective immunity against the virulent virus challenge, as revealed by majorly reduced levels of pneumonia. This was evidenced by the fact that, as compared to unvaccinated challenged controls, vaccinated animals exhibited a significantly lessened extent of gross lung pathology, a close to normal lung density, and a significantly higher level of peripheral capillary oxygen saturation (SpO2). Moreover, while all of the mock-vaccinated pigs exhibited high levels of viremia 12 days after the virus challenge, five of the eight pigs immunized with the inactivated virus were no longer viremic at this time. All of the pigs immunized with the MLV ceased to be viremic 10 days after the challenge.

Conclusions

Although not as effective as the MLV vaccine, the results obtained indicate that the prototype inactivated PRRS virus vaccine tested is effective against a heterologous PRRS virus challenge.

Financial Support

National Pork Board

PRRS 39

Porcine dermatitis and nephropathy syndrome in Russia: a case-report

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Objective
The aim of this investigation was to set up correlation between clinical findings and PCV-2 status by cross-sectional study.

Methods
Porcine dermatitis and nephropathy syndrome (PDNS) outbreak was recently observed at industrial PCV-2a vaccinated farm in Russia. Clinical manifestations of PDNS were observed in 120-140 day old animals (morbidity 1%, mortality 100%). Samples were collected from animals at 60, 90, 120, 150 and 190 days of life. Serum samples were analyzed by PCR (PCV2, PCV3, PRRSV) and ELISA (PCV-2 (IgG\IgM), PRRSV (IgG)).

Results
Proportion of PCV-2 ELISA positive animals increased from 0 (days 60-80) to 100% (190 day); proportion of piglets with viremia increased from 20 (day 60) to 100% (day 120) and subsequently decreased to 50 and 20% (days 150 and 190, respectively). Proportion of PRRSV PCR-positive animals remained almost unchanged: 40-60% during experiment, while proportion of PRRSV IgG-positive animals increased from 0 on day 60 to 60% on day 120, than subsequent decreased. In tissue samples PCV2 and PRRSV were detected in 100 and 45%. Presence of PCV-3 was not detected in serum samples but it was found in 2 out of 20 tissue samples.

Conclusions
Our study revealed that emergence of PDNS correlated with increase in circulation of PCV-2d. To our knowledge, this is the first published description of PDNS in Russia.

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PRRS 40

Complete genome of a "Russian group" isolate of subtype 1 PRRSV-1 is different from the "classic" PRRSV-1 strains.

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Objective

Russian isolates of subtype 1 PRRSV-1 form a separate clade based on partial genomic phylogenetic analyses. In the present study, we report a complete genome sequencing of a strain from the "Russian group" subtype 1 PRRSV-1. The virus was isolated from lung tissue of dead weaning piglets from the endemic farm in West Siberia, Russia in 2018 and was named Tyu18.

Methods

Total RNA was fragmented, converted into cDNA using random primers and subjected to second strand synthesis using NEBNext® Ultra II Non-Directional RNA Second Strand Synthesis Module. Resulting dsDNA was used for library preparation with NEBNext® Fast DNA Library Prep Set for Ion Torrent™ and sequenced on Ion S5™ XL System, occupying a part of Ion 530 Chip with mean 200-bp read length option. Resulting raw reads were analyzed with CLC Genomics Workbench 11, including quality control steps and *de novo* assembly, yielding several contigs with major one representing the viral genome with approximately 500-fold average coverage.

Results

The full sequence of the PRRSV Tyu18 isolate genome was 14941 nucleotides in length excluding poly (A) tail. The Tyu18 PRRSV isolate was compared with the prototype strains of genotype 1 subtypes 1, 2 and 3, (Lelystad, WestSib13 and Lena). Full sequence alignment revealed 78.1, 78.1 and 77.7 % homology, respectively. Comparison of ORF1a sequences of the Tyu18 and Lelystad isolates revealed a deletion of 51 amino acids in the variable region of the NSP2 protein (positions 655-706) and a deletion of one amino acid at position 570. The 3' end of the ORF3 sequence of the Tyu18 isolate is shorter by 3, 13 and 29 amino acids than that of the WestSib13, Lena and Lelystad strains respectively.

Conclusions

We sequenced and analyzed the complete genome sequence of the PRRSV Tyu18 isolate, which belongs to "Russian group" subtype 1 of European type PRRSV. To our knowledge, this is the first complete genome sequence of a "Russian group" subtype 1 PRRSV-1. Continuous circulation of subtype 1 PRRSV-1 in Russia resulted in formation of the new genetic group reflecting growing diversity of PRRSV-1.

Financial Support

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PRRS 41

Identification of host proteins that interact with non-structural proteins-1α and -1β of prrsv-1

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Objective

Porcine reproductive and respiratory syndrome viruses (PRRSV) are rapidly evolving and existing vaccines are failing to control the PRRS panzootic. PRRSV produces 16 non-structural proteins (NSPs) that are involved in viral replication and/or modulating the host immune response. Previous studies have shown that PRRSV NSP1α and NSP1β modulate host cell responses; however, the underlying molecular mechanisms remain to be fully elucidated. Therefore, this project aims to identify and characterise novel PRRSV-1 NSP1-host protein interactions.

Methods

NSP1α and NSP1β from a representative PRRSV-1 field strain were screened for interactions using a protein expression library generated from the primary target cell of PRRSV-1, porcine alveolar macrophages, and the yeast-2-hybrid (y-2-h) system, a method of detecting protein-protein interactions.

Results

The screens identified 62 and 127 putative binding partners for NSP1α and NSP1β, respectively. Three interactions from the NSP1α screen and 27 from the NSP1β screen were confirmed using y-2-h; these proteins are involved in either interferon signalling, the NF-κB pathway, ubiquitination or nuclear transport.

Conclusions

Identifying and characterising these novel interactions will increase our understanding of how PRRSV-1 NSP1α/β modulates the host cellular immune response, which could subsequently be exploited to rationally attenuate PRRSV-1 as a basis for improved vaccines.

Financial Support

Biotechnology and Biological Sciences Research Council

PRRS 42

Development of a bespoke PRRSV genomic toolkit

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Objective
Porcine reproductive and respiratory syndrome viruses (PRRSVs) are positive ssRNA viruses that are the causative etiological agent of PRRS; a syndrome that is responsible for the largest health-related losses in the US swine industry. Despite the research effort allocated to PRRS in the US, key aspects of the disease evolution, diagnosis and control are yet-to-be resolved. In addition, affordable tools are not readily available for the rapid description of the PRRSV landscape at the genomic level. We hypothesize that PRRSVs exists as a quasispecies and therefore the development of a rapid NGS approach to better describe the true quasispecies nature of PRRSV is both warranted and timely.

Methods
We selected three PRRSV isolates and a suite of PRRSV RT-PCR positive biological samples as our source material. A custom sequence library protocol coupled with the application of the Nanopore MinION sequencing was chosen for rhe creation of a bespoke PRRSV genomic toolkit.

Results
We confirmed that the bespoke PRRSV genomic toolkit can capture the true PRRSV genomic diversity within the sample types tested. We anticipate that when applied, it could be used to reveal biologically relevant genomic modifications across the genome of PRRSV. The quasispecies nature of PRRSV was also confirmed.

Conclusions
We show that the bespoke PRRSV genomic toolkit has the potential to reveal new insights into the evolution of PRRSV.

