

APPENDIX D
SAES-422
Format for Multistate Research Activity
Accomplishments Report

Project/Activity Number: NC-229. Title: PRRSV and other emerging viral diseases of swine

Period Covered: November 30 2016 to December 1, 2017

Date for This Report to be submitted to NIMSS: March 2, 2018

Annual Meeting Date: December 3, 2017

Participants:

The following stations were represented at the meeting:

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Table 1:

<p style="text-align: center;">NC229 2017 Annual Meeting: “<i>New Science: Insights for Control of Swine Viral Diseases</i>”</p> <p style="text-align: center;">Sunday December 3 2017, 1-5 PM</p> <p style="text-align: center;">Denver-Houston Room ,Marriot Downtown Hotel, Chicago Illinois</p>	
1:00-1:05 David Benfield, OSU, administrative advisor NC229 “Welcome”	3:00-3:20 Kelly Lager NADC USDA/ARS “Update on Senecavirus pathogenesis”
1: 05-1:25 “Differential rates of PRRSV replication in antigen presenting cells: potential implication on adaptive immunity” Joe Darbellay and Volker Gerdtz Univ Saskatchewan, Canada	3:20-3:40 Andres Perez UMN “Weaned pigs source of genetic diversity of swine influenza virus , implications for SIV vaccination
1:25-1:45 Hiep Vu, Univ Nebraska	3:40-4:00 Wenju Ma KSU

“Following a strategy to broaden the protective capacity for PRRSV MLV vaccines”	“SIV: The pig a mixing vessel, in vitro and in vivo”
1:45-2:05 Federico Zuckerman Univ Illinois UC “Unfolded protein response to PRRSV : enhancement and suppression , contrasting effects on cytokine production”	4:00-4:20 Heather Wilson VIDO-Intervac Saskatoon , Canada “Uterine mucosal immunization in pigs: Porcine parvovirus model”
2:05-2:25 Crystal Loving NADC USDA/ARS “Metabolism, homeostasis and PRRSV acquired immune response”	4:20-4:40 Peter Johnson/Margo Holland USDA NIFA “Update from NIFA”
2:25-2:45 Aradhya Gourapura OSU Advances on intranasal mucosal immunization of swine.	4:40-5:00 NC-229 business meeting, renewal of authorities for executive technical committee
2:45-3:00 Break	5:00 Adjourn

Brief summary of minutes of annual meeting:

The 2017 NC229 meeting was held on the afternoon of December 3, 2017, at the Marriot Downtown Hotel in Chicago Illinois. Meeting attendance exceeded 80 persons and participating stations represented are listed above. The meeting Agenda is shown in Table 1 (see above). The business meeting centered on the topics noted below:

- 1) **Dr S. Ramamoorthy** (NDSU) was nominated and unanimously elected as the incoming NC-229 Vice–Chair.
- 2) There was strong group support for submitting an NC229 renewal proposal in 2018. Efforts in early 2018 will include discussion among members on proposal focus and emphasis and on assembling a representative writing team for proposal preparation.
- 3) Planning and scheduling of future NC-229 annual meetings was briefly discussed. It was suggested that closer alignment of the meeting with CRWAD, perhaps involving dedicated NC-229 sessions within the CRWAD program might be advantageous for maintaining NC-229 identity and avoiding excessive overlap with the NA PRRSV symposium and other weekend events. The need for a formal registration fee to cover costs of the meeting was also raised. A survey soliciting opinions on the future of NC-229 meeting structure will be circulated to participating stations in early 2018.
- 4) Meeting adjourned 5:30 PM

Accomplishments by objective :

Objective 1. Control of PRRSV

In objective 1, the major areas of focus/achievements by the NC-229 group during 2017 included:

1.1 Innate immunity against PRRSV. Studies were conducted on the effect of PRRSV NSPs on innate immunity mechanisms, on apoptosis, and the capacity for PRRSV viruses to modulate overall immune response by stimulating IFN rather than suppressing it. Also included were the effect of PRRSV of macrophages and cytokines modulation. The stations with studies in this area were: UCONN, UIUC, KSU, OSU, China Agr U, NE, NADC, SDSU and UMD

1.2 PRRSV immunity and vaccinology. Work to understand correlates of immunity and mechanisms to broaden protection, including neutralizing antibodies, developing of naturally occurring or synthetic strains of PRRSV inducing broader protection, alternative vectors for delivering PRRSV antigens or epitopes, DIVA marker systems, mechanism of attenuation and immunogenic potential of NSPs etc. was conducted. The stations with studies in this area were: UMN, UMD, VPI, NADC, UNL, UIUC, UWI, ISU, NE and KSU

1.3 Virulence of PRRSV. Studies aimed at understanding virulence factors/markers and impact of bacterial co-infection on disease severity were performed by stations: NADC and China Agr U

1.4 Mapping genetic of resistance to PRRSV infection (ISU and KSU) , genetic modification of receptors (KSU) were conducted.

1.5 Epidemiology of PRRSV transmission, which may include aerobiology, and virus evolution was conducted by: UMN, ISU, VNIIVViM-Russia and UWI, Detection of PRRSV in studs (ISU)

1.6 Economic Impact of PRRSV control; UMN, ISU

1.7 Outbreaks investigations for breeding herds and oral fluids monitoring (ISU)

Objective 2 Developing effective and efficient approaches for detection, prevention and control of emerging viral diseases of swine.

In objective 2, the major areas of focus/achievements by the NC-229 group during 2017 included:

2.1 ascertaining pathogenesis and transmission of and establishing diagnostics and reagents for **PEDV**:(ISU, UMN, OSU, KSU, SDSU, VNIIVViM-Russia, Purdue) Studying the protective immune response to PEDV: OSU

2.2 Genomics and replication of **PCV and novel ss DNA viruses** of swine (ISU, NDSU, NADC)

2.3 Genetic and antigenic evolution of swine influenza virus (SIV) and epidemiology of transmission of SIV (NADC UMN, ISU, SDSU, CENSA-Cuba) testing of SIV vaccines in vivo (NADC) and in vitro models (Purdue) testing of adjuvants for SIV inactivated immunogens (NADC)

2.4 Characterizing the ongoing outbreak of **Seneca valley virus (SVV)**, development of diagnostic tools and characterization of pathogenesis, fulfillment of Koch's postulates: ISU, SDSU, UMN, KSU

- 2.5 Characterization of diagnostic reagents for **Atypical Pestivirus of Swine** (KSU, ISU).
- 2.6 **Classical swine fever** pathogenesis & epidemiology (UCON) and vaccinology (CENSA-Cuba)
- 2.7 **African Swine Fever Virus**, epidemiology (VNIIVViM-Russia, UIUC) and protective immunity/vaccinology (VNIIVViM-Russia, UIUC, KSU, TexA& M)
- 2.8 **Swine vesicular disease virus** (VNIIVViM-Russia)
- 2.9 **New vaccines for swine parainfluenza type 1** (ISU)
- 2.10 **Rapid response vaccinology for emerging diseases of swine** (NDSU, ISU)

A complete description of all research work conducted by participating stations (submitting reports in 2017) is attached.

Impacts:

General impacts of the NC-229 program in 2017

- The NC-229 annual meeting continues to positively impact researchers in the area of swine viral disease. The meeting is widely attended by active and engaged research scientists. This year, the high quality scientific presentations under the general theme: ***“New Science: Insights for Control of Swine Viral Diseases”*** resulted in discussions of considerable value for the research community as a whole.
- Outputs of peer-reviewed publications in 2017 were notable; the NC-229 group has published a total of 178 refereed journal publications this year (see “2017 NC229 Publications”).

Some selected examples of NC-229 research impacting viral diseases of swine in 2017 follow:

Impacts for PRRSV Control:

- Possible role of IFN-positive PRRSV strain on vaccine improvement (UMD)
- Advances in understanding virulence of highly pathogenic PRRSV (CHINA Agr U)
- Focus on broadly neutralizing antibodies and swine genetics may provide a bio-marker for broadly protective vaccine (KSU)
- Initial experiments in North America to approach intertypic cross protection using MLVs (OSU and KSU). License of a new concept MLV to a company (NE)
- Extensive analysis of the role of recombination and genomics of PRRSV and its effect on virulence (NADC, China Agr U)

Impacts for PEDV and other endemic swine viruses research

- Development of diagnostic immunoreagents and techniques for senecavirus serology (SDSU and ISU)
- PEDV pathogenesis, and SVV pathogenesis and diagnostic tools (ISU, MN, SDSU, KSU)
- Methods for the development of rapid-response serological diagnostics were developed for PEDV (NDSU) PEDV, and
- Evidence that composting represents an effective and bio-secure approach to inactivate PEDV in porcine carcasses (NEB)
- Risk assessment of feed transmission for PEDV (SDSU, NEB)
- Swine health monitoring program for monitoring swine influenza (SIV) transmission (MN) and molecular classification and public health implications (NADC and UGA)
- Evaluation of viral strains and platforms to improve current vaccines (NADC, ISU, MN, SDSU).

Publications/funding sources: (see attached “2017 NC229 Publications”).

Authorization: Submission by an AES or CES director or administrative advisor through NIMSS constitutes signature authority for this information.

*Limited to three pages or less exclusive of publications, details may be appended.

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: December 1 2016 to November 30 2017

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

OBJECTIVE 1. Control of PRRSV.

Refer to publications listed in Section D.

OBJECTIVE 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Refer to publications listed in Section D.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS (500 words):

Research advances over the last year by this research group have continued to expand our understanding of PRRSV, PEDV, PCV2, IAV, ASFV, SVA and other emerging viral diseases of swine and provide new ideas for preventing, countering and/or eliminating these infections. Extensive work has been done on the mechanisms of host-pathogen(s) interactions. Likewise new work on the ecology and epidemiology of these agents provide insight into the mechanisms by which they maintain endemicity. Continued assessment and research in diagnostic technology is contributing to the improvement and refinement of our ability to surveil, detect, and diagnose PRRSV, PEDV, PCV2, IAV, ASFV, SVA, and other emerging viral infections. On-going work on new methods of surveillance promise to provide new, highly cost-effective methods of tracking infection and implementing area elimination/eradication programs. Accomplishments in these areas linked with research in viral ecology/epidemiology and improvements in vaccinology will lead to the development of approaches that will make possible the control of PRRSV and other viral infections on farms and in regions.

D. PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

- Abente EJ, Gauger PC, Walia RR, Rajao DS, Zhang J, Harmon KM, Killian ML, Vincent AL. 2017. Detection and characterization of an H4N6 avian-lineage virus in pigs in the Midwestern United States. *Virology* 511:56-65.
- Abente EJ, Kitikoon P, Lager KM, Gauger PC, Anderson TK, Vincent A. 2016. A highly pathogenic avian influenza virus H5N1 with 2009 pandemic H1N1 internal genes demonstrates increased replication and transmission in pigs. *J Gen Virol* 98:18-30.
- Baker KL, Mowrer C, Canon A, Linhares DCL, Rademacher C, Karriker LA, Holtkamp DJ. 2016. Systematic epidemiological investigations of cases of Senecavirus A in U.S. swine breeding herds. *Transbound Emerg Dis* 64:11–18.
- Baker KL, Thomas PR, Karriker LA, Ramirez A, Zhang J, Wang C, and Holtkamp DJ. 2017. Evaluation of an accelerated hydrogen peroxide disinfectant to inactivate porcine epidemic diarrhea virus in swine feces on aluminum surfaces under freezing conditions. *BMC Vet Res* 81:100-107.
- Canning P, Ruston C, Madson D, Bates J, Skoland K, Davenport J, Gaul S, Wang C, Chen Q, Zhang J, Karriker L. 2017. Effect of direct-fed microbial *Bacillus subtilis* C-3102 on

- enteric health in nursery pigs after challenge with porcine epidemic diarrhea virus. *J Swine Health Prod* 25:129-137.
- Cao D, Cao QM, Subramaniam S, Yugo DM, Heffron CL, Rogers AJ, Kenney SP, Tian D, Matzinger SR, Overend C, Catanzaro N, LeRoith T, Wang H, Piñeyro P, Lindstrom N, Clark-Deener S, Yuan L, Meng, X-J. 2017. Pig model mimicking chronic hepatitis E virus infection in immunocompromised patients to assess immune correlates during chronicity. *Proc Natl Acad Sci USA* 114:6914-6923.
- Cochrane RA, Schumacher LL, Dritz SS, Woodworth JC, Huss AR, Stark CR, DeRouchey JM, Tokach MD, Goodband RD, Bai J, Chen Q, Zhang J, Gauger PC, Derscheid RJ, Magstadt DM, Main RG, Jones CK. 2017. Effect of pelleting on survival of porcine epidemic diarrhea virus (PEDV)-contaminated feed. *J Anim Sci* 95:1170-1178.
- Curry S, Schwartz KJ, Yoon KJ, Gabler NK, Burrough ER. 2017. Effect of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity. *Vet Microbiol* 211:58-66.
- Curry SM, Burrough ER, Schwartz KJ, Yoon K-J, Lonergan SM, Gabler NK. 2017. Porcine epidemic diarrhea virus reduces feed efficiency in nursery pigs. *J Anim Sci* (*in press*).
- Curry SM, Gibson KA, Burrough ER, Schwartz KJ, Yoon KJ, Gabler NK. 2017. Nursery pig growth performance and tissue accretion modulation due to porcine epidemic diarrhea virus or porcine deltacoronavirus challenge. *J Anim Sci* 95:173-181.
- Evans AB, Dong P, Loyd H, Kraus G, Zhang J, Carpenter S. 2017. Identification and characterization of small molecule inhibitors of porcine reproductive and respiratory syndrome virus. *Antiviral Res* 146:28-35.
- Ferreira FM, Arruda B, Stevenson G, Schwartz K, Madson D, Yoon KJ, Zhang J, Pineyro P, Chen Q, Arruda P. 2017. Development of polioencephalomyelitis in cesarean-derived colostrum-deprived pigs following experimental inoculation with either Teschovirus A serotype 2 or serotype 11. *Viruses* 9:179.
- Gillam F, Zhang J, and Zhang C. 2017. Hepatitis B core antigen based novel vaccine against porcine epidemic diarrhea virus. *J VirolMethods* (*in press*).
- Giménez-Lirola LG, Zhang J, Carrillo JA, Chen Q, Magtoto R, Poonsuk K, Baum DH, Piñeyro P, Zimmerman J. 2017. Reactivity of porcine epidemic diarrhea virus structural proteins to antibodies against porcine enteric coronaviruses: diagnostic implications. *J Clin Microbiol* 55:1426-1436.
- Gonzalez W, Giménez-Lirola LG, Holmes A, Lizano S, Goodell C, Poonsuk K, Sitthicharoenchai P, Sun Y, Zimmerman J. 2017. Detection of *Actinobacillus pleuropneumoniae* ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated under experimental conditions. *J Vet Res* 61:163-171.
- Holtkamp DJ, Myers J, Thomas P, Karriker L, Ramirez A, Zhang J, Wang C. 2017. Efficacy of an accelerated hydrogen peroxide disinfectant to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces. *Can J Vet Res* 81:100-107.
- Kraft JB, Woodard K, Giménez-Lirola L, Setness B, Ju J, Lasley P, Nelson E, Zhang J, Baum D, Gauger P, Main R, Zimmerman J. 2017. Serum and mammary secretion antibody responses in PEDV-immune gilts following PEDV vaccination. *J Swine Health Prod* (*in press*).

- Lee K, Polson D, Lowe E, Main R, Holtkamp D, Martínez López B. 2017. Unraveling the contact patterns and network structure of pig shipments in the United States and its association with porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks. *Prev Vet Med* 138:113–123.
- Linhares DCL, Betlach C, Morrison RB. 2017. Effect of immunologic solutions on sows and gilts on time to stability, and production losses in breeding herds infected with 1-7-4 PRRSV. *Prev Vet Med* doi: 10.1016/j.prevetmed.2017.05.024
- Lopez WA, Angulo J, Zimmerman JJ, Linhares DCL. 2017. PRRS monitoring in breeding herds using processing fluids. *J Swine Health Prod* (*in press*).
- Martin BE, Sun H, Carrel M, Cunningham FL, Baroch JA, Hanson-Dorr KC, Young SG, Schmit B, Nolting J, Yoon K-J, Lutman MW, Pedersen K, Lager K, Bowman A, Slemons R, Smith DR, DeLiberto T, Wan X-F. 2017. US feral swine were exposed to both avian and swine influenza A viruses. *Appl Environ Microbiol* (*in press*).
- Matias-Ferreira F, Arruda B, Stevenson G, Schwartz K, Madson D, Yoon K-J, Zhang J, Piñeyro P, Chen Q, Arruda P. 2017. Development of polioencephalomyelitis in cesarean-derived colostrum-deprived pigs following experimental inoculation with either Teschovirus A serotype 2 or serotype 11. *Viruses* 9:e179.
- Opriessnig T, Gerber PF, Shen H, Castro A, Zhang J, Chen Q, Halbur PG. 2017. Evaluation of the efficacy of a commercial inactivated genogroup 2b based porcine epidemic diarrhea virus vaccine and experimental live genogroup 1b exposure against 2b challenge. *Vet Res* 48:69.
- Palinski R, Piñeyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM. 2017. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *J Virol* 91(1):e01879-16.
- Park JY, Welch M, Harmon KM, Zhang J, Piñeyro PE, Li G, Gauger PC. 2017. Complete genome sequence of porcine respirovirus 1 strain USA/MN25890NS/2016, isolated in the United States. *Genome Announc* 5(42):e01139-17.
- Piñeyro PE, Lozada MI, Alarcón LV, Sanguinetti R, Cappuccio JA, Pérez EM, Vannucci F, Armocida AD, Madson DM, Perfumo CJ, Quiroga MA. 2017. First retrospective studies with etiological confirmation of porcine transmissible gastroenteritis virus infection in Argentina. *BMC Vet Res* (*in press*).
- Piñeyro PE, Subramaniam S, Kenney SP, Heffron CL, Giménez-Lirola LG, Meng XJ. 2016. Modulation of proinflammatory cytokines in monocyte-derived dendritic cells by porcine reproductive and respiratory syndrome virus through interaction with the porcine intercellular-adhesion-molecule-3-grabbing nonintegrin. *Viral Immunol* 29:546-556
- Poonsuk K, Zhang J, Chen Q, Gonzalez W, Correa da Silva Carrion L, Sun Y, Ji J, Wang C, Main R, Zimmerman J, Giménez-Lirola L. 2016. Quantifying the effect of lactogenic antibody on porcine epidemic diarrhea virus infection in neonatal piglets. *Vet Microbiol* 197:83-92.
- Poonsuk K, Zimmerman J. 2017. Historical and contemporary aspects of maternal immunity in swine. *Anim Health Res Rev* doi.org/10.1017/S1466252317000123

- Rajao DS, Loving CL, Waide EH, Gauger PC, Dekkers JC, Tuggle CK, Vincent AL. 2017. Pigs with severe combined immunodeficiency are impaired in controlling Influenza A virus infection. *J Innate Immun* 9:193-202.
- Rajão DS, Walia R, Campbell B, Gauger PC, Janas-Martindale A, Killian ML, Vincent AL. 2017. Reassortment between swine H3N2 and 2009 pandemic H1N1 in the United States resulted in influenza A viruses with diverse genetic constellations with variable virulence in pigs. *J Virol* 91:e01763-16.
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- Senthilkumaran C, Yang M, Bittner H, Ambagala A, Lung O, Zimmerman J, Giménez-Lirola LG, Nfon C. 2017. Detection of genome, antigen and antibodies in oral fluids from pigs infected with foot-and-mouth disease virus. *Can J Vet Res* 81:82-90.
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- Sparks JW, Karriker LA, Day DN, Wulf LW, Zhang J, Stock ML, Bates JL, Gehring R, and Coetzee JF. 2017. Vaccination mitigates the impact of PRRSV infection on the pharmacokinetics of ceftiofur crystalline-free acid in pigs. *J Vet Pharmacol Ther* 40:363-369.
- Subramaniam S, Piñeyro P, Derscheid RJ, Madson DM, Magstadt DR, Meng XJ. 2017. Dendritic cell-targeted porcine reproductive and respiratory syndrome virus (PRRSV) antigens adjuvanted with polyinosinic-polycytidylic acid (poly (I: C)) induced non-protective immune responses against heterologous type 2 PRRSV challenge in pigs. *Vet Immunol Immunopathol* 190:18-25.
- Sun Y, Wang C, Meeker WQ, Morris M, Rotolo M, Zimmerman J. 2017. A latent spatial piecewise exponential model for interval-censored disease surveillance data with time-varying covariates and misclassification. *Statistics and Its Interface (in press)*.
- Temeeyasen G, Madapong A, Saeng-Chuto K, Stott CJ, Piñeyro P, Nilubol D. 2017. Mucosal immune response in pigs following the primary and secondary exposure to porcine epidemic diarrhea virus genogroups 1 and 2. *BMC Vet Res (in press)*.
- Temeeyasen G, Sinha A, Giménez-Lirola LG, Zhang JQ, Piñeyro PE. 2017. Differential gene modulation of pattern-recognition receptor TLR and RIG-I-like and downstream mediators on intestinal mucosa of pigs infected with PEDV non S-INDEL and PEDV S-INDEL strains. *J Virol (in press)*.

van Geelen AGM, Anderson TK, Lager KM, Das PB, Otis NJ, Montiel NA, Miller LC, Kulshreshtha V, Buckley AC, Brockmeier SL, Zhang J, Gauger PC, Harmon KM, Faaberg KS. 2017. Porcine reproductive and respiratory disease virus: evolution and recombination yields distinct ORF5 RFLP 1-7-4 viruses with individual pathogenicity. *Virology* 513:168-179.

Zhang J, Zheng Y, Xia X, Chen Q, Abate SA, Yoon K-J, Harmon KM, Gauger PC, Main RG, Li G. 2017. High-throughput whole genome sequencing of porcine reproductive and respiratory syndrome virus from cell culture materials and clinical samples using next-generation sequencing technology. *J Vet Diagn Invest* 29:41-50.

2) Abstracts or Proceedings

Almeida MN, Zimmerman J, Linhares D. November 2017. PRRSV monitoring at the abattoir using oral fluids. Proc 2017 James D. McKean Swine Disease Conference. Ames, Iowa. pp. 72-73.

Almeida MN, Zimmerman JJ, Holtkamp D, Rademacher C, Linhares DCL. November 2017. Maximizing herd sensitivity to detect PRRSV in due-to-wean pigs using family oral fluids sampling. Proc 2017 James D. McKean Swine Disease Conference. Ames, Iowa. p 66-68.

Anderson TK, Gauger PC, Souza CK, Walia RR, Venkatesh D, Zeller MA, Rajao DS, Lewis NS, Abente EJ, Zhang J, Vincent AL. 2017. Spatial dissemination and evolution of human-origin H3 influenza A virus in US swine. 10th Annual NIAID Centers of Excellence for Influenza Research and Surveillance Network Meeting.

Anderson TK, Gauger PC, Souza CK, Walia RR, Venkatesh D, Zeller MA, Rajao DS, Lewis NS, Abente EJ, Zhang J, Vincent AL. June 2017. Spatial dissemination and evolution of human-origin H3 influenza A virus in US swine. 36th Annual Meeting of American Society for Virology. Madison, Wisconsin.

Baker K, Mowrer C, Zhang J, Ramirez A, Karriker L, Holtkamp D.J. March 2017. Evaluation of a peroxygen-based disinfectant to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces under freezing conditions. Proc 48th Ann Meet Am Assoc Swine Veterinarians. Denver, Colorado. p. 53.

Baker S, Linhares DCL. November 2017. Assessment of production impact following attenuated PRRS virus vaccination in endemically infected breeding herds. Proc 2017 James D. McKean Swine Disease Conference. Ames, Iowa. p. 70-71.

Baum DH, Giménez-Lirola LG, Zimmerman JJ, Main RG. November 2017. Serology overview. Proc 2017 James D. McKean Swine Disease Conference. Ames, Iowa. pp. 115-117.

Bhandari M, Hoang H, Sun D, Shi K, Labios L, Madson D, Magstadt D, Arruda P, Yoo D, Yoon K-J. December 2016. Humoral immune ontogeny in weaned pigs following experimental porcine epidemic diarrhea virus (PEDV) infection/reinfection. Proc 97th Ann Meet Conference of Research Workers in Animal Diseases. Chicago, Illinois.

Buckley A, Guo B, Kulshreshtha V, van Geelen A, Yoon K-J, Lager K. December 2017. Comparison of historic and contemporary strains of Senecavirus A. Proc 98th Ann Meet Conference of Research Workers in Animal Diseases. Chicago, Illinois.

- Buckley A, Guo B, Montiel N, Kulshreshtha V, van Geelen A, Yoon KJ, Lager K. December 2016. Senecavirus A infection in sows, neonates, and market weight gilts with subsequent protective immunity. Proc 97th Ann Meet Conference of Research Workers in Animal Diseases. Chicago, Illinois.
- Buckley A, Kulshreshtha V, van Geelen A, Guo B, Yoon K-J, Lager K. September 2017. Infectious dose of Senecavirus A in market-weight swine and neonatal piglets. Proc Allen D. Leman Swine Conference. St. Paul, Minnesota.
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3) Book chapters or monographs

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E. FUNDING SOURCES FOR RESEARCH

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- Holtkamp D.J., Linhares D.C. Monitoring and updating the value of productivity losses due to porcine reproductive and respiratory syndrome virus. National Pork Board. \$84,237. November 1, 2015. 3 years.
- Holtkamp D.J., Linhares D.C., Karriker L. Development of PRRS outbreak investigation and data management/analysis program for breeding herds in regional PRRSV projects in Iowa. Iowa Pork Producers Association. \$37,628. May 1, 2016. 1 year, continuation.
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Holtkamp, D.J., Zhang J. Effect of disinfectants and treatment conditions on the molecular detection of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV). Virox. \$26,475. June 1, 2017. 6 Months.

Linhares D.C., Holtkamp D.J., Arruda A., Morrison B., Silva G., Vilalta C. Description of biosecurity aspects of herds with low or high PRRS incidence and comparison within and between production systems. Swine Health Information Center. \$40,619. October 1, 2016. 1 year.

Linhares D.C., Johnson C., Holtkamp D.J. Effect of attenuated PRRSV on short term and long term whole herd productivity. American Association of Swine Veterinarians Foundation. \$11,824. June 1, 2017, 1 year.

Linhares D.C., Zimmerman J.J., Rademacher C., Holtkamp D.J. Herd sensitivity of PRRSV-monitoring schemes on sow herds undergoing virus elimination. Boehringer Ingelheim Vetmedica, Inc. \$35,000. June 1, 2016, 1 year.

Miller C, Yoon KJ. 7/1/15-6/30/17. Development of novel reovirus-based mucosal vaccine vectors for PEDV antigen production. Iowa Pork Producers Association, \$25,000 for 1st year.

Xu W, Yoon K-J. 7/1/15-12/31/17. Development of T-cell based vaccine against African swine fever virus. Center of Excellence for Emerging and Zoonotic Animal Diseases, \$79,977.

Yoon K-J. 9/1/2016-8/30/2018. Investigate the pathogenesis and biology of emerging and re-emerging swine viral diseases. USDA ARS Cooperative Agreement, \$275,000.

Zhang J, Gauger P, and Harmon K. Development and evaluation of a real-time PCR and an insulated isothermal PCR for the detection of Senecavirus A. Swine Health Information Center. \$27,333. 2016-2017.

Zhang J, Gauger P, Harmon K, Main R, and Wang C. Comparison of PRRSV virus isolation in different cell lines towards improving success of isolating PRRSV from clinical samples. American Association of Swine Veterinarians Foundation. \$30,000. 2017-2018.

Zhang J, Schumacher L, Chen Q, Gauger P, Giménez-Lirola L, Magstadt D, and Arruda P. Pathogenicity and antibody responses of different U.S. PEDV strains in pigs of different ages. Iowa Pork Producers Association. \$108,087. 2017-2018.

Zhang J. Charoen Pokphand Foods (CPF) Fellowship. Charoen Pokphand Foods (CPF) Thailand. \$165,860. 2016-2020.

F. WORK PLANNED FOR NEXT YEAR

Refer to funded projects.

Objective 1. Control of PRRSV

HOLTKAMP: PRRS Outbreak Investigation Program. Continue to develop and pilot the PRRS Outbreak Investigations Program for the Iowa Pork Producers Association. The program is now entering its fourth year. The objective of the PRRS outbreak investigations program for breeding herds is to improve biosecurity and reduce the geographic spread of the virus. The program is

being piloted on 30 breeding herds in the Buchanan County, Southeast Iowa and Southwest Iowa regional PRRSV projects in Iowa (USA). Six PRRS outbreak investigations were conducted in 2016 / 2017. The investigations were facilitated by me, with help from Rita Neat, Kimberley Gerardy and Chris Mowrer. In addition, the outbreak investigation forms were previously adapted to conduct a porcine epidemic diarrhea virus (PEDV) outbreak investigations. The forms have also been adapted for seneca virus A (SVA).

LINHARES - Disease detection / monitoring:

1. Processing fluids to detect PRRSV/PCV2 at low prevalence in neonates (3-5 days old).
 - a. Using PF to screen farms for PRRSV
 - b. Monitoring herds undergoing elimination (documenting time to test PF-negative)
 - c. Correlating PF results with downstream performance
 - d. Testing conditions (time/temperature before testing, extraction, PCR conditions)
2. Family oral fluids to detect PRRS at low prevalence in due-to-wean (DTW) pigs
 - a. Conditions to improve success rate to obtain fluids
 - b. FOF vs blood
3. Production data for automated, ongoing monitoring of swine herds
 - a. Automated SPC application for breeding herds to detect early signals of significant disease outbreaks
 - b. Automated SPC application for growing pigs
4. Predictors of growing pig performance
 - a. Consolidating source farm data (health and production data), growing pig data (e.g. feed mill, supervisor, stocking density/flow), biosecurity, and demographic data to correlate/predict closeout ADG/mortality
5. Domestic swine disease reporting system
 - a. Dashboard with consolidated/aggregated data from VDLs to report disease over time and space, by age group, specimen, state.
 - b. Veterinary council group
6. Sentinel farm approach for regional surveillance

Objective 2. Detection, prevention, and control of emerging viral diseases of swine.

HOLTKAMP: Rapid Response Program for Epidemiological Investigations of emerging and transboundary diseases. In August of 2016, the Swine Health and Information Center (SHIC) funded development of a rapid response program for epidemiological investigations of emerging and transboundary swine diseases. A six-member advisory group was formed to provide input regarding the responsibilities of RRC leaders and members, the content and delivery of RRC training, the design of disease investigation forms, and any other matters related to the program. The foundation of the program will be a Rapid Response Corps (RRC) consisting of a nationwide network of veterinary consultants, state animal health officials, epidemiologists and, when appropriate, federal animal health officials. A critical aspect of the program will be the development and use of a standardized approach and methodology for conducting epidemiological investigations. Standard forms and summary reports developed for the PRRS outbreak investigation pilot project funded by the Iowa Pork Producers (IPPA) will be used for training purposes. In the event of an emerging or transboundary disease outbreak, forms and reports will be adapted as necessary. While RRC members will be trained to ask open-ended

questions during the investigations, specific closed-ended questions will be embedded in the investigation form to capture a consistent set of information that can be accumulated in a database. The database will serve as a primary source of information to help meet the objectives for a rapid response in the event of a novel emerging or transboundary disease.

LINHARES

1. Field investigations of emerging diseases (Porcine Sapelovirus, Porcine Astrovirus type 3, Porcine Teschovirus)
2. Comparison of changes in productivity of herds using killed vs attenuated PRRS vaccine
3. Within and between production system comparison of PRRS impact of breeding herd productivity

GAUGER

1. Development of a vaccine challenge model for porcine parainfluenza virus type 1.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

N/A

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Progress on efforts to develop a PRRSV vaccine with enhanced immunogenicity and DIVA capabilities included a) the development of 2 vaccine constructs in which selected structural proteins were re-engineered in the backbone of an infectious clone to test the hypothesis that the mutations would enhance B cell mediated immunity b) expression of a DIVA marker in the modified infectious clone and c) introduction of selected mutations to target suicidal replication of the modified live vaccine to enhance vaccine safety. The vaccine constructs were tested recently in pigs and data is under analysis.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

1. Proprietary methods for the development of first generation, rapid-response vaccines for RNA viruses were developed using PEDV as a model. The processes were intended to be a hybrid between inactivated and attenuated vaccines, such that the safety and efficacy advantages respectively, could be combined. The methods developed are also highly relevant to the autogenous vaccine industry where vaccine safety is a large concern. Testing of the vaccine candidate in 3-4 week old pigs elicited strong spike protein specific antibody responses. Vaccinated pigs were completely protected against challenge with the virulent virus, while unvaccinated controls showed clinical signs and viral shedding in feces. The vaccine virus was not detected in fecal matter, prior to challenge; nor did vaccination induce any clinical signs. Hence, the approach for first-response vaccine development was both highly safe and effective. A grant has been submitted to NIFA for funding to test the vaccine in sows and measure lactogenic immunity.