Financial Support
U.S. Department of Agriculture

PRRS 43

Assessment of the protective efficacy of a cold-adapted attenuated genotype 2b PEDV

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Objective

Considering efficacy issue of modified live virus (MLV) vaccines for classical G1a PEDV, there is a high priority for the development of a next-generation MLV vaccine against G2b epizootic or related strains prevalent in the field, which can replicate to high titer in the gut and also boost lactogenic immune responses without giving rise to clinical illnesses. In general, the attenuated virus utilized to prepare for the MLV vaccine can be achieved by traditional cell culture adaptation procedures of the virulent wild-type virus in non-host cell lines, but this process is impeded by difficulties in performing laboratory procedures, such as numerous time-consuming, and repetitive passages in non-host cell lines. In contrast, adaptation to growth at low temperatures by short-term serial passages *in vitro* has been frequently used to generate several attenuated DNA and RNA viruses.

Methods

In this study, we generated a cold-adapted live attenuated vaccine candidate (Aram-P29-CA) by short-term passage of a virulent PEDV isolate at successively lower temperatures in Vero cells.

Results

Whole genome sequencing identified 12 amino acid changes in the cold-adapted strain with no insertions and deletions throughout the genome. Animal inoculation experiments confirmed the attenuated phenotype of Aram-P29-CA virus in the natural host. Pregnant sows were orally administered P29-CA live vaccines two doses at 2-week intervals prior to parturition, and the newborn piglets were challenged with the parental virus. The oral homologous prime-boost vaccination of P29-CA significantly improved the survival rate of the piglets and notably mitigated the severity of diarrhea and PEDV fecal shedding after the challenge. Furthermore, strong antibody responses to PEDV were detected in the sera and colostrum of immunized sows and in the sera of their offspring.

Conclusions

These results demonstrated that the cold-adapted attenuated virus can be used as a live vaccine in maternal vaccination strategies to provide durable lactogenic immunity and confer passive protection to litters against PEDV.

Financial Support

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PRRS 44

Genotypic and phenotypic characteristics of novel emerging lineage 1 PRRSV nsp2 deletion variants in South Korea

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Objective

We recently isolated two lineage 1 strains of PRRSV-2 from vaccinated pig farms experiencing respiratory problems and identified novel discontinuous deletions of 140 amino acids (111-1-19-9) in the nonstructural protein 2 (nsp2) region of both isolates. Although natural large deletions in the middle HV2 of nsp2 have also been identified in field isolates of PRRSV-2 in South Korea, the origin and pathogenicity of the novel PRRSV nsp2 deletion (DEL) mutants recently emerging in Korean pig farms remain largely unknown.

Methods

The present study was conducted to determine the complete genome sequences and pathogenicity of two PRRSV-2 nsp2 deletion DEL isolates, KNU-1901-OS and KNU-1902-BY.

Results

The full-length genomes of KNU-1901-OS and KNU-1902-BY were identically determined to be 14,989 nucleotides in length, excluding the poly(A) tail, which were 423-nucleotide shorter than that of the PRRSV-2 prototype strain VR-2332 due to the presence of notable discontinuous deletions within the nsp2 gene. The genomes of KNU-1901-OS and KNU-1902-BY consisted of a 190-nucleotide 5′ untranslated region (UTR), a 14,677-nucleotide protein-coding region, and a 151-nucleotide 3′-UTR. Whole genome evaluation revealed that the nucleotide sequences of KNU-1901-OS and KNU-1902-BY are most similar to each other (3.8% sequence divergence), but distantly close to the Korean (13.7–18.7% sequence divergence) and global NADC30-like lineage 1 strains (15.1–17.4% sequence divergence). To evaluate the *in vitro* immunity of nsp2 deletion variants, we sought to explore alteration of inflammatory cytokine and chemokine expression in immortalized PAM-KNU cells infected with each virus strain using quantitative real-time RT-PCR. A variety of antiviral genes, including cytokines and chemokines, were found to be significantly altered in nsp2 DEL variant-infected PAM cells.

Conclusions

In order to understand the impact of the large nsp2 DEL on viral pathogenesis, the pathogenicity of two isolates was examined in pigs. Results of animal inoculation studies to assess the virulence of the PRRSV-2 nsp2 DEL strains will be discussed.

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PRRS 45

Characterization of PRRSV1 transmission routes in an endemic farm identifies conserved phylogenetic clusters

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Objective

The main objective of the present work was to characterize the transmission chains of PRRSV1 in piglets and weaners of an endemically infected farm using whole genome sequencing.

Methods

Samples were obtained from a positive unstable farm, where a blanket vaccination protocol with a PRRSV1 MLV was being applied. Blood samples from sixty randomly selected piglets from 10 sows were taken at 2, 4, 6 and 9 weeks of age. The presence of PRRSV1 was assessed by RT-qPCR. Positive samples were single-passaged in porcine alveolar macrophages and characterized by NGS in an Illumina Miseq platform, from which the viral quasi-species and the complete consensus genome sequence was obtained.

Results

The phylogenetic trees based on complete genome sequences consistently identified several transmission lines and clusters that could be linked to particular sows and pens. Within the PRRSV1 genome, the segments Nsp2 and Nsp9 were the ones that better identified the phylogenetic groups. Furthermore, the whole genome p-distances ranged between 0.2 and 0.7% within clusters, with values around 1% being the highest reported among them. Within PRRSV1 genome the segments showing the highest p-distances were ORF4 (2.5%) and ORF5a (3.0%).

Conclusions

The results highlight the importance of the sow in the PRRSV1 strain transmitted to the offspring. Also, they indicate virus diversification both during the transmission event, between the transmissible and the founder viral quasi-species, and throughout the infection within the host.

Financial Support

Ministry of Science of Innovation Spain

PRRS 46

Host factors involved in porcine reproductive and respiratory syndrome virus entry

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Objective

Porcine reproductive and respiratory syndrome virus (PRRSV), one of the most important animal pathogens of global significance, causes porcine reproductive and respiratory syndrome. The disease is found in most pig-producing countries and affects the swine industry and food security worldwide, causing enormous economic losses each year. The goal of this project is to discover the porcine cellular factors that are crucial for elucidating the mechanisms of PRRSV entry and dissect the discrete entry steps that are regulated by specific host factors.

Methods

This work involves the use of two complementary, technologically advanced genome-scale genetic screens for gain- and loss-of-function of PRRSV entry. For the gain-of-function screen, we are using a cyclical packaging rescue strategy with a retroviral cDNA library, derived from the PRRSV-susceptible porcine macrophage cell line ZMAC, to identify one or more cellular genes that confer susceptibility to PRRSV infection on the PRRSV-nonsusceptible porcine kidney cell line PK-15. For the loss-of-function screen, we are using a multiplexed RNAi screen strategy with a lentiviral porcine shRNA library to identify one or more cellular genes that play an important role in PRRSV entry into the PRRSV-susceptible porcine macrophage cell line ZMAC.

Results

We are currently screening both the iterative cDNA library and the multiplexed RNAi library.

Conclusions

The outcomes of this research will not only shed new light on the cell/tissue tropism and pathogenesis of PRRSV, but also significantly advance the development of novel antiviral interventions capable of inhibiting the early stages of the PRRSV life cycle.

Financial Support

U.S. Department of Agriculture, National Institute for Food and Agriculture

PRRS 47

Medium chain fatty acids to reduce Porcine Reproductive Respiratory Syndrome Virus replication in MARC-145 cells

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Objective

PRRSv has been the most economically significant pathogen to the swine industry for over 30 years. The US swine industry estimates annual production losses of over $600 million. The objective of this study was to evaluate the efficacy of medium chain fatty acids (MCFA) to reduce viral replication of PRRSv.

Methods

In this study, PRRSv were cultured on MARC-145 cells to evaluate the impact of MCFA on viral replication of Type I and Type II PRRSv strains. Individual MCFA concentrations were tested ranging from 1000µg/ml to 1µg/ml and compared to control cells. $TCID_{50}$/ml was calculated for all treatment concentrations. PPRSv replication was detected using the FITC labeled IgG anti-PRRSv monoclonal antibody in MARC-145 cells. MARC-145 cells were cultured for 48 hours before exposure to MCFA and virus inoculation.

Results

The results of this study demonstrated that MCFA were able to reduce replication of both strains (type I and type II) of PRRSv in the MARC-145 cells. Based on data from this study, there are statistical differences in the efficacy of individual MCFAs to reduce viral replication of PRRSv strains.

Conclusions

Further research is needed to determine the mode of action of individual as well as combinations of MCFA on PRRSv replication. Future studies should be conducted to confirm these results in an in vivo model.

PRRS 48

In vivo characterization of an emerging PRRSV variant with novel -2/-1 PRF signal in nsp2 region

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Objective

The recent emergence of PRRSV variants caused higher mortality in nursery pigs and increased rate of reproductive failure in sows. In our previous study, we isolated an emerging PRRSV strain, KS17-C1, which is phylogenetically grouped with other emerging PRRSV isolates reported from pigs with severe clinical manifestations. Genome sequence analysis showed that the -2/-1 programmed ribosome frameshifting (PRF) signal located within nsp2 differs the most from historical PRRSV strains. The -2/-1 PRF generates two frameshifting products, nsp2TF and nsp2N, respectively. In the historical PRRSV strains, -1 PRF immediately encounters a stop codon, which terminates the translation of -1 reading frame to produce nsp2N. However, KS 17-C1 isolate contains mutations disrupting the -1 PRF stop codon, extending the translation of nsp2N to additional 23 amino acids (aa) at 3’-end (nsp2N+23aa). Since the PRF products, especially nsp2N, have been identified as innate immune antagonists, the present study was aimed at characterizing the pathological features of this emerging virus and determine whether nsp2N extension correlates with the increased pathogenicity of the virus in infected pigs.

Methods

A recombinant virus (KS17-C1-Mut) with restored -1 PRF stop codon was generated. Groups of 4-week-old pigs were experimentally infected with wild-type (WT) virus, KS17-C1-Mut or mock-infected.

Results

The WT virus-infected group showed severe clinical signs (high fever and labored breathing). Pathological lesions, including lung pneumonia, and enlarged lymph nodes, were also observed. In contrast, pigs infected with KS17-C1-Mut showed moderate clinical signs and pathological lesions. During the early time of infection (3 days post infection), there was 25% reduced viral load in serum from KS17-C1-Mut infected pigs in comparison to that of WT-virus infected pigs.

Conclusions

These results confirmed the high pathogenic nature of the virus; and the attenuated phenotype of KS17-C1-Mut suggests a possible link between nsp2N extension and pathogenicity of this group of newly emerging PRRSV variants in the US.

Financial Support

U.S. Department of Agriculture

PRRS 49

Single-cell RNA-sequencing to identify transcriptional signatures in a PRRSV persistent cell line

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Objective

PRRSV persistent infection is a significant problem impeding disease control and prevention. Recently, we established a cell culture model of PRRSV persistence. In PRRSV persistent cells (MARC/PRRSV-P109), we identified three cell sub-populations: non-infected cells, infected cells with only viral dsRNA, and infected cells with both viral dsRNA and proteins. The objective of this study was to identify transcriptional associated with PRRSV persistence.

Methods

Single-cell RNA-sequencing (scRNA-seq) technology was applied to PRRSV persistent MARC/PRRSV-P109 and mock-infected MARC-145 cells.

Results

Similar gene clusters were detected in infected and mock-infected cells, however, a distinct gene cluster (PRRSV-C03) was identified in MARC/PRRSV-P109 cells. In a comparison of MARC/PRRSV-P109 and mock-infected cells, 6 differentially expressed genes (DEGs) were identified, of which *GPS2*, *TUSC5*, and *ENSCSAG00000011825* were predominantly expressed at higher levels in the PRRSV-C03 cluster. These 3 genes were also among 12 DEGs in the PRRSV-C03 cluster when comparing with other clusters in MARC/PRRSV-P109 cells. Further analysis of the PRRSV-C03 cluster revealed 3 distinct sub-clusters, in which sub-cluster B was distinguished from sub-clusters A and C, based on its lower expression levels of *GPS2* and *TUSC5*, and higher expression levels of *NDRG1* and *FXYD2*. *GPS2* encodes a protein that functions as the signal repressor in G protein-mitogen-activated protein kinase signal cascades related to signal transduction pathways of immune gene expression. Overexpression of the *GPS2* gene could suppress host immune responses. Based on these data, we suspect that sub-clusters A and C could comprise cells that contain only viral dsRNA, while sub-cluster B could consist of cells that contain both viral dsRNA and protein.

Conclusions

Current research is directed at validating these DEGs. Our studies have established a PRRSV persistent cell model system and demonstrated the feasibility of using scRNA-seq technology to identify molecular signatures of PRRSV persistence.

Financial Support

USDA National Institute of Food and Agriculture

PRRS 50

Development and characterization of monoclonal antibodies against African Swine Fever virus

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Objective

Current outbreaks of ASFV in Asia and some European countries pose the potential pandemic threat to global swine industry. Highly sensitive and specific diagnostic reagents and assays are urgently needed for rapid detection and implementation in quarantine and elimination of infected animals. The objective of this study is to develop and characterize a panel of specific monoclonal antibodies (mAbs) for ASFV diagnostics and research.

Methods

ASFV proteins were selected based on their known or predicted properties of being present on the surface of intracellular mature virion or extracellular viral particles. Antigenic regions of these proteins were expressed for mice immunization, and spleenocytes from immunized mouse were fused with mouse myeloma cells to generate hybridomas. ASFV specific mAbs from hybridoma culture were initially screened using *in vitro* expression system and then confirm the reactivity in ASFV-infected cells.

Results

MAbs against ASFV p10, p22, p30, CD2V, p49, and p54 proteins have been generated. Reactivity of these mAbs was confirmed by immunofluorescent assay (IFA), immunoprecipitation and Western blot analysis in Vero cells expression the specific ASFV protein. Currently, these mAbs are being characterized in ASFV-infected Vero cells and swine macrophages. The p30 mAbs, 47-3, 62-35, and 142-4, were determined by IFA to recognize ASFV strains BA71 V and Georgia/2007. MAb 47-3 detected p30 expression by immunohistochemistry in tissue samples from Georgia/2007-infected pigs. Epitope mapping revealed that mAb 47-3 recognized a linear epitope within N-terminus of p30, in which the epitope is potentially associated with important immunological functions. In contrast, mAbs 62-35 and 142-4 only recognized a large immunodominant region in the C-terminus, which is highly hydrophilic and predicted to contain an intrinsically disordered protein region.

Conclusions

This panel of mAbs and mAb-based diagnostic assays represent valuable tools for ASFV detection and surveillance. They are also important tools for basic mechanism studies toward developing vaccines and antiviral agents.