2. Methods to improve the delivery and immunogenicity of peptide antigens encoding specific epitopes was developed in collaboration with scientists with expertise in polymeric material science. Three 2009 H1N1 influenza viral epitopes were expressed as a string using a bacterial expression system. The highly hydrophobic peptide did not enter cells when incubated alone on MDCK cells. When conjugated with a proprietary polymer, the antigen was detected intracellularly, with negligible cytotoxicity. Vaccination of pigs with the conjugated peptide vaccine elicited strong anti-peptide antibody responses. Upon challenge with the virulent homologous virus, pigs vaccinated with the conjugated peptide or peptide alone showed enhanced viral replication in day 3 post-challenge, when compared to unvaccinated controls or pigs administered the polymer alone. However, at day 6 post-challenge the trend was rapidly reversed with vaccinated pigs clearing the virus rapidly while unvaccinated pigs showed an increasing viral titer. Hence, the conjugation of the peptide to the polymer was effective in enhancing delivery in vitro and protection in vivo. The mechanisms of protection did not appear to involve neutralizing antibody responses and remain to be elucidated

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

1. One PhD student was trained in vaccine development methods and provided to present his work at a regional conference where he won the second-place award.

2. Methods for the development of rapid-response serological diagnostics were developed for PEDV

3. Methods for rapid-response vaccine development were optimized and tested for PEDV. The rapid-response vaccine was highly safe and effective in 3-4 week old piglets and had broad applicability to other RNA viruses. A patent to cover the technology was filed in Feb 2017.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

N/A

2) Abstracts or Proceedings

1. Karsky, J, Singh, P and Ramamoorthy, S. 7th Euro Global Summit on Clinical Microbiology, Quantification of the PEDV virus with a colorimetric assay. Amsterdam, Netherlands (2017). Invited presentation.

2. Gagandeep Singh, Pankaj Singh, Angela Pillatzki, Eric Nelson, Brett Webb, Steven Dillberger-Lawson and Sheela Ramamoorthy. PEDV: A Model for rapid response vaccines. North Dakota Academy of Sciences (2017), Grand Forks, ND. (2nd place award).

3. Singh, G., Zholobko, O., Pillatzki, A., Nelson, E., Webb, B., Voronov, A., and Ramamoorthy, S. Vaccination of Pigs with improved HA and M2e Epitope Based Amphiphilic Invertible Polymeric Peptide Vaccine against Swine Influenza Viruses (SIVs). NDSU-KU Joint Symposium on Biotechnology, Nanomaterials, and Polymers. Fargo, ND (2017).

4. Singh. G., Zholobko. O., Pillatzki. A., Nelson. E., Webb. B., Voronov. A., and Ramamoorthy. S. Enhancing Delivery and Immune Response of Peptide Vaccine by Polymer-Peptide Mixed Micellar Assemblies. 2nd International Symposium on Materials from Renewables (ISMR). Athens, GA (2017).

5. Gagandeep Singh, Pankaj Singh, Angela Pillatzki, Eric Nelson, Brett Webb, Steven Dillberger-Lawson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases Rapid response vaccine against the porcine epidemic diarrhea virus (PEDV). Chicago, IL. (2017).

6. Gagandeep Singh, Oksana Zholobko, Angela Pillatzki, Brett Webb, Eric Nelson, Andriy Voronov and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Improved delivery of a HA and M2e-based peptide vaccine against swine influenza viruses. Chicago, IL. (2017).

7. Pankaj Singh, Gagandeep Singh, Jenna Karsky, Eric Nelson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Quantifying porcine epidemic diarrhea virus-specific neutralizing antibodies with a rapid colorimetric assay. Chicago, IL. (2017).

8. Oleksandr Kolyvushko, Gagandeep Singh, Brett Webb, Angela Pillatzki, Diego Diel, Steven Dillberger-Lawson, Eric Nelson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Efficacy of a commercial PCV2 vaccine against the contemporary PCV2d strain. Chicago, IL. (2017).

3) Book Chapters or Monographs

Joint research and training initiatives between East African and North American Universities. John Baligwamunsi Kaneene, Margaret Loy Khaita, John David Kabasa, Florence Wakoko, William Sicho, Douglas Freeman, Claire Card, Teresa Bergholz, Sheela Ramamoorthy, Ayele Teshome, Jesca Nakavuma, Samuel Majalija, Stevens Kisaka, Paul Ssajjakambwe, Sam Okech, Micheal Muleme, Sylvia Angubua Baluka, Herbert Kazoora, Patrick Vudriko *Pan Afr Med J.* 2017; 27(Suppl 4): 4, 24 August 2017

D. FUNDING SOURCES

1. Porcine Model for Torque Teno Virus Infections – NIH R21. Impact Score – 19. Funding release awaited
2. First response vaccines for emergency preparedness – USDA NIFA. Pending.

E. WORK PLANNED FOR NEXT YEAR:

1. The current efforts to develop improved PCV2 and PRRSV vaccine with DIVA capabilities will be completed.
2. Rapid response vaccine for swine influenza viruses and testing of the developed rapid-response PEDV vaccines in sows will be targeted.
3. A porcine coinfection model of TTV and SIV coinfections will be developed to determine if and how TTV infections shift the immune response profile in influenza infections.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: University of Nebraska Lincoln

A. Personnel

1) NC-229 STATION REPRESENTATIVE:

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Studies on protective PRRSV immunity: role of innate immunity induction in an effective acquired immunity; strategies of broadening protective efficacy of live vaccines

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Studies on biosecure inactivation of PEDV in carcasses and in manure

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

PRRSV:

Effective technology transfer of new synthetic live vaccine technology to industry through signature of multi-year contract with a vaccine company based in the US

PEDV:

Evidence that composting represents an effective and biosecure approach to inactivate PEDV in porcine carcasses, providing a method to reduce transmission and control virus spread on farms.

Treatment of PEDV infected manure with alkaline lime slurry was shown to inactivate PEDV using a bioassay, thus providing an intervention for producers and manure handlers to minimize risk of PEDV transmission during manure handling.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Stevens E.E., Miller D., Brittenham B.A., Vitosh-Sillman S.J., Brodersen B.W., Jin V.L., **Loy J.D.**, and Schmidt A.M. Alkaline stabilization of manure slurry inactivates porcine epidemic diarrhea virus (PEDV). *Journal of Swine Health and Production*. Accepted/In Press

Vitosh-Sillman S., **Loy J. D.**, Brodersen B.W., Kelling C.K., Eskridge K., and Millmier Schmidt A. Effectiveness of composting as a biosecure mortality disposal method for porcine epidemic diarrhea virus (PEDV)-infected pig carcasses. (2017) *Porcine Health Management*. Vol 3 (22) DOI: 10.1186/s40813-017-0068-z

Vitosh-Sillman S. **Loy J. D.**, Brodersen B.W., Doster A.R., Kelling C., Topliff C., Nelson E., Bai J., Schirtzinger E., Poulsen E., Meadors B., Anderson J., Hause B., Anderson G., and Hesse, R. (2016) Experimental infection of conventional nursing pigs and their dams with porcine deltacoronavirus. *Journal of Veterinary Diagnostic Investigation*. Vol 28 (5) 486-497*
DOI: 10.1177/1040638716654200

Sun H, Workman A, Osorio FA, Steffen D, Vu HLX. Development of a broadly protective modified-live virus vaccine candidate against porcine reproductive and respiratory syndrome virus. *Vaccine*. 2018 Jan 2;36(1):66-73. doi: 10.1016/j.vaccine.2017.11.028. Epub 2017 Nov 22. PubMed PMID: 29174314.

Kimpston-Burkgren K, Correas I, Osorio FA, Steffen D, Pattnaik AK, Fang Y, Vu HLX. Relative contribution of porcine reproductive and respiratory syndrome virus open reading frames 2-4 to the induction of protective immunity. *Vaccine*. 2017 Aug 3;35(34):4408-4413. doi: 10.1016/j.vaccine.2017.06.061. Epub 2017 Jul 6. PubMed PMID: 28689650.

Correas I, Osorio FA, Steffen D, Pattnaik AK, Vu HLX. Cross reactivity of immune responses to porcine reproductive and respiratory syndrome virus infection. *Vaccine*. 2017 Feb 1;35(5):782-788. doi: 10.1016/j.vaccine.2016.12.040.

Epub 2017 Jan 3. PubMed PMID: 28062126.

Sun H, Pattnaik AK, Osorio FA, Vu HLX. Identification of viral genes associated with the interferon-inducing phenotype of a synthetic porcine reproductive and respiratory syndrome virus strain. *Virology*. 2016 Dec;499:313-321. doi: 10.1016/j.virol.2016.09.018. Epub 2016 Oct 11. PubMed PMID: 27736706.

Vu HLX, Pattnaik AK, Osorio FA. Strategies to broaden the cross-protective efficacy of vaccines against porcine reproductive and respiratory syndrome virus. *Vet Microbiol*. 2017 Jul;206:29-34. doi: 10.1016/j.vetmic.2016.09.014. Epub 2016 Sep 21. PubMed PMID: 27692670.

D. FUNDING SOURCES

On-farm Remediation and Prevention of Swine Enteric Diseases. USDA-AFRI, Foundational Program, 2016-68008-25043

Pathogenesis Studies with Porcine Epidemic Diarrhea Virus (PEDV): Generation and Characterization of Infectious Clone-Derived Viruses", Pattnaik, A. (Principal Investigator), **Loy, J.** (Investigator),

Investigation of host genetic role in porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) susceptibility USDA-AFRI, Foundational Program,

PD: Daniel Ciobanu, Co-PD: Hiep Vu

Amount : \$ 459,200

2017-2019

Determine the correlates of cross-protective immunity to PRRSV USDA NIFA Grant No. 2016-67015-24922

PD: Vu, Hiep Co-PD: Osorio, F

Amount: \$477,635

2016-2019

E. WORK PLANNED FOR NEXT YEAR

Work continues on developing proteomics based approaches to enteric coronavirus characterization and differentiation using mass spectrum biomarker based approach.

Work continues on experimental vaccinology: broadening protection for live vaccines against PRRSV and centralized antigenic subunit immunization against swine influenza

Use of PRRSV model to investigate host genetics

Developmental research on PEDV reverse genetics

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

USDA, Agricultural Research Service, National Animal Disease Center
1920 Dayton Avenue, Ames, IA 50010

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Nicholson, Tracy; Research Microbiologist, NADC; tracy.nicholson@ars.usda.gov
Vincent, Amy; Veterinary Medical Officer, NADC; amy.vincent@ars.usda.gov

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- **Miller:** applied RNA analyses on infected and control monocyte-derived cells. Such research uncovered networks of predicted protein-protein interactions and biological processes related to both low virulence and highly pathogenic PRRSV infection. The analysis revealed the ability of PRRSV to affect cell activation. Genes showing variability in expression were related to cellular structure and inflammatory immune responses. These results supply novel insight into the interplay of PRRSV pathogenicity and immune system evasion.
- **Miller:** to identify mechanisms that modulate innate and adaptive immune responses to swine viral pathogens, conducted genome-wide RNA profiling of signature genes in activated porcine monocytic innate immune cells. From this research, the diverse antiviral properties that interferon and interferon-stimulated gene families have on swine viral pathogens were determined. The data revealed different expression levels of inflammatory cytokines, chemokines, receptors, interferon-regulatory factors and interferon-stimulated gene families in PRRSV-infected macrophages setting the stage for development of novel therapies and vaccine strategies.
- **Miller:** expression analysis of the type and quantity of small non-coding RNAs was completed comparing healthy and PRRSV-infected pigs to elucidate when the largest change in gene expression occurs, and if all categories of small non-coding RNAs are

affected. Transfer RNA fragments experienced a lower reduction in number than the microRNAs and appear to be more stable across time points than microRNA or other non-coding RNAs. This information helps in understanding how gene function in the pig can become dysregulated by PRRSV, in conjunction with how the pig's immune system responds to the virus.

- **Faaberg:** a modified attenuated vaccine of PRRSV was used to prepare novel candidate vaccine constructs. One region of the attenuated vaccine was amplified and will be used to join to another section of the genome of more contemporary viruses found in production systems.
- **Faaberg, Lager:** sequenced the entire genome of 17 PRRSV isolates prepared by scientists at Iowa State University and discovered that the isolates, originally thought to be similar based on a small region of the genome, were very dissimilar. The isolate genomes were analyzed for evidence of viral recombination using index prototype strain genomes representative of different lineages. Several instances of viral recombination were detected in most of the 17 isolates, showing that viral recombination occurs at a high frequency in infected swine herds. Four genomically distinct isolates were chosen for swine infection experiments and resulted in a spectrum of diseases, two of which were much more pathogenic than the others, and one which produced very mild disease.
- **Lager:** conducted animal studies to investigate field observations that traditional use of live-virus inoculation in breeding age gilts to induce PRRSV protection is now failing because of some inherit change in contemporary field isolates.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

- **Faaberg and collaborator:** studied the enzymatic activity of the papain-like protease 2 domain in nonstructural protein 3 of porcine epidemic diarrhea virus (PEDV) and porcine delta coronavirus (PDCoV). The research found striking differences in this domain between the two viruses, and will be used to further investigate viral virulence traits.
- **Faaberg and collaborator:** Developing infectious clones of PEDV and PDCoV for vaccine generation
- **Lager:** Demonstrated that 1) wild-type SVA infection can induce a protective immune response with a duration for at least 4-5 months, 2) SVA transmission can occur for at least 2 weeks post infection to age-matched sows, and 3) environmental contamination may be a likely source of SVA detected in sows moving from farm to eventual slaughter. This information will help in developing response strategies at slaughter house. which can help in developing control programs on the farm.
- **Miller:** GEO ID: GSE74473 Organism/cell line/tissue: Sus scrofa domesticus/tracheobronchial lymph nodes (TBLN). Raw Digital Gene Expression Tag Profiling sequences. A major goal of this study was to profile the biological and molecular networks involved in the pathological response caused by Pseudorabies virus infected porcine tracheobronchial lymph node. Gene Expression Omnibus is a public functional genomics data repository supporting MIAME-compliant data submissions for free access by scientists which increases usability and visibility. The resource supports archiving of raw data, processed data and

metadata which are indexed, cross-linked and searchable. All data are freely available for download in a variety of formats. GEO also provides several web-based tools and strategies to assist users to query, analyse and visualize data. There is evidence that more scientists are using a data-driven approach to research, whereby the first step in a project is to combine and re-analyse public data sets to reveal previously unknown relations or uncover ever more subtle trends in the data.

- **Nicholson:** To identify genomic differences between virulent and non-virulent *Haemophilus parasuis* isolates, the closed whole-genome sequence and genome-wide methylation patterns for the highly virulent Nagasaki strain and for the non-virulent D74 strain were obtained. 366 genes unique to Nagasaki and 324 genes unique to D74, including several putative Type I and Type III restriction modification systems, hemolysins, and other putative virulence-associated genes were identified. Fourteen methylation motifs were identified in the Nagasaki genome and fifteen methylation motifs were identified in the D74 genome, with only one motif shared between the two genomes. To evaluate the contribution of gene expression differences, RNA sequencing was performed on Nagasaki and D74 after growth with and without 5% CO₂. 284 genes were differentially expressed in strain D74 in response to 5% CO₂, while only 36 genes were differentially expressed in strain Nagasaki. These data demonstrate that strain D74 is more transcriptionally responsive to carbon dioxide levels that mimic in vivo conditions within the respiratory tract and suggest that non-virulent *H. parasuis* strains may be more adaptive to colonization within the respiratory tract than virulent strains. Collectively, the unique genomic and transcriptional features identified in this study provide a foundation for understanding the genomic attributes responsible for the spectrum of virulent phenotypes that exist among *H. parasuis* isolates. This information is paramount to designing effective vaccines needed by the swine industry to mitigate *H. parasuis* disease burden.
- **Vincent, Abente:** to investigate host-pathogen interactions at cellular or molecular levels, host gene expression profiles were examined using a PCR array targeting 168 genes associated with the swine antiviral response and cytokine and chemokine pathways. Differential gene expression patterns were observed.
- **Vincent, Abente and collaborators:** to examine virus, host, and population factors that influence interspecies transmission in swine, work continued on a recently established human-like H3 virus lineage in swine to study its genetic and antigenic evolution. Representative human and swine human-like viruses were used to perform virus histochemistry on swine tissue and in vitro replication assays. A pathogenesis and transmission study with a North American 2017 H7N9 low pathogenic avian influenza virus was completed.
- **Vincent, Abente:** to identify emerging IAV and monitor genetic and antigenic evolution in swine, subtype and genetic patterns were monitored to identify changing patterns or emerging viruses. H1N1, H1N2, and H3N2 with molecular signatures suggesting antigenic changes were identified and virus isolates obtained from the USDA IAV-S surveillance repository for antigenic and pathogenic characterization.
- **Vincent, Abente and collaborators:** to develop and implement an automated clade tool for H1 with standardized global nomenclature, a phylogenetic based method for classifying H1 IAV was developed and validated on a large global dataset of hemagglutinin gene

sequences. The automated tool was demonstrated to be highly accurate and was implemented on the Influenza Research Database (fludb.org).

- **Vincent, Abente:** to identify genetic changes important for antigenic drift or pathogenicity in swine or other hosts, IAV subtype H1 and H3 viruses with unique antigenic motifs, predicted to be antigenically distinct, were obtained and tested in vitro to characterize their antigenic phenotypes. New antigenic motif patterns in H3 were shown to be distinct from previous H3 and changed in frequency of detection over time.
- **Vincent, Abente:** to identify genetic changes important for antigenic drift or pathogenicity in swine or other hosts, IAV subtype H1 and H3 viruses with unique antigenic motifs, predicted to be antigenically distinct, were obtained and tested in vitro to characterize their antigenic phenotypes. New antigenic motif patterns in H3 were shown to be distinct from previous H3 and changed in frequency of detection over time.
- **Vincent, Abente:** to investigate adjuvants or immune-modulatory agents that result in robust immune responses (mucosal delivered, long lived, broadly cross-protective, and/or reduce the number of vaccine boosters), a study was conducted to test the effect of sequential heterologous infection in imprinting the humoral immune response. The order of infection significantly impacted the humoral immune response to each of the viruses and certain exposure patterns led to increased lung pathology.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

1. **Identified the effect that porcine reproductive and respiratory syndrome virus (PRRSV) infection has on the display of signature genes of activated mononuclear cells.**
Monocytic cells are one of the cell types that are intricately involved in the animal's response to disease. Following infection, the monocytic cell becomes activated which can occur by direct contact with an infectious agent, or indirectly through stimulation of the cell by specific proteins produced by other cells in the body. Activated monocytic cells then become polarized (meaning the cell has developed a certain response against a virus or bacteria). ARS researchers studied the direct involvement of polarization of monocytes during infection. Understanding the complex nature of the protective immune response may be critical to improving vaccines.
2. **Analyzed gene expression changes during pseudorabies virus (PRV) infection.** PRV causes severe disease in swine and is an economically important disease, or disease threat in most swine producing countries. As the pig responds to a PRV infection, changes in metabolism reflect changes in the expression of specific genes. Gene expression describes the regulation of the pig's metabolic processes, and gene expression profiling is the process of determining which genes are active in a specific cell or group of cells. Variation in gene expression profiles can act as an important indicator of disease or predisposition to disease. Characterizing core gene changes gives insight to how the virus affects the host, and how the host is trying to combat the infection which can lead to a greater understanding of how to build better vaccines which may help in the control of pseudorabies.

3. **Annotation of IFN gene families in swine and across 155 animal genomes.** Innate immune interferons (IFNs), particularly type I IFNs, are primary mediators regulating antiviral immunity. These antiviral cytokines have evolved remarkable molecular and functional diversity to confront ever-evolving viral threats. ARS researchers showed that pigs have the largest and an expanding type I IFN family, consisting of nearly 60 functional genes that encode seven IFN subtypes including multigene subtypes of one class of IFN (IFN- α). Whereas subtypes such as IFN- α and - β have been widely studied, the unconventional IFN- ω subtype has barely been investigated. Cross-species comparison revealed the molecular and functional novelty of porcine interferon-omega subtype (ω), which has evolved several novel features: a signature multi-gene subtype, emerging isoforms that have much higher antiviral potency than typical IFN- α , high antiviral (but little antiproliferative) activity in cells of humans and other mammalian species, and potential action through unusual signaling pathways. This study revealed the antiviral potency of porcine IFN- ω and potential use of novel IFN-based antivirals against devastating viral diseases.
4. **Described the interaction of type I IFNs (IFN- α and - β) and a specific pathway of signaling (mTOR-mechanistic target of rapamycin) that underlie PRRSV infection.** Targeting on macrophages, ARS researchers elaborated the direct involvement of the mTOR signaling pathway during PRRSV infection. Comprehensive understanding of the immunological impact may become increasingly important to understand host-virus interactions of existing and emerging pathogens, with application to the development of novel therapies and vaccine strategies.
5. **Described recombination within a set of diverse PRRSV field isolates.** ARS scientists processed 17 isolates that had emerged in the United States in 2015 for next generation sequencing and assembled them into complete viral genomes. Results revealed that the viruses were very dissimilar in all parts of their genomes. Further evolutionary analyses, comparing the isolates to unique prototype index genomes, revealed several common areas where the viruses had recombined. The data indicates the remarkable ability of PRRSV to undergo high frequency recombination in the field. Three viral isolates were used to challenge swine. One isolate was shown to produce enhanced clinical disease. The viral strain will be used in our formulation of new vaccine candidates.
6. **Demonstrated the utility and differences between PRRSV genome modifications in two different regions of nonstructural protein 2 (nsp2).** ARS researchers investigated the stability of mutant viruses. Next generation sequencing showed that three inserted small tags were all stable (except for one mutant) over ten passages in susceptible cells. The rate of viral replication of all mutants in cells was not inhibited and the viral plaque size for the mutants was not decreased. However, detailed analyses showed that insertion of any of the tags near the beginning of the protein could be detected in genome length and multiple smaller viral RNAs, whereas tag insertion near the end of the protein only was detected in genome length viral RNA. In addition, infected cell immunofluorescence examination suggests that the two different nsp2 insertions resulted in proteins localizing to discrete areas around the cell nucleus. The mutant viruses will be used to investigate the role of nsp2 in pathogenesis.

7. **Investigated the ecology and protective immune response of Senecavirus A (SVA), a swine virus that has recently emerged as a problem in US swine.** Demonstrated that 1) wild-type SVA infection can induce a protective immune response with a duration for at least 4-5 months, 2) SVA transmission can occur for at least 2 weeks post infection to age-matched sows, and 3) environmental contamination may be a likely source of SVA detected in sows moving from farm to eventual slaughter. This information will help in developing response strategies at slaughter house. which can help in developing control programs on the farm.
8. **Biofilm plays a role in persistence of *Bordetella bronchiseptica* in the lung.** *B. bronchiseptica* is a bacterial respiratory swine pathogen that routinely infects pigs for long periods of time. This holds true despite the use of vaccines, where *B. bronchiseptica* is frequently isolated from the nose of vaccinated animals. Like many bacteria, *B. bronchiseptica* can form biofilms, which protects the bacteria from a variety of host clearance mechanisms and antimicrobial compounds. ARS scientists tested a known biofilm factor produced by bacteria termed Bps for its role in biofilm formation of swine isolates of *B. bronchiseptica* and its role in swine respiratory disease. Results indicated that Bps was required for biofilm formation and for infecting the lungs or lower respiratory tract of swine. These findings provide critical information needed to design improved vaccines and intervention strategies to control or eliminate chronic carriage of *B. bronchiseptica* and other bacterial pathogens in swine.
9. **Antimicrobial resistance in swine livestock-associated (LA), methicillin-resistant *Staphylococcus aureus* (MRSA) is lower than in human MRSA isolates.** *S. aureus* is a common and sometimes devastating human pathogen that has the ability to acquire resistance to antibiotics resulting in MRSA. Swine can carry strains of MRSA that do not appear to cause disease in swine, but it is unclear whether these swine LA-MRSA are a risk for humans. ARS scientists determined the antimicrobial resistance profiles and genetic mechanisms of antimicrobial resistance among swine LA-MRSA and human clinical MRSA isolates. Swine LA-MRSA isolates exhibited resistance to fewer antibiotics than MRSA isolates from humans with no swine contact. Distinct genomic antimicrobial resistance elements were harbored by each subgroup, with little overlap in shared antimicrobial resistance genes between swine LA-MRSA and human clinical MRSA isolates. These results indicate there are distinct populations of MRSA in swine and humans, and antibiotic resistance is more prevalent in human strains, suggesting that human to human spread is more of a risk than swine to human transmission.
10. **Use of a granulocyte-colony stimulating factor (G-CSF) to prevent *Streptococcus suis* infection in swine.** The use of immunomodulators is a promising alternative to the use of antibiotics to prevent and combat infectious disease. Previously ARS scientists demonstrated a replication-defective adenovirus vector that expresses G-CSF elicited a sustained increase in circulating neutrophils, a type of white blood cell that is beneficial in preventing bacterial diseases. In new studies, pigs given the vectored G-CSF had an improved outcome when infected with *Streptococcus suis*, the leading cause of meningitis in weaned pigs. Thus, the use of G-CSF in pigs to induce an increase in circulating neutrophil numbers may be a useful alternative to antibiotics for prevention

of Streptococcal and other bacterial diseases, especially during times of stress and pathogen exposure such as post-weaning.

11. **Zinc Resistance within Swine Associated Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates in the USA is Associated with MLST Lineage.** Zinc resistance in livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) sequence type (ST) 398 is primarily mediated by the *czrC* gene co-located with the *mecA* gene, encoding methicillin resistance, within the type V SCC*mec* element. Because *czrC* and *mecA* are located within the same mobile genetic element, it has been suggested that the use of in feed zinc as an antidiarrheal agent has the potential to contribute to the emergence and spread of MRSA in swine through increased selection pressure to maintain the SCC*mec* element in isolates obtained from pigs. To test this assumption, the prevalence of zinc resistance in US swine associated LA-MRSA ST5 isolates, MRSA ST5 isolates from humans with no swine contact, and US swine associated LA-MRSA ST398 isolates was evaluated. The data suggest that selection pressure associated with zinc supplementation in feed is unlikely to have played a significant role in the emergence of LA-MRSA ST5 in the US swine population. The data also indicate that zinc resistance is associated with MLST lineage suggesting a potential link between genetic lineage and carriage of resistance determinants.
12. **Developed a computational tool that automatically classifies global swine H1 subtype HA gene sequences.** Infection with influenza A virus (IAV) is one of the most important respiratory diseases of swine and is the second most common viral diagnosis of respiratory disease in the United States. The USDA IAV swine surveillance system initiated in 2009 has increased the amount of publically available sequence data on swine viruses circulating in the United States. A significant barrier for swine producers to make timely vaccine interventions and for researchers to use relevant viruses in studies is having the computational expertise to analyze and characterize the HA gene. The HA protein is a major component of vaccines and target for immune responses. In collaboration with an international network of influenza experts, ARS researchers developed a computational tool that can automatically classify swine H1 subtype HA gene sequences. An important component of the tool is the harmonization of H1 HA nomenclature, as well as a standardized technique for genetically characterizing the HA gene. This open-access tool will aid swine producers, veterinarians, vaccine manufacturers, and IAV vaccine researchers in selecting vaccine strains to match the strains that are currently circulating. Properly matching vaccines to field strains is a critical part of managing swine influenza.
13. **Reassortant influenza A virus (IAV) with highly pathogenic avian influenza H5N1 surface genes had modestly increased replication and transmission in pigs.** Following the introduction of the 2009 pandemic H1N1 virus (H1N1pdm09), many animal species have been shown to be infected due to human to animal transmission. The IAV genome is composed of 8 gene segments, and mixing of gene segments from distinct parental viruses can result in progeny viruses with improved capability of infecting a host, ability to evade immunity, or with distinct pathogenic phenotypes. ARS scientists demonstrated that a laboratory generated reassortant virus with highly pathogenic avian influenza H5N1 surface genes and internal genes from H1N1pdm09 virus had

modestly increased replication and transmission in pigs when compared to the parental H5N1 virus. Although not yet detected in pigs from natural events, this finding highlights the importance of maintaining a robust surveillance program to detect spillover events into swine and suggests that interspecies transmission barriers may partially be overcome by reassortment. Interspecies transmission into pigs is a risk to swine production as well as human pandemic risk.

14. **Demonstrated properties of H3N2 influenza A virus (IAV) strains isolated from swine varied depending on the genome constellation.** Following the introduction of the 2009 pandemic H1N1 (H1N1pdm09) from humans to swine, mixing of IAV gene segments between H1N1pdm09 and swine viruses occurred. By studying genomes of IAV detected in swine, a large number of gene segment combinations (genomes) among H3 subtype swine viruses were shown to be circulating in commercial herds. ARS researchers selected IAV with genomes representing observed patterns in viruses circulating in swine farms to investigate in experimental challenge studies. Infection properties of viral strains varied depending on the genome constellation and may explain why some combination of genes have been more successful in the U.S. swine population. This underscores the importance of surveillance and assessing whole-genome sequence data to better understand the disease properties of circulating IAV strains in the field. This information will help guide intervention strategies and improved choices in vaccine design.
15. **Demonstrated pigs with severe combined immunodeficiency (SCID) were impaired in controlling influenza A virus (IAV) infection.** Influenza A virus infections tend to be acute and relatively short in duration due to rapid induction of the immune response. Study of the immune response to IAV can reveal new ways to prevent or treat infections. Humans and animals may have genetic disorders that interrupt normal immune responses. In collaboration with scientists at Iowa State University, ARS researchers showed that pigs with SCID that do not have B-cell or T-cell immunity were impaired in controlling IAV infection. The delayed clearance of infection was despite an intact innate immune response. These SCID pigs provide a valuable model to understand the immune mechanisms associated with protection and recovery in a natural host for influenza.
16. **Mammals captured near infected poultry farms lack evidence of exposure to 2014-2015 highly pathogenic avian influenza virus.** In 2014 and early 2015, a Eurasian strain of highly pathogenic avian influenza A (HPAI) virus was detected in poultry in Canada and the United States, causing a large economic loss to the poultry industry and tremendous investment by the industry and USDA officials to control the outbreak. In an effort to understand the spread of the Eurasian H5 virus, epidemiologic investigations occurred at poultry facilities. Synanthropic birds and mammals were sampled at infected and uninfected poultry farms in northwest Iowa, and in collaboration with APHIS scientists, ARS researchers tested for evidence of infection with HPAI H5. No mammal species showed evidence of infection or exposure, but a very small number of European starlings were found to have evidence of infection. These results indicate species that cohabitate with humans and their domestic animals merit further scrutiny to better understand potential biosecurity risks to HPAI outbreaks.