Financial Support

National Pork Board

Long and short reads sequencing of total RNA for detection of pathogens in chicken respiratory clinical swabs

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Objective
Co-infections of poultry with different RNA viruses and bacteria are often misdiagnosed and affect the accurate diagnosis of clinical disease. An unbiased agnostic approach with rapid and precise characterization of these pathogens is crucial for respiratory disease management. The ability of long and short reads sequencing approaches to rapidly detect viral and bacterial pathogens from clinical samples was evaluated.

Methods
Three sample sets (12 oral swabs per set); 1) experimental samples comprising of Infectious Bronchitis Virus (IBV), Avian Influenza Virus (AIV) and *Mycoplasma synoviae* (MS); 2) field-collected clinical samples from commercial poultry in Pakistan, and 3) field collected clinical samples from live bird markets in Kenya were sequenced with MinION-random sequencing approach in comparison to MiSeq short read sequencing platform. Briefly, total RNA was extracted, randomly reverse transcribed, barcoded double-stranded cDNA libraries and pooled for sequencing. For quantitative detection of pathogens, read-based analyses of the MinION and MiSeq data were performed using BLAST on customized databases.

Results
In each of the sample sets, microbial reads were detected within 30 minutes of MinION sequencing and accurately characterized for their genetic types. In experimental swabs with coinfections, the MinION read counts for each of the pathogen (AIV, IBV and MS) were comparable with the respective RT-qPCR Ct values in all samples. In field collected samples, MinION and MiSeq approaches detected NDV in all the Kenyan and Pakistani samples. Bioinformatics analysis of MinION and MiSeq data lead to the assembly of multiple NDV complete genomes (genotype Vd) from Kenya samples and multiple NDV (genotype VIIi) and AIV (H9N2) genomes from Pakistani samples.

Conclusions

Together, these results suggest that MinION provides a rapid, multiplexed, and cost-effective alternative for the rapid detection of respiratory pathogens in clinical samples with coinfections and pathogen identification may be comparable to MiSeq platform in some cases.

Financial Support
U.S. Department of Agriculture

PRRS 52

Epitope mapping of the ASFV p30 protein

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Objective

African swine fever (ASF) is a highly contagious viral disease of swine which causes high mortality, approaching 100%, in domestic pigs. ASF is the only member of the *Asfarviridae* family, genus *Asfivirus*. Identification of the most antigenic viral proteins is highly relevant for the improvement of serological diagnostic tests. Structural p30 protein was identified as highly antigenic during infection. Furthermore, antibodies against this protein are involved in virus neutralization and internalization. The goal of this work was to identify immunodominant peptides in the structure of p30 protein of ASFV which could be used as antigens to detect ASFV specific antibodies following natural infection in pigs.

Methods

Translated p30 gene of the virus was analyzed for prediction of linear B-cell epitopes. Surface accessibility, epitope prediction, hydrophilicity and antigenicity of the protein were estimated by using software modules available at the Immune Epitope Database and Analysis Resource (IEDB) web page (http://tools.iedb.org). Six peptides covering the full length of the p30 protein were synthesized and used as the antigens in indirect ELISA test.

Results

Serological reactivity of synthesised peptides was evaluated on the panel of positive serum samples. Serological reacttivity was detected with the peptide no. 6 only, spanning amino acids 100-120. Weak reactivity was also detected with peptide no 1. covering aminoacids 1-20 of the protein.

Conclusions

Identified immunodominant peptide originating from different virus serotypes will further be tested as antigens for serological detection of ASFV specific antibodies. The proposed approach could increase specificity and sensitivity of current serological tests.

Financial Support

This work was supported by the National agency for agricultural research (NAZV), project number QK1920187.

PRRS 53

Preventing PRRS through modifications in the virus receptor CD163

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Objective
The extracellular region of CD163 is composed of 9 scavenger receptor cysteine-rich (SRCR) domains along with two proline serine threonine (PST) domains. A large body of work shows that pigs lacking SRCR5 of CD163 are resistant to infection with PRRSV-1 and PRRSV-2. The ultimate goal of this research is to construct a pig possessing the smallest modification in CD163 sufficient to prevent PRRSV infection while retaining normal CD163 functions. The first objective is the use of an in vitro system to map the CD163 peptide sequences important for PRRSV infection. For the second objective, the results will be used to develop and test the same CD163-modifications in pigs. The final objective is to understand the participation of CD163 in inflammation and immunity.

Methods
For Objective 1, HEK293T (HEK) cells were transfected with domain-deleted plasmid constructs fused to EGFP. Cell surface expression of modified CD163 was measured by flow cytometry of cells stained with anti-CD163 antibody. Transfected cells were infected with a PRRSV isolate expressing a red fluorescent protein (RFP) and viewed under a fluorescence microscope. Insertion of proline-arginine (PR) dipeptides along the SRCR5 peptide sequence was used to probe peptide sequences and secondary structures within SRCR5 involved in virus recognition.

Results
Cells expressing a deletion of SRCR5 or PSTII did not support infection. The deletion of SRCR8 and 9 had a lesser effect on infection. PR insertions in SRCR5 possessing the greatest effect on infection were identified for both PRRSV-1 and PRRSV-2 isolates.

Conclusions
The results from this study identify likely contact regions and structural requirements in CD163 involved in PRRSV infection and will be incorporated into the construction of CD163-modified pigs. USDA NIFA Award # 2017-67015-26774.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture

PRRS 54

Detection of atypical porcine pestivirus (APPV) by RNAscope method and electron microscopy

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Objective

A new Pestivirus, the atypical porcine pestivirus (APPV) was discovered in 2015 in the USA by next-generation sequencing and it has been linked to A-II type congenital tremor (CT). CT is a neurological syndrome of newborn piglets which is characterized by tremors of the head and limbs. On affected farms, the virus can be responsible for 10 % of piglet mortality. Since then, its presence has been reported from various countries of Europe, America, and Asia. Different studies have shown that pregnant sows inoculated with APPV positive blood-or tissue-suspension farrowed piglets affected by congenital tremors. CT affected piglets may become asymptomatic by weaning, and together with the subclinically infected piglets might serve as a source of infection for naive animals.

Methods

Our research group has developed a novel RNA based in situ hybridization method (RNAscope) that targets the viral RNA in formalin-fixed paraffin-embedded (FFPE) tissues of APPV infected pigs. 3-3 one-day-old piglets were selected from two different APPV infected herds for the study. 2-2 animals were showing symptoms of CT and the third ones were clinically healthy and served as negative controls. To assess the distribution of the virus in the nervous system and the ultrastructure of the affected cells, we also performed transmission electron microscopy on cerebral and cerebellar samples of PCR positive and negative control animals.

Results

APPV was detected in the inner granular cell layer, in the Purkinje-cells and in the molecular layer of the cerebellum, and also in degrading neurons surrounded by glial cells of the cerebrum and brainstem. Our ultrastructural studies in the cerebellum have likely detected the virus in the vicinity of overactive ribosomes; however, overactivity is common in the case of newborn animals. As no previous studies revealed the exact morphology of the virus, we are performing further analyses for the verification of our findings.

Conclusions

Our findings contribute to the biology of atypical porcine pestivirus and the cause of the generalized tremor observed in the affected piglets.