17. **The 2014-2015 highly pathogenic H5NX avian influenza virus that emerged in North America demonstrated limited replication in experimentally challenged pigs.** The susceptibility of pigs to HPAI H5N1, H5N2, and H5N8 clade 2.3.3.3 the recently emerged in North America were assessed. Pigs and trachea explants were inoculated with a representative panel of H5NX clade 2.3.4.4 HPAI viruses from North America. Limited virus replication was restricted to the lower respiratory tract of challenged pigs, though absent in the nasal passages and trachea cultures, as determined by RRT-PCR in all samples. Seroconversion of inoculated pigs was detected by NP ELISA but was not reliably detected by antigen-specific hemagglutination inhibition. Boost with adjuvanted virus was required for the production of neutralizing antibodies to assess cross-reactivity between wild-type avian strains. All RRT-PCR and serology tests were negative for contact animals indicating a failure of transmission from primary inoculated pigs. Collectively, our data show HPAI H5NX clade 2.3.4.4 viruses to be poorly adapted for replication and transmission in swine.
18. **A recently emerged avian-origin canine influenza A viruses does not replicate efficiently in experimentally challenged pigs.** A genetically and antigenically distinct avian-origin H3N2 canine influenza was detected in March of 2015 in Chicago, Illinois and subsequently caused widespread outbreaks in dogs across the country. Within the first 5 months of its original detection, over 1000 dogs in the Midwest were affected followed by positive detections in 23 additional states. We observed that the US canine H3N2 strain does not replicate efficiently in experimentally challenged swine, especially the upper respiratory tract. Low titers of virus were detected in the lungs of 4/5 pigs. Although virus was detected by RT-PCR in NS of 2/10 pigs, infectious virus was not isolated. Consistent with the limited replication detected in the upper respiratory tract, there was no evidence of transmission, suggesting a low risk of sustained infection in pigs.
19. **An H4N6 avian influenza A virus isolated from a clinically ill pig does not transmit efficiently in an experimental challenge and transmission study.** In late 2015, an avian-origin H4N6 influenza A virus was isolated from pigs in the United States during a routine diagnostic investigation of clinical respiratory disease in the herd. Serological analysis from additional pigs at the farm and other pigs within the swine production system indicated that the virus did not efficiently transmit from pig-to-pig and the mode of transmission to swine could not be determined. The isolate was characterized at the molecular level and the pathogenesis and transmission was experimentally evaluated in pigs. Although the virus replicated in the lungs of pigs and caused mild pulmonary lesions, there was no evidence of replication in the upper respiratory tract or transmission to indirect contacts, supporting the findings on the farm. Despite the lack of transmission and replication in the upper respiratory tract, efficient replication in the lung could lead to the emergence of a novel reassortant. Continued surveillance efforts are important to monitor and better understand the dynamics of cross-species spread of IAV.
20. **The molecular determinants of antigenic drift in the H3 hemagglutinin of swine influenza A virus were identified.** Six of the 7 positions previously identified in human seasonal H3 (positions 145, 155, 156, 158, 159, 189, and 193) were also

indicated in swine H3 antigenic evolution. To experimentally test the effect on virus antigenicity of these 7 positions, substitutions were introduced into the HA of an isogenic swine lineage virus. We tested the antigenic effect of these introduced substitutions by using hemagglutination inhibition (HI) data with monovalent swine antisera and antigenic cartography to evaluate the antigenic phenotype of the mutant viruses. Combinations of substitutions within the antigenic motif caused significant changes in antigenicity. One virus mutant that varied at only two positions relative to the wild type had a >4-fold reduction in HI titers compared to homologous antisera. Potential changes in pathogenesis and transmission of the double mutant were evaluated in pigs. Although the double mutant had virus shedding titers and transmissibility comparable to those of the wild type, it caused a significantly lower percentage of lung lesions. Elucidating the antigenic effects of specific amino acid substitutions at these sites in swine H3 IAV has important implications for understanding IAV evolution within pigs as well as for improved vaccine development and control strategies in swine.

21. Identified and characterized a novel reassortant human-like H3N2 and

H3N1 Influenza A Viruses isolated from pigs. Human-like swine H3 influenza A viruses were detected by the USDA surveillance system. The swine human-like H3N2 and H3N1 viruses encoded hemagglutinin genes similar to those in human seasonal H3 strains and internal genes closely related to those of 2009 H1N1 pandemic viruses. The H3N2 neuraminidase was of the contemporary human N2 lineage, while the H3N1 NA was of the classical swine N1 lineage. Both viruses were antigenically distant from swine H3 viruses that circulate in the United States and from swine vaccine strains and also showed antigenic drift from human seasonal H3N2 viruses. Their pathogenicity and transmission in pigs were compared to those of a human H3N2 virus with a common HA ancestry. Both swine human-like H3 viruses efficiently infected pigs and were transmitted to indirect contacts, whereas the human H3N2 virus did so much less efficiently. To evaluate the role of genes from the swine isolates in their pathogenesis, reverse genetics-generated reassortants between the swine human-like H3N1 virus and the seasonal human H3N2 virus were tested in pigs. The contribution of the gene segments to virulence was complex, with the swine HA and internal genes showing effects *in vivo*. The experimental infections indicate that these novel H3 viruses are virulent and can sustain onward transmission in pigs, and the naturally occurring mutations in the HA were associated with antigenic divergence from H3 IAV from humans and swine. Consequently, these viruses could have a significant impact on the swine industry if they were to cause more widespread outbreaks, and the potential risk of these emerging swine IAV to humans should be considered.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Abente, E.J., Kitikoon, P., Lager, K.M., Gauger, P.C., Anderson, T.K., Vincent, A.L. 2017. A highly pathogenic avian-derived influenza virus H5N1 with 2009 pandemic H1N1 internal genes

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- Brockmeier, S.L., Loving, C.L., Palmer, M.V., Spear, A., Nicholson, T.L., Faaberg, K.S., Lager, K.M. 2017. Comparison of Asian porcine high fever disease isolates of porcine reproductive and respiratory syndrome virus to United States isolates for their ability to cause disease and secondary bacterial infection in swine. *Vet. Microbiol*. 203, 6-17.
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- Fleming, D.S., Miller, L.C. (2016). Leading edge analysis of transcriptomic changes during Pseudorabies Virus infection. *Genomics Data* 10:104–106.
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2) Abstracts or Proceedings

- Faaberg, K. S., Wang, F.-X., M. A. Kappes, Das, P. B., Spear, A. 2017. Porcine reproductive and respiratory syndrome virus nonstructural protein 2 – a replicase protein that is packaged into virions and performs multiple functions. *Viruses & Cells: Gordon Research Conference, Biology, Pathogenesis, and Treatment of Viral Infections*, May 14-19, 2017, Lucca, Italy.
- Faaberg, K. S., Wang, F.-X., M. A. Kappes, Das, P. B., Spear, A. 2017. Porcine reproductive and respiratory syndrome virus nonstructural protein 2: a replicase protein that is packaged into virions and performs multiple functions. Abstract S2P-2. XIVth International Nidovirus Symposium, June 4-9, 2017, Kansas City, MO, USA.
- Fleming, D.S., Miller, L.C. (2017) Small non-coding RNAs (sncRNA) regulate gene silencing and modify homeostatic status in animals faced with porcine reproductive and respiratory syndrome virus (PRRSV) [abstract]. 36th International Society for Animal Genetics Conference (2017), July 16-19 2017, Dublin Ireland.
- Fleming, D.S., Miller, L.C. (2017) Small non-coding RNAs (sncRNA) regulate gene silencing and modify homeostatic status in animals faced with porcine reproductive and respiratory syndrome virus (PRRSV) [abstract]. XIVth International Nidovirus Symposium (Nido2017), June 4-9, 2017, Kansas City, Missouri.
- Fleming, D.S., Miller, L.C. (2017). (w)HOL(e)ISTIC gene ontology and pathway analysis using open source web tools [abstract]. International Plant & Animal Genome XXV, January 14-18, 2017, San Diego, California

- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes [abstract]. 36th International Society for Animal Genetics Conference (2017), July 16-19 2017, Dublin Ireland.
- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes [abstract]. XIVth International Nidovirus Symposium (Nido2017), June 4-9, 2017, Kansas City, Missouri.
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- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals potential of HP-PRRSV to modulate immune system activation related to host pathogen and damage associated signaling in infected porcine monocytes [abstract]. International Plant & Animal Genome XXV, January 14-18, 2017, San Diego, California.
- Nicholson T.L., Brunelle B.W., Bayles D.O., Alt D.P., Shore, S.M. Comparative Genomic and Transcriptional Analysis of Virulent and Non-virulent *Haemophilus parasuis* isolates. Abstract. ASM Microbe 2017 (American Society for Microbiology General Meeting) June 1-5, 2017. New Orleans, LA. USA.
- Sang, Y., Liu, Q., Miller, L.C., Lee, J., Ma, W., Blecha, F. (2017) Cross-Species Comparison Reveals Molecular and Functional Novelty of Porcine Interferon-Omega Subtype [abstract]. The American Association of Immunologists 2017, May 12-16, 2017, Washington DC.
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- Van Geelen, A.G.M., Faaberg, K.S., Phani, D., Montiel, N., Miller, L.C., Kulshreshtha, V., Buckley, A., and Lager, K.M. (2016). Comparative pathogenesis and characterization of contemporary 1-7-4 PRRSV isolates in weanling age piglets.[abstract]. American Association of Swine Veterinarians Annual Meeting February 25-28, 2017, Denver, Colorado.
- Wang, F., Otis, N., Chenge, J., Bester, S., Pegan, S. D., Faaberg, K. S. 2017. Nonstructural Protein 2 and Papain-like Protease 2 Chimeras between Highly-Pathogenic JXwn06 and Ingelvac PRRS® MLV Strains of Porcine Reproductive and Respiratory Syndrome Virus. Abstract S8. O-08. XIVth International Nidovirus Symposium, June 4-9, 2017, Kansas City, MO, USA.

3) Book Chapters or Monographs

D. FUNDING SOURCES

- Faaberg, Lager, Miller, Brockmeier, Nicholson, Vincent, Abente - USDA ARS Research Funds
- Sang, Rowland, Blecha, Miller – NIFA-AFRI - Antiviral regulation underlying the activation status of porcine monocytic innate immune cells
- Faaberg, Pegan – National Pork Board - Role of the viral ovarian domain protease in PRRSV pathogenesis
- Faaberg, Anderson, Lager – National Pork Board - United States Swine Pathogen Database
- Lager - Animal And Plant Health Inspection Service (APHIS), U.S. Department of Agriculture - Emerging Swine Disease Studies: Porcine Epidemic Diarrhea Virus (PEDV)
- Lager - Animal And Plant Health Inspection Service (APHIS), U.S. Department Of Agriculture - Identify Mechanisms of Viral Pathogenesis, Transmission, and Immunity of Porcine Epidemic Diarrhea Virus and Other Emerging Swine Coronaviruses
- Nicholson- Iowa Pork Producers Association (IPPA)-Comparative genomic and virulence analysis of *Streptococcus suis* isolates
- Vincent-NIAID-NIH CEIRS, USDA-APHIS

E. WORK PLANNED FOR NEXT YEAR

Miller:

- Establish that gene response pathways altered by PRRSV infection in monocytic cells provide a framework for identification of genes and gene products critical for anti-PRRSV regulation.
- Show that small non-coding RNAs (sncRNA) are a significant regulator of gene silencing when animals are faced with a pathogen that may modify their homeostatic status.
- Determine the diverse antiviral properties that IFN and ISG families have on swine viral pathogens.
- Maintain Surveillance for emerging swine diseases.

Faaberg:

- In vitro and vivo analysis of engineered PRRSV strains
- PEDV and PDCoV pathogenesis
- Swine Pathogen Database

Lager:

- Pathogenesis of Seneca virus A
- Pathogenesis of PEDV and PDCoV
- PEDV Immunology

Brockmeier:

- Use functional genomics to determine virulence mechanisms of *Streptococcus suis* and *Haemophilus parasuis*.
- Establish what effects antibiotic usage or infection with common pathogens has on the respiratory microbiome and carriage of common bacterial pathogens.

- Identify immunogenic, protective, and conserved proteins of *Streptococcus suis* and *Haemophilus parasuis* through immunoproteomics that will be cross protective against multiple serotypes.

Nicholson:

- Obtain complete whole-genome sequence of virulent and non-virulent *Streptococcus suis* isolates.
- Complete comparative genomic and transcriptional analysis of virulent and non-virulent *Streptococcus suis* isolates.
- Identify the genetic determinants that differentiate human and swine methicillin-resistant *Staphylococcus aureus* (MRSA) strains.
- Determine the role of biofilms in persistence of pathogens in the respiratory tract of swine.

Vincent:

- Perform routine sequence analysis of influenza A virus in swine surveillance sequence data to monitor for genetic and potential antigenic evolution. Select isolates for in vitro and in vivo studies.
- Test amino acid substitutions in H3 hemagglutinin genes of influenza A viruses to examine antigenic evolution.

Abente:

- Characterize swine innate and adaptive host immune gene profiles to wild type swine IAV infection.
- Test predicted antigenic targets in WIV, LAIV and vectored vaccine platforms against influenza A virus challenge in pigs.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

No specific progress – Plans to initiate studies in 2018

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Ongoing efforts involving swine influenza virus include utilizing contemporary isolates from North America, we are interrogating the zoonotic potential of these viruses as well as assessing virulence determinants. Studies include assessing antigenic relatedness of existing commercial vaccines with contemporary isolates.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

These studies primarily have value regarding public health impact – what is the zoonotic potential of circulating swine influenza viruses? However, this has ancillary impact for pork producers, informing risk and enabling de-risking of production. Also, analysis of potential efficacy of existing commercial vaccines through antigenic analysis can directly inform vaccination practices for producers.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Molecular epidemiology of swine influenza A viruses in the Southeastern United States, highlights regional differences in circulating strains. (2017) Kyriakis CS, Zhang M, Wolf S, Jones LP, Shim BS, Chocallo AH, Hanson JM, Jia M, Liu D, Tripp RA. *Vet Microbiol.* 211:174-179. doi: 10.1016/j.vetmic.2017.10.016. PMID: 29102115

Influenza D in Italy: towards a better understanding of an emerging viral infection in swine. (2017) Foni E, Chiapponi C, Baioni L, Zanni I, Merenda M, Rosignoli C, Kyriakis CS, Luini MV, Mandola ML, Bolzoni L, Nigrelli AD, Faccini S. *Sci Rep.* 7(1):11660. doi: 10.1038/s41598-017-12012-3. PMID: 28916759

2) Abstracts or Proceedings

Assessing of the zoonotic potential of swine influenza viruses in a primary respiratory cell culture model. Constantinos S. Kyriakis, Madelyn Krunkosky, and S. Mark Tompkins. CRWAD 2017 — The 98th Annual Conference of Research Workers in Animal Diseases December 1-5, 2017. Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois

3) Book Chapters or Monographs

none

D. FUNDING SOURCES

HHSN272201400004C

4/1/2014 – 3/31/2021

Emory University/NIH/NIAID

NIAID CENTERS OF EXCELLENCE FOR INFLUENZA RESEARCH AND SURVEILLANCE

The major goal of this project is to understand zoonotic potential of currently circulating swine influenza viruses.

E. WORK PLANNED FOR NEXT YEAR

We will continue work assessing zoonotic potential of swine influenza viruses, using primary human and swine cell culture systems. Ongoing studies include utilizing established murine models of infection to assess virulence, viral determinants of virulence, and mechanisms of severe disease (i.e. immune responses to infection). In addition, a subset of viruses will be assessed for virulence in swine. Moving forward we will be exploring evolutionary potential of viruses using in vivo and in vitro infection models, assessing reassortment of viruses. Of interest to stakeholders will be new collaborative studies exploring point of care sequence analysis of swine virus isolates, an approach to dramatically improve swine influenza surveillance. This will eventually expand beyond influenza.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Goldberg, Tony. University of Wisconsin-Madison. tgoldberg@vetmed.wisc.edu

2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

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Thomas Friedrich, Associate Professor of Pathobiological Sciences, thomasf@primate.wisc.edu

Jens Kuhn, Virology Lead, NIH Integrated Research Facility at Fort Detrick (IRF-Frederick), kuhnjens@niaid.nih.gov

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

N/A

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Research at the University of Wisconsin-Madison funded by NIH has focused on discovering and characterizing viruses of the family Arteriviridae. This work has focused on simian hemorrhagic fever virus and its relatives, which are related to PRRSV. This research is not part of any USDA-funded study but is relevant to the central biology of arteriviruses.

Specifically, we have deployed metagenomic methods for generating full-genome sequences of arteriviruses directly from infected host tissues. Using these methods, we have discovered and characterized 12 novel simian arteriviruses. These discoveries have helped inform a taxonomic reclassification that will soon be applied to the nidoviruses by the International Committee on the Taxonomy of Viruses. Research on the specific viruses is elucidating common determinants of arterivirus pathogenesis and immunity, which will inform the detection, prevention and control of PRRSV.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Understanding the PRRSV requires a comparative perspective. Our studies of the family Arteriviridae place PRRSV in a comparative perspective with its relatives. Our findings show that PRRSV is not the most diverse of the arteriviruses, and that it should probably be split into two species, corresponding to Type 1 and Type II PRRSV, and that patterns of evolution and host-switching that we have documented for the arteriviruses also apply to PRRSV, as well as to other RNA viruses of swine that may not yet have been discovered.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications (recent)

Moncla, L. H., A. M. Weiler, G. Barry, J. T. Weinfurter, J. M. Dinis, O. Charlier, M. Lauck, A. L. Bailey, V. Wahl-Jensen, C. W. Nelson, J. C. Johnson, Y. Cai, T. L. **Goldberg**, D. H. O'Connor, P. B. Jahrling, J. H. Kuhn and T. C. Friedrich (in press). Within-host evolution of simian arteriviruses in crab-eating macaques. *J Virol* 91(4).

Yu, S. Q., Y. Cai, C. Lyons, R. F. Johnson, E. Postnikova, S. Mazur, J. C. Johnson, S. R. Radoshitzky, A. L. Bailey, M. Lauck, T. L. **Goldberg**, D. H. O'Connor, P. B. Jahrling, T. C. Friedrich and J. H. Kuhn (2016). Specific detection of two divergent simian arteriviruses using RNAscope *in situ* hybridization. *PLoS One* 11(3): e0151313.

Bailey, A. L., M. Lauck, R. R. Ghai, C. W. Nelson, K. Heimbruch, A. L. Hughes, T. L. **Goldberg**, J. H. Kuhn, A. J. Jasinska, N. B. Freimer, C. Apetrei and D. H. O'Connor (2016). Arteriviruses, pegiviruses, and lentiviruses are common among wild African monkeys. *J Virol* 90(15): 6724-6737.

Wahl-Jensen, V., J. C. Johnson, M. Lauck, J. T. Weinfurter, L. H. Moncla, A. M. Weiler, O. Charlier, O. Rojas, R. Byrum, D. R. Ragland, L. Huzella, E. Zommer, M. Cohen, J. G. Bernbaum, Y. Cai, H. B. Sanford, S. Mazur, R. F. Johnson, J. Qin, G. F. Palacios, A. L. Bailey, Peter B. Jahrling, T. L. **Goldberg**, D. H. O'Connor, T. C. Friedrich and J. H. Kuhn (2016). Divergent simian arteriviruses cause simian hemorrhagic fever of differing severities in macaques. *mBio* 7: e02009-15.

Kuhn, J. H., M. Lauck, A. L. Bailey, A. M. Shchetinin, T. V. Vishnevskaya, Y. Bào, T. F. F. Ng, M. LeBreton, B. S. Schneider, A. Gillis, U. Tamoufe, J. L. D. Diffo, J. M. Takuo, N. O. Kondov, L. L. Coffey, N. D. Wolfe, E. Delwart, A. N. Clawson, E. Postnikova, L. Bollinger, M. G. Lackemeyer, S. R. Radoshitzky, G. Palacios, J. Wada, Z. V. Shevtsova, P. B. Jahrling, B. A. Lapin, P. G. Deriabin, M. Dunowska, S. V. Alkhovsky, J. Rogers, T. C. Friedrich, D. H. O'Connor and T. L. **Goldberg** (2015). Reorganization and expansion of the nidoviral family *Arteriviridae*. *Archives of Virology*: 161: 755-768.

2) Abstracts or Proceedings (recent)

Simons, N.D., Eick, G., Ruiz-Lopez, M. J., Chapman, C.A., Goldberg, T. L., Sterner, K.N., Ting, N., (2017). Host immune gene expression and viral infection status from whole blood transcriptomics in the Ugandan red colobus. American Association of Physical Anthropologists, New Orleans, Louisiana, USA.

Lester, J., Sibley, S. D., Hyeroba, D., Tumukunde, A., Wen, G., Dearlove, B., Jones, J. H., Switzer, W., Chapman, C. A., Ting, N., Frost, S. D., Goldberg, T. L. (2016). Patterns of infection and transmission within a wild non-human primate zoonotic reservoir. Wellcome Genome Conference: Exploring Human Host-Microbiome Interactions in Health and Disease, Hinxton, United Kingdom.

Simons, N.D., Christie, D. M., Eick, G., Ruiz-Lopez, M. J., Chapman, C.A., Goldberg, T. L., Ting, N., Sterner, K.N., (2016). Disease-associated genetic variation drives differential expression of MHC-DQA1 *in vitro*: A role for cis-regulatory variation in disease susceptibility in wild primates. American Association of Physical Anthropologists, Atlanta Georgia, USA.

Goldberg, T. L., Chapman, C. A., O'Connor, D. H., Friedrich, T., Lauck, M., Sibley, S., Bailey, A., Hyeroba, D., Tumukunde, A., Wen, G., Ting, N., Kuhn, J., Ghai, R., Simons, N., Dinis, J., Thurber, M. (2014). Cross-species transmission of taxonomically diverse pathogens in a community of wild primates. American Society for Tropical Medicine and Hygiene. New Orleans, Louisiana, USA.

3) Book Chapters or Monographs

[none]

D. FUNDING SOURCES

R01AI098420 (NIH-NIAID; *Biological and Human Dimensions of Primate Retroviral Transmission*) and related sources of internal and external support at NIH and UW-Madison and the Wisconsin National Primate Research Center.

E. WORK PLANNED FOR NEXT YEAR

Continue to characterize the diversity and pathogenesis of the arteriviruses in their natural hosts and in experimental systems.

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INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection: UMN continued work on mechanisms of immune protection and correlates of immunity, particularly in the area of neutralizing antibodies.
- Host genetic control of anti-PRRSV infection and vaccination responses:
- UMN, in collaboration with cooperating veterinarians and producers, characterized individual variation in anti-PRRSV antibody responses that may have a genetic basis.
- UMN characterized gene expression variation in that contributes to age-dependent immune variation in response to PRRSV.
- PRRSV Pathogenesis. UMN investigated highly pathogenic PRRSV from U.S. outbreaks.
- Association between PRRS incidence and epidemiological factors was quantified in sow farms
- Role of animal movement networks in PRRS epidemiology
- UMN assisted in epidemiological investigations of the introduction of PRRSV in Chile, 2013-2015.
- UMN developed methods to assess the efficacy of biosecurity methods to decrease the viability of airborne PRRSV

- UMN tested biosecurity methods to inactivate airborne PRRS virus.
- Characterized size of airborne particles associated with PRRSV under field conditions
- Developed a model to estimate PRRS virus introduction into filtered farms with negative-pressure

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

- Viral diseases of swine of recent origin. High-throughput nucleic acid sequencing and data analysis was applied to diagnostic lab cases from recent and novel rotavirus types.
- Analytical models were developed to estimate risk for transmission of porcine coronaviruses via contaminated feed and feed ingredients
- Epidemiological models to forecast the hypothetical transmission of FMDv within different types of swine farms were formulated and parameterized
- Investigated the seasonality of influenza A virus in breed to wean farms, and assessed the impact of climatic conditions on influenza infections at weaning
- Reported that multiple genome constellations of similar and distinct influenza A viruses co-circulate during epidemics in swine which may serve as a mechanism of virus persistence in growing pig populations
- Investigated the origin and persistence of influenza A virus in a live animal market in Minnesota
- Through complete genome sequencing of influenza A viruses isolated from farrow to wean farms, we revealed the emergence, persistence and subsidence of diverse viral genotypes and proposed mechanisms of virus introduction and persistence in pigs
- Evaluated biosecurity measures directed at preventing the indirect transmission of porcine epidemic diarrhea virus.
- Developed and assessed methods of air sampling and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms
- Established and validated novel sampling methods to conduct surveillance of influenza virus
- Developed a GMR biosensor chip to detect Influenza A virus
- Developed an in vivo passaged PEDV isolate for potential vaccine development
- Characterized the mucosal immune response to PEDV infection at the GI epithelium

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

- Quantifying the association between epidemiological factors and PRRSV incidence is pre-requisite for developing predictive models of PRRSV spread through systems and regions
- Advancement in the understanding of neutralizing antibody responses of swine to PRRSV and variation in individual animal responses is expected to provide new opportunities for genetic improvement of resistance to PRRSV as well as in the area of mechanisms of protective immunity.

- Molecular understanding of age-dependent resistance to PRRSV may lead to improved immunological tools for stimulation of immunological PRRSV resistance and improved vaccine prevention.
- Genetic analysis of rotavirus strain variation will aid in identification of conserved and variable regions associated with immune protection that is expected to improve prevention of rotaviral diarrhea.
- A risk analysis of transmission of PEDV was useful to qualitatively assessing virus transmission in important feed ingredients of porcine origin. These ingredients (meat and bone meal, spray dried porcine plasma) represent an important strategy to increase recycle of nutrients into animal feed that otherwise can increase environmental impact of food production. We used a combination of empirical evidence, expert advice, and mathematical models to answer these important questions. Therefore, these studies conducted at UMN are key to sustain food production.
- Epidemiological models of viral diseases exotic to the U.S. swine industry, such as the FMDV, help to develop preventive and control strategies to mitigate the impact of hypothetical epidemics
- Novel methods of sampling pigs may lead to more cost effective surveillance of influenza A virus
- Application of in depth sequencing of influenza viruses in farms evidences the high degree of co-circulation of genetically and antigenically distinct strains within farms Information on seasonality patterns observed for influenza infections may help target timing of vaccination strategies to decrease prevalence at weaning
- Modeling approaches to predict risk of PRRSV infections into filtered farms should help producers make biosecurity investment decisions
- Investigations into the transmission of influenza viruses within farms are providing new information in terms of dynamics, mechanisms and patterns of transmission in both, sow farms and growing pigs which should aid in the control of influenza virus
- Investigations into the influenza viruses isolated in winter and summer in an animal market in St. Paul, MN are indicating that viruses do not persist in the markets between seasons but that they originate from commercial pigs.
- Investigations into risk factors of influenza infections in piglets at weaning indicated that both, sow vaccination and gilt influenza status at entry are factors associated with influenza detection at weaning
- Prototype of a pen-side influenza virus test using GMR technology was developed in the laboratory

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Alba A, Morrison R, Cheeran A, Rovira A, Alvarez J, Perez AM. Optisample™: Open web-based application to optimize sampling strategies for active surveillance activities at the herd level. Porcine Respiratory Reproductive Syndrome (PRRS) as a working example. Plos One

- Alkhamis M, Arruda A, Morrison R, Perez A. Novel approaches for Spatial and Molecular Surveillance of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) in the United States. *Nature Scientific Reports*.
- Alkhamis M, Arruda A, Vilalta C, Morrison RB, Perez AM. Surveillance of porcine reproductive and respiratory syndrome virus in the United States using risk mapping and species distribution modeling. *Preventive Veterinary Medicine*
- Alonso C, Raynor PC, Goyal S, Olson BA, Alba A, Davies PR, Torremorell M (2017). Assessment of air sampling methods and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms. *J Vet Diagn Invest*, 29(3):298-304. doi: 10.1177/1040638717700221.
- Anderson BD, Lednicky JA, Torremorell M, and Gray GC. (2017). A Review of Bioaerosol Sampling in Swine Production Facilities. *Front Vet Sci*, <https://doi.org/10.3389/fvets.2017.00121>
- Arruda AG, Alkhamis MA, VanderWaal K, Morrison RB, Perez AM. Estimation of time-dependent reproduction numbers for porcine reproductive and respiratory syndrome (PRRS) across different regions and production systems of the United States. *Frontiers in Veterinary Science*.
- Arruda AG, Vilalta C, Perez A, Morrison R. Land Altitude, Slope, and Coverage as Risk Factors for Porcine Reproductive and Respiratory Syndrome (PRRS) Outbreaks in the United States. *Plos One*.
- Arruda P., Schwartz K., Arruda B., Rovira A., Vannucci F., Resende T., Nietfeld J., Sundberg P., Hause B. 2017 "Detection of a Novel Sapelovirus in Central Nervous Tissue of Pigs with Polioencephalomyelitis in the U.S". *Transboundary and Emerging Diseases*, 64:311-315.
- Chamba Pardo FO, Alba-Casals A, Nerem J, Morrison RB, Puig P, Torremorell M (2017). Influenza herd-level prevalence and seasonality in breed-to-wean pig farms in the Midwestern United States. *Front Vet Sci*. 4:167, <https://doi.org/10.3389/fvets.2017.00167>
- Cottingim, K M, H Verma, PE Urriola, F Sampedro, GC Shurson, and S Goyal. 2017. Feed additives decrease survival of delta coronavirus in nursery pig diets. *Porcine Health Management* 3:1-7 doi: 10.1186/s40813-016-0048-8
- Cottingim, K.M., L.J. Johnston, A.M. Hilbrands, P.E. Urriola, and G.C. Shurson. 2017 Ultraviolet irradiation of spray-dried porcine plasma does not affect the growth performance of nursery pigs when compared with nonirradiated bovine plasma. *J. Anim. Sci*. 95:3120-3128
- Diaz A, Marthaler D, Corzo C, Muñoz-Sanzi C, Sreevatsan S, Culhane M, Torremorell M (2017). Multiple genome constellations of similar and distinct influenza A viruses co-circulate during epidemics in swine. *Scientific reports*, 19;7(1):11886. doi: 10.1038/s41598-017-11272-3.
- Diaz A, Marthaler D, Culhane M, Sreevatsan S, Alkhamis M, Torremorell M (2017). Complete Genome Sequencing of Influenza A Viruses within Swine Farrow-to-Wean Farms Reveals the Emergence, Persistence, and Subsidence of Diverse Viral Genotypes. *J Virol pii: JVI.00745-17*. doi: 10.1128/JVI.00745-17

- Dvorak, C.M.T, Z. Akkutay-Yoldar, S.R. Stone; S.J. Tousignant, F. Vannucci, DVM, and M.P. Murtaugh. 2017. An indirect enzyme-linked immunosorbent assay for the identification of antibodies to Senecavirus A in swine. *BMC Vet Res.* 13:50. doi: 10.1186/s12917-017-0967-x.
- Dvorak, C.M.T., Y. Yang, C. Haley, N. Sharma and M.P. Murtaugh. 2016. National reduction in porcine circovirus type 2 prevalence following introduction of vaccination. *Vet. Micro.* 189:86-90.
- Gillespie, T., Q. Song, M. Inskeep, S. Stone and M.P. Murtaugh. 2017. Effect of booster vaccination with inactivated porcine epidemic diarrhea virus on neutralizing antibody response in mammary secretions. *Viral Immunol.* DOI: 10.1089/vim.2017.0023
- Kim Y&, Yang M, Goyal SM, Cheeran M C-J, Torremorell M (2017). Evaluation of biosecurity measures to prevent indirect transmission of porcine epidemic diarrhea virus. *BMC Vet Res*, 13(1):89.doi:10.1186/s12917-017-1017-4
- Kinsley AC, Patterson G, VanderWaal KL, Craft ME, Perez AM. Parameters values for epidemiological models of foot-and-mouth disease in swine. *Frontiers in Veterinary Sciences.*
- Macedo N, Cheeran MC-J, Rovira A, Holtcamp A, Torremorell M. Effect of Enrofloxacin on *Haemophilus parasuis* infection, disease and immune response. *Veterinary Microbiology* 2017; 199:91-99. doi:10.1016/j.vetmic. 2016.12.032
- Neira V, Brito B, Mena J, Culhane M, Apel MI, Max V, Perez P, Moreno V, Mathieu C, Johow M, Badia C, Torremorell M, Medina RA, Ortega R (2017). Epidemiological investigations of the introduction of porcine reproductive and respiratory syndrome virus in Chile, 2013-2015. *PLoS ONE* 12(7): e0181569. <https://doi.org/10.1371/journal.pone.0181569>
- Nelson M, Culhane M, Trovão N, Patnayak D, Halpin R, Lin X, Shilts M, Das S, Detmer S. (2017) The emergence and evolution of influenza A (H1 α) viruses in swine in Canada and the United States. *J. Gen. Virol.* 98(11):2663-2675 doi:10.1099/jgv.0.000924
- Perez AM, Willeberg PW. Foot-and-Mouth Disease in Swine. *Frontiers in Veterinary Science.*
- Rahe, M.C. and M.P. Murtaugh. 2017. Effector mechanisms of humoral immunity to porcine reproductive and respiratory syndrome virus. *Vet. Immunol. Immunopathol.* 186:13-17.
- Rahe, M.C. and M.P. Murtaugh. 2017. Interleukin-21 drives proliferation and differentiation of porcine memory B cells into antibody secreting cells. *PLoS One.* 12:e0171171.
- Rahe, M.C. and M.P. Murtaugh. 2017. Mechanisms of adaptive immunity to porcine reproductive and respiratory syndrome virus. *Viruses.* 9:148; doi:10.3390/v9060148.
- Rahe, M.C., K. Gustafson, and M.P. Murtaugh. 2017. B cell tetramer development for veterinary vaccinology. *Viral Immunol.* doi: 10.1089/vim.2017.0073.
- Sasaki Y, Alvarez J, Sekiguchi S, Sueyoshi M, Otake S, Perez A. Spatial dynamics of porcine epidemic diarrhea (PED) spread in Miyazaki prefecture, Japan. *Preventive Veterinary Medicine*
- Shephard F, Chen F, Culhane M, Murtaugh M, Marthaler M. 2017. Longitudinal surveillance of porcine rotavirus B strains from the United States and Canada and in silico identification of antigenically important sites. *Pathogens.* In press.
- Trudeau, MP, H Verma, F Sampedro, PE Urriola, GC Shurson, and S Goyal. 2017. Environmental persistence of porcine coronavirus in feed and feed ingredients. *PLOS ONE* 12(5):e0178094.

- Trudeau, MP, H Verma, F Sampedro, PE Urriola, GC Shurson, J McKelvey, SD Pillai, and S Goyal. 2016. Comparison of thermal and non-thermal processing of swine feed and the use of selected feed additives on inactivation of porcine epidemic diarrhea virus PEDV. *PLOS ONE* 11:6-e0158128
- Trudeau, MP, H Verma, PE Urriola, F Sampedro, GC Shurson. 2017. Survival of porcine epidemic diarrhea virus (PEDV) in thermally treated feed ingredients and on surfaces. *Porcine Health Management* 3:17 doi.org/10.1186/s40813-017-0064-3
- Valdes-Donoso P, VanderWaal K, Jarvis LS, Wayne S, Perez AM. Using machine learning to predict swine movements with application to the control of infectious diseases. *Frontiers in Veterinary Science*.
- VanderWaal, K., R. Morrison, C. Neuhauser, C. Vilalta, A. Perez. 2017. Translating big data into smart data for veterinary epidemiology. *Frontiers in Veterinary Science* 4: 110.
- Vilalta Sans C, Arruda AG, Tousignant SJP, Valdes-Donoso P, Muellner P, Muellner U, Alkhamis MA, Morrison RB, Perez AM. A review of quantitative tools used to assess the epidemiology of porcine reproductive and respiratory syndrome (PRRS) in U.S. swine farms using the Swine Health Monitoring Program (SHMP) data. *Frontiers in Veterinary Science*.
- Wu K, Klein T, Krishna VD, Su D, Perez AM, Wang JP. Portable GMR handheld platform for the detection of influenza A virus. *ACS Sensors*.

2) Abstracts or Proceedings

- Alba A, Morrison RE, Cheeran A., Rovira A, Alvarez J, Perez AM. OptiSample tool for demonstration of freedom from disease in swine farms. International conference on animal health surveillance, NZ-Rotorua. New Zealand Veterinary Association Apr 2017.
- Alkhamis MA, Arruda AG, Morrison RB, Perez AM. Ecological niche and phylogeography of Porcine Reproductive and Respiratory Syndrome Virus in the Midwest of United States. GEOVET, Valdivia, Chile, November 2016
- Alkhamis MA, Vilalta C, Perez AM and Morrison RB. Maximum Entropy Ecological Niche Modelling for Surveillance of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) in the United States. Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), 2017, Inverness, Scotland, United Kingdom.
- Alonso C, Torremorell M, Davies P, Raynor P (2017). Concentration, size distribution, and infectivity of airborne particles carrying PRRS, influenza A, and PED viruses emitted by acutely infected animals. *Proc Int Pig Vet Soc*, PO-PF3-087, p 472.
- Alonso C, Torremorell M, Davies P, Raynor P, Goyal S (2017). Airborne transmission of highly pathogenic avian influenza virus: Preparedness considerations for the swine industry. *Proc Int Pig Vet Soc*, PO-PW1-294, p 442.
- Alonso C, Torremorell M, Goyal S, Davies P, Raynor P (2017). Particle size distribution of airborne PRRS and PED viruses emitted by infected animals inside and outside swine facilities. *Proc Int Pig Vet Soc*, PO-PW1-099, p 575.

- Brito B, Mena J, Torremorell M, Culhane M, Johow M, Mathieu C, Neira Ramirez VM, Ortega R (2017). Analysis of the recent re-introduction of PRRSV in Chile. *Proc Int Pig Vet Soc*, PO-PW1-095, p 555.
- Chamba F, Allerson M, Culhane M, Davies P, Morrison R, Perez A, Torremorell M (2017). Effect of sow vaccination on the detection of influenza A virus in pigs at weaning. *Proc Int Pig Vet Soc*, O-VVD5-014, p 158.
- Chamba F, Diaz A, Culhane M, Torremorell M (2017). Effect of maternally-derived antibodies on influenza infection and antibody response in pigs after weaning. *PO-PCO1-006*, p 602.
- Chamba F, Nerem J, Alba A, Torremorell M (2017). Influenza A virus prevalence and seasonality in Midwestern US breeding herds. *Proc 48th Am Assoc Swine Vet*, p 278.
- Chamba F, Wayne S, Culhane M, Perez A, Torremorell M (2017). Effect of influenza prevalence at weaning on transmission, clinical signs and performance after weaning. *Proc 48th Am Assoc Swine Vet*, p 42.
- Chamba F, Wayne S, Perez A, Culhane M, Torremorell M. Effect of influenza prevalence at weaning on transmission, clinical signs and performance after weaning. *American Association of Swine Veterinarians (AASV) 48th Annual Meeting*. February 26th 2017. Denver, CO.
- Chamba F, Wayne S, Perez A, Culhane M, Torremorell M. Impact of influenza prevalence at weaning on transmission in the nursery. *Minnesota Veterinary Medical Association (MVMA) 120th Annual Meeting*. February 23rd 2017. Minneapolis, MN.
- Chamba F, Wayne S, Perez A, Culhane M, Torremorell M. The association between influenza immune status and infection patterns in nursery. *2017 Allen D. Leman Swine Conference*. September 19th 2017. St. Paul, MN.
- Diaz A, Torremorell M, Culhane M, Sreevatsan S (2017). The antigenic diversity of influenza A viruses during infection of weaned pigs. *Proc Int Pig Vet Soc*, PO-PT2-02, p 601.
- Diaz A, Torremorell M, Culhane M, Sreevatsan S (2017). The persistence of influenza A viruses in swine breeding herds. *Proc Int Pig Vet Soc*, PO-PT2-020, p 601.
- Dvorak CMT, Akkutay-Yoldar Z, Stone SR, Tousignant SJ, Vannucci F, Murtaugh MP. 2016. Development of an indirect enzyme-linked immunosorbent assay for the identification of antibodies to Senecavirus A. *Proc Allen D Leman Swine Conf*. Abst 49
- Dvorak, C.M.T. and M.P. Murtaugh*. 2016. PCV2 natural infection, immunity and genetic diversity. *China Animal Health Inspection*. 33:64-70. (in Chinese).
- Dvorak, C.M.T., B.J. Payne, J.L. Seate, M.P. Murtaugh. 2016. Project Update: examination of colostral PCV2 neutralizing antibody levels and viremia in sows. *SDEC Partners Research Update*. Vol 5, Issue 6.
- Haley C, Dvorak C, Murtaugh M. 2016. An update on the U.S. national porcine circovirus type 2 prevalence: before and after wide scale vaccination. *24th IPVS Abstract book*. p 495.
- Iglesias I, VanderWaal K, Sampedro F, Kinsley A, Goldsmith T, Perez A.. Effectiveness of alternative control measures for foot-and-mouth disease in swine farms in the USA. *International Meeting on Emerging Diseases and Surveillance (IMED)*. Vienna, Austria, November 2016.

- Janni KA, Torremorell M, Jacobson LD, Hetchler B (2017). Modeling airborne virus concentrations in filtered swine barns with negative-pressure ventilating systems. Proc ASABE.
- Jarvis M, Lam H, Nelson M, Murtaugh M, Marthaler D. 2016. Evolution and protein changes in porcine epidemic diarrhea virus from the United States. 24th IPVS Abstract book. p 510
- Jarvis M, Lam H, Nelson M, Murtaugh M, Marthaler D. 2016. Evolution and protein changes in porcine epidemic diarrhea virus in the United States. Proc Allen D Leman Swine Conf. Abst 51
- Kinsley, A, VanderWaal, K., M.E. Craft, A. Perez. When enough is enough? Exploring complexity in FMD models for swine. Poster session at: 15th Annual Ecology and Evolution of Infectious Diseases Conference. Santa Barbara, CA, June 25-27, 2017.
- Kinsley, A. VanderWaal, K., Craft, M.E., Morrison, B., Perez, A. How can farm structure and demography influence pathogen persistence within a herd? Minnesota Veterinary Medical Association Annual Meeting. Minneapolis, Minnesota.
- Kinsley, A., VanderWaal K., Craft M., Perez A. 2016. UMN CVM Points of Pride Research Day. St. Paul, MN. Managing Complexity: Simplifying assumptions of FMD models for swine.
- Kinsley, A., VanderWaal, K., Craft, M.E., Morrison, B., Perez, A. 2017. Leman Conference. St. Paul, MN. Managing Complexity: Simplifying assumptions of FMD models for swine.
- Kinsley, A., VanderWaal, K., R. Morrison, M. Craft, A. Perez. Managing complexity: simplifying assumptions of FMD models for swine. Invited oral presentation Allen D. Leman Conference. Sept 18, 2017, St. Paul, MN.
- Kinsley, A., VanderWaal, K. Craft, M., Morrison R., Perez, A. 2017. "Managing Complexity: Simplifying assumptions of foot-and-mouth disease models for swine. 15th Annual Ecology and Evolution of Infectious Disease meeting. Santa Barbara, CA.
- Kinsley, A.C., K. VanderWaal, M. Craft, R.B. Morrison, A.M. Perez. Managing complexity: simplifying assumptions of foot-and-mouth disease models for swine. Poster presentation at University of Minnesota College of Veterinary Medicine Points of Pride Research Day. October 4, 2017, St. Paul, MN.
- Krishna VD, Kim Y, Yang M, Vannucci F, Torremorell M, Cheeran MC-J (2017). Systemic and Mucosal Immune Response to Porcine Epidemic Diarrhea Virus (PEDV) in swine. Institute of Virology Symposium, Minneapolis MN.
- Krishna VD, Wu, K, Klien T, Perez A, Wang JP, Cheeran MC-J (2017). Influenza A virus detection using a giant magnetoresistance (GMR) biosensing portable handheld device Leman Swine Conference, St Paul, MN.
- Marthaler, D. and M.P. Murtaugh. 2016. Interpreting PRRS ORF5 sequencing, can we do better? Swine Health Monitoring Project. SHMP@umn.edu. 8/19/2016.
- Marthaler, D. and M.P. Murtaugh. 2016. Phylogenetic relationships and percent sequence identity in combination with RFLP to interpret virus relationships. Swine Health Monitoring Project. SHMP@umn.edu. 8/26/2016.
- Murtaugh MP. 2016. Broadly neutralizing antibodies for PRRSV. USDA-NIFA Animal Health and Animal Well-being (and Related Programs) Project Director Meeting. Program book p.5.

- Neira Ramirez VM, Brito B, Saavedra M, Tapia R, Tapia K, Garcia V, Torremorell M, Medina R (2017). Genetic diversity of sine origin influenza A virus in Chile and the Latin American perspective. Proc Int Pig Vet Soc, PO-PT2-007, p 599.
- Payne B, Jacobs A, Dvorak C, Murtaugh M. 2016. PCV2 vaccine cross-protection: identification of sequences in successfully vaccinated field cases. 24th IPVS Abstract book. p 503
- Perez AM. Keynote presentation: Swine Health Monitoring Program in the US. GEOVET, Valdivia, Chile, November 2016
- Perez, A., & VanderWaal, K. Present and future of the Dr. Morrison Swine Health Monitoring Project (MSHMP). Invited oral presentation Allen D. Leman Conference. 2017 Sept 18, St. Paul, MN.
- Perez, A., & VanderWaal, K. Present and future of the Dr. Morrison Swine Health Monitoring Project (MSHMP). Invited oral presentation Allen D. Leman Conference. 2017 Sept 18, St. Paul, MN.
- Rahe M, Borgerding AJ, Wiseman B, Cenatti L, Martin D, Murtaugh MP. 2016. Establishment and characterization of a porcine lymphoma cell line. J Immunol 196 (1 Supplement) 216.18.
- Trudeau, MP, H Verma, F Sampedro, PE Urriola, GC Shurson, and SM Goyal. 2016. Environmental Persistence of Porcine Epidemic Diarrhea Virus (PEDV), Porcine Delta Corona Virus (PDCoV), and Transmissible Gastroenteritis (TGEV) in Feed Ingredients. iCOMOS - 2nd International Conference on One Medicine One Science. University of Minnesota – Minneapolis MN
- Valdes-Donoso P, VanderWaal K, Wayne S, Perez AM. Using machine learning to predict swine movements in a regional program (RCP) to control infectious diseases. GEOVET, Valdivia, Chile, November 2016
- VanderWaal, K. Data integration: Dynamic risk for swine diseases. Invited oral presentation at: Allen D. Leman Conference. 2017 Sept 16, St. Paul, MN.
- Yang Z, L. Galina, T. Knutson, A. Rovira, D. Marthaler. “Investigating porcine circovirus associated disease (PCVAD) in commercial swine herds by next generation sequencing.” AASV Annual Meeting, 2017. Denver, CO, USA.

3) Book Chapters or Monographs

- Torremorell M (2016). Immunity, diagnosis and intervention strategies of influenza infections in pigs. In: Animal Influenza. Ed. David Swayne, 2nd Edition. Wiley-Blackwell, pp: 452-458. Contribution: Wrote chapter 17.

D. FUNDING SOURCES

Dates	Title	Funding source	PI
2/15/16-2/14/19	Broadly neutralizing antibodies to PRRSV	USDA NIFA	Murtaugh

5/1/16-4/30/17	Pen-side respiratory pathogen identification	Boehringer Ingelheim Vetmedica	Murtaugh
5/1/16-4/30/17	Toward animal challenge-free prediction of vaccine efficacy	American Association of swine Veterinarians Foundation	Murtaugh
8/26/15-8/25/16	Energetics of B cell activation	Puretein Bioscience	Murtaugh
7/1/16-6/30/17	PRRS multistate project	UMN CVM Hatch	Murtaugh
7/1/17-30/17	In vitro vaccine testing model for evaluating the quality of humoral protection	UMN AES	Murtaugh
07/01/2015-06/30/2017	Management and analysis of big data for near real-time detection and early response to food animal health threats	Mn Drive GFV	Perez
07/01/2016-06/30/2018	Development of epidemiological tools for PRRS outbreak investigations	UMN Hatch funds	Perez
10/01/2017-09/30/2019	Developing multiplex Giant magnetoresistance (GMR) biosensors for the detection of swine respiratory pathogens.	CVM Emerging and Zoonotic Diseases	co-PI (Cheeran)
08/01/2017- 07/31/2018	A near-real time global surveillance system for swine diseases	Swine Health Information Center	Perez
05/01/2017- 06/30/2018	Using Swine Health Monitoring Project to Facilitate Business Continuity	MN Board of Animal Health	Perez
10/15/2017- 10/14/2018	Development and implementation of a domestic swine bio-surveillance monitoring and surveillance	Swine Health Information Center	Torrison

	system. Part 2: Multiple data streams integration and reporting		
10/15/2017- 10/14/2018	Enhancing Dr. Bob Morrison's Swine Health Monitoring Program (MSHMP) capacity and preparedness through the integration with outputs from a SHIC-led disease surveillance programs and research integration with US higher education institutions.	Swine Health Information Center	Corzo
11/01/2017 – 10/31/2018	Dynamic mapping of PRRS and PED infection risk across space and time	Swine Health Information Center	VanderWaal
07/17-06/19	A comprehensive surveillance system to control influenza in pigs	Rapid Agricultural Response Fund (renewal)	Torremorell, M
07/15-06/17	A comprehensive surveillance system to control influenza in pigs	Rapid Agricultural Response Fund	Torremorell, M
03/15-02/18	Characterization of influenza diversity in piglets and risk factors for diversity	USDA-AFRI-NIFA	Torremorell, M
01/01/17-04/30/17	Demonstration of airborne PRRSV inactivation by a non-thermal plasma	NPB (subcontract with Michigan)	Torremorell, M
09/30/16-09/20/21	Optimizing assessment of virus containing particles in animal agriculture	NIOSH/NIH	Raynor, P
09/30/16-09/20/21	Longitudinal study of infectious disease risks at the human-swine interface	NIOSH/NIH	Davies, P
07/15-06/17	Does prevalence of influenza A virus at weaning influence disease transmission rates, clinical manifestation of disease, and production performance?	NPB	Torremorell, M
09/14-08/17	Detection and control of PRRS virus and emerging viral diseases of swine	Minnesota Agricultural Experimental Station	Torremorell, M

01/13-12/17	Genetically improving resistance of pigs to PRRSV infection	NIFA-USDA	Dekkers, J
04/17-03/18	Association of the Presence of Influenza A Virus in Pigs at Weaning with Post-Weaning Performance and Cost of Production	BOEHRINGER INGLEHEIM VETMEDICA, INC.	Culhane, M
07/2015 – 08/2017	Development of a live attenuated PEDV vaccine for weanling pigs	Emerging and Zoonotic Infectious disease signature program grant	Cheeran

E. WORK PLANNED FOR NEXT YEAR

- To investigate the role of neutralizing antibodies in PRRSV cross-protection.
- To investigate host factors associated with PRRSV susceptibility and resistance.
- To investigate host factors associated with PRRSV susceptibility and resistance.
- To determine infection incidence in growing pigs, specifically to identify when and how often new PRRSV infections happen in wean-to-finish pigs
- To evaluate risk factors associated to PRRSV infection in growing pigs.
- To associate production and economic impact of PRRSV infections in growing pigs. to investigate host factors associated with PCV2 susceptibility and resistance.
- To build a risk based model of porcine virus transmission in feed ingredients.
- To formulate models for forecasting risk for PRRSV spread
- To formulate models for between-farm transmission of exotic viruses
- To evaluate mechanisms of influenza virus transmission and persistence in piglets
- To evaluate the effect of maternally derived antibodies against influenza A virus on infection dynamics in growing pigs
- To investigate patterns and dynamics of influenza A virus transmission in growing pigs
- To investigate farm factors associated with influenza A virus detection in piglets at weaning
- To investigate the bi-directional transmission of influenza A virus between pigs and people
- To evaluate the impact of vaccination on influenza A virus genetic and antigenic diversity in piglets
- To evaluate strategies of vaccination to control influenza in piglets at weaning
- To investigate methodologies and approaches to inactivate airborne viruses
- To develop and optimize methods to assess virus containing particles in animal agriculture
- To develop antibody reagents that can distinguish *Mycoplasma hyopneumonia* in a standard ELISA test.
- To develop a diagnostic GMR biosensor array that can detect influenza, PRRSV and *Mycoplasma hyopneumoniae* in clinical samples.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

In a collaborative research project with Dr. Ying Fang, Kansas State University, we evaluated the efficacy of concurrent but consecutive vaccination of type 1 and type 2 PRRSV in pigs

In the US, both North American (Type 2) and European (Type 1) PRRSV are circulating in swine herds. Our collaborative study has evaluated the efficacy of consecutive and concurrent vaccination of pigs with modified live Type 1 and Type 2 PRRSV vaccine candidates. Results indicated that vaccination of pigs with both PRRSV genotypes at 3 days apart (type 1 MLV followed by type 2 MLV) provides better immune protection and clearance of both the viral infections than those pigs vaccinated simultaneously with both type 1 and type 2 MLVs.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

This study demonstrated that the consecutive vaccination with modified PRRSV Type 1 followed by Type 2 provides satisfactory protection against both the viruses.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

1. Dhakal, S, J. Hiremath, K. Bondra, Y.S. Lakshmanappa, D. Shyu, K. Oyuang, K. Kang, B. Binjawadagi, J. Goodman, K. Tabynov, S. Krakowka, B. Narasimhan, C.W. Lee and **G.J. Renukaradhya** (2017). Biodegradable nanoparticle delivery of inactivated swine influenza

virus vaccine provides heterologous cell-mediated immune response in pigs. J Control Release, 247:194-205. PMID: 28057521

2. Dhakal, S, J. Goodman, K. Bondra, Y.S. Lakshmanappa, J. Hiremath, D. Shyu, K. Oyuang, K. Kang, S. Krakowka, M.J. Wannemuehler, C.W. Lee, B. Narasimhan and **G.J. Renukaradhya** (2017). Polyanhydride nanovaccine against swine influenza virus in pigs. Vaccine, 35(8): 1124-1131. PMID: 28117173

2) Abstracts or Proceedings

1. Lunney J.K, M. Bailey, J. Manirarora, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang and L. Wooldridge. US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies. Abstract #263, 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
2. Dhakal, S and **G.J. Renukaradhya**. PLGA nanoparticle delivery of inactivated swine influenza virus vaccine provides heterologous protection through cell-mediated immunity in pigs. Abstract #102, AAVI mini-symposium and 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
3. Dhakal, S, J. Goodman, K. Bondra, Y.S. Lakshmanappa, J. Hiremath, D. Shyu, K. Oyuang, K. Kang, S. Krakowka, M.J. Wannemuehler, C.W. Lee, B. Narasimhan and **G.J. Renukaradhya** (2016). Polyanhydride nanovaccine against swine influenza virus in pigs. Abstract #117, AAVI mini-symposium and 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
4. Francis, O, M. Bailey, L. Wooldridge, J. Manirarora, G.J. Renukaradhya, S. Kenney, J. Labresh, Y. Sang, J.K. Lunney. Development of new immune reagents for swine health, vaccine and disease studies. British Society for Immunology and Dutch Society for Immunology, Annual Congress meeting, Liverpool, UK, December 6-9, 2016.
5. Lee, C.W and **G.J. Renukaradhya**. Universal Influenza Vaccine. Represented OARDC, The Ohio State University to the US Capital Hill Washington DC meeting attended by US senators and USDA team, April 5, 2017.
6. Dhakal, S, K. Bondra, D. Shyu, K. Tabynov, C.W. Lee and **G.J. Renukaradhya**. Intramuscular route of delivery of PLGA-nanoFlu vaccine improves antibody response in pigs. OARDC Research Conference, The Ohio State University, Wooster, Ohio. April 20, 2017.
7. Dhakal, S, J. Hiremath, K. Bondra, Y.S. Lakshmanappa, D. Shyu, K. Oyuang, K. Kang, B. Binjawadagi, J. Goodman, K. Tabynov, S. Krakowka, B. Narasimhan, C.W. Lee and **G.J. Renukaradhya**. Biodegradable nanoparticle delivery of inactivated swine influenza virus vaccine provides heterologous cell-mediated immune response in pigs. Abstract # 263, Immunology 2017, AAI meeting, Washington Convention Center, Washington DC, May 12-16, 2017.
8. Manirarora, J, M. Bailey, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang, O. Francis, L. Wooldridge, and J.K. Lunney. US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies. Immunology 2017, AAI meeting, Washington Convention Center, Washington DC, May 12-16, 2017.
9. Lakshmanappa, Y.S, P. Shang, S. Dhakal, S. Renu, B. Hogshead, P. Bernardo, X. Yan, Y. Fang and **G.J. Renukaradhya**. Concurrent but consecutive vaccination of modified live type 1

and type 2 PRRSV provides better protection in nursery pigs. Abstract #44, North American PRRS Symposium Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference, December 1-3, 2017, Chicago, IL.

10. Lunney J.K, M. Bailey, J. Manirarora, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang, O. Francis and L. Wooldridge. US-UK Collaborative Swine Immune Toolkit Initiative: Development and characterization of immune reagents for swine health, vaccine and disease studies. North American PRRS Symposium Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference, December 1-3, 2017, Chicago, IL.

3) Book Chapters or Monographs

1. **Book Chapter:** Garmendia, A.E, W. Mwangi and **G. J. Renukaradhya**. Chapter 29 – Porcine Reproductive and Respiratory Syndrome. Veterinary Vaccines for Livestock - 1st Edition, FAO. Editors - Samia Metwally, Ahmed Elldrissi and Gerrit Viljoen, Elsevier Publication 2017.

D. FUNDING SOURCES

1. **Funding agency:** USDA-AFRI, 2013-67015-20476 (MPI) (\$2,351,639) (RG share \$600,000)
Period: 11/01/2012 – 01/31/2018
Role: Multiple Principal Investigators; Chang-Won Lee (contact) and Renukaradhya Gourapura
Title of the project: Universal Flu Vaccine by a Norovirus P Particle Platform
2. **Funding agency:** USDA-AFRI US-UK grant (\$500,000) (RG share \$69,568), 2015-67015-23216 and BBSRC grant BB/M028232/1
Period: 04/1/2015 to 03/31/2018
Role: **PI:** Lunney, JK; **Co-PIs:** Bailey, M; Gourapura, RJ; LaBresh, JW; Sang, Y; Kenney, S.
Title of the project: Swine Immune Toolkit: Development of new immune reagents for swine health, vaccine and disease studies
3. **Funding agency:** USDA-AFRI, 2017-67015-26909, \$500,000 (RG share \$88,119)
Period: 08/15/2017 – 08/14/2020
Role: Co-Principal Investigator, PI: Diego Diel
Title of the project: A Multi-Species Vaccine Delivery Platform for Infectious Disease Prevention and Control in Livestock

E. WORK PLANNED FOR NEXT YEAR

1. Investigate the mechanisms involved in induction of protective mucosal response by nanoparticle based influenza virus vaccine candidates delivered intranasally in pigs.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: Kansas State University

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Role of nsp2 frameshifting (Fang). Our previous studies identified two novel PRRSV proteins, nsp2TF and nsp2N, which were expressed by novel -2/-1 programmed ribosomal frameshifting (PRF) mechanism. During the past year, we performed in depth analysis on the role of nsp2TF/nsp2N in suppressing host innate immune responses. We also assessed the potential application of nsp2TF-deficient mutants in MLV vaccine development. In a nursery pig model, the mutant virus-immunized pigs showed reduced lung lesion and also lower levels of viral loads in lung and tonsil at 14 days post challenge.

Novel PRRS vaccine (Fang). New PRRS vaccine construction strategies have been explored during the past two years. Collaborating with Dr. Biao He at University of Georgia, parainfluenza virus 5 (PIV5) vector-based PRRS vaccine is under development.

Knockout of maternal CD163 protects fetuses from infection (Rowland). CD163-positive fetuses, recovered between 109 days of gestation or 20 days after maternal infection, were completely protected from PRRSV in dams possessing a complete knockout of the CD163 receptor. The results demonstrate a practical means to eliminate PRRSV-associated reproductive disease, a major source of economic hardship to agriculture.

Peptide sequences in SRCR domain 5 of porcine CD163 involved in infection with PRRSV. HEK293T (HEK) cells transfected with domain-deleted constructs fused to enhanced green fluorescent protein (EGFP) were infected with a PRRSV-2 isolate expressing a red fluorescent protein (RFP). The results showed that cells expressing a deletion of the 101 amino acid SRCR5 or the 16 amino acid PSTII domain did not support infection. Insertion of proline-arginine (PR) dipeptides along the SRCR5 polypeptide was used to probe secondary and tertiary structures within SRCR5 involved in infection. The results from this study identify likely contact regions in

SRCR5 involved in forming the interaction between CD163 and the corresponding PRRSV protein.

Fecal microbiota transplantation improves outcome in nursery pigs (Niederwerder).

Previous work demonstrated an association between increased microbiome diversity and improved outcome characteristics following co-infection with PRRSV and PCV2, including reduced virus replication, improved weight gain, and decreased clinical disease. The current work focuses on modulating the microbiome composition through fecal microbiota transplantation (FMT). Morbidity and mortality due to PCVAD was reduced in pigs receiving FMT from a healthy high parity sow. The FMT pigs also possessed high antibody titers and reduced lung lesions. FMT represents a new strategy for improving outcomes following co-infections with PRRSV.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

E2 vaccine and companion ELISA for classical swine fever (Shi). We are developing and testing a novel E2-subunit vaccine for classical swine fever (CSF) that can be produced safely and cost-effectively in CSF free countries. We are also developing a unique ELISA that can differentiate CSF virus infected pigs from the pigs vaccinated with C-strain vaccines or E2 subunit vaccines.

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

Adenovirus-vectored novel African Swine Fever Virus multi-antigen cocktail elicit strong but non-protective immune responses in commercial pigs (Mwangi). Previous work focused on demonstrating the immunogenicity of seven adenovirus-vectored novel ASFV antigens formulated as a single vaccine. The cocktail primed strong ASFV antigen-specific IgG responses, which were recalled upon boosting. However, upon challenge with ASFV Georgia, vaccinated pigs had higher mean clinical scores, mean body temperatures, and decreased WBC counts as compared to the controls. Overall, the data suggest that the ASFV-antigen specific antibodies induced in the pigs enhanced ASF disease. The development of a protective ASFV subunit vaccine will require an immunization strategy that will elicit strong cytotoxic T lymphocyte response while limiting humoral immunity.

Risk of transboundary movement of ASFV via contaminated feed ingredients

(Niederwerder and Rowland). In collaboration with Scott Dee at Pipestone, we developed a model to study whether viruses, such as ASFV, CSFV and others, when mixed with feed ingredients could remain viable under the time and environmental conditions encountered during a trans-Atlantic shipment to the US. By using this model, we have shown that ASFV is capable of surviving the journey, suggesting that certain feed ingredients could serve as vehicles for infectious agents, thus posing a significant threat to the US swine industry.

Risk of African swine fever virus (ASFV) transmission in feed (Niederwerder and Rowland).

It is known that ASFV can be transmitted via the oral route through ingestion of swill or experimental inoculation. However, very little is known about the risk of ASFV Georgia 2007 transmission in contaminated feed. One important possibility is that the Georgia isolate may possess unique properties related to the stability of the virus in the environment. The goal of this work is to determine the median infectious dose (ID₅₀) for ASFV Georgia 2007 through oral exposure via natural drinking and eating behavior. Progress relates to the establishment of protocols for the propagation and detection of ASFV.

Mitigation of foreign animal disease introduction in feed (Niederwerder and Rowland). The goals of this project are to: 1) develop baseline data for the effectiveness of mitigants on the inactivation of ASFV, CSFV and Chinese PRV; 2) test candidate mitigants in a pig oral inoculation model via natural feeding behavior; and 3) evaluate the effectiveness of mitigants on inactivation of viruses in a transboundary model that simulates conditions when feed ingredients are shipped from another country. Progress to date includes the testing of medium chain fatty acids on their ability to inhibit ASFV infection *in vitro*.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Non-MLV CSF vaccines based on E2 that are effective create the opportunity to vaccinate pigs in CSF-free countries

The highly efficient -2/-1 programmed ribosomal frameshifting (PRF) mechanism, by which PRRSV efficiently produces novel proteins, nsp2TF and nsp2N can be applied to the development of new MLV vaccines for PRRS.

Diagnostic reagents and assays developed in our recent studies, including monoclonal antibodies, the pathogen array system, and diagnostic assays, provide important tools in emerging pathogen discovery, control and prevention.

Blocking PRRSV infection through the genetic modification of CD163 demonstrates a practical means to prevent PRRS.

The manipulation of the pig microbiome creates opportunities to improve animal health and provide alternatives to antibiotics and other growth promoters.