Financial Support

European Social Fund

Design and development of photonic biosensors for swine viral diseases detection

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Objective

The SWINOSTICS project aims to develop a novel field diagnostic device, based on advanced, proven, bio-sensing and photonics technologies to tackle emerging and endemic viruses causing epidemics in swine farms

Methods

The SWINOSTICS approach is based on the combination of photonic integrated circuit (PIC) technology and bio-sensing technology to detect different swine viruses (each virus presence will be detected by one of the functionalized resonant rings forming the PIC). The targeted viruses in the project are: African swine fever virus (ASFV), classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PPRSV) [20], porcine parvovirus (PPV), porcine circovirus 2 (PCV2), and Swine Influenza A virus (SIV). In the present work the detection of PCV2 was aimed.

Results

Our biosensor PIC comprises three main building blocks: sensing ring resonators, light coupling block (grating couplers), and optical power distribution block. The ring resonators are used as sensor elements (using one resonant ring for each targeted virus), the light coupling section allows to introduce and extract the optical signals into/out of the PIC whilst the power distribution block is necessary to feed all the resonator rings of the PIC with a common laser source input. Several photonic sensors PICs were functionalized with the aim of detecting PCV2. On top of the surface, commercial rabbit polyclonal antibodies against PCV2 virus (Thermo Fisher Scientific #PA5-34969) were covalently immobilized in an oriented manner.

Conclusions

One of the fabricated PICs was used in a preliminary experiment for detection of PCV2 virus. After the rings functionalization with specific antibodies and attaching a microfluidic layer on the PIC, positive detection of several virus concentrations was achieved, demonstrating the good performance and feasibility of the proposed detection technique.

Financial Support

H2020 SWINOSTICS project

PRRS 56

Replication of zoonotic genotype 3 hepatitis E virus (HEV) is enhanced by the ORF4 protein of human genotype 1 HEV

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Objective

Hepatitis E virus (HEV) is a zoonotic pathogen with several genotypes (g) known to cross between humans and animals, while others are solely restricted to human infection. HEV causes acute hepatitis resulting in 44,000 deaths per year worldwide. One of the most at risk populations for HEV-induced mortality are pregnant women in their second and third trimesters. In this pregnant population the mortality rates increase to 30% due to acute liver failure, up from 1% seen in otherwise healthy infected patients. Reasons contributing to enhanced pregnancy related pathology are not fully understood. Recently, a novel fourth open reading frame (ORF4) overlapping ORF1 was discovered in g-1 HEV. Expression of this ORF4 protein is regulated via an IRES-like RNA element and was shown to enhance g-1 HEV replication. We hypothesized that ORF4 may act as a replication enhancer across multiple genotypes of HEV.

Methods

To experimentally determine whether g-3 HEV contains an ORF4 similar to g-1, g-1 and g-3 sequence comparisons were performed. To assess whether ORF4 protein could enhance g-3 replication, Huh7 cell lines constitutively expressing ORF4 were created. Replication of the Kernow-C1 g-3 HEV was assessed in ORF4-expressing and ORF4-lacking Huh7 cell lines.

Results

Despite not naturally encoding ORF4, replication of g-3 HEV was enhanced by the presence of g-1 ORF4 protein. These results suggest the function of ORF4 protein from g-1 HEV is transferrable, enhancing the replication of g-3 HEV.

Conclusions

ORF4 may be utilized to enhance replication of difficult to propagate HEV genotypes in cell culture. Additionally, viruses in which ORF4 is inserted into the g-3 viral backbone may provide a useful swine model for studying pregnancy pathology associated with ORF4

Financial Support

Ohio State University Start Up Package

Improved methods for African Swine Fever Virus whole genome sequencing and genotyping

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Objective

Whole genome sequencing (WGS) of African Swine Fever Virus (ASFV) from clinical samples is complicated by its large genome and host genome contamination, and routine sequencing is generally limited to short regions of genetic diversity within the ASFV genome. The objectives of the current study were (i) to develop improved methods for WGS of ASFV from clinical samples, and (ii) to expand the genomic regions used for PCR-based ASFV genotyping, thus enhancing our ability to monitor the ongoing spread of ASFV.

Methods

Viral DNA was extracted from the plasma or serum of pigs experimentally infected with ASFV strains E70 (Genotype I), Armenia07 (Genotype II), or Ken05/Tk1 (Genotype X). qPCR-based selection was used to identify samples with low host genomic contamination and samples were sequenced using a NextSeq. In addition, long amplification PCR was performed with the samples to generate five separate amplicons with an average length of 12 kilobases that include all established ASFV genotyping regions. The amplicons were sequenced using a MiSeq and a portable MinIon Nanopore sequencer.

Results

The whole genome sequences of E70 and Armenia07 strains were determined from serum and plasma samples, respectively. The E70 sequence had 99.9% identity with the BA71 stain and Armenia07 had 99.9% identity with the Georgia07 strain. Long PCR amplicons were generated from E70, Armenia07, and Ken05/Tk1 clinical samples and sequenced, resulting in sequencing coverage of >60 kilobases (approximately 30%) of the ASFV genome. The sequenced segments include all established regions used for ASFV genotyping, including EP402R, B602L, B646L, E183L, I73R/I329L junction, and the multigene family (MGF) regions.

Conclusions

The study demonstrates that ASFV WGS can be performed using clinical samples with a relatively simple method of qPCR-screening and high-depth sequencing. Moreover, the employment of long amplification PCR genotyping can significantly increase the genetic information routinely collected from clinical samples and may be used to identify genomic regions involved in ASFV evolution and virulence.

Financial Support

State of Kansas

PRRS 58

Comprehensive transcriptomic analysis of African Swine Fever Virus infected pigs

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Objective
African Swine Fever Virus (ASFV) is a large double-stranded DNA virus which causes up to 100% mortality in domestic and feral swine. Currently, a virulent genotype II ASFV strain is circulating and spreading through European and Asian countries, and threatening naïve territories including the United States. There is no vaccine available for ASFV, and the development of an ASFV vaccine is of high priority. However, there has been limited success towards the development of a vaccine due to the complex nature of the virus and our limited understanding of its replication strategies. Studies that identify important virus and host factors and pathways involved in ASFV infection will greatly advance our understanding of ASFV-host interaction and ASFV biology, and facilitate the development of rationally designed vaccines against ASFV.

Methods
A comprehensive transcriptomic approach was used to characterize both virus and host total gene expression during the course of ASFV infection in pigs. Whole blood specimens, derived from pigs infected with virulent ASFV genotype I or II strains, were collected on multiple days over the course of ASFV infection. Total RNA was extracted and prepared for RNA sequencing using an Illumina Nextseq 500 platform. Analyses of the resulting transcriptomic data included standard RNAseq methods as well as a novel discrete mathematical approach from which a transcriptomic death signature was characterized.

Results
Differential expression of ASFV and host genes at different times after infection were evaluated. Despite similarities in clinical disease outcomes, the results suggest unique host gene expression in pigs infected with genotype I versus II ASFV strains and at different days post infection. Nonetheless, a series of commonly expressed host genes were identified after infection with the virulent ASFV genotypes.

Conclusions
The data from these transcriptomic studies will contribute to our understanding of ASFV-host protein interaction, ASFV pathogenesis and host immune responses, as well as to the development of rationally designed ASFV vaccines.

Financial Support
State of Kansas

PRRS 59

An overview of research at the National Animal Disease Center on endemic and emerging viral diseases of swine

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Objective

Our ARS project has four objectives: identify pathogenic mechanisms of swine *Nidovirales*, including those of porcine respiratory and reproductive syndrome virus (PRRSV) and emerging porcine coronaviruses; discover vaccines, including identifying mechanisms to modulate innate and adaptive immune responses; identify and monitor genetic and antigenic evolution in *Nidovirales* and emerging viral pathogens; and identify mechanisms of pathogenesis, transmission, and immunity for emerging viral diseases of swine.