Understanding how pathogens are transmitted in feed and the development of interventions may prevent the introduction of the next “PEDV” –like outbreak.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

Refereed Publications:

Burakova, Y., Madera, R., McVey, S.; Schlup, J.R., Shi, J., (2017) Adjuvants for animal vaccines; *Viral Immunology*, available online 06/15/2017

Burakova, Y., Shi, J., Schlup, J.R. (2017) Impact of oil composition on formation and stability of emulsions produced by spontaneous emulsification; *Journal of Dispersion Science and Technology*, vol. 38 (12), 1749-1754

- Li, X., A. Galliher-Beckley, L. Wang, J. Nietfeld, W. Feng, J. Shi. 2017. Comparison of Immune Responses in Pigs Infected with Chinese Highly Pathogenic PRRS Virus Strain HV and North American Strain NADC-20. *Open Virol J* 11:73-82.
- Guo R., D. Davis, Y. Fang, 2017. Intercellular transfer of mitochondria rescues virus-induced cell death but facilitates cell-to-cell spreading of porcine reproductive and respiratory syndrome virus. *Virology*. In press.
- Li Y., P. Shang, D. Shyu, A. C. Carrillo, P. Naraghi-Arani, C. J. Jaing, R. Gourapura, A. E. Firth, E. J. Snijder, Y. Fang, 2017. Nonstructural proteins nsp2TF and nsp2N of porcine reproductive and respiratory syndrome virus (PRRSV) play important roles in suppressing host innate immune responses. *Virology*. In press.
- Shang P, S. Misra, B. Hause, Y. Fang. 2017. A Naturally Occurring Recombinant Enterovirus Expresses a Torovirus Deubiquitinase. *Journal of Virology*, Jun 26;91(14). Spotlight Feature.
- Kimpston-Burkgren K, I. Correias, F. A. Osorio, D. Steffen, A. K. Pattnaik, Y. Fang, H. L. X. Vu. 2017. Relative contribution of porcine reproductive and respiratory syndrome virus open reading frames 2-4 to the induction of protective immunity. *Vaccine*. Aug 3;35(34):4408-4413.
- Fowler V. L., R. H. Ransburgh, E. G. Poulsen, J. Wadsworth, D. P. King, V. Mioulet, N. J. Knowles, S. Williamson, X. Liu, G. A. Anderson, Y. Fang, J. Bai. 2017. Development of a novel real-time RT-PCR assay to detect Seneca Valley virus-1 associated with emerging cases of vesicular disease in pigs. *Journal of Virological Methods*. Jan;239:34-37.
- Niederwerder, M.C. 2017. Role of the microbiome in swine respiratory disease. *Vet Microbiol*. 209: 97-106.
- Niederwerder, M, RRR Rowland. 2017. Is there a risk for introducing porcine reproductive and respiratory syndrome virus (PRRSV) through legal trade in pork? *Food Environ Virol*. 9:1-13.
- Dunkelberger, JR, NVL Serão, Z Weng, EH Waide, MC Niederwerder, MA Kerrigan, JK Lunney, RRR Rowland, JCM Dekkers. 2017. Genomic regions associated with host response to porcine reproductive and respiratory syndrome vaccination and co-infection in nursery pigs. *BMC Genomics*. 18:865
- Prather, RS, KD Wells, KM Whitworth, MA Kerrigan, MS Samuel, A Mileham, LN Popescu, RRR Rowland. 2017. Knockout of maternal CD163 protects fetuses from infection with porcine reproductive and respiratory syndrome virus (PRRSV). *Sci Rep*. In press.
- Jaing, C, RRR Rowland, JE Allen, A Certoma, JB Thissen, J Bingham, B Rowe, JR White, J Wynne, D Johnson, N Gaudreault, DT Williams. 2017. Gene expression analysis of whole blood RNA from pigs infected with low and high pathogenic African swine fever viruses. *Nature Sci Rep*. in press.
- Heimerman, M, MV Murgia, P Wu, AD Lowe, W Jia, RRR Rowland. 2017. Linear epitopes in African swine fever virus (ASFV) p72 recognized by monoclonal antibodies prepared against baculovirus expressed antigen. *J Vet Diagn Invest*. In press.
- Popescu, LN, BR Tribble, N Chen, RRR Rowland. 2017. GP5 of porcine reproductive and respiratory syndrome virus (PRRSV) as a target for homologous and broadly neutralizing antibodies. *Vet Microbiol*. In press.
- Dekkers, J, Rowland RR, JK Lunney, G Plastow. 2017. Host genetics of response to porcine reproductive and respiratory syndrome in nursery pigs. *Vet Microbiol*. In press.
- Dunkelberger JR, VN Serão, MC Niederwerder, MA Kerrigan, JK Lunney, RR Rowland, JC Dekkers. 2017. Effect of a major quantitative trait locus for porcine reproductive and

- respiratory syndrome (PRRS) resistance on response to coinfection with PRRS virus and porcine circovirus type 2b (PCV2b) in commercial pigs, with or without prior vaccination for PRRS. *J Anim Sci.* 95:584-598.
- Waide, EH, CK Tuggle, NV Serão, M Schroyen, A Hess, RR Rowland, JK Lunney, G Plastow, JC Dekkers. 2017. Genomewide association of piglet responses to infection with one of two porcine reproductive and respiratory syndrome virus isolates. *J Anim Sci.* 95:16-38.
- Wells, KD, R Bardot, K M Whitworth, BR Tribble, Y Fang, A Mileham, MA Kerrigan, MS Samuel, RS Prather, RRR Rowland. 2017. Substitution of porcine CD163 SRCR domain 5 with a CD163-like homolog confers resistance of pigs to genotype 1 but not genotype 2 porcine reproductive and respiratory syndrome (PRRS) viruses. *J Virol.* In press.
- Popescu, L, NN Gaudreault, K Whitworth, M Murgia, JC Nietfeld, A Mileham, M Samuel, KD Wells, RS Prather, RR Rowland. 2017. Genetically edited pigs lacking CD163 show no resistance following infection with the African swine fever virus isolate, Georgia 2007/1. *Virology.* 501:102-106.
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Abstracts or Proceedings

- Burakova, Y., Madera R., Wang L., Schlup, J., Shi, J. Combination adjuvants in subunit veterinary vaccines. 3rd International Conference on Vaccine Research and Development, Washington D.C., November 14th, 2017 (Oral presentation).
- Wang, L., R. Madera, Y. Burakova, S. Buist, Y. Sang, J. Nietfeld, J. Henningson, W. Gong, C. Tu, J. Shi. "Recombinant PRRSV P129 expressing CSFV E2 glycoprotein enhanced pathogenesis of CSFV in pigs"; 3rd International Conference on Vaccine Research and Development, Washington D.C., November 14th, 2017 (Oral presentation).
- Madera R, Gong W, Wang L, Burakova Y, Llellish K, Galliher-Beckley A, Nietfeld J, Henningson J, Jia K, Li P, Bai J, Schlup J, McVey S, Tu C, Shi J. Towards the development of a one-dose classical swine fever sub-unit vaccine; 3rd International Conference on Vaccine Research and Development, Washington D.C., November 14th, 2017 (Oral presentation).
- Rui Guo, Benjamin B. Katz, John M. Tomich, Duane Davis, Tom Gallagher and Ying Fang. 2017. Tunneling nanotubes provide a unique conduit for intercellular spread of PRRSV infection: A novel pathways for nidovirus transmission. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Ian Brierley, Andrew E. Firth, Jens Kuhn, Ying Fang. 2017. Characterization of a -2/-1 programmed ribosomal frameshifting in simian arteriviruses. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Duan-Liang Shyu, Pengcheng Shang, Jianfa Bai, Kang Ouyang, Santosh Dhakal, Jagadish Hireamt, Basavaraj Binjawadagi, Gourapura J. Renukaradhya, Ying Fang. 2017. Mutations in a highly conserved motif of nsp1beta protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV). XIV International Nidovirus Symposium, Kansas City, KS.

- Xinyu Yan, Tao Wang, Ying Fang. 2017. Generation and characterization of monoclonal antibodies against simian hemorrhagic fever virus nonstructural protein 2. XIV International Nidovirus Symposium, Kansas City, KS.
- Pengcheng Shang, Saurav Misra, Ben Hause, Ying Fang. 2017. A naturally occurring recombinant enterovirus expresses torovirus protease. XIV International Nidovirus Symposium, June 1-6, 2017, Kansas City, KS; North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Rui Guo, Pengcheng Shang, Celena A. Carrillo, Xingyu Yan, Tao Wang, Crystal J. Jaing, Ying Fang. 2017. Double-stranded viral RNA as a potential mediator for the persistence of porcine reproductive and respiratory syndrome virus. XIV International Nidovirus Symposium, Kansas City, KS.
- Pengcheng Shang, Yanhua Li, Ian Brierley, Andrew E. Firth and Ying Fang. 2017. RNA stem-loop structures and conserved region in PRRSV ORF6 are important for virus replication. XIV International Nidovirus Symposium, June 1-6, 2017, Kansas City, KS; North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Fangfeng Yuan, Rui Guo, Yanhua Li and Ying Fang. 2017. Potential role of porcine reproductive and respiratory syndrome virus structural protein nsp1alpha in mitochondrion dysfunction. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Sawsan Naphthine, Emmely E. Treffers, Pengcheng Shang, Ali Tas, Andrew E. Firth, Ian Brierley, Eric J. Snijder and Ying Fang. 2017. A novel mechanism of protein-stimulated trans-activation of ribosomal frameshifting in porcine reproductive and respiratory syndrome virus: implication in improved vaccine development. XIV International Nidovirus Symposium, Kansas City, KS.
- Emmely E. Treffers, Sawsan Naphthine, Yanhua Li, Ali Tas, Susanne Bell, Brian Mark, Andrew Firth, Ying Fang, Ian Brierley, Eric J. Snijder. 2017. A viral protein and poly-(C) binding proteins direct efficient -2 and -1 programmed ribosomal frameshifting at the same site in arterivirus genomes. EMBO conference: Protein Synthesis and Translational Control. Heidelberg, Germany.
- Yanhua Li, Andrew E. Firth, Ian Brierley, Eric Snijder, Jens Kuhn, Ying Fang. 2017. A dual ribosomal frameshifting mechanism transactivated by an arterivirus protein and host cellular factors. USDA project director meeting, December 1, 2017; North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Yashavanth Shaan Lakshmanappa, Pengcheng Shang, Santosh Dhakal, Sankar Renu, Bradley Hogshead, Pauline Bernardo, Xinyu Yan, Ying Fang and Renukaradhya J Gourapura. 2017. Concurrent but consecutive vaccination of modified live type 1 and type 2 PRRSV provides better protection in nursery pigs. North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Rui Guo, Pengcheng Shang, Celena A. Carrillo, Xingyu Yan, Tao Wang, Crystal J. Jaing, Megan Niederwerder, Raymond R. Rowland, Ying Fang. 2017. Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV. North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Pengcheng Shang, Rui Guo, Celena A. Carrillo, Crystal J. Jaing, Ying Fang. 2017. Multiplexed digital mRNA profiling of immune responses in pigs persistently infected with porcine

- reproductive and respiratory syndrome virus. North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Yanhua Li, Ying Fang, Chase Stahl, Jianfa Bai, Xuming Liu, Lalitha Peddireddi, Gary Anderson. 2017. Development of a real-time RT-PCR assay for detecting porcine parainfluenza virus 1 infection in pigs. 2017 North American PRRS Symposium on PRRS, Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference. December 1-3, 2017, and 98th Conference of Research Workers in Animal Disease. December 3-5, 2017, Chicago, IL.
- Yin Wang, Fangfeng Yuan, Wanglong Zheng, Elizabeth Poulsen, Xuming Liu,, Lalitha Peddireddi, Richard Hesse, Ying Fang, Gary Anderson, and Jianfa Bai. 2017. Genetic diversity and molecular detection assay development for PCV3 and PCV2 strains. 98th Conference of Research Workers in Animal Disease. December 3-5, 2017. Chicago, IL.
- Yin Wang, Wanglong Zheng, Elizabeth Poulsen, Xuming Liu, Jianqiang Zhang, Kyoung-Jin Yoon, Lalitha Peddireddi, Richard Hesse, Ying Fang, Gary Anderson, and Jianfa Bai. 2017. Development of a Luminex Multiplex Assay for the Detection and Differentiation of Type 2 PRRSV Field Strains and the Four US Vaccine Strains. 2017 North American PRRS Symposium on PRRS, Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference. December 1-3, 2017, and 98th Conference of Research Workers in Animal Disease. December 3-5, 2017, Chicago, IL.
- Yin Wang, Bin Yang, Wanglong Zheng, Elizabeth Porsen, Xuming Liu, Ying Fang, Gary Anderson and Jianfa Bai. 2017. Development and validation of a multiplex real-time PCR assay for the detection and differentiation of FMDV and SVV strains. 98th Conference of Research Workers in Animal Disease. December 3-5, 2017. Chicago, IL.
- Xuming Liu, Hewei Zhang, Yin Wang, Yanhua Li, Yuekun Lang, Elizabeth Poulsen, Lance Noll, Nanyan Lu, Russell Ransburgh, Wanglong Zheng, Wenjun Ma, Lalitha Peddireddi, Ying Fang, Gary Anderson, Jianfa Bai. A multiplex real-time RT-PCR assay for simultaneous detection and differentiation of Influenza D, C, B and A viruses in swine and cattle. 2017 North American PRRS Symposium on PRRS, Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference. December 1-3, 2017, and 98th Conference of Research Workers in Animal Disease. December 3-5, 2017, Chicago, IL.
- X. Liu, Y. Wang, X. Shi, R. Ransburgh, H. Zhang, L. Noll, E. Poulsen, V. Pasupuleti, A. Karczmarek, T. Nagaraja, D. Renter, Y. Fang, G. Anderson, J. Bai. 2017. Multiplex molecular serotyping of *Salmonella* using Luminex xMAP assay and Check&Trace *Salmonella* kit comparing with serological method. 98th Conference of Research Workers in Animal Disease. December 3-5, 2017. Chicago, IL.
- Fangfeng Yuan, Xuming Liu, Jianfa Bai, Gary Anderson, Ying Fang, Bailey Arruda, Paulo Arruda, Lalitha Peddireddi. 2017. Development and validation of a real time RT-PCR assay for the detection of Atypical Porcine Pestivirus. 2017 North American PRRS Symposium on PRRS, Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference. December 1-3, 2017, and 98th Conference of Research Workers in Animal Disease. December 3-5, 2017, Chicago, IL.
- Yin Wang, Feangfeng Yuan, Elizabeth Porsen, Russel Ransburgh, Wanglong Zheng, Xuming Liu, Nanyan Lu, Lalitha Peddireddi, Ying Fang, Gary Anderson and Jianfa Bai. 2017. Development and validation of a multiplex real-time PCR assay for the detection and

- differentiation of PCV2 and PCV3 strains. 2017 AAVLD / USAHA Annual Meeting. October 12-18, 2017. San Diego, CA.
- Peddireddi. 2017. Development of a quantitative real time RT-PCR assay for sensitive detection of emerging Atypical Porcine Pestivirus associated with congenital tremor in pigs. 2017 AAVLD / USAHA Annual Meeting. October 12-18, 2017. San Diego, CA.
- Huiling Wei, Pengcheng Shang, Yanhua Li, Sarah Zaiser, Pratik Katwal, Victor C. Huber, Ying Fang, Biao He. 2017. Developing a parainfluenza virus 5 (PIV5)-based PRRSV vaccine. USDA project director meeting, December 1, 2017, Chicago, IL.
- Popescu, L, BR Tribble, N Chen, RRR Rowland. 2017. GP5 of porcine reproductive and respiratory syndrome virus (PRRSV) as a target for homologous and broadly neutralizing antibodies. XIVth International Nidovirus Symposium, June 4-9, Kansas City, MO.
- Stoian, A, J Springfield, RRR Rowland. 2017. CD163 SRCR5 and PSTII domains are involved in recognition by Type II Porcine Reproductive and Respiratory Syndrome virus (PRRSV). XIVth International Nidovirus Symposium, June 4-9, Kansas City, MO.
- Gaudreault NN, MV Murgia, LN Popescu, V Petrovan, M Phillips, A Stoian, M Mogler, RRR Rowland. 2017. Novel prime boost approach incorporating vector based and modified live vaccines to improve protection against African swine fever virus. International Conference on Emerging Zoonoses. May 7-10, Manhattan, KS.
- Stoian, A, LN Popescu, NN Gaudreault, MV Murgia, RRR Rowland. 2017. CD163 on macrophages is a receptor for porcine reproductive and respiratory syndrome virus but not for African swine fever virus. 8th International Conference on Zoonoses, May 7-10, Manhattan, KS.
- Rowland, RRR, C Jaing, J Allen, A Certoma, JB Thissen, J Bingham, B Rowe, JR White, JW Wynne, D Johnson, DT Williams. 2017. Gene expression analysis of whole blood from pigs infected with low and high pathogenic African swine fever viruses. 8th International Conference on Emerging Zoonoses, May 7-10, Manhattan, Kansas.
- Jaing, C, J Allen, JB Thissen, RRR Rowland, D Williams. 2016. Whole Transcriptome Analysis of Pigs Infected with African Swine Fever Virus. 2016 Global African Swine Fever Alliance (GARA), Ploufragan, France.
- Niederwerder, M, CJ Jaing, RA Oberl, JB Thissen, AG Cino-Ozuna, KS McLoughlin, RRR Rowland. 2016. The role of the microbiome in PRRS. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Hossain, M, RRR Rowland. 2016. Development of a new strategy for detection of classical swine fever virus antibodies in alphavirus based replicon particle derived E2 and Erns vaccinated swine. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Petrovan, V, P Shang, F Yuan, MV Murgia, Y Fang, RRR Rowland. 2016. Development, characterization and diagnostic application of monoclonal antibodies against ASFV p30. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Popescu, L, N Gaudreault, RRR Rowland. 2016. Oral fluids diagnostic methods for chronic Classical swine fever virus. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Chen Z, F Yuan, Y Li, P Shang, R Schroeder, K Lechtenberg, J Henningson, B Hause, J Bail, RRR Rowland, A Clavijo, Y Fang. 2016. Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A. 2016 North American PRRSV Symposium, December 3-4, Chicago.

- Constance, LM, B Bloomberg, JK Lunney, JCM Dekkers, RRR Rowland, MC Niederwerder. 2016. Comparison of morbidity and mortality after challenge with two North American PRRS virus isolates shows marked variation in time course and prevalence of clinical disease between isolates. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Dong, J, J Lunney, E Fritz-Waters, Y Nguyen, RRR Rowland, A Hess, J Reecy, J Dekkers. 2016. The effect of PRRS viral level and isolate on tonsil gene expression 42 days after infection. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Popescu, L, NN Gaudreault, KM Whitworth, MV Murgial, JC Nietfeld, A Mileham, M Samuel, KD Wells, RS Prather, RRR Rowland. 2016. Genetically edited pigs lacking CD163 show no resistance following infection with the African Swine Fever Virus isolate, Georgia 2007/1. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Ke, H, M Kerrigan, M Han, RRR Rowland, D Yoo. 2016. SAP domain in nsp1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) correlates with interferon suppression in cells and pathogenesis in pigs. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Liu, Q, J Lee, W Ma, RRR Rowland, F Blecha, Y Sang. 2016. Antiviral potency and functional novelty of porcine Interferon-Omega subtype. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Murgia, MV, NN Gaudreault, RRR Rowland. 2016. Identification of African swine fever virus p30 antigenic epitopes after experimental infection. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Shang, P, A Avila, R Guo, SK Whitaker, Y Li, J Tomich, RRR Rowland, Y Fang. Development of novel chimeric vaccine and delivery system for classical swine fever virus. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Stoian, A, RRR Rowland. 2016. Identification of CD163 domain involved in the infection with Type II porcine reproductive and respiratory viruses. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Dee, S., F.V. Bauermann, M.C. Niederwerder, A. Singrey, T. Clement, M. de Lima, G. Patterson, M.A. Sheahan, Ana M.M. Stoian, Vlad Petrovan, C.K. Jones, J. DeJong, J. Ji, G.D. Spronk, J. Christopher-Hennings, J.J. Zimmerman, R.R.R. Rowland, E. Nelson, P. Sundberg, and D.G. Diel. 2017. Evaluation of the survival of viral pathogens in contaminated feed ingredients using transboundary shipment models. Oral presentation, 98th Conference of Research Workers in Animal Diseases, Chicago, IL. Abstract #78.
- Guo, R., P. Shang, C.A. Carrillo, X. Yan, T. Wang, C.J. Jaing, M. Niederwerder, R.R.R. Rowland, and Y. Fang. 2017. Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV. Oral and poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Dunkelberger, J.R., N.V.L. Serão, M. Niederwerder, M. Kerrigan, M. Schroyen, C.K. Tuggle, J. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Genomic prediction of a PRRS-vaccinated training population to predict host response to PRRS virus-only or PRRS virus/PCV2b co-infection. Poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Constance, L.A., J.B. Thissen, C.J. Jaing, K.S. McLoughlin, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Pre-challenge microbiome composition is associated with improved weight gain in pigs after vaccination with a porcine reproductive and respiratory syndrome (PRRS) modified live virus (MLV) vaccine followed by challenge with PRRSV

- and porcine circovirus type 2 (PCV2b). Poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C., R.R.R. Rowland, L.A. Constance, M.L. Potter, M.A. Kerrigan, R.A. Hesse, and A.G. Cino-Ozuna. 2017. Fecal microbiota transplantation improves outcome in nursery pigs following co-infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2d. Oral and poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C., D. Diel and S. Dee. 2017. Novel approaches for assessing the risks of importing viruses from other countries through feed and feed ingredients. Invited program talk, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C. 2017. Role of the Microbiome in Porcine Respiratory Disease. Invited program talk, Swine Day, Animal Sciences and Industry, Kansas State University, Manhattan, KS.
- Niederwerder, M.C. 2017. Risk of Virus Introduction and Transmission in Feed. Invited program talk, Swine Day, Animal Sciences and Industry, Kansas State University, Manhattan, KS.
- Constance, L.A., J.B. Thissen, C.J. Jaing, K.S. McLoughlin, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Role of the gut microbiome in response to vaccination and viral respiratory infection in growing pigs. Poster presentation, Research and the State Forum, Kansas State University, Manhattan, KS. *Awarded as a Top 10 Presentation for showcase at the state capitol.
- Dunkelberger, J.R., N.V.L. Serão, M. Niederwerder, E. Waide, M. Kerrigan, M. Schroyen, C.K. Tuggle, J.K. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Pigs selected for increased natural resistance to PRRS are more resistant to PRRSV/PCV2b co-infection. Poster Presentation, Allen D. Leman Swine Conference, St Paul, MN.
- Niederwerder, M.C. 2017. Role of the microbiome in respiratory disease. Invited general session talk and Proceedings, 50th Annual Conference of the American Association of Bovine Practitioners, Omaha, NE.
- Ober, R.A., J.B. Thissen, C.J. Jaing, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Increased microbiome diversity is associated with improved growth rates of pigs after co-infection with PRRSV/PCV2. Poster presentation, XIVth International Nidovirus Symposium, Kansas City, MO.
- Jaing, C.J., J.B. Thissen, K.S. McLoughlin, M.C. Niederwerder, R.R.R. Rowland. 2017. Rapid disease diagnostics and surveillance using a broad-spectrum microbial detection array. Oral presentation, XIVth International Nidovirus Symposium, Kansas City, MO.
- Dunkelberger, J.R., N.V. Serão, Z.Q. Weng, M.C. Niederwerder, E.H. Waide, M.A. Kerrigan, J.K. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Biological evidence for genomic regions associated with host response to co-infection with PRRS virus and PCV2b in commercial nursery pigs. Oral presentation, Breeding and Genetics session, American Society of Animal Science Midwest Meeting, Omaha, NE. *J Anim Sci* 95: supplement 2: 16-16. doi:10.2527/asasmw.2017.034.
- Constance, L.A., B. Bloomberg, J.K. Lunney, J.C.M. Dekkers, R.R.R. Rowland, and M.C. Niederwerder. 2017. Comparison of morbidity and mortality after challenge with two North American PRRS virus isolates shows marked variation in time course and prevalence of clinical disease between isolates. Selected as top 15 poster submitted; presented in the

Veterinary Student Poster Competition, American Association of Swine Veterinarians Annual Meeting, Denver, CO.

Niederwerder, M.C., C.J. Jaing, J.B. Thissen, A.G. Cino-Ozuna, K.S. McLoughlin, and R.R. Rowland. 2017. Microbiome associations in pigs with the best and worst clinical outcomes following co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Poster presentation, Research Topics Session, American Association of Swine Veterinarians Annual Meeting, Denver, CO.

Book Chapters or Monographs- none to report

D. FUNDING SOURCES

Fang, Y. A novel arterivirus protein and expression mechanism: implication in vaccine and companion diagnostic assay development (USDA-NIFA, 01/01/2015 – 12/31/2019; \$472,179).

Biao, H., Y. Fang. Developing a Parainfluenza Virus 5 (PIV5)-based PRRS Vaccine (USDA-NIFA, 1/1/2016 – 12/31/2018, \$450,000).

Fang, Y., G. Anderson. Generation of reagents for differentiation of swine pathogens (private company, 04/01/2015-03/31/2018, \$250,000).

Fang, Y., S. Baker. The XIV International Nidovirus Symposium (USDA-NIFA conference grant, 06/01/2017-05/31/2018, \$15,000).

Baker, S., Y. Fang. International Nidovirus Symposium (NIH R13 AI129358, 12/01/2016-12/30/2017, \$5,000).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li, M. Potter and G. Anderson. Development and evaluation of antibody detection assay for PCV3 virus (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$71,600).

Marthaler, D., Y. Fang, M. Murtaugh. Development and validation of a rapid ELISA against porcine teschovirus in serum and oral fluid (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$66,033).

Marthaler, D., Y. Fang, M. Murtaugh. Development and validation of ELISA against porcine sapelovirus in serum and oral fluid (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$66,033).

Liu, X., J. Bai, Y. Fang, W. Ma, L. Peddireddi, Y. Li and G. Anderson. Development of antibody detection assays for swine influenza B, C, and D viruses. (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$106,100).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li and G. Anderson. Detection and differentiation of Seneca Valley virus (SVV) from foot-and-mouth disease virus (FMDV). (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$65,700).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li and G. Anderson. Detection and differentiation of PCV3 from PCV2a, PCV2b and the highly prevalent PCV2d mutant strains. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$56,700).

Peddireddi, L., J. Bai, Y. Fang, X. Liu, R. Hesse, B. Hause, G. Anderson, B. Arruda and P. Arruda. Development of sensitive and reliable diagnostic assay to detect atypical porcine pestivirus (APPV) in swine. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$55,267).

Liu, X., J. Bai, Y. Fang, L. Peddireddi, Y. Li and G. Anderson. Multiplex real-time RT-PCR assay for simultaneous detection and differentiation of swine influenza C, D, and B viruses. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$58,500).

Li, Y., Y. Fang, J. Bai, X. Liu, L. Peddireddi, G. Anderson and C. Stahl. Development of a TaqMan quantitative RT-PCR test for porcine parainfluenza virus 1. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$56,500).

Bai, J., Y. Fang, X. Liu, J. Zhang, KJ. Yoon. Detection and Differentiation of Field Strains and Commonly used Vaccine Strains of Type 2 PRRSV in the U.S. (National Pork Board, 11/15/2016 – 11/14/2017, \$79,080).

Niederwerder. 2017 – 2018. “Fecal microbiota transplantation as an alternative tool for increasing porcine reproductive and respiratory syndrome (PRRS) vaccine efficacy and reducing the effects of PRRS.” College of Veterinary Medicine Success For Young Investigators Grant Program. Total Awarded Funding Amount: \$15,000.

Jones, Niederwerder, Rowland et al., 2017 – August 2018. “Validation of a low-cost tool for Senecavirus A detection, and surveillance of viral prevalence in United States feed mills.” Swine Health Information Center. Total Awarded Funding Amount: \$21,500.

Jones, Niederwerder et al., 2017 – April 30, 2018. “Assessing the role of medium chain fatty acids as an alternative to medically important antibiotics.” National Pork Board. Total Awarded Funding Amount: \$73,597.

Dee, Niederwerder, Rowland et al., Cassie Jones, and Steve Dritz. April 5, 2017 – April 4, 2018. “Evaluation of chemical mitigants for neutralizing the risk of foreign animal diseases in contaminated feed ingredients.” Swine Health Information Center. Total Awarded Funding Amount: \$120,000.

Niederwerder, Hesse. 2016 –2017. “Comprehensive Literature Review on the current knowledge for Porcine Epidemic Diarrhea Virus (PEDV) and Porcine Deltacoronavirus (PDCoV).” National Pork Board. Total Awarded Funding Amount: \$10,000.

Niederwerder. 2016 –2017. “Assessing microbiome diversity as a tool for the mitigation of viral disease in nursery pigs.” Kansas State University College of Veterinary Medicine Intramural Success for Young Investigators Grant. Total Awarded Funding Amount: \$14,995.

Niederwerder, Rowland, et al., Swine health Information Center (SHIC) and Kansas NBAF matching funds, 2017-2018, Assessing tools for the mitigation of foreign animal disease introduction and transmission in feed \$275,000.

Niederwerder, Rowland, et al., National Pork Board, NPB#17-057, 2017-2018 and Kansas NBAF matching funds, Assessing the risk of African swine fever virus (ASFV) transmission in feed. \$290,000

Dee (Niederwerder, Rowland) et al., Swine Health Information Center (SHIC) and Kansas NBAF matching funds, 2017-2018, Evaluation of the risk of transboundary movement of ASFV via contaminated feed ingredients. \$140,000.

Rowland, National Pork Board, NPB Project No. 17-160, 2017-2018, Adaptation of PRRSV to genetic modifications in CD163, \$66,000.

Rowland, Fang and Prather, USDA AFRI 2016-09462, 2017-2020, Preventing porcine reproductive and respiratory syndrome (PRRS) through modifications in the virus receptor, CD163, \$330,000.

Rowland and Prather, National Pork Board, NPB, 2016-2018, Genetic modifications in CD163 that confer complete resistance of pigs to infection with PRRSV, \$128,000.

Mwangi and Rowland, National Pork Board, 2017, Efficacy of prototype live-vectored polyvalent African swine fever virus vaccines, approximately \$200,000.

Rowland, NPPC, 2016-2017, Risk if SVA transmission by pig meat. \$40,000.
Mwangi and Rowland, USDA NIFA, 2016-2019 Protective efficacy of an adenovirus-vectored ASFV multi-antigen cocktail, Rowland budget = \$80,000
Shi. Evaluation of a plant-made CSFV vaccine during a challenge study in swine. iBio CMO, LLC. Bryan, TX 77807.
Shi. Characterization of mammalian inflammatory and innate immune responses to *Culicoides* *Sonorensis* cellular lipids and evaluate use of adjuvants. USDA ARS, AR9865

E. WORK PLANNED FOR NEXT YEAR

Continue to develop E2 CSF vaccines
To test PRF manipulation in highly pathogenic PRRSV field strains
Explore the new vector platform(s) for PRRS vaccine development
Develop diagnostic reagents and assays for emerging swine pathogens
Continue to work on the interaction between CD163 and PRRSV-1 and PRRSV-2 isolates
Seek additional resources and funding to evaluate the effect of microbiome manipulations on pig health following infection with PRRSV
Understand the risk and mitigation of ASFV and other transboundary diseases in pigs

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Zhang, Yanjin; University of Maryland; zhangyj@umd.edu

2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

Zhu, Xiaoping, UMD

Xiao, Zhengguo, UMD

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

1. We continued studying the atypical PRRSV strain, A2MC2, which is able to induce type I interferons in cultured cells. A2MC2 was found to induce higher level of neutralizing antibodies *in vivo* compared with the Ingelvac PRRS MLV and VR-2385. We discovered that the middle half of the A2MC2 genome is needed for triggering the IFN synthesis. First, a cDNA infectious clone of this atypical strain was constructed as a DNA-launched version. Virus recovery was achieved from the infectious clone and the recovered virus, rA2MC2, was characterized. The rA2MC2 retained the feature of interferon induction in cultured cells. Infection of pigs with the rA2MC2 virus caused viremia similar to that of the wild type virus. Chimeric infectious clones were constructed by swapping genomic fragments with a cDNA clone of a moderately virulent strain VR-2385 that antagonizes IFN induction. Analysis of the rescued chimeric viruses demonstrated that the middle two fragments, ranging from nt4545 to nt12709 of the A2MC2 genome, were needed for the IFN induction, whereas the chimeric viruses containing any one of the two A2MC2 fragments failed to do so. The results and the cDNA infectious clone of the IFN-inducing A2MC2 will facilitate further study of its biology, ultimately leading towards the development of an improved vaccine against PRRS.
- We have also continued our study on PRRSV interaction with the JAK/STAT pathway. We studied PRRSV effect on signal transducer and activator of transcription 3 (STAT3). STAT3 is known to play critical roles in cell growth, proliferation, differentiation, immunity and inflammatory responses. We discovered that PRRSV infection led to significant reduction of STAT3 protein level but had minimum effect on its transcripts. Further study showed that non-structural protein 5 (nsp5) of PRRSV induced the STAT3 degradation by increasing its polyubiquitination level and shortening its half-life from 24 h to approximately 3.5 h. The C-terminal domain of nsp5 was shown to be required for the STAT3 degradation. Moreover, the STAT3 signaling in the cells transfected with nsp5 plasmid was significantly inhibited. This study provides insight into the PRRSV

interference with the JAK/STAT signaling, leading to perturbation of the host innate and adaptive immune responses.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Our studies on the interferon-inducing PRRSV A2MC2 and construction of infectious cDNA clone are beneficial for vaccine development and biology study of this strain. Better protective immunity against PRRS is expected from an optimized A2MC2.