Methods

In vivo and *in vitro* research has focused on investigating mechanisms of pathogenesis, transmission, immunity, evolution, and methods of intervention for PRRSV, porcine coronaviruses, and senecavirus A.

Results

Transmission and pathology studies with SVA demonstrated viral shedding patterns and optimal times for sample collection for diagnosis. Vaccine formulations for PRRSV, PEDV and PDCoV were developed and tested. Network analyses on mRNA and miRNA data revealed multiple gene expression pathways are altered following PRRSV infection. The US Swine Pathogen Database has established a resource for 31,000 PRRSV, 5000 PEDV, 4000 ASFV, and 224 SVA sequences with convenient tools to retrieve, display, and select data for analyses. Our PRRSV data reveal multiple cocirculating clades of viruses in gene and genome trees that were divergent from available vaccines.

Conclusions

Evolutionary analyses show the dynamic landscape of PRRSV evolution with the cocirculation of multiple genetic clades that are not controlled by current vaccine strategies. Our animal pathogenesis studies aid swine veterinarians and producers in making on-farm decisions to prevent disease and/or reduce transmission if farms become infected. Our immunology studies reveal inhibition and activation of networks involved in viral entry, proliferation, and pro-inflammatory signaling may underlie the ability of PRRSV to hinder host homeostasis. This demonstrates the challenges facing swine agriculture, and how integrated programs that include surveillance with *in silico* and *in vivo* studies can result in applied solutions.

Financial Support

USDA ARS

PRRS 60

Development of a novel multiplex real-time PCR for the detection and differentiation of SADS-CoV, an emerging swine coronavirus.

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Objective
A coronavirus variant known as Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV) has emerged in China where the first clinical signs in pigs were observed in late December 2016. In 2017, an outbreak in several Guangzhou farms was associated with fatal acute diarrhea in neonatal piglets. The introduction of this new swine enteric coronavirus in the US, could have significant economic consequences to the swine industry. Therefore, rapid detection methods are required for the early diagnosis of this pathogen. Here we report the development of a new real-time PCR assay for SADS-CoV that will be multiplexed with two other swine coronaviruses. The final configuration for the test in development will be PEDV / SADS-CoV / PDCoV / IC.

Methods
Currently there are only a few SADS sequences available on Genbank. These sequences were downloaded and imported into the Sequencher 5.4.5 Software. The software was used to assemble the sequences into a contig. The sequences were visually inspected for conserved region. For Corona Viruses mainly the Nucleocapsid (N) and RNA-dependent RNA polymerase (RdRp) gene regions seem to be highly conserved. For the new SADS rtRT-PCR assay, the N region was chosen. An In Vitro Transcript was designed, which included the target sequences for the SADS assay. The process to create the IVT began with designing a syntemp oligo. The oligo includes a T7 promotor which is used by the MEGAscript® T7 Kit to transcribe the In Vitro Transcript (IVT).

Results
The IVT is then tested with the primer, probes and a chosen One-Step RT-PCR kit on the ABI 7500 Real-Time PCR Instrument. The results show the slope of -3.321280 and R2 value of 0.984144 and demonstrate that the chosen design of the rtRT-PCR SADS assay is efficient. The analytical specificity of the SADS-CoV PCR assay was evaluated against a panel of enteric swine corona viruses at SDSU to ensure that the test specifically amplifies SADS-CoV viruses and does not cross-react with closely related non-SADS swine coronaviruses. A commercial set of research reagents to detect swine coronaviruses has been on the market since 2013 from Tetracore. The PEDV-TGEV-SDCoV multiplex PCR has a three-year track record of consistent performance, although TGEV is very seldom seen in the US. Since the practical limit of multiplex real-time PCR is four fluorochromes; it is necessary to replace the TGEV assay with the SADS-CoV assay for a product that is a multiplex rRT-PCR consisting of PEDV, SADS-CoV, PDCoV and an internal positive control. The stand-alone SADS-CoV and multiplex PEDV-SADS-CoV-PDCoV-IC rRT-PCR was tested with collaborative partners, with a collection of swine coronaviruses including SADS-CoV in China.

Conclusion
It is important to the Pork industry that there be well validated, commercially available tests that can be mass-produced under cGMP conditions and deployed to veterinary diagnostic laboratories for an outbreak contingency should this novel swine coronavirus make its way to the US or any other country.

PRRS 61

First report of Pestivirus D (Border Disease Virus) identification in small ruminants in Tajikistan

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Objective
Pestivirus D (BDV) is one of the 11 main viral species of the genus Pestivirus in the Flaviviridae family. Pestiviruses infect a variety of livestock species including pigs and are an important source of respiratory and reproductive diseases. In addition, emerging and re-emerging pestiviruses a major concern. BDV is a positive single stranded RNA pestivirus which was first reported in 1959 and now causes significant economic losses to the sheep industry worldwide. The objective of this research was to characterize Pestivirus D as a new source of respiratory and reproductive failures of small ruminants in the Republic of Tajikistan.

Methods
Serum samples of sheep and goats with symptoms of respiratory and reproductive failure from 13 regions of Tajikistan were tested by AGID. Samples from the lungs, liver, spleen, heart and stomach from all kids and aborted fetuses were analyzed by RT-PCR and AGID. For virus isolation, PK-15, Vero, lamb kidney and goat testicles cell cultures were used. The presence of a non-cytopathogenic virus was determined by direct immunofluorescence. Viruses were sequenced and phylogenetic analysis carried out using MEGA 7.0.

Results
During the serological assay it has been found that the prevalence of BDV infection varies between different districts. BDV was isolated from the lungs, liver, spleen and heart samples of aborted fetuses on PK-15 and lamb kidney cell lines and shown to be non-cytopathogenic biotype. The phylogenetic analysis showed that sheep were infected with BDV genotype 3, which possessed a 90-91% identity with strain "297" from Slovakia and strain AH12-02 from China but form a separate subclade in BDV-3 genotype. Also during experimental studies we have detected the Pestivirus H (Hobi virus) as a contaminant of vaccine against PPRV which has been used for immunization. The sequences of detected viruses were deposited in GenBank under accession numbers KX900607 and KX 900608.

Conclusions
This is the first description of an infection with BDV in small ruminants herds in Central Asian district – Tajikistan. It has also confirmed the role of BDV in the pathology of respiratory and reproductive diseases.

PRRS 62

Immunohistochemical diagnostics of porcine reproductive and respiratory syndrome

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Objective

Porcine Reproductive and Respiratory Syndrome (PRRS) causes significant economic impact on the swine industry. The confirmation of the etiological role of PRRS virus in respiratory pathology remains a relevant task. As the disease caused by the PRRS virus belongs to the group of respiratory pathologies, the clinical signs are similar to the diseases with a different etiological origin. In other cases, the circulation of the PRRS virus in adult pigs can be asymptomatic. In this study immunohistochemical studies of tissues were used to diagnose infection with a Russian PRRSV isolate.

Methods

Blood serum and parenchymal organs from 40 piglets age 21 to 240 days experimentally infected West Siberian 13 (4.66 log TCID 50/cm3) and a reference strain, Lelystad virus (5.0 log TCID50/cm3). Virus was inoculated in a dose of 2 cm3 intramuscularly and 3 cm3 intranasally. The negative control group was inoculated with an uninfected MARK-145 cell culture media. Tissues were also obtained from naturally infected pigs from farms in Moscow, Smolensk, Rostov and Tyumen regions were used. Serum was assayed for virus using PCR (Vetbiochem, Russia). For PCR-positive samples, the infectious activity was determined in the porcine alveolar macrophage cell culture. A PRRS – Serotest plus kit (Vetbiochem, Russia) was used for the detection of PRRSV antibodies. IHC was performed using in-house antibodies 3h9, 4h7h9, which are specific to the nucleocapsid protein. Tissues were fixed in a 10% solution of neutral buffered formalin. For cryotomic techniques, fresh tissues were delivered to the laboratory on ice no later than 36 hours after sampling.

Results

This study confirmed the importance of IHC in the diagnosis of PRRSV-infection. In the experimental pigs, antigen was primarily located in alveolar macrophages and bronchial lymph nodes, which also possessed significant pathmorphological changes. However, pathological changes in other organs were not associated with the presence of PRRSV antigen.

Conclusions

This study reaffirms the importance of IHC in diagnosing PRRSV infection as a source of respiratory disease on Russian farms and identifies the best tissues for obtaining an accurate diagnosis.

PRRS 63

NC-229 Multistate Committee: Iowa State University Annual Report (2018 - 2019)

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Objective
Established in 1999, the NC-229 Multistate Committee (Detection and Control of PRRSV and Emerging Viral Diseases of Swine) was originally composed exclusively of US research institutions and focused exclusively on porcine reproductive and respiratory syndrome virus research. Over time, NC-229 expanded to include international institutions and broadened its focus to include a variety of infectious diseases of swine.

Methods
Research by the Iowa State University (ISU) NC-229 research group has continued to add to our understanding of African swine fever virus, atypical porcine pestivirus, classical swine fever virus, foot-and-mouth disease virus, influenza A virus, porcine astroviruses, porcine circoviruses, porcine epidemic diarrhea virus, porcine sapelovirus, porcine sapoviruses, PRRSV, pseudorabies virus, Senecavirus A, and other emerging viral diseases of swine and providing new approaches for preventing, countering and/or eliminating these infections.

Results
Extensive work has been done on the mechanisms of host-pathogen(s) interactions. New work on the ecology and epidemiology of these agents provides insight into their mechanisms of maintenance and transmission. Continued assessment and research in sample matrices and diagnostic technology is improving and refining our ability to surveil, detect, and diagnose viral infections.

Conclusions
On-going work on new methods of surveillance promises to provide new, cost-effective methods of detecting infections and implementing area elimination/eradication programs. According to Google Scholar, publications by ISU NC-229 Research Leaders have been cited an aggregate of 37,561 times since 2014. Cumulatively, research productivity by ISU faculty in 2018 included > 144 peer-reviewed manuscripts and > 103 research grants ($12.3 million).

PRRS 64

Virginia NC229: PRRS virus and other emerging viral diseases of swine

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Objective
The research efforts have focused on 4 main topics:

Immunology of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with identification of cytotoxic T cell epitopes (Meng lab).

Immonopathogenesis of PRRSV with PRRSV-induced stress granules associated with viral replication complexes and suppression of host translation (Meng lab).

Vaccine strategies for porcine epidemic diarrhea virus utilizing the Hepatitis B core antigen (Zhang lab).

PRRSV detection and genotyping using MinION-based sequencing (Lahmers lab).

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Detection and control of PRRS virus and emerging viral diseases of swine (NC-229 South Dakota)

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Objective

The overall objective of the study was to develop effective and efficient methods for detection, prevention and control of pressing viral diseases of swine including porcine reproductive and respiratory syndrome virus (PRRSV), senecavirus A and influenza virus.

Methods

Molecular, cellular, metagenomic, and immunological methods were used to achieve the objective.

Results

Research efforts to develop new diagnostic reagents and assays for Senecavirus A and influenza virus D have resulted in new monoclonal antibodies and virus neutralization test (FFN), immunofluorescence staining (IFA), and immunohistochemistry staining (IHC) for these viruses. Additionally, metagenomics and DNA sequencing technologies were used to identify the presence of bastrovirus in swine in the US and PRRSV 1-7-4 type strains in Peru. To better understand virus host interaction, we examined the role of interferon-induced genes such as interferon induced transmembrane protein 3 (IFITM3) in PRRSV replication. The interaction between PRRSV and stress granule resident protein G3BP1 was investigated. We have also shown that senecavirus A modulates apoptosis response and 3C protease plays a role in this process. In vivo studies on the pathogenesis of senecavirus A showed that virus can be spread by close contact and virus can persist in infected hosts. Finally, a novel live attenuated vaccine was developed for senecavirus A and animal immunization showed protective response against heterologous challenge.

Conclusions

Overall, new monoclonal antibodies and new or improved diagnostic assays have been developed and evaluated. New knowledge on virus host interaction and potential vaccine candidates were obtained and provides foundation for future studies.

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NC-229 Multistate Research Project: Nebraska Station Update

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Objective

Founded in 1999, the NC-229 Multistate Committee initial goal was to address the topic "Porcine Reproductive and Respiratory Disease Syndrome: Methods for the integrated control, prevention and elimination of PRRS in United States Swine Herds". Since 2009, the Committee expanded its goals to include other important emerging diseases of swine. At Nebraska Station, we focus on improving the safety and protective efficacy of vaccines against two important swine viruses: PRRSV and influenza virus of swine (IAV-S).

For PRRSV, we recently generated a fully synthetic live-attenuated PRRSV vaccine candidate carrying a consensus genome sequence (designated CON90) that confers broad levels of heterologous protection against different divergent PRRSV strains. In 2019, our main focus was to characterize the immune responses of pigs after being vaccinated with the live-attenuated PRRSV strain CON90 to elucidate the mechanisms of protection. We found that virus-neutralizing antibodies were meagerly detected from pigs vaccinated with CON90. IFN-ϒ secreting cells started to appear at 18 dpi and peaked at 32 dpi. CD4+CD8+ double positive T cells were the major population that secret IFN-ϒ, followed by CD4+ T cells. Only low levels of CD8+ T cells were detected at 25 and 32 dpi respectively. The data suggested that T cell immunity is more likely a correlate of the immune protection conferred by CON90.

For IAV-S, substantial genetic diversity represents the main challenge for the development of a broadly protective vaccine against this important pathogen. To overcome this challenge, we created a consensus HA gene (designated H3-CON.1) from a large set of H3 sequences of IAV-S. Pigs vaccinated with H3-CON.1 immunogen elicited broader levels of cross-reactive neutralizing antibodies and interferon gamma secreting cells than those vaccinated with a naturally occurring H3 immunogen. Collectively, the data provide a proof-of-evidence that the consensus immunogen approach may be effectively employed to develop a broadly protective vaccine against IAV-S.

NC229 Kansas State University Station Report

Raymond (Bob) Rowland, Kansas State University, Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine. Other participants: Ying Fang, Jishu Shi, Megan Niederwerder

The overriding economic importance of PRRSV to North American agriculture, plus the significance of other emerging diseases and foreign animal diseases, such as ASF and CSF, represent the driving force for the research conducted at K-state. The overall goal is to develop new and improved control strategies for swine diseases. Since there are no vaccines for ASF, novel vaccine approaches are being pursued, including novel vaccines, diagnostic tests, and risk mitigation.