Our studies on STAT3 may contribute to our understanding of PRRSV interference of host immune response.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

1. Eve Fontanella, Zexu Ma, **Yan-Jin Zhang**, Alessandra Castro, Huigang Shen, Patrick G Halbur, Tanja Opriessnig: An interferon inducing porcine reproductive and respiratory syndrome virus vaccine candidate elicits protection against challenge with a heterologous virulent type 2 strain in pigs. *Vaccine*. 2017 Jan 3;35(1):125-131.
2. L. Yang, R. Wang, Z. Ma, Y. Xiao, Y. Nan, Y. Wang, S. Lin, and **Y. Zhang**: Porcine Reproductive and Respiratory Syndrome Virus Antagonizes JAK/STAT3 Signaling via Inducing STAT3 Degradation. *Journal of Virology* 2017, 91:e02087-16.
3. L. Yang and **Y. Zhang**. Antagonizing Cytokine-Mediated JAK-STAT Signaling by Porcine Reproductive and Respiratory Syndrome Virus. *Veterinary Microbiology* 209C (2017) pp. 57-65. *Review*
4. Z. Ma, Y. Yu, Y. Xiao, T. Opriessnig, R. Wang, L. Yang, Y. Nan, S. Samal, P. Halbur, and **Y. Zhang**: The Middle Half Genome of Interferon-Inducing Porcine Reproductive and Respiratory Syndrome Virus Strain A2MC2 Is Indispensable for Host Recognition. *Journal of General Virology* 2017 Jul;98(7):1720-1729.
5. Yuchen Nan, Chunyan Wu, Guoqian Gu, Weiyao Sun, **Yanjin Zhang** and En-Min Zhou Improved Vaccine against PRRSV: Current progress and future perspective. *Front. Microbiol.* 8:1635. *Review*

2) Abstracts or Proceedings

1. L. Yang, Z. Ma, and **Y. Zhang**: PRRSV Interference with the Cytokine-mediated JAK/STAT Signaling. *2016 PRRS Symp.*
2. E. Fontanella, Z. Ma, **Y. Zhang**, A. Castro, H. Shen, P. Halbur, T. Opriessnig: An interferon inducing PRRSV vaccine candidate protects against challenge with a heterologous virulent type 2 strain in a conventional pig model. *2016 PRRS Symp.*

3) Book Chapters or Monographs

Zexu Ma, Liping Yang, and **Yan-Jin Zhang**. Porcine Reproductive and Respiratory Syndrome Virus: Propagation and Quantification. *Current Protocols in Microbiology*. Book chapter. *In press*.

D. FUNDING SOURCES

Maryland Agricultural Experiment Station

E. WORK PLANNED FOR NEXT YEAR

We will continue to characterize the mechanism of PRRSV A2MC2 in inducing production of type I interferons and explore passaged A2MC2 for vaccine development. We will also continue to study the mechanism of PRRSV interference with innate immune response and examine PRRSV-host interactions.

Project Initiation

Title: Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine			
Accession No.	1006533	Sponsoring Institution	National Institute of Food and Agriculture
Project No.	VA-136308	Project Status	ACTIVE
Funding Source	Hatch/Multi State	Multistate No.	NC229
Start Date	07/01/2015	DUNS Number	003137015
Submitted By	Robin Williams	End Date	09/30/2019
		Date Submitted to NIFA	11/13/2017

Project Director

Kevin Lahmers

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Performing Organization/Institution

SAES - VIRGINIA POLYTECHNIC INSTITUTE AND
1880 PRATT DR STE 2006
BLACKSBURG, VIRGINIA 24060-3580

Performing Department

College of Vet Medicine

Co-Project Directors

Meng, X. J.

Collaborating Departments

College of Vet Medicine

Collaborating/Partnering States

CONNECTICUT

ILLINOIS

INDIANA

IOWA

KANSAS

MARYLAND

MINNESOTA

NEBRASKA

NORTH DAKOTA

OHIO

SOUTH DAKOTA

VIRGINIA

WISCONSIN

WYOMING

Collaborating/Partnering Countries

{NO DATA ENTERED}

Collaborating/Partnering Organizations

{NO DATA ENTERED}

Non-Technical Summary

Currently singular vaccines against either PRRSV or PCV2 are available but a bi-valent vaccine against both PRRSV and PCV2 are lacking. The objective of this project is to evaluate the use of non-pahtogenic PCV1 and the vaccine virus PCV1-2 as potential vaccine delivery vectors for the development of a bi-valent vaccine against both PRRSV and PCV2. We expect that the project will validate the use of PCV1 as a useful vaccine delivery vector for other swien pathogens, and we also expect that we will demonstarte that the vaccine virus PCV1-2 can serve as a vaccine delivery vector for creating bi-valent vaccines against other swine viruses.

United States Department of Agriculture
Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Goals / Objectives

(1)

The overall objective for this five-year NC-229 project is to reduce the impact PRRS has on producers, and to assess the feasibility and financial acceptability of PRRS area control and/or elimination for producers. To that end, we focus on the following major points, which faithfully represent the current research priorities of the US swine industry (Pork Check off NPB) :
1.1) PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection, 1.2) PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA), 1.3). Economic Impact of Interventions: determining the economic benefit of vaccination in positive herds

(2)

Develop effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence, which includes the following: 2.1) Porcine Epidemic Diarrhea Virus, 2.2) Swine Influenza Virus, 2.3) African Swine Fever, 2.4) Emerging serotypes of swine rotaviruses

Methods

We plan to evaluate the potential use of the non-pathogenic porcine circovirus type 1 (PCV1) as a vaccine delivery vector against other swine pathogens such as PRRSV. Immunogenic epitopes from swine pathogens such as PRRSV will be cloned into the infectious clone of PCV1, and viable chimeric viruses will be generated and their immunogenicity and potential use as a vectored vaccine will be tested in pigs.

We also plan to determine if the commercial vaccine against PCV2, the chimeric PCV1-2 virus, can be used as a vector to develop a bi-valent vaccine against both PCV2 and PRRSV. PRRSV antigenic epitopes will be cloned into the backbone of the vaccine virus PCV1-2, and viable chimeric viruses will be recovered and characterized for the ability to induce protective immunity in pigs against both PCV2 and PRRSV.

The project will be evaluated based on the outcomes such as potential vaccine candidates, journal publications as well as scientific meeting presentations.

Target Audience

The target audiences are swine veterinarians, and research scientists through scientific meeting presentations of the research results as well as scientific journal publications of the research data.

Products

- *Publications in peer-reviewed journals
- *Graduate PhD students in agricultural sciences
- *Scientific presentations in national and international conferences

Expected Outcomes

- *Increase in the knowledge regarding our understanding the mechanisms of pathogenesis of PRRSV and PCV2.
- *Increase in the knowledge of understanding the protective immunity and vaccine design against PRRSV and PCV2.

Keywords

Porcine circovirus type 1 ~Porcine circovirus type 2 ~Porcine reproductive and respiratory syndrome virus ~Vaccine vector

Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.3	0.0	0.1	0.0	0.4
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.1	0.0	0.0	0.0	0.1
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.4	0.0	0.1	0.0	0.5

Animal Health Component 100 %

Is this an AREERA Section 204 Integrated Activity? No

Activities

Research 100 %
 Extension 0 %
 Education 0 %

Research Effort Categories

Basic 50 %
 Applied 50 %
 Developmental 0 %

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
311	3510	1101	34
311	1030	1101	33
722	3510	1090	33

Knowledge Area

311 - Animal Diseases; 722 - Zoonotic Diseases and Parasites Affecting Humans

Subject Of Investigation

1030 - Papaya; 3510 - Swine, live animal

Field Of Science

1090 - Immunology; 1101 - Virology

Associated Planned Programs

Plan Year	Program Name	Percentage
2015	Agriculture Profitability and Sustainability	80
2015	Food, Nutrition, and Health	20

Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Assurance Statements

1. Are Human Subjects Involved? ☒ No ☐ Yes

If YES to Human Subjects

Is the Project Exempt from Federal regulations?

☐ Yes

If yes, select the appropriate exemption number.

☐ No

If no, is the IRB review Pending?

☐ Yes☐ No IRB Approval Date

Human Subject Assurance Number

2. Are Vertebrate Animals Used? ☐ No ☒ Yes

If YES to Vertebrate Animals

Is the IACUC review Pending?

☐ Yes☒ No IACUC Approval Date July 07, 2016

Animal Welfare Assurance Number 16-097 (CVM)

Project Signature Panel

Dr. Saied Mostaghimi

Director

Virginia Agricultural Experiment Station

Assurance Statement Panel

Dr. Saied Mostaghimi

Director

Virginia Agricultural Experiment Station

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: University of Connecticut

A. NC-229 REPRESENTATIVE:

Guillermo Risatti, University of Connecticut, guillermo.risatti@uconn.edu

Other PRINCIPAL LEADERS associated with the projects

Antonio Garmendia, University of Connecticut, Antonio.garmendia@uconn.edu

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV (Risatti; Garmendia).

We plan to test whether IFN β levels correlate with protection from PRRS. For this purpose, ongoing vaccination/challenge studies in swine will be a source of samples to examine IFN β and downstream ISGs responses *in vivo*. It is expected that bioactive IFN β will be produced by PAMs of swine infected with PRRSV in a strain-dependent manner. These studies will include evaluation of IFN β expression, Mx and ISG15 expression as a measure IFNAR-mediated signaling and overall anti-viral bioactivity in BAL fluids, virus-stimulated PAMs, serum. A series of anti-swine IFN β monoclonal antibodies (mAbs) were developed to be utilized to assess IFN β in immunoassays and bioassays (Garmendia). Emily Morse an honor's student tested whether envelope proteins devoid of viral nucleic acid extracted from CsCl purified PRRSV induced IFN β in normal porcine alveolar macrophages (PAMs). At two concentrations of envelope proteins tested to stimulate PAMs there were relatively low but significant increases in IFN β mRNA expression when compared to baseline levels ($p < 0.05$) as measured by quantitative RT-PCR. The data suggest that replication of virus may not be strictly necessary for induction of IFN β . In fact virus replication may result in inhibition of IFN β induction with some strains of virus as some NS proteins known to inhibit such induction will be produced. Research conducted to test IFN β and downstream ISGs responses of PAMs to infection with PRRSV showed that bioactive IFN β was produced although this was variable. The study also showed that Mx1 protein was expressed and indicated as IFNAR-mediated signaling and roughly followed the IFN β responses. In conclusion, IFN β induction/signaling do occur variably upon infection of natural host cells with PRRSV. Interestingly, Mx-1 expression by infected PAMs generally correlated with IFN β production (The activation of the IFN β induction/signaling pathway in porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus is variable Overend C., J. Cui, M. Grubman, A.E. Garmendia Vet Res Commun 41(1):15-22. 2017 (Garmendia).

We are developing an ELISA DIVA test for differentiating animals vaccinated from Classical Swine Fever Virus (CSFV) infected animals as a companion assay for a modified live marker vaccine that our group have designed (*Development of an improved live attenuated antigenic*

marker CSF vaccine strain candidate with an increased genetic stability. Holinka LG, Fernandez-Sainz I, Sanford B, O'Donnell V, Gladue DP, Carlson J, Lu Z, Risatti GR, Borca MV. 2014. Virology. Dec; 471-473:13-8). The test is based on the use of a CSFV E2 modified glycoprotein expressed in baculovirus/insect cell system. When added to a commercially available CSFV antibody ELISA detection test together with swine sera, the E2 modified protein competes with those antibodies elicited by the marker vaccine. However, the modified protein is unable to compete with antibodies elicited by a natural infection with wild-type viruses. We have been able to confirm the working hypothesis. A MS student Yuxiang Wang has been mentored under this project. (Risatti).

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

The objective of the study is to examine the role of IFN beta in protective immunity against PRRS. Investigating IFN beta will contribute to gain a better understanding of the innate response to PRRSV which in turn will be useful to the overall knowledge of mechanisms of general pathogenesis, immune evasion and protection or lack thereof. (The activation of the IFN β induction/signaling pathway in porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus is variable Overend C., J. Cui, M. Grubman, A.E. Garmendia Vet Res Commun 41(1):15-22. 2017 (Garmendia).

The project is aimed to improve/produce biological control tools against CSF. A DIVA companion test is needed for the vaccine candidate develop in collaboration with Plum Island Animal Disease Center, ARS, USDA (*Development of an improved live attenuated antigenic marker CSF vaccine strain candidate with an increased genetic stability. Holinka LG, Fernandez-Sainz I, Sanford B, O'Donnell V, Gladue DP, Carlson J, Lu Z, Risatti GR, Borca MV. 2014. Virology. Dec; 471-473:13-8).* (Risatti).

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1. Publications in press
2. Abstracts or Proceedings

E. FUNDING SOURCES FOR PRRSV RESEARCH

Hatch Multistate Project, NC229. Storrs Agricultural Experiment Station (Risatti).

Hatch Project, Storrs Agricultural Experiment Station (Garmendia).

Polyvalent T cell Mosaic Vaccine to Cross-Protect Swine against Heterologous PRRSV Strains. USDA/NIFA Grant Number 2011 67015-30176 (Garmendia).

F. WORK PLANNED FOR NEXT YEAR

- 1) This year we plan to retest levels and bioactivity of IFN β in representative archival samples from a recent vaccine study. Additionally samples collected in an ongoing vaccine/challenge study are included in the testing for IFN β levels and bioactivity to determine how these correlate with protection outcomes. Measurements of IFN β will be made during vaccination, after challenge and at necropsy in serum, culture fluids of PBMNC stimulated *in vitro* with virus or viral antigens, BAL fluids and PAMs stimulated as the PBMNC. ELISAs, flow cytometry and bioassays will be utilized to do the

evaluation. In addition, the induction of IFN β by stimulation of PAMs with detergent extracts of viral proteins will be extended to proteins extracted from different strains of virus and will be compared with induction outcomes resulting from infection with the corresponding infectious viruses. (Garmendia).

- 2) Studies on ASFV virulence and protection, CSFV DIVA ELISA as companion test for an experimental modified-live marker vaccine (Risatti).

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence. (Risatti)

We are engaged in a collaboration with Plum Island Animal Disease Center (PIADC), ARS, USDA, in a project entitled “Development of recombinant African Swine Fever Virus (ASFV) attenuated viruses containing multiple deletions for use as vaccine candidates.” (Risatti).

We are in the process of developing collaborative work (e.g. Uganda) for establishing ASF surveys among domestic pigs and for assessing features of circulating viruses in the that country.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Basic research on the role of specific ASFV genes in virus virulence is under investigation. The purpose is to identify virus targets that once modified render attenuated virus that might be used as vaccine candidates.

A communicable disease surveillance system such as for ASF is aimed to detect early presence of the disease and to estimate risks associated with disease spread. Active surveillance refers to the systematic collection, analysis, and interpretation of disease data (i.e.: ASF) for use in planning, implementing and evaluating animal disease control measures.

Improving biologicals tools for better control of CSFV. We will continue working on developing of an ELISA test that can be used as companion assay for an experimentally developed modified-live marker vaccine.

D. ASF PUBLICATIONS ISSUED OR “IN PRESS”

1) Publications

Holinka LG, O'Donnell V, **Risatti GR**, Azzinaro P, Arzt J, Stenfeldt C, Velazquez-Salinas L, Carlson J, Gladue DP, Borca MV. Early protection events in swine immunized with an experimental live attenuated classical swine fever marker vaccine, FlagT4G. *PLoS One*. 2017 May 24; 12(5):e0177433. doi: 10.1371/journal.pone.0177433. eCollection 201.

Borca MV, O'Donnell V, Holinka LG, Sanford B, Azzinaro PA, **Risatti GR**, Gladue DP. Development of a fluorescent ASFV strain that retains the ability to cause disease in swine. *Sci Rep*. 2017 Apr 24; 7:46747. doi: 10.1038/srep46747.

O'Donnell V, **Risatti GR**, Holinka LG, Krug PW, Carlson J, Velazquez-Salinas L, Azzinaro PA, Gladue DP, Borca MV. Simultaneous Deletion of the 9GL and UK Genes from the African Swine Fever Virus Georgia 2007 Isolate Offers Increased Safety and Protection against Homologous Challenge. *J Virol*. 2016 Dec 16; 91(1).

2) Abstracts or Proceedings

University of Connecticut, College of Agriculture, Health and Natural Resources, **Graduate Research Forum**, March 25th 2017, Storrs, CT. **“Development of ELISA-based test for serological differentiation of vaccinated from Classical Swine Fever Virus infected animals”**. Yuxiang Wang¹, Manuel V. Borca² and Guillermo R. Risatti¹. (1) Department of Pathobiology and Veterinary Science, College of Agriculture Health and Natural Resources, University of Connecticut. (2) Plum Island Animal Disease Center, Agricultural Research Service, US Department of Agriculture.

Conference of Research Workers in Animal Diseases, Chicago, IL, USA, December 1-5, 2017. **“Understanding the diverse roles of viroporin activity of classical swine fever virus protein p7”**. M. Borca¹, E. Largo², N. Huarte², L. Holinka¹, K. Berggren¹, E. Ramirez-Medina^{1,3}, G. Risatti³, J. Nieva², D.P. Gladue¹. (1) PIADC, ARS, USDA, USA; (2) University of the Basque Country, Bilbao, Spain; (3) University of Connecticut, USA.

E. FUNDING SOURCES FOR ASF and CSF RESEARCH

Plum Island Animal Disease Center, ARS, USDA.

F. WORK PLANNED FOR NEXT YEAR

Active surveillance of ASF is planned to continue next year in both countries.

Collaborative research with PIADC on development of recombinant African Swine Fever Virus (ASFV) attenuated viruses containing multiple deletions for use as vaccine candidates.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30, 2016 to December 1, 2017

INSTITUTION OR STATION: South Dakota State University

A. Personnel:

1) NC-229 STATION REPRESENTATIVE: Eric A. Nelson; SDSU; eric.nelson@sdstate.edu

2) Other PRINCIPAL LEADERS associated with the projects:

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- **PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection**

Research efforts directed toward PRRSV control primarily focused on innate immunity in PRRSV pathogenesis, virus host interactions, and a virus-like particle (VLP) approach for PRRSV vaccine development. Studies lead by the X. Wang lab, suggested that PRRSV may have evolved strategies to overcome the formation and anti-viral activity of stress granules (SGs) during viral infection. One possible mechanism mediated by PRRSV may be to modulate the expression of G3BP1, a key component of SGs. The efficacy of PRRSV VLPs together with the use of a novel 2', 3'-cGAMP VacciGrade™ adjuvant in an animal challenge model was also explored. PRRSV nucleocapsid protein specific antibody was detected in all animals at day 10 after challenge, but no significant difference was observed among vaccinated and control groups. Surprisingly, a significantly higher viremia was observed in the VLPs and VLPs plus adjuvant groups compared to the control group. The increased viremia correlated with a higher interferon- α induction in the serum of the VLPs and the VLPs plus adjuvant groups. PRRSV VLPs and PRRSV VLPs plus adjuvant failed to provide protection against PRRSV challenge.

- **Host genetic control of anti-PRRSV infection and vaccination responses**
- **PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA)**
- **Economic Impact of Interventions: determining the economic benefit of vaccination in positive herds**

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

- **PEDV Diagnostics, immunity and vaccinology**

Neutralizing monoclonal antibodies (mAbs) against the spike protein of porcine epidemic diarrhea virus (PEDV) were used to map neutralizing epitopes. Epitope mapping by peptide ELISAs revealed that seven of these mAbs recognized linear neutralizing epitopes located in the N-terminus of the S2 glycoprotein subunit. Additionally, one mAb recognized a neutralizing epitope located in the C-terminus of S2, while only one neutralizing mAb reacted against a region of the S1 glycoprotein subunit. The mAbs that recognized epitopes within the S2 subunit presented the highest neutralizing activity, suggesting the S2 glycoprotein subunit contains immunodominant neutralizing epitopes of PEDV.

Additional mAbs were developed against the PLP2 region of PEDV in support of research efforts led by scientists at USDA-NADC. These reagents will be valuable for studying the interaction of non-structural proteins to better understand how they contribute to PEDV pathogenesis.

A recombinant ORFV-based vaccine candidate for PEDV was developed and its immunogenicity and protective efficacy was evaluated in pregnant gilts. Animals were immunized with the ORFV-based recombinant alone or immunized and exposed orally to live PEDV. Immunization with ORFV-PEDV-S alone or with ORFV-PEDV-S + live PEDV elicited the development of PEDV specific antibodies in serum, colostrum and milk of immunized sows. Upon challenge, reduced mortality was observed in animals born to immunized gilts, when compared to sham-immunized controls.

Another approach under investigation involves development of a nano-particle based vaccine platform for PEDV. Codon-optimized PEDV spike gene expression constructs were generated and fused into a ferritin nanoparticle scaffold plasmid. Expression and antigenicity of these nanoparticle constructs is being assessed *in vitro* prior to producing a Newcastle Disease Virus (NDV) vector expressing the PEDV spike-ferritin nanoparticles and conducting *in vivo* mouse experiments.

- **Senecavirus A epidemiology, diagnostics and pathogenesis**

Senecavirus A (SVA) is a re-emerging pathogen of swine that causes vesicular disease that is indistinguishable from Foot and Mouth Disease (FMD) in affected animals. Since its re-emergence in the US in July 2015, over 250 outbreaks have been confirmed. Our group has been actively working in different aspects of SVA epidemiology, infection immunity and pathogenesis, and on diagnostic assay development and validation. To date, we have obtained over 40 complete genome sequences of contemporary US and Brazilian SVA isolates and prepared a manuscript to assess the evolution and genetic diversity of these isolates in comparison with historical isolates. We have also conducted comprehensive studies to characterize the pathogenesis and immunity to SVA infection.

Additionally, diagnostic assays and reagents are currently under development and some in final stages of validation.

- **Swine influenza virus(SIV) evolution and detection**

Influenza is another significant pathogen of swine. PCR assays for influenza A are well established, but pigs can also be infected with influenza B, C and D. Therefore, we are developing assays designed to provide cost efficient testing, promoting the continued surveillance for all swine influenza viruses. Prototype assays have been developed for influenza B, C and D. These assays are being combined in panels and more fully validated. Well validated and rapid diagnostic tools such as these new multiplex real-time PCR assays will be vital for continued swine health and production while enhancing the One Health Initiative.

- **SIV Control by vaccination or other interventions**
- **SIV at the human-animal interface**
- **African Swine Fever (ASF): Vaccine Design and Development**
- **CSFV vaccination, diagnosis epidemiology**
- **Assessing pathogen survival in feed**

Since the emergence of PEDV in the US in 2013, the team at SDSU has been working closely with Pipestone Applied Research to assess potential risk factors that may have contributed to emergence of the virus in the US. Results from the initial study, demonstrating that PEDV survives in different feed matrices under transportation conditions simulating a trip from Asia to the US led to an expansion of this study. Our group, together with Pipestone Applied Research and collaborators from Kansas State University assessed the survival of 11 additional pathogens in feed ingredients. Results from this study showed that several other pathogens of importance to swine and/or surrogate viruses also survive the journey in the feed matrix. A report of the results from this study has recently been submitted for publication and is currently under review.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Innate immunity is the first line of defense against virus infections. A better understanding of innate immunity against PRRSV and PEDV will allow us to better understand viral pathogenesis, which in turn may facilitate the development of novel prophylactic strategies against these devastating swine diseases.

New monoclonal antibody-based reagents for Senecavirus A and a fluorescence-based virus neutralization assay for the detection of neutralizing antibodies are now available to researchers and diagnosticians throughout the US. Availability of these tools should provide substantial benefit to the swine industry in the control of Senecavirus A.

New knowledge generated from our studies on Senecavirus A has directly impacted the swine industry by providing critical information on the pathogenesis and immune responses of this important pathogen. We expect that this information will have an even broader impact in the future by allowing the design of improved prevention and control strategies.

Research on novel vector platforms and vaccine candidates for livestock species has had a significant impact on our understanding of novel approaches to vaccine design. Preliminary data generated as a part of this project was used to obtain two large grants from NIFA-USDA (Standard-Foundational) and from the South Dakota Governor's Office of Economic Development (Established the South Dakota Center for Biologics Research and Commercialization, SD-CBRC).

The transboundary risk of feed ingredients contaminated with high consequence pathogens and surrogate viruses representing foreign animal diseases was evaluated in a model simulating shipment from China to the US. Results demonstrate the ability of multiple viral pathogens to survive in certain feed ingredients, including soybean meal. This study suggests that contaminated feed ingredients could present transboundary risk factors for high consequence pathogens.

D. PERTINENT PUBLICATIONS ISSUED OR "IN PRESS"

1) Refereed publications

Maggioli, M.F., Lawson, S., de Lima, M., Joshi, L.R., Faccin, T.C., Bauermann, F.V., Diel, D.G. Adaptive immune responses following Senecavirus A infection in pigs. Accepted. *Journal of Virology*. November 2, 2017.

Martins, M., Joshi, L.J., Rodrigues, F.S., Anziliero, D., Frandoloso, R., Kutish, G.F., Rock, D.L., Weiblen, R., Flores, E.F., Diel, D.G. 2017. Immunogenicity of ORFV-based vectors expressing the rabies virus glycoprotein in livestock species. *Virology*, 511:229-239. doi:10.1016/j.virol.2017.08.027.

Okda, F.A., Lawson, S., Singrey, A., Nelson, J., Hain, K.S., Joshi, L.R., Christopher-Hennings, J., Nelson, E.A., Diel, D.G. 2017. The S2 glycoprotein subunit of porcine epidemic diarrhea virus contains immunodominant neutralizing epitopes. *Virology*, 509:185-194. doi:10.1016/j.virol.2017.06.013.

Van Noort, A., Nelsen, A., Pillatzki, A.E., Diel, D.G., Li, F., Nelson, E., Wang, X. 2017. Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2', 3'-cGAMP VacciGrade™ adjuvant exacerbates viremia after virus challenge. *Virology Journal*, 14(1):76. doi: 10.1186/s12985-017-0746-0.

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2) Abstracts or Proceedings

Dee S., Niederwerder M., Diel D.G. 2017. Modeling the transboundary survival of foreign animal and endemic disease pathogens via contaminated feed ingredients. United States Animal Health Association (USHA) Annual 121st Meeting. Subcommittee on Global Health and Trade, Oct 12-18, 2017, San Diego, CA.

Diel D.G., 2017. Emerging pathogens of swine: Implications for diagnostics and lessons learned from Senecavirus A. American Association of Swine Veterinarians (AASV) Annual Meeting. Feb 25-27, 2017, Denver, CO.

Joshi, L.R., Fernandes, M.V.H., Clement, T., Lawson, S., Resende, T.P., Vannucci, F.A., Nelson, E.A., Diel D.G. 2016. Pathogenesis and infection dynamics of Senecavirus A in pigs. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Fernandes, M.H.V., Okda, F., Joshi, L.R., Hain, K.S. Nelson, E.A., Christopher-Hennings, J., Osorio, F.A., Vu, H., Diel, D.G. 2016. Evaluation of immunodominant B- and T-cell epitopes as inducers of protective immunity against porcine reproductive and respiratory syndrome virus. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Lawson, S., Maggioli, M.F., Joshi, L.R., Fernandes, M.H.V., Christopher-Hennings, J., Nelson, E.A., Diel, D.G. 2016. Immune Responses to Senecavirus A in Pigs. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Hain, K.S., Joshi, L.R., Okda, F., Nelson, J., Singrey, A., Lawson, S., Martins, M., Pillatzki, A., Kutish, G.F., Nelson, E.A., Flores, E.F., Diel, D.G. 2016. Development of a recombinant parapoxvirus expressing the spike protein of porcine epidemic diarrhea virus. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Joshi, L.R., Mohr, K.A., Gava, D., Kutish, G., Piñeyro, P., Zhang, J., Caron, L., Schaefer, R., Diel, D.G. 2016. Genetic characterization and phylogenetic analysis of Senecavirus A. North American PRRSV and other emerging viruses Symposium. Dec 3-4, 2016. Chicago, IL.

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Tignon, M., I Christiaens, H. Nauwynck, D. Ojkic, E. Nelson, A.B. Cay. 2017. European Symposium of Porcine Health Management. Comparative study of serological methods for diagnosis of porcine epidemic diarrhea virus (PEDV) infection. May 3-5, 2017. Prague, Czech Republic.

Kraft, J., K. Woodard, L.G. Gimenez-Lirola, B. Setness, J. Ji, P. Lasley, E.A. Nelson, J. Zhang, D. Baum, P. Gauger, J. Zimmerman, R. Main. 2017. Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination. American Association of Swine Veterinarians. Feb 25-28, 2017. Denver, CO.

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Van Noort, A. M., Wang, X., A. V. N., Pillatzki, A. E., Diel, D. G., Li, F., Nelson, E. A., Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2'3'-cGAMP VacciGrade adjuvant exacerbates viremia after virus challenge. 2016 PRRSV Symposium. Dec 4, 2016. Chicago, IL.

3) Book chapters or monographs

Fernandes, M.H.V., Diel, D.G. 2017. Emerging viral diseases: Porcine epidemic diarrhea virus. *In*: Flores, E.F. Veterinary Virology. 3 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 36, p.571.

Canal, C.V., Diel, D.G. 2017. Poxviridae. *In*: Flores, E.F. Veterinary Virology. 3 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 19, p.571.

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4) Theses/Dissertations Published

Okda, F.A. 2017. PhD Dissertation, Surveillance of emerging livestock viruses. South Dakota State University, Brookings, SD.

Hain, K.S. 2017. M.S. Thesis. Development and characterization of a recombinant Orf virus expressing the spike protein of porcine epidemic diarrhea virus. South Dakota State University, Brookings, SD.

Joshi, L.R. 2017. M.S. Thesis. Senecavirus A: Epidemiology, pathogenesis and infection dynamics. South Dakota State University, Brookings, SD.

E. FUNDING SOURCES FOR SWINE VIROLOGY RESEARCH

Diel D.G., Maggioli M.F., Joshi L.R., Bauermann, F.V., Nelson E.A., Hennings. J. Investigating the effect of stressors on Senecavirus A pathogenesis and the potential occurrence of the “carrier state” in sows. National Pork Board. 12/17-11/18.

Diel D.G. Maggioli M.F., Bauermann F.V., Nelson E. Young, A., Gourapura, R. A multi-species vaccine delivery platform for infectious disease prevention and control in livestock species. USDA-NIFA. 9/2017-3/2021.

Diel D.G., Bauermann, F.V., Singrey A., Nelson E. Evaluating survival of viral pathogens in porcine plasma. Sonac-Darling. 9/2017-8/2018.

Diel D.G., Dee, S., Bauermann F.V., Singrey A., Nelson E. Evaluation of chemical mitigants for neutralizing the risk of foreign animal diseases in contaminated feed ingredients. Swine Health Information Center. 8/2017-7/2018.

Niederwerder, M., Rowland R.R., Dee, S., Diel D.G. Evaluation of the risk of transboundary movement of ASFV via contaminated feed ingredients. Swine Health Information Center. 3/2017-2/2018.

Ma, W., D. Wang. Study influenza B in pigs with PRRSV infection. NIH R21. 12/2016 - 11/2018.

Christopher-Hennings, J., Nelson, E., Diel, D. G., Li, F., Scaria, J., Wang, D., Chaussee, M., Herrmann, S. South Dakota Center for Biologics Research (SD-CBRC), South Dakota Governor’s Office of Economic Development. 6/2017-6/2022.

Diel D., Clement. T., Bauermann, F.V., Nelson E.A., Christopher-Hennings, J. Development of rapid diagnostic capability for encephalomyocarditis virus - (EMCV). Swine Health Information Center. 11/2016 - 10/2017.

Rauh, R., Diel D., Christopher-Hennings, J. Development of a high throughput real-time RT-PCR assay (dry, room temperature stable and fluid formats) for the detection and discrimination of Senecavirus A (SVA), Foot-and-mouth disease virus (FMDV) and Swine Vesicular Disease virus (SVDV). Swine Health Information Center. 11/2016 - 10/2017.