Progress summary

Rowland: Characterization of virus receptors. In collaboration with University of Missouri. Pigs possessing virus receptor CRISPR knockouts for PRRSV, coronaviruses, and senecavirus are being evaluated for disease resistance. Progress to date includes the identification of domains in CD163 that confer resistance to PRRSV. Pigs lacking aminopeptidase N (APN) are resistant to TGEV, but not PEDV. And, pigs lacking the gene for anthrax toxin receptyor-1 (ANTXR-1), show resistance to senecavirus A. Publication: Whitworth et al. 2018. Resistance to coronavirus infection in amino peptidase N-deficient pigs. Transgenic Res. In press.

Fang: In support of ASF diagnostics, several immunogenic ASFV proteins have been cloned and expressed. The preparation of mAbs create the opportunity to develop blocking ELISAs that possess increased specificity. A p30 blocking ELISA has been evaluated for specificity and sensitivity using sera from virus-infected and p30 immunized pigs. Publication: Guo et al. 2018. Double-stranded viral RNA persists in vitro and in vivo during prolonged infection of porcine reproductive and respiratory syndrome virus. Virology. 524:78-89.

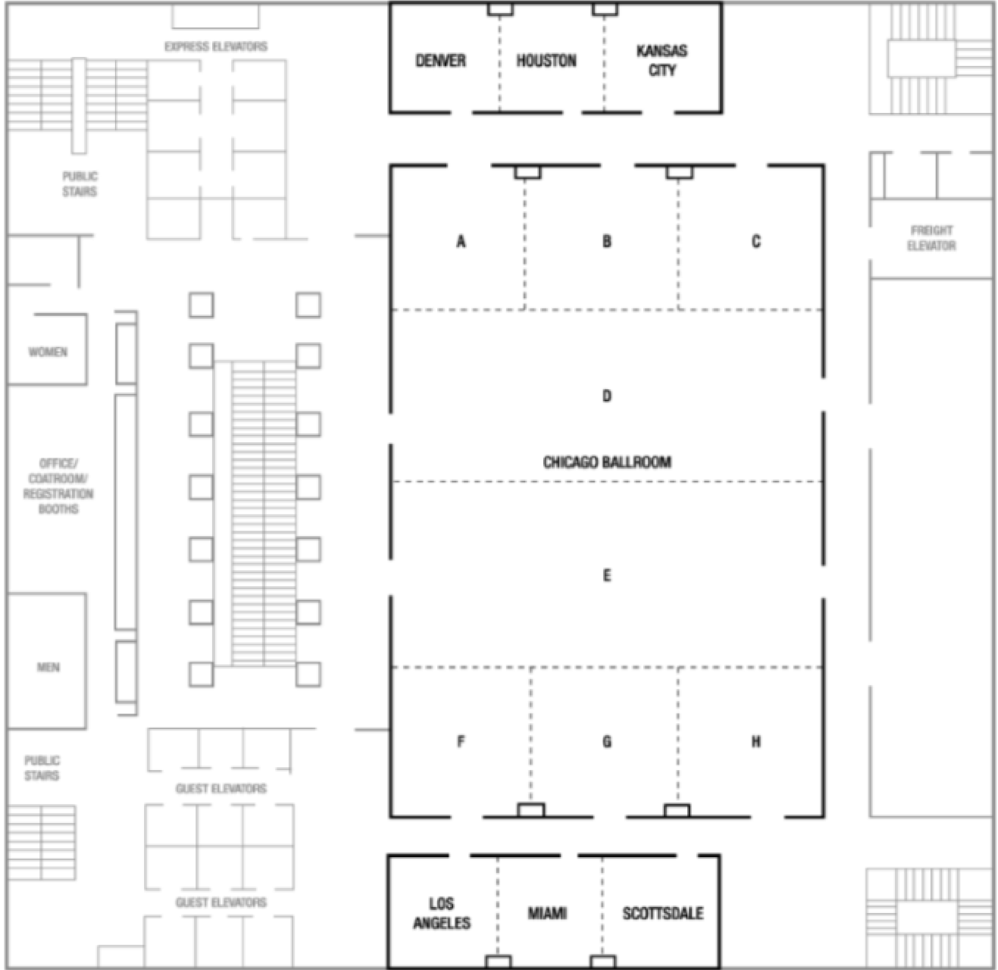
Niederwerder: Since ASFV is stable in the environment, work is being conducted to measure the stability of virus in feed and the minimum dose of ASFV needed to infect a pig under natural conditions. This information is important for testing novel feed additives that can inactivate the virus. Publications: Niederwerder et al., Infectious dose of African swine fever virus when consumed naturally in liquid or feed. Emerg Infect Dis. 25:891-897; Stoian et al., Half-life of African swine fever virus in shipped feed. Emerg Infect Dis. In Press.

Shi: The virus neutralization test (VNT) is widely used for serological survey of classical swine fever (CSF) and thje efficacy evaluation of CSF vaccines. The C-strain CSF vaccine is the most frequently used vaccine in the field. For this project, neutralizing mAb-based competitive ELISA is being developed as a substitute for the classical VNT.

CHICAGO MARRIOTT MAGNIFICENT MILE

4TH FLOOR MEETING ROOMS

5TH FLOOR MEETING ROOMS



2019 North American PRRS Symposium
Program At A Glance

Friday, November 1, 2019

1:00 PM - 5:00 PM	**Onsite registration** – Preregistered badge/program pickup *5th Floor – Registration Desk*

Saturday, November 2, 2019

7:00 AM - 5:00 PM	**Onsite registration** – Preregistered badge/program pickup *5th Floor – Registration Desk/Kiosk*
8:00 AM - 12:00 PM	**Session 1 – *Recent Advances in the Control of Swine Diseases*** 5th Floor - Chicago Ballrooms A, B, C, D
10:00 AM - 10:30 AM	**Session 1 Refreshment Break** 5th Floor – Chicago Ballrooms Foyer
10:30 AM - 12:00 PM	***Special Session on the Control of PRRS in the Field*** Shifting the PRRS research focus to grow/finish phase of production – What are we learning and implications for making progress on PRRS control Sponsored by *Boehringer-Ingelheim*
12:00 PM - 1:15 PM	**Lunch**
1:00 PM - 5:00 PM	**Poster Set-up Begins** 4th Floor – Avenue Ballroom
1:15 PM - 5:00 PM	**Session 2 - Invited Talks and Presentations from Selected**
3:00 PM - 3:30 PM	**Session 2 Refreshment Break** 5TH Floor – Chicago Ballrooms Foyer
4:30 PM - 5:00 PM	**NC-229 Business Meeting** 5th Floor – Chicago Ballrooms A, B, C, D
5:30 PM - 7:30 PM	**Special Reception and Poster Session Abstracts - Organized by NC-229** 5th Floor – Chicago Ballrooms A, B, C, D
7:30 PM - 8:00 PM	**Poster Removal**

Sunday, November 3, 2019

8:00 AM - 12:00 PM	**Session 3 - CRWAD Sessions 2 and 8 on Swine Diseases Organized by CRWAD** 5th Floor, Chicago Ballroom E

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We invite you to use the Whova App for a full schedule of all presentations, poster sessions and general sessions.

The Event Invitation Code is: **AnimalDiseases**

NA CRWAD/NAPRRSS Conference WiFi Network Name and Password is printed on the back of all name badges.

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