Dee, S., E.A. Nelson, D. Diel, C, Neill. Evaluating the survival of FAD viral surrogate pathogens in ingredients shipped from China to the US. Swine Health Information Center. 4/2016 - 4/2017.

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Dilberger-Lawson, S. R., Nelson, E. A., Singrey, A. R., Development of monoclonal and polyclonal antibodies against PEDV PLP2, USDA NADC, 03/2017-12/2017.

Dilberger-Lawson, S. R., Diel, D. G., Singrey, A. R., Clement, T. J., Hennings, J., Nelson, E. A. Development of a bELISA for serological diagnostics and surveillance of SVA infection, National Pork Board, 06/2017-06/2018.

Li, F., Clement, T. J., Hennings, J., Nelson, E. A., Rauh, R., Hause, B., Detection and differentiation of influenza Types A, B, C and D in swine, Swine Health Information Center, 11/16-11/2017.

Voronov, A., S. Ramamoorthy, S. Stafslie, E. Nelson. Polymeric adjuvants for peptide vaccines. ND-APUC 6/2015-6/2017.

Wang, D., E.A. Nelson, R. Kaushik, F. Li. Novel porcine epidemic diarrhea virus vaccine pipeline. USDA AFRI. 12/2015-12/2017.

F. WORK PLANNED FOR NEXT YEAR

Objective 1: Control of PRRSV.

We will continue to investigate the role of stress granules (SGs) in PRRSV and PEDV replication and host innate immunity. We will primarily focus on the kinetics and mechanistic basis of viruses and SGs interaction.

Objective 2: Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

One goal for the next year will be to utilize new, recently developed expressed protein antigens and monoclonal antibodies to formulate improved competitive ELISA-based assays for Senecavirus A serology. FMIA-based assays will also be evaluated.

We will continue our efforts to develop and fully validate new real-time PCR assays for high impact viral diseases of swine. With funding from the Swine Health Information Center and industry partners, we will continue focus on full validation of real-time PCR assays for rapid diagnosis of encephalomyocarditis virus (EMCV) and detection and differentiation of influenza Types A, B, C and D in swine.

We will continue efforts related to the development and evaluation of recombinant vaccine candidates for endemic and emerging viral pathogens of swine. Further study will focus on understanding basic aspects of SVA innate immune evasion and pathogenesis; along with development of vaccine candidates for Senecavirus A. Additional efforts will focus on improved vaccine strategies for swine influenza.

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: December 1 2016 to December 30, 2017

INSTITUTION OR STATION:
China Agricultural University (CAU)

A. NC-229 REPRESENTATIVE:

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Other PRINCIPAL LEADERS associated with the projects

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Per station for ALL Accomplishment = Maximum 3,000 characters including spaces;

Full NC229 report for ALL Accomplishment = Maximum 30,000 characters):

This section focuses on intended activities, outputs, and short-term outcomes. The report should also reflect on the items that stakeholders want to know, or want to see. The accomplishments should cover only the current year of the project.

Objective 1. Control of PRRSV

Indicate progress in any the following areas, as appropriate in each case/station

PRRSV Immunity and Vaccinology : understanding correlates of immunity and mechanisms to broaden protection,

Host genetic control of anti-PRRSV infection and vaccination responses

(1) We have proved DDX18, which is a member of DEAD-box RNA helicases (DDXs) family, participated in viral replication. Previously, we found the DDX18 interacts with both nsp2 and nsp10 of PRRSV by Co-Immunoprecipitation (Co-IP). In the present study, we demonstrated the interactions of DDX18 with nsp2 and nsp10, and located DDX18's binding regions as the N-terminus of nsp2 and both the N-terminus and C-terminus of nsp10. The expression of the nsp2 or nsp10 in MARC-145 cells and primary PAM cells redistributed DDX18 from the nucleus to the cytoplasm, and promoted the viral replication, but silencing of the DDX18 gene in MARC-145 cells down-regulated the replication of PRRSV.

(2) The interaction of interleukin-2 enhancer binding factor 2 (ILF2) with nsp9 or nsp2 was first demonstrated in 293FT cells co-transfected with ILF2-expressing plasmid and nsp9-expressing plasmid or nsp2-expressing plasmid. The interaction of endogenous ILF2 with the nsp9 or nsp2 of PRRSV was further confirmed in MARC-145 cells transduced with GFP-nsp9-expressing lentiviruses or infected with PRRSV JXwn06. The RdRp domain of nsp9 was shown to be responsible for its interaction with ILF2, while three truncated nsp2 were shown to interact with ILF2. Moreover, we observed that ILF2 partly translocated from the nucleus to the cytoplasm and co-localized with nsp9 and nsp2 in PRRSV-infected MARC-145 cells and PAMs.

(3) In our researches, we first predicted by software that the multiple proteins of porcine reproductive and respiratory syndrome virus (PRRSV) could be sumoylated. Next, we confirmed that Nsp1 β , Nsp4, Nsp9, Nsp10 and nucleocapsid (N) protein of PRRSV could interact with the sole SUMO E2 conjugating enzyme Ubc9, and Ubc9 could be co-localized with Nsp1 β , Nsp4, Nsp9 and Nsp10 in the cytoplasm, while with N protein in both the cytoplasm and nucleus. Finally, we demonstrated that N protein could be sumoylated by either SUMO1 or SUMO2/3. In addition, the overexpression of Ubc9 could inhibit viral genomic replication at early period of PRRSV infection and the knockdown of Ubc9 by siRNA could promote the virus replication.

(4) In the present study, the pathogenicity of a NADC30-like strain CHsx1401 for piglets was analyzed, and the potential cross-protective efficacy of three MLV vaccines including two commercial MLV vaccines and an attenuated low pathogenic PRRSV against this virus was further evaluated in piglets. The NADC30-like CHsx1401 was shown to cause fever, respiratory clinical signs, and lung gross and microscopic lesions of the inoculated piglets, suggesting that this virus is moderate virulent for piglets. Vaccination of piglets with the MLV vaccines could not reduce the clinical signs and lung lesions, and was partially efficacious in the reduction of viral loads in sera upon NADC30-like CHsx1401 challenge, indicating that these three MLV vaccines provide extremely limited cross-protection efficacy against the NADC30-like virus infection. Additionally, Ingelvac PRRS MLV appeared to exert some beneficial efficiency in shortening the period of clinical fever and in improving the growth performance of the challenged pigs.

PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA)

(1) In the present study, the genetic characterization of a recombinant type 2 PRRSV (designated TJnh1501) was analyzed and its pathogenicity for piglets was examined. Our study showed that each region of TJnh1501 genome had 96.67–100% nucleotide and 96.5–100% amino acid identities with a Chinese highly pathogenic PRRSV-derived modified-live virus (MLV)-like except for its nonstructural protein 2 (nsp2)-coding region; while its nsp2-coding region shared higher nucleotide (84.44–85.85%) and amino acid (82.44–84.79%) identities with NADC30 and NADC30-like CHsx1401, and in particular, the highly variable region of nsp2 exhibited characteristic 131-aa deletion identical to NADC30 and NADC30-like CHsx1401. Meanwhile, we identified two recombination breakpoints located in the nt1737 and nt3506 of nsp2-coding region, which had higher nucleotide homology with NADC30 and NADC30-like CHsx1401. Moreover, TJnh1501 infection could cause persistent fever, moderate respiratory clinical signs, higher viremia, and obvious gross and microscopic lung lesions in piglets. The virus was shown to have lower pathogenicity than HP-PRRSV JXwn06, but higher than NADC30-like CHsx1401 for piglets.

Economic Impact of Interventions: determining the economic benefit of vaccination in positive

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

Indicate progress in the following areas: as appropriate in each case/station:

- PEDV Diagnostics
- PEDV immunity and vaccinology. .
- Swine influenza virus(SIV) evolution and detection
- SIV Control by vaccination or other interventions
- SIV at the human-animal interface
- African Swine Fever (ASF): Vaccine Design and Development
- CSFV vaccination, diagnosis epidemiology

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Impact statements (500 characters per statement)

This section focuses on actual or intended potential long-term outcomes and impacts, covering only the current year of the project. The report should also reflect on the items that stakeholders want to know, or want to see. List any grants, contracts, and/or other resources obtained by one

or more project members as a result of the project's activities. Include the recipients, funding source, amount awarded and term if applicable.

- (1) Our findings proved that the cellular RNA helicase DDX18 plays a role in the replication of PRRSV, and provides insights into the understanding of PRRSV replication.
- (2) Our analysis indicated that knockdown of ILF2 favored the replication of PRRSV, while over-expression of ILF2 impaired the viral replication in MARC-145 cells. It also gives us another insight into the understanding of PRRSV replication.
- (3) These findings revealed the SUMOylation property of PRRSV N protein and the involvement of Ubc9 in PRRSV replication through interaction with multiple proteins of PRRSV. To our knowledge, this is the first study indicating the interplay between SUMO modification system and PRRSV.
- (4) Our findings gave valuable guidance for the choice and use of PRRSV MLV vaccines to control NADC30-like virus infection in the field.
- (5) Our findings revealed that TJnh1501 is a recombinant type 2 PRRSV from the recombinant event between NADC30-like and MLV-like derived from the Chinese highly pathogenic PRRSV, and it exhibits intermediate virulence for pigs. This study adds valuable evidence for understanding the role of genomic recombination in the evolution of PRRSV.
- (6) In the review “Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus”, we summarized the recent advances in our understanding of the pathogenesis, evolution and ongoing field practices on the control of this troubling virus in China.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

- a) Jin H, Zhou L, Ge X, et al. *Cellular DEAD-box RNA helicase 18 (DDX18) promotes the PRRSV replication via interaction with virus nsp2 and nsp10*. Virus research, 2017, 238: 204-212.
- b) Wen X, Bian T, Zhang Z, et al. *Interleukin-2 enhancer binding factor 2 interacts with the nsp9 or nsp2 of porcine reproductive and respiratory syndrome virus and exerts negatively regulatory effect on the viral replication*. Virology journal, 2017, 14(1): 125.

- c) Wang C, Zeng N, Liu S, et al. *Interaction of porcine reproductive and respiratory syndrome virus proteins with SUMO-conjugating enzyme reveals the SUMOylation of nucleocapsid protein*. PloS one, 2017, 12(12): e0189191.
- d) Zhou L, Yang B, Xu L, et al. *Efficacy evaluation of three modified-live virus vaccines against a strain of porcine reproductive and respiratory syndrome virus NADC30-like*. Veterinary Microbiology, 2017.
- e) Bian T, Sun Y, Hao M, et al. *A recombinant type 2 porcine reproductive and respiratory syndrome virus between NADC30-like and a MLV-like: Genetic characterization and pathogenicity for piglets*. Infection, Genetics and Evolution, 2017, 54: 279-286.
- f) Han J, Zhou L, Ge X, et al. *Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus*. Veterinary Microbiology, 2017.

2) Abstracts or Proceedings

Cite authors, year, title, meeting (use abbreviations, e.g., Proc., CRWAD, AASV, 2008 PRRS Symp., etc.) Do not give full dates.

- a) Identification Critical Amino Acids in Nsp9 and Nsp10 Determining the Fatal Virulence of the Chinese Highly Pathogenic PRRSV. **Lei Xu**, Lei Zhou, Weifeng Sun, Pingping Zhang, Xinna Ge, Xin Guo, Jun Han, Hanchun Yang. XIVth International Nidovirus Symposium, 2017, Kansas City, USA.

3) Book chapters or monographs

Give full citation

E. FUNDING SOURCES FOR SWINE VIROLOGY RESEARCH

1) Current

- a. Major Program of National Natural Science Foundation of China (31490603)
- b. The earmarked fund for Modern Agro-industry Technology Research System of China (CARS-36) from the Ministry of Agriculture of the People's Republic of China.
- c. National Basic Research Program of China 481 (2014CB542700) from the Chinese Ministry of Science and Technology
- d. Key Program of National Natural Science Foundation of China (31330077)

F. WORK PLANNED FOR NEXT YEAR

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: December 1 2016 to November 30 2017

INSTITUTION OR STATION: Iowa State University

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

OBJECTIVE 1. Control of PRRSV.

Refer to publications listed in Section D.

OBJECTIVE 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Refer to publications listed in Section D.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS (500 words):

Research advances over the last year by this research group have continued to expand our understanding of PRRSV, PEDV, PCV2, IAV, ASFV, SVA and other emerging viral diseases of swine and provide new ideas for preventing, countering and/or eliminating these infections. Extensive work has been done on the mechanisms of host-pathogen(s) interactions. Likewise new work on the ecology and epidemiology of these agents provide insight into the mechanisms by which they maintain endemicity. Continued assessment and research in diagnostic technology is contributing to the improvement and refinement of our ability to surveil, detect, and diagnose PRRSV, PEDV, PCV2, IAV, ASFV, SVA, and other emerging viral infections. On-going work on new methods of surveillance promise to provide new, highly cost-effective methods of tracking infection and implementing area elimination/eradication programs. Accomplishments in these areas linked with research in viral ecology/epidemiology and improvements in vaccinology will lead to the development of approaches that will make possible the control of PRRSV and other viral infections on farms and in regions.

D. PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

- Abente EJ, Gauger PC, Walia RR, Rajao DS, Zhang J, Harmon KM, Killian ML, Vincent AL. 2017. Detection and characterization of an H4N6 avian-lineage virus in pigs in the Midwestern United States. *Virology* 511:56-65.
- Abente EJ, Kitikoon P, Lager KM, Gauger PC, Anderson TK, Vincent A. 2016. A highly pathogenic avian influenza virus H5N1 with 2009 pandemic H1N1 internal genes demonstrates increased replication and transmission in pigs. *J Gen Virol* 98:18-30.
- Baker KL, Mowrer C, Canon A, Linhares DCL, Rademacher C, Karriker LA, Holtkamp DJ. 2016. Systematic epidemiological investigations of cases of Senecavirus A in U.S. swine breeding herds. *Transbound Emerg Dis* 64:11–18.
- Baker KL, Thomas PR, Karriker LA, Ramirez A, Zhang J, Wang C, and Holtkamp DJ. 2017. Evaluation of an accelerated hydrogen peroxide disinfectant to inactivate porcine epidemic diarrhea virus in swine feces on aluminum surfaces under freezing conditions. *BMC Vet Res* 81:100-107.
- Canning P, Ruston C, Madson D, Bates J, Skoland K, Davenport J, Gaul S, Wang C, Chen Q, Zhang J, Karriker L. 2017. Effect of direct-fed microbial *Bacillus subtilis* C-3102 on

- enteric health in nursery pigs after challenge with porcine epidemic diarrhea virus. *J Swine Health Prod* 25:129-137.
- Cao D, Cao QM, Subramaniam S, Yugo DM, Heffron CL, Rogers AJ, Kenney SP, Tian D, Matzinger SR, Overend C, Catanzaro N, LeRoith T, Wang H, Piñeyro P, Lindstrom N, Clark-Deener S, Yuan L, Meng, X-J. 2017. Pig model mimicking chronic hepatitis E virus infection in immunocompromised patients to assess immune correlates during chronicity. *Proc Natl Acad Sci USA* 114:6914-6923.
- Cochrane RA, Schumacher LL, Dritz SS, Woodworth JC, Huss AR, Stark CR, DeRouchey JM, Tokach MD, Goodband RD, Bai J, Chen Q, Zhang J, Gauger PC, Derscheid RJ, Magstadt DM, Main RG, Jones CK. 2017. Effect of pelleting on survival of porcine epidemic diarrhea virus (PEDV)-contaminated feed. *J Anim Sci* 95:1170-1178.
- Curry S, Schwartz KJ, Yoon KJ, Gabler NK, Burrough ER. 2017. Effect of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity. *Vet Microbiol* 211:58-66.
- Curry SM, Burrough ER, Schwartz KJ, Yoon K-J, Lonergan SM, Gabler NK. 2017. Porcine epidemic diarrhea virus reduces feed efficiency in nursery pigs. *J Anim Sci* (*in press*).
- Curry SM, Gibson KA, Burrough ER, Schwartz KJ, Yoon KJ, Gabler NK. 2017. Nursery pig growth performance and tissue accretion modulation due to porcine epidemic diarrhea virus or porcine deltacoronavirus challenge. *J Anim Sci* 95:173-181.
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- Zhang J, Zheng Y, Chen Q, Bade S, Yoon KJ, Harmon K, Gauger P, Main R, Li G. March 2017. Application of next-generation sequencing technology to whole genome sequencing of PRRSV under diagnostic testing. Proc 48th Ann Meet Am Assoc Swine Veterinarians. Denver, Colorado. pp. 35-38.
- Zhang J, Zimmerman JJ. May 2017. Overview of emerging, re-emerging and/or zoonotic swine viruses. Proc 8th Asian Pig Veterinary Society Congress. Wuhan, China, pp. 45-56.
- Zhu Y, Huang H, Johnson E, Meyerhofer A, Bade S, Harmon K, Gauger P, Zhang J. October 2017. Comparison of isolation of swine influenza A virus in human colorectal adenocarcinoma (CACO-2) cell line and Madin-Darby canine kidney (MDCK) cell line. Proc 60th Ann Conf Am Assoc Vet Lab Diagn. San Diego, California. p. 216.
- Zimmerman J. February 2017. Swine medicine in the 21st century: Immovable object meets unstoppable force. Proc 48th Ann Meet Am Assoc Swine Veterinarians. Denver, Colorado. pp. 12-19.
- Zimmerman J. July 2017. Desafios en el control de las enfermedades. Memorias LI Congreso Nacional de la Asociación Mexicana de Veterinarios Especialistas en Cerdos (AMVEC). Querétaro, México. pp. 178-183.

3) Book chapters or monographs

- Balasuriya UBR and Zhang J. 2017. Development and characterization of an infectious cDNA clone of equine arteritis virus. In: Perez DR (ed). Reverse Genetics of RNA Viruses: Methods and Protocols. Methods Mol Biol 1602:11-28.

E. FUNDING SOURCES FOR RESEARCH

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- Bai J, Fang Y, Zhang J, Yoon KJ, Jaing C, and Liu X. Detection and differentiation of field strains and commonly used vaccine strains of Type 2 PRRS virus in the US. National Pork Board. \$79,080. 2016-2017.
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- Gauger P. Development of serological assays for porcine parainfluenza virus type 1 in swine. SHIC 2017-18, \$30,000
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- Holtkamp D.J., Linhares D.C. Comparison of a standard entry and a bench entry protocol for prevention of environmental contamination from personnel entry in a commercial swine facility. American Association of Swine Veterinarians Foundation. \$12,500. June 1, 2016, 6 months.
- Holtkamp D.J., Linhares D.C. Monitoring and updating the value of productivity losses due to porcine reproductive and respiratory syndrome virus. National Pork Board. \$84,237. November 1, 2015. 3 years.
- Holtkamp D.J., Linhares D.C., Karriker L. Development of PRRS outbreak investigation and data management/analysis program for breeding herds in regional PRRSV projects in Iowa. Iowa Pork Producers Association. \$37,628. May 1, 2016. 1 year, continuation.
- Holtkamp D.J., Linhares D.C., Karriker L., Ramirez A. Development and support of an industry rapid response program for epidemiological investigations of emerging, transboundary and endemic swine diseases with known etiology. Swine Health Information Center. \$191,353. September 1, 2016. 1 year.

Holtkamp, D.J., Zhang J. Effect of disinfectants and treatment conditions on the molecular detection of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV). Virox. \$26,475. June 1, 2017. 6 Months.

Linhares D.C., Holtkamp D.J., Arruda A., Morrison B., Silva G., Vilalta C. Description of biosecurity aspects of herds with low or high PRRS incidence and comparison within and between production systems. Swine Health Information Center. \$40,619. October 1, 2016. 1 year.

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Linhares D.C., Zimmerman J.J., Rademacher C., Holtkamp D.J. Herd sensitivity of PRRSV-monitoring schemes on sow herds undergoing virus elimination. Boehringer Ingelheim Vetmedica, Inc. \$35,000. June 1, 2016, 1 year.

Miller C, Yoon KJ. 7/1/15-6/30/17. Development of novel reovirus-based mucosal vaccine vectors for PEDV antigen production. Iowa Pork Producers Association, \$25,000 for 1st year.

Xu W, Yoon K-J. 7/1/15-12/31/17. Development of T-cell based vaccine against African swine fever virus. Center of Excellence for Emerging and Zoonotic Animal Diseases, \$79,977.

Yoon K-J. 9/1/2016-8/30/2018. Investigate the pathogenesis and biology of emerging and re-emerging swine viral diseases. USDA ARS Cooperative Agreement, \$275,000.

Zhang J, Gauger P, and Harmon K. Development and evaluation of a real-time PCR and an insulated isothermal PCR for the detection of Senecavirus A. Swine Health Information Center. \$27,333. 2016-2017.

Zhang J, Gauger P, Harmon K, Main R, and Wang C. Comparison of PRRSV virus isolation in different cell lines towards improving success of isolating PRRSV from clinical samples. American Association of Swine Veterinarians Foundation. \$30,000. 2017-2018.

Zhang J, Schumacher L, Chen Q, Gauger P, Giménez-Lirola L, Magstadt D, and Arruda P. Pathogenicity and antibody responses of different U.S. PEDV strains in pigs of different ages. Iowa Pork Producers Association. \$108,087. 2017-2018.

Zhang J. Charoen Pokphand Foods (CPF) Fellowship. Charoen Pokphand Foods (CPF) Thailand. \$165,860. 2016-2020.

F. WORK PLANNED FOR NEXT YEAR

Refer to funded projects.

Objective 1. Control of PRRSV

HOLTKAMP: PRRS Outbreak Investigation Program. Continue to develop and pilot the PRRS Outbreak Investigations Program for the Iowa Pork Producers Association. The program is now entering its fourth year. The objective of the PRRS outbreak investigations program for breeding herds is to improve biosecurity and reduce the geographic spread of the virus. The program is

being piloted on 30 breeding herds in the Buchanan County, Southeast Iowa and Southwest Iowa regional PRRSV projects in Iowa (USA). Six PRRS outbreak investigations were conducted in 2016 / 2017. The investigations were facilitated by me, with help from Rita Neat, Kimberley Gerardy and Chris Mowrer. In addition, the outbreak investigation forms were previously adapted to conduct a porcine epidemic diarrhea virus (PEDV) outbreak investigations. The forms have also been adapted for seneca virus A (SVA).

LINHARES - Disease detection / monitoring:

1. Processing fluids to detect PRRSV/PCV2 at low prevalence in neonates (3-5 days old).
 - a. Using PF to screen farms for PRRSV
 - b. Monitoring herds undergoing elimination (documenting time to test PF-negative)
 - c. Correlating PF results with downstream performance
 - d. Testing conditions (time/temperature before testing, extraction, PCR conditions)
2. Family oral fluids to detect PRRS at low prevalence in due-to-wean (DTW) pigs
 - a. Conditions to improve success rate to obtain fluids
 - b. FOF vs blood
3. Production data for automated, ongoing monitoring of swine herds
 - a. Automated SPC application for breeding herds to detect early signals of significant disease outbreaks
 - b. Automated SPC application for growing pigs
4. Predictors of growing pig performance
 - a. Consolidating source farm data (health and production data), growing pig data (e.g. feed mill, supervisor, stocking density/flow), biosecurity, and demographic data to correlate/predict closeout ADG/mortality
5. Domestic swine disease reporting system
 - a. Dashboard with consolidated/aggregated data from VDLs to report disease over time and space, by age group, specimen, state.
 - b. Veterinary council group
6. Sentinel farm approach for regional surveillance

Objective 2. Detection, prevention, and control of emerging viral diseases of swine.

HOLTKAMP: Rapid Response Program for Epidemiological Investigations of emerging and transboundary diseases. In August of 2016, the Swine Health and Information Center (SHIC) funded development of a rapid response program for epidemiological investigations of emerging and transboundary swine diseases. A six-member advisory group was formed to provide input regarding the responsibilities of RRC leaders and members, the content and delivery of RRC training, the design of disease investigation forms, and any other matters related to the program. The foundation of the program will be a Rapid Response Corps (RRC) consisting of a nationwide network of veterinary consultants, state animal health officials, epidemiologists and, when appropriate, federal animal health officials. A critical aspect of the program will be the development and use of a standardized approach and methodology for conducting epidemiological investigations. Standard forms and summary reports developed for the PRRS outbreak investigation pilot project funded by the Iowa Pork Producers (IPPA) will be used for training purposes. In the event of an emerging or transboundary disease outbreak, forms and reports will be adapted as necessary. While RRC members will be trained to ask open-ended

questions during the investigations, specific closed-ended questions will be embedded in the investigation form to capture a consistent set of information that can be accumulated in a database. The database will serve as a primary source of information to help meet the objectives for a rapid response in the event of a novel emerging or transboundary disease.

LINHARES

1. Field investigations of emerging diseases (Porcine Sapelovirus, Porcine Astrovirus type 3, Porcine Teschovirus)
2. Comparison of changes in productivity of herds using killed vs attenuated PRRS vaccine
3. Within and between production system comparison of PRRS impact of breeding herd productivity

GAUGER

1. Development of a vaccine challenge model for porcine parainfluenza virus type 1.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Sheela Ramamoorthy
Assoc Prof
Sheela.ramamoorthy@ndus.edu

2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

N/A

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Progress on efforts to develop a PRRSV vaccine with enhanced immunogenicity and DIVA capabilities included a) the development of 2 vaccine constructs in which selected structural proteins were re-engineered in the backbone of an infectious clone to test the hypothesis that the mutations would enhance B cell mediated immunity b) expression of a DIVA marker in the modified infectious clone and c) introduction of selected mutations to target suicidal replication of the modified live vaccine to enhance vaccine safety. The vaccine constructs were tested recently in pigs and data is under analysis.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

1. Proprietary methods for the development of first generation, rapid-response vaccines for RNA viruses were developed using PEDV as a model. The processes were intended to be a hybrid between inactivated and attenuated vaccines, such that the safety and efficacy advantages respectively, could be combined. The methods developed are also highly relevant to the autogenous vaccine industry where vaccine safety is a large concern. Testing of the vaccine candidate in 3-4 week old pigs elicited strong spike protein specific antibody responses. Vaccinated pigs were completely protected against challenge with the virulent virus, while unvaccinated controls showed clinical signs and viral shedding in feces. The vaccine virus was not detected in fecal matter, prior to challenge; nor did vaccination induce any clinical signs. Hence, the approach for first-response vaccine development was both highly safe and effective. A grant has been submitted to NIFA for funding to test the vaccine in sows and measure lactogenic immunity.

2. Methods to improve the delivery and immunogenicity of peptide antigens encoding specific epitopes was developed in collaboration with scientists with expertise in polymeric material science. Three 2009 H1N1 influenza viral epitopes were expressed as a string using a bacterial expression system. The highly hydrophobic peptide did not enter cells when incubated alone on MDCK cells. When conjugated with a proprietary polymer, the antigen was detected intracellularly, with negligible cytotoxicity. Vaccination of pigs with the conjugated peptide vaccine elicited strong anti-peptide antibody responses. Upon challenge with the virulent homologous virus, pigs vaccinated with the conjugated peptide or peptide alone showed enhanced viral replication in day 3 post-challenge, when compared to unvaccinated controls or pigs administered the polymer alone. However, at day 6 post-challenge the trend was rapidly reversed with vaccinated pigs clearing the virus rapidly while unvaccinated pigs showed an increasing viral titer. Hence, the conjugation of the peptide to the polymer was effective in enhancing delivery in vitro and protection in vivo. The mechanisms of protection did not appear to involve neutralizing antibody responses and remain to be elucidated

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

1. One PhD student was trained in vaccine development methods and provided to present his work at a regional conference where he won the second-place award.

2. Methods for the development of rapid-response serological diagnostics were developed for PEDV

3. Methods for rapid-response vaccine development were optimized and tested for PEDV. The rapid-response vaccine was highly safe and effective in 3-4 week old piglets and had broad applicability to other RNA viruses. A patent to cover the technology was filed in Feb 2017.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

N/A

2) Abstracts or Proceedings

1. Karsky, J, Singh, P and Ramamoorthy, S. 7th Euro Global Summit on Clinical Microbiology, Quantification of the PEDV virus with a colorimetric assay. Amsterdam, Netherlands (2017). Invited presentation.

2. Gagandeep Singh, Pankaj Singh, Angela Pillatzki, Eric Nelson, Brett Webb, Steven Dillberger-Lawson and Sheela Ramamoorthy. PEDV: A Model for rapid response vaccines. North Dakota Academy of Sciences (2017), Grand Forks, ND. (2nd place award).

3. Singh, G., Zholobko, O., Pillatzki, A., Nelson, E., Webb, B., Voronov, A., and Ramamoorthy, S. Vaccination of Pigs with improved HA and M2e Epitope Based Amphiphilic Invertible Polymeric Peptide Vaccine against Swine Influenza Viruses (SIVs). NDSU-KU Joint Symposium on Biotechnology, Nanomaterials, and Polymers. Fargo, ND (2017).

4.Singh. G., Zholobko. O., Pillatzki.A., Nelson. E., Webb. B., Voronov. A., and Ramamoorthy. S. Enhancing Delivery and Immune Response of Peptide Vaccine by Polymer-Peptide Mixed Micellar Assemblies. 2nd International Symposium on Materials from Renewables (ISMR). Athens, GA (2017).

5.Gagandeep Singh, Pankaj Singh, Angela Pillatzki, Eric Nelson, Brett Webb, Steven Dillberger-Lawson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases Rapid response vaccine against the porcine epidemic diarrhea virus (PEDV). Chicago, IL. (2017).

6.Gagandeep Singh, Oksana Zholobko, Angela Pillatzki, Brett Webb, Eric Nelson, Andriy Voronov and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Improved delivery of a HA and M2e-based peptide vaccine against swine influenza viruses. Chicago, IL. (2017).

7.Pankaj Singh, Gagandeep Singh, Jenna Karsky, Eric Nelson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Quantifying porcine epidemic diarrhea virus-specific neutralizing antibodies with a rapid colorimetric assay. Chicago, IL. (2017).

8.Oleksandr Kolyvushko, Gagandeep Singh, Brett Webb, Angela Pillatzki, Diego Diel, Steven Dillberger-Lawson, Eric Nelson and Sheela Ramamoorthy. 95th Annual Meeting of the Council of Research Workers in Animal Diseases. Efficacy of a commercial PCV2 vaccine against the contemporary PCV2d strain. Chicago, IL. (2017).

3) Book Chapters or Monographs

Joint research and training initiatives between East African and North American Universities. John Baligwamunsi Kaneene, Margaret Loy Khaita, John David Kabasa, Florence Wakoko, William Sicho, Douglas Freeman, Claire Card, Teresa Bergholz, Sheela Ramamoorthy, Ayele Teshome, Jesca Nakavuma, Samuel Majalija, Stevens Kisaka, Paul Ssajjakambwe, Sam Okech, Micheal Muleme, Sylvia Angubua Baluka, Herbert Kazoora, Patrick Vudriko *Pan Afr Med J.* 2017; 27(Suppl 4): 4, 24 August 2017

D. FUNDING SOURCES

1. Porcine Model for Torque Teno Virus Infections – NIH R21. Impact Score – 19. Funding release awaited
2. First response vaccines for emergency preparedness – USDA NIFA. Pending.

E. WORK PLANNED FOR NEXT YEAR:

1. The current efforts to develop improved PCV2 and PRRSV vaccine with DIVA capabilities will be completed.
2. Rapid response vaccine for swine influenza viruses and testing of the developed rapid-response PEDV vaccines in sows will be targeted.
3. A porcine coinfection model of TTV and SIV coinfections will be developed to determine if and how TTV infections shift the immune response profile in influenza infections.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: University of Nebraska Lincoln

A. Personnel

1) NC-229 STATION REPRESENTATIVE:

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Studies on protective PRRSV immunity: role of innate immunity induction in an effective acquired immunity; strategies of broadening protective efficacy of live vaccines

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Studies on biosecure inactivation of PEDV in carcasses and in manure

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

PRRSV:

Effective technology transfer of new synthetic live vaccine technology to industry through signature of multi-year contract with a vaccine company based in the US

PEDV:

Evidence that composting represents an effective and biosecure approach to inactivate PEDV in porcine carcasses, providing a method to reduce transmission and control virus spread on farms.

Treatment of PEDV infected manure with alkaline lime slurry was shown to inactivate PEDV using a bioassay, thus providing an intervention for producers and manure handlers to minimize risk of PEDV transmission during manure handling.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Stevens E.E., Miller D., Brittenham B.A., Vitosh-Sillman S.J., Brodersen B.W., Jin V.L., **Loy J.D.**, and Schmidt A.M. Alkaline stabilization of manure slurry inactivates porcine epidemic diarrhea virus (PEDV). *Journal of Swine Health and Production*. Accepted/In Press

Vitosh-Sillman S., **Loy J. D.**, Brodersen B.W., Kelling C.K., Eskridge K., and Millmier Schmidt A. Effectiveness of composting as a biosecure mortality disposal method for porcine epidemic diarrhea virus (PEDV)-infected pig carcasses. (2017) *Porcine Health Management*. Vol 3 (22) DOI: 10.1186/s40813-017-0068-z

Vitosh-Sillman S. **Loy J. D.**, Brodersen B.W., Doster A.R., Kelling C., Topliff C., Nelson E., Bai J., Schirtzinger E., Poulsen E., Meadors B., Anderson J., Hause B., Anderson G., and Hesse, R. (2016) Experimental infection of conventional nursing pigs and their dams with porcine deltacoronavirus. *Journal of Veterinary Diagnostic Investigation*. Vol 28 (5) 486-497*
DOI: 10.1177/1040638716654200

Sun H, Workman A, Osorio FA, Steffen D, Vu HLX. Development of a broadly protective modified-live virus vaccine candidate against porcine reproductive and respiratory syndrome virus. *Vaccine*. 2018 Jan 2;36(1):66-73. doi: 10.1016/j.vaccine.2017.11.028. Epub 2017 Nov 22. PubMed PMID: 29174314.

Kimpston-Burkgren K, Correas I, Osorio FA, Steffen D, Pattnaik AK, Fang Y, Vu HLX. Relative contribution of porcine reproductive and respiratory syndrome virus open reading frames 2-4 to the induction of protective immunity. *Vaccine*. 2017 Aug 3;35(34):4408-4413. doi: 10.1016/j.vaccine.2017.06.061. Epub 2017 Jul 6. PubMed PMID: 28689650.

Correas I, Osorio FA, Steffen D, Pattnaik AK, Vu HLX. Cross reactivity of immune responses to porcine reproductive and respiratory syndrome virus infection. *Vaccine*. 2017 Feb 1;35(5):782-788. doi: 10.1016/j.vaccine.2016.12.040.

Epub 2017 Jan 3. PubMed PMID: 28062126.

Sun H, Pattnaik AK, Osorio FA, Vu HLX. Identification of viral genes associated with the interferon-inducing phenotype of a synthetic porcine reproductive and respiratory syndrome virus strain. *Virology*. 2016 Dec;499:313-321. doi: 10.1016/j.virol.2016.09.018. Epub 2016 Oct 11. PubMed PMID: 27736706.

Vu HLX, Pattnaik AK, Osorio FA. Strategies to broaden the cross-protective efficacy of vaccines against porcine reproductive and respiratory syndrome virus. *Vet Microbiol*. 2017 Jul;206:29-34. doi: 10.1016/j.vetmic.2016.09.014. Epub 2016 Sep 21. PubMed PMID: 27692670.

D. FUNDING SOURCES

On-farm Remediation and Prevention of Swine Enteric Diseases. USDA-AFRI, Foundational Program, 2016-68008-25043

Pathogenesis Studies with Porcine Epidemic Diarrhea Virus (PEDV): Generation and Characterization of Infectious Clone-Derived Viruses", Pattnaik, A. (Principal Investigator), **Loy, J.** (Investigator),

Investigation of host genetic role in porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) susceptibility USDA-AFRI, Foundational Program,

PD: Daniel Ciobanu, Co-PD: Hiep Vu

Amount : \$ 459,200

2017-2019

Determine the correlates of cross-protective immunity to PRRSV USDA NIFA Grant No. 2016-67015-24922

PD: Vu, Hiep Co-PD: Osorio, F

Amount: \$477,635

2016-2019

E. WORK PLANNED FOR NEXT YEAR

Work continues on developing proteomics based approaches to enteric coronavirus characterization and differentiation using mass spectrum biomarker based approach.

Work continues on experimental vaccinology: broadening protection for live vaccines against PRRSV and centralized antigenic subunit immunization against swine influenza

Use of PRRSV model to investigate host genetics

Developmental research on PEDV reverse genetics

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

USDA, Agricultural Research Service, National Animal Disease Center
1920 Dayton Avenue, Ames, IA 50010

A. Personnel

1) NC-229 STATION REPRESENTATIVE:

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Nicholson, Tracy; Research Microbiologist, NADC; tracy.nicholson@ars.usda.gov
Vincent, Amy; Veterinary Medical Officer, NADC; amy.vincent@ars.usda.gov

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- **Miller:** applied RNA analyses on infected and control monocyte-derived cells. Such research uncovered networks of predicted protein-protein interactions and biological processes related to both low virulence and highly pathogenic PRRSV infection. The analysis revealed the ability of PRRSV to affect cell activation. Genes showing variability in expression were related to cellular structure and inflammatory immune responses. These results supply novel insight into the interplay of PRRSV pathogenicity and immune system evasion.
- **Miller:** to identify mechanisms that modulate innate and adaptive immune responses to swine viral pathogens, conducted genome-wide RNA profiling of signature genes in activated porcine monocytic innate immune cells. From this research, the diverse antiviral properties that interferon and interferon-stimulated gene families have on swine viral pathogens were determined. The data revealed different expression levels of inflammatory cytokines, chemokines, receptors, interferon-regulatory factors and interferon-stimulated gene families in PRRSV-infected macrophages setting the stage for development of novel therapies and vaccine strategies.
- **Miller:** expression analysis of the type and quantity of small non-coding RNAs was completed comparing healthy and PRRSV-infected pigs to elucidate when the largest change in gene expression occurs, and if all categories of small non-coding RNAs are

affected. Transfer RNA fragments experienced a lower reduction in number than the microRNAs and appear to be more stable across time points than microRNA or other non-coding RNAs. This information helps in understanding how gene function in the pig can become dysregulated by PRRSV, in conjunction with how the pig's immune system responds to the virus.

- **Faaberg:** a modified attenuated vaccine of PRRSV was used to prepare novel candidate vaccine constructs. One region of the attenuated vaccine was amplified and will be used to join to another section of the genome of more contemporary viruses found in production systems.
- **Faaberg, Lager:** sequenced the entire genome of 17 PRRSV isolates prepared by scientists at Iowa State University and discovered that the isolates, originally thought to be similar based on a small region of the genome, were very dissimilar. The isolate genomes were analyzed for evidence of viral recombination using index prototype strain genomes representative of different lineages. Several instances of viral recombination were detected in most of the 17 isolates, showing that viral recombination occurs at a high frequency in infected swine herds. Four genomically distinct isolates were chosen for swine infection experiments and resulted in a spectrum of diseases, two of which were much more pathogenic than the others, and one which produced very mild disease.
- **Lager:** conducted animal studies to investigate field observations that traditional use of live-virus inoculation in breeding age gilts to induce PRRSV protection is now failing because of some inherit change in contemporary field isolates.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

- **Faaberg and collaborator:** studied the enzymatic activity of the papain-like protease 2 domain in nonstructural protein 3 of porcine epidemic diarrhea virus (PEDV) and porcine delta coronavirus (PDCoV). The research found striking differences in this domain between the two viruses, and will be used to further investigate viral virulence traits.
- **Faaberg and collaborator:** Developing infectious clones of PEDV and PDCoV for vaccine generation
- **Lager:** Demonstrated that 1) wild-type SVA infection can induce a protective immune response with a duration for at least 4-5 months, 2) SVA transmission can occur for at least 2 weeks post infection to age-matched sows, and 3) environmental contamination may be a likely source of SVA detected in sows moving from farm to eventual slaughter. This information will help in developing response strategies at slaughter house. which can help in developing control programs on the farm.
- **Miller:** GEO ID: GSE74473 Organism/cell line/tissue: Sus scrofa domesticus/tracheobronchial lymph nodes (TBLN). Raw Digital Gene Expression Tag Profiling sequences. A major goal of this study was to profile the biological and molecular networks involved in the pathological response caused by Pseudorabies virus infected porcine tracheobronchial lymph node. Gene Expression Omnibus is a public functional genomics data repository supporting MIAME-compliant data submissions for free access by scientists which increases usability and visibility. The resource supports archiving of raw data, processed data and

metadata which are indexed, cross-linked and searchable. All data are freely available for download in a variety of formats. GEO also provides several web-based tools and strategies to assist users to query, analyse and visualize data. There is evidence that more scientists are using a data-driven approach to research, whereby the first step in a project is to combine and re-analyse public data sets to reveal previously unknown relations or uncover ever more subtle trends in the data.

- **Nicholson:** To identify genomic differences between virulent and non-virulent *Haemophilus parasuis* isolates, the closed whole-genome sequence and genome-wide methylation patterns for the highly virulent Nagasaki strain and for the non-virulent D74 strain were obtained. 366 genes unique to Nagasaki and 324 genes unique to D74, including several putative Type I and Type III restriction modification systems, hemolysins, and other putative virulence-associated genes were identified. Fourteen methylation motifs were identified in the Nagasaki genome and fifteen methylation motifs were identified in the D74 genome, with only one motif shared between the two genomes. To evaluate the contribution of gene expression differences, RNA sequencing was performed on Nagasaki and D74 after growth with and without 5% CO₂. 284 genes were differentially expressed in strain D74 in response to 5% CO₂, while only 36 genes were differentially expressed in strain Nagasaki. These data demonstrate that strain D74 is more transcriptionally responsive to carbon dioxide levels that mimic in vivo conditions within the respiratory tract and suggest that non-virulent *H. parasuis* strains may be more adaptive to colonization within the respiratory tract than virulent strains. Collectively, the unique genomic and transcriptional features identified in this study provide a foundation for understanding the genomic attributes responsible for the spectrum of virulent phenotypes that exist among *H. parasuis* isolates. This information is paramount to designing effective vaccines needed by the swine industry to mitigate *H. parasuis* disease burden.
- **Vincent, Abente:** to investigate host-pathogen interactions at cellular or molecular levels, host gene expression profiles were examined using a PCR array targeting 168 genes associated with the swine antiviral response and cytokine and chemokine pathways. Differential gene expression patterns were observed.
- **Vincent, Abente and collaborators:** to examine virus, host, and population factors that influence interspecies transmission in swine, work continued on a recently established human-like H3 virus lineage in swine to study its genetic and antigenic evolution. Representative human and swine human-like viruses were used to perform virus histochemistry on swine tissue and in vitro replication assays. A pathogenesis and transmission study with a North American 2017 H7N9 low pathogenic avian influenza virus was completed.
- **Vincent, Abente:** to identify emerging IAV and monitor genetic and antigenic evolution in swine, subtype and genetic patterns were monitored to identify changing patterns or emerging viruses. H1N1, H1N2, and H3N2 with molecular signatures suggesting antigenic changes were identified and virus isolates obtained from the USDA IAV-S surveillance repository for antigenic and pathogenic characterization.
- **Vincent, Abente and collaborators:** to develop and implement an automated clade tool for H1 with standardized global nomenclature, a phylogenetic based method for classifying H1 IAV was developed and validated on a large global dataset of hemagglutinin gene

sequences. The automated tool was demonstrated to be highly accurate and was implemented on the Influenza Research Database (fludb.org).

- **Vincent, Abente:** to identify genetic changes important for antigenic drift or pathogenicity in swine or other hosts, IAV subtype H1 and H3 viruses with unique antigenic motifs, predicted to be antigenically distinct, were obtained and tested in vitro to characterize their antigenic phenotypes. New antigenic motif patterns in H3 were shown to be distinct from previous H3 and changed in frequency of detection over time.
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- **Vincent, Abente:** to investigate adjuvants or immune-modulatory agents that result in robust immune responses (mucosal delivered, long lived, broadly cross-protective, and/or reduce the number of vaccine boosters), a study was conducted to test the effect of sequential heterologous infection in imprinting the humoral immune response. The order of infection significantly impacted the humoral immune response to each of the viruses and certain exposure patterns led to increased lung pathology.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

1. **Identified the effect that porcine reproductive and respiratory syndrome virus (PRRSV) infection has on the display of signature genes of activated mononuclear cells.**
Monocytic cells are one of the cell types that are intricately involved in the animal's response to disease. Following infection, the monocytic cell becomes activated which can occur by direct contact with an infectious agent, or indirectly through stimulation of the cell by specific proteins produced by other cells in the body. Activated monocytic cells then become polarized (meaning the cell has developed a certain response against a virus or bacteria). ARS researchers studied the direct involvement of polarization of monocytes during infection. Understanding the complex nature of the protective immune response may be critical to improving vaccines.
2. **Analyzed gene expression changes during pseudorabies virus (PRV) infection.** PRV causes severe disease in swine and is an economically important disease, or disease threat in most swine producing countries. As the pig responds to a PRV infection, changes in metabolism reflect changes in the expression of specific genes. Gene expression describes the regulation of the pig's metabolic processes, and gene expression profiling is the process of determining which genes are active in a specific cell or group of cells. Variation in gene expression profiles can act as an important indicator of disease or predisposition to disease. Characterizing core gene changes gives insight to how the virus affects the host, and how the host is trying to combat the infection which can lead to a greater understanding of how to build better vaccines which may help in the control of pseudorabies.

3. **Annotation of IFN gene families in swine and across 155 animal genomes.** Innate immune interferons (IFNs), particularly type I IFNs, are primary mediators regulating antiviral immunity. These antiviral cytokines have evolved remarkable molecular and functional diversity to confront ever-evolving viral threats. ARS researchers showed that pigs have the largest and an expanding type I IFN family, consisting of nearly 60 functional genes that encode seven IFN subtypes including multigene subtypes of one class of IFN (IFN- α). Whereas subtypes such as IFN- α and - β have been widely studied, the unconventional IFN- ω subtype has barely been investigated. Cross-species comparison revealed the molecular and functional novelty of porcine interferon-omega subtype (ω), which has evolved several novel features: a signature multi-gene subtype, emerging isoforms that have much higher antiviral potency than typical IFN- α , high antiviral (but little antiproliferative) activity in cells of humans and other mammalian species, and potential action through unusual signaling pathways. This study revealed the antiviral potency of porcine IFN- ω and potential use of novel IFN-based antivirals against devastating viral diseases.
4. **Described the interaction of type I IFNs (IFN- α and - β) and a specific pathway of signaling (mTOR-mechanistic target of rapamycin) that underlie PRRSV infection.** Targeting on macrophages, ARS researchers elaborated the direct involvement of the mTOR signaling pathway during PRRSV infection. Comprehensive understanding of the immunological impact may become increasingly important to understand host-virus interactions of existing and emerging pathogens, with application to the development of novel therapies and vaccine strategies.
5. **Described recombination within a set of diverse PRRSV field isolates.** ARS scientists processed 17 isolates that had emerged in the United States in 2015 for next generation sequencing and assembled them into complete viral genomes. Results revealed that the viruses were very dissimilar in all parts of their genomes. Further evolutionary analyses, comparing the isolates to unique prototype index genomes, revealed several common areas where the viruses had recombined. The data indicates the remarkable ability of PRRSV to undergo high frequency recombination in the field. Three viral isolates were used to challenge swine. One isolate was shown to produce enhanced clinical disease. The viral strain will be used in our formulation of new vaccine candidates.
6. **Demonstrated the utility and differences between PRRSV genome modifications in two different regions of nonstructural protein 2 (nsp2).** ARS researchers investigated the stability of mutant viruses. Next generation sequencing showed that three inserted small tags were all stable (except for one mutant) over ten passages in susceptible cells. The rate of viral replication of all mutants in cells was not inhibited and the viral plaque size for the mutants was not decreased. However, detailed analyses showed that insertion of any of the tags near the beginning of the protein could be detected in genome length and multiple smaller viral RNAs, whereas tag insertion near the end of the protein only was detected in genome length viral RNA. In addition, infected cell immunofluorescence examination suggests that the two different nsp2 insertions resulted in proteins localizing to discrete areas around the cell nucleus. The mutant viruses will be used to investigate the role of nsp2 in pathogenesis.

7. **Investigated the ecology and protective immune response of Senecavirus A (SVA), a swine virus that has recently emerged as a problem in US swine.** Demonstrated that 1) wild-type SVA infection can induce a protective immune response with a duration for at least 4-5 months, 2) SVA transmission can occur for at least 2 weeks post infection to age-matched sows, and 3) environmental contamination may be a likely source of SVA detected in sows moving from farm to eventual slaughter. This information will help in developing response strategies at slaughter house. which can help in developing control programs on the farm.
8. **Biofilm plays a role in persistence of *Bordetella bronchiseptica* in the lung.** *B. bronchiseptica* is a bacterial respiratory swine pathogen that routinely infects pigs for long periods of time. This holds true despite the use of vaccines, where *B. bronchiseptica* is frequently isolated from the nose of vaccinated animals. Like many bacteria, *B. bronchiseptica* can form biofilms, which protects the bacteria from a variety of host clearance mechanisms and antimicrobial compounds. ARS scientists tested a known biofilm factor produced by bacteria termed Bps for its role in biofilm formation of swine isolates of *B. bronchiseptica* and its role in swine respiratory disease. Results indicated that Bps was required for biofilm formation and for infecting the lungs or lower respiratory tract of swine. These findings provide critical information needed to design improved vaccines and intervention strategies to control or eliminate chronic carriage of *B. bronchiseptica* and other bacterial pathogens in swine.
9. **Antimicrobial resistance in swine livestock-associated (LA), methicillin-resistant *Staphylococcus aureus* (MRSA) is lower than in human MRSA isolates.** *S. aureus* is a common and sometimes devastating human pathogen that has the ability to acquire resistance to antibiotics resulting in MRSA. Swine can carry strains of MRSA that do not appear to cause disease in swine, but it is unclear whether these swine LA-MRSA are a risk for humans. ARS scientists determined the antimicrobial resistance profiles and genetic mechanisms of antimicrobial resistance among swine LA-MRSA and human clinical MRSA isolates. Swine LA-MRSA isolates exhibited resistance to fewer antibiotics than MRSA isolates from humans with no swine contact. Distinct genomic antimicrobial resistance elements were harbored by each subgroup, with little overlap in shared antimicrobial resistance genes between swine LA-MRSA and human clinical MRSA isolates. These results indicate there are distinct populations of MRSA in swine and humans, and antibiotic resistance is more prevalent in human strains, suggesting that human to human spread is more of a risk than swine to human transmission.
10. **Use of a granulocyte-colony stimulating factor (G-CSF) to prevent *Streptococcus suis* infection in swine.** The use of immunomodulators is a promising alternative to the use of antibiotics to prevent and combat infectious disease. Previously ARS scientists demonstrated a replication-defective adenovirus vector that expresses G-CSF elicited a sustained increase in circulating neutrophils, a type of white blood cell that is beneficial in preventing bacterial diseases. In new studies, pigs given the vectored G-CSF had an improved outcome when infected with *Streptococcus suis*, the leading cause of meningitis in weaned pigs. Thus, the use of G-CSF in pigs to induce an increase in circulating neutrophil numbers may be a useful alternative to antibiotics for prevention

of Streptococcal and other bacterial diseases, especially during times of stress and pathogen exposure such as post-weaning.

11. **Zinc Resistance within Swine Associated Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates in the USA is Associated with MLST Lineage.** Zinc resistance in livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) sequence type (ST) 398 is primarily mediated by the *czrC* gene co-located with the *mecA* gene, encoding methicillin resistance, within the type V SCC*mec* element. Because *czrC* and *mecA* are located within the same mobile genetic element, it has been suggested that the use of in feed zinc as an antidiarrheal agent has the potential to contribute to the emergence and spread of MRSA in swine through increased selection pressure to maintain the SCC*mec* element in isolates obtained from pigs. To test this assumption, the prevalence of zinc resistance in US swine associated LA-MRSA ST5 isolates, MRSA ST5 isolates from humans with no swine contact, and US swine associated LA-MRSA ST398 isolates was evaluated. The data suggest that selection pressure associated with zinc supplementation in feed is unlikely to have played a significant role in the emergence of LA-MRSA ST5 in the US swine population. The data also indicate that zinc resistance is associated with MLST lineage suggesting a potential link between genetic lineage and carriage of resistance determinants.
12. **Developed a computational tool that automatically classifies global swine H1 subtype HA gene sequences.** Infection with influenza A virus (IAV) is one of the most important respiratory diseases of swine and is the second most common viral diagnosis of respiratory disease in the United States. The USDA IAV swine surveillance system initiated in 2009 has increased the amount of publically available sequence data on swine viruses circulating in the United States. A significant barrier for swine producers to make timely vaccine interventions and for researchers to use relevant viruses in studies is having the computational expertise to analyze and characterize the HA gene. The HA protein is a major component of vaccines and target for immune responses. In collaboration with an international network of influenza experts, ARS researchers developed a computational tool that can automatically classify swine H1 subtype HA gene sequences. An important component of the tool is the harmonization of H1 HA nomenclature, as well as a standardized technique for genetically characterizing the HA gene. This open-access tool will aid swine producers, veterinarians, vaccine manufacturers, and IAV vaccine researchers in selecting vaccine strains to match the strains that are currently circulating. Properly matching vaccines to field strains is a critical part of managing swine influenza.
13. **Reassortant influenza A virus (IAV) with highly pathogenic avian influenza H5N1 surface genes had modestly increased replication and transmission in pigs.** Following the introduction of the 2009 pandemic H1N1 virus (H1N1pdm09), many animal species have been shown to be infected due to human to animal transmission. The IAV genome is composed of 8 gene segments, and mixing of gene segments from distinct parental viruses can result in progeny viruses with improved capability of infecting a host, ability to evade immunity, or with distinct pathogenic phenotypes. ARS scientists demonstrated that a laboratory generated reassortant virus with highly pathogenic avian influenza H5N1 surface genes and internal genes from H1N1pdm09 virus had

modestly increased replication and transmission in pigs when compared to the parental H5N1 virus. Although not yet detected in pigs from natural events, this finding highlights the importance of maintaining a robust surveillance program to detect spillover events into swine and suggests that interspecies transmission barriers may partially be overcome by reassortment. Interspecies transmission into pigs is a risk to swine production as well as human pandemic risk.

14. **Demonstrated properties of H3N2 influenza A virus (IAV) strains isolated from swine varied depending on the genome constellation.** Following the introduction of the 2009 pandemic H1N1 (H1N1pdm09) from humans to swine, mixing of IAV gene segments between H1N1pdm09 and swine viruses occurred. By studying genomes of IAV detected in swine, a large number of gene segment combinations (genomes) among H3 subtype swine viruses were shown to be circulating in commercial herds. ARS researchers selected IAV with genomes representing observed patterns in viruses circulating in swine farms to investigate in experimental challenge studies. Infection properties of viral strains varied depending on the genome constellation and may explain why some combination of genes have been more successful in the U.S. swine population. This underscores the importance of surveillance and assessing whole-genome sequence data to better understand the disease properties of circulating IAV strains in the field. This information will help guide intervention strategies and improved choices in vaccine design.
15. **Demonstrated pigs with severe combined immunodeficiency (SCID) were impaired in controlling influenza A virus (IAV) infection.** Influenza A virus infections tend to be acute and relatively short in duration due to rapid induction of the immune response. Study of the immune response to IAV can reveal new ways to prevent or treat infections. Humans and animals may have genetic disorders that interrupt normal immune responses. In collaboration with scientists at Iowa State University, ARS researchers showed that pigs with SCID that do not have B-cell or T-cell immunity were impaired in controlling IAV infection. The delayed clearance of infection was despite an intact innate immune response. These SCID pigs provide a valuable model to understand the immune mechanisms associated with protection and recovery in a natural host for influenza.
16. **Mammals captured near infected poultry farms lack evidence of exposure to 2014-2015 highly pathogenic avian influenza virus.** In 2014 and early 2015, a Eurasian strain of highly pathogenic avian influenza A (HPAI) virus was detected in poultry in Canada and the United States, causing a large economic loss to the poultry industry and tremendous investment by the industry and USDA officials to control the outbreak. In an effort to understand the spread of the Eurasian H5 virus, epidemiologic investigations occurred at poultry facilities. Synanthropic birds and mammals were sampled at infected and uninfected poultry farms in northwest Iowa, and in collaboration with APHIS scientists, ARS researchers tested for evidence of infection with HPAI H5. No mammal species showed evidence of infection or exposure, but a very small number of European starlings were found to have evidence of infection. These results indicate species that cohabitate with humans and their domestic animals merit further scrutiny to better understand potential biosecurity risks to HPAI outbreaks.

17. **The 2014-2015 highly pathogenic H5NX avian influenza virus that emerged in North America demonstrated limited replication in experimentally challenged pigs.** The susceptibility of pigs to HPAI H5N1, H5N2, and H5N8 clade 2.3.3.3 the recently emerged in North America were assessed. Pigs and trachea explants were inoculated with a representative panel of H5NX clade 2.3.4.4 HPAI viruses from North America. Limited virus replication was restricted to the lower respiratory tract of challenged pigs, though absent in the nasal passages and trachea cultures, as determined by RRT-PCR in all samples. Seroconversion of inoculated pigs was detected by NP ELISA but was not reliably detected by antigen-specific hemagglutination inhibition. Boost with adjuvanted virus was required for the production of neutralizing antibodies to assess cross-reactivity between wild-type avian strains. All RRT-PCR and serology tests were negative for contact animals indicating a failure of transmission from primary inoculated pigs. Collectively, our data show HPAI H5NX clade 2.3.4.4 viruses to be poorly adapted for replication and transmission in swine.
18. **A recently emerged avian-origin canine influenza A viruses does not replicate efficiently in experimentally challenged pigs.** A genetically and antigenically distinct avian-origin H3N2 canine influenza was detected in March of 2015 in Chicago, Illinois and subsequently caused widespread outbreaks in dogs across the country. Within the first 5 months of its original detection, over 1000 dogs in the Midwest were affected followed by positive detections in 23 additional states. We observed that the US canine H3N2 strain does not replicate efficiently in experimentally challenged swine, especially the upper respiratory tract. Low titers of virus were detected in the lungs of 4/5 pigs. Although virus was detected by RT-PCR in NS of 2/10 pigs, infectious virus was not isolated. Consistent with the limited replication detected in the upper respiratory tract, there was no evidence of transmission, suggesting a low risk of sustained infection in pigs.
19. **An H4N6 avian influenza A virus isolated from a clinically ill pig does not transmit efficiently in an experimental challenge and transmission study.** In late 2015, an avian-origin H4N6 influenza A virus was isolated from pigs in the United States during a routine diagnostic investigation of clinical respiratory disease in the herd. Serological analysis from additional pigs at the farm and other pigs within the swine production system indicated that the virus did not efficiently transmit from pig-to-pig and the mode of transmission to swine could not be determined. The isolate was characterized at the molecular level and the pathogenesis and transmission was experimentally evaluated in pigs. Although the virus replicated in the lungs of pigs and caused mild pulmonary lesions, there was no evidence of replication in the upper respiratory tract or transmission to indirect contacts, supporting the findings on the farm. Despite the lack of transmission and replication in the upper respiratory tract, efficient replication in the lung could lead to the emergence of a novel reassortant. Continued surveillance efforts are important to monitor and better understand the dynamics of cross-species spread of IAV.
20. **The molecular determinants of antigenic drift in the H3 hemagglutinin of swine influenza A virus were identified.** Six of the 7 positions previously identified in human seasonal H3 (positions 145, 155, 156, 158, 159, 189, and 193) were also

indicated in swine H3 antigenic evolution. To experimentally test the effect on virus antigenicity of these 7 positions, substitutions were introduced into the HA of an isogenic swine lineage virus. We tested the antigenic effect of these introduced substitutions by using hemagglutination inhibition (HI) data with monovalent swine antisera and antigenic cartography to evaluate the antigenic phenotype of the mutant viruses. Combinations of substitutions within the antigenic motif caused significant changes in antigenicity. One virus mutant that varied at only two positions relative to the wild type had a >4-fold reduction in HI titers compared to homologous antisera. Potential changes in pathogenesis and transmission of the double mutant were evaluated in pigs. Although the double mutant had virus shedding titers and transmissibility comparable to those of the wild type, it caused a significantly lower percentage of lung lesions. Elucidating the antigenic effects of specific amino acid substitutions at these sites in swine H3 IAV has important implications for understanding IAV evolution within pigs as well as for improved vaccine development and control strategies in swine.

21. Identified and characterized a novel reassortant human-like H3N2 and

H3N1 Influenza A Viruses isolated from pigs. Human-like swine H3 influenza A viruses were detected by the USDA surveillance system. The swine human-like H3N2 and H3N1 viruses encoded hemagglutinin genes similar to those in human seasonal H3 strains and internal genes closely related to those of 2009 H1N1 pandemic viruses. The H3N2 neuraminidase was of the contemporary human N2 lineage, while the H3N1 NA was of the classical swine N1 lineage. Both viruses were antigenically distant from swine H3 viruses that circulate in the United States and from swine vaccine strains and also showed antigenic drift from human seasonal H3N2 viruses. Their pathogenicity and transmission in pigs were compared to those of a human H3N2 virus with a common HA ancestry. Both swine human-like H3 viruses efficiently infected pigs and were transmitted to indirect contacts, whereas the human H3N2 virus did so much less efficiently. To evaluate the role of genes from the swine isolates in their pathogenesis, reverse genetics-generated reassortants between the swine human-like H3N1 virus and the seasonal human H3N2 virus were tested in pigs. The contribution of the gene segments to virulence was complex, with the swine HA and internal genes showing effects in vivo. The experimental infections indicate that these novel H3 viruses are virulent and can sustain onward transmission in pigs, and the naturally occurring mutations in the HA were associated with antigenic divergence from H3 IAV from humans and swine. Consequently, these viruses could have a significant impact on the swine industry if they were to cause more widespread outbreaks, and the potential risk of these emerging swine IAV to humans should be considered.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Abente, E.J., Kitikoon, P., Lager, K.M., Gauger, P.C., Anderson, T.K., Vincent, A.L. 2017. A highly pathogenic avian-derived influenza virus H5N1 with 2009 pandemic H1N1 internal genes

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2) Abstracts or Proceedings

- Faaberg, K. S., Wang, F.-X., M. A. Kappes, Das, P. B., Spear, A. 2017. Porcine reproductive and respiratory syndrome virus nonstructural protein 2 – a replicase protein that is packaged into virions and performs multiple functions. *Viruses & Cells: Gordon Research Conference, Biology, Pathogenesis, and Treatment of Viral Infections*, May 14-19, 2017, Lucca, Italy.
- Faaberg, K. S., Wang, F.-X., M. A. Kappes, Das, P. B., Spear, A. 2017. Porcine reproductive and respiratory syndrome virus nonstructural protein 2: a replicase protein that is packaged into virions and performs multiple functions. Abstract S2P-2. XIVth International Nidovirus Symposium, June 4-9, 2017, Kansas City, MO, USA.
- Fleming, D.S., Miller, L.C. (2017) Small non-coding RNAs (sncRNA) regulate gene silencing and modify homeostatic status in animals faced with porcine reproductive and respiratory syndrome virus (PRRSV) [abstract]. 36th International Society for Animal Genetics Conference (2017), July 16-19 2017, Dublin Ireland.
- Fleming, D.S., Miller, L.C. (2017) Small non-coding RNAs (sncRNA) regulate gene silencing and modify homeostatic status in animals faced with porcine reproductive and respiratory syndrome virus (PRRSV) [abstract]. XIVth International Nidovirus Symposium (Nido2017), June 4-9, 2017, Kansas City, Missouri.
- Fleming, D.S., Miller, L.C. (2017). (w)HOL(e)ISTIC gene ontology and pathway analysis using open source web tools [abstract]. International Plant & Animal Genome XXV, January 14-18, 2017, San Diego, California

- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes [abstract]. 36th International Society for Animal Genetics Conference (2017), July 16-19 2017, Dublin Ireland.
- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes [abstract]. XIVth International Nidovirus Symposium (Nido2017), June 4-9, 2017, Kansas City, Missouri.
- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals the potential of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus to modulate immune system activation related to host-pathogen and damage associated signaling in infected porcine monocytes [abstract]. The American Association of Immunologists 2017, May 12-16, 2017, Washington DC.
- Miller, L.C., Fleming, D.S., Li, X., Bayles, D. O., Blecha, F., Sang, Y. (2017). Transcriptomic analysis reveals potential of HP-PRRSV to modulate immune system activation related to host pathogen and damage associated signaling in infected porcine monocytes [abstract]. International Plant & Animal Genome XXV, January 14-18, 2017, San Diego, California.
- Nicholson T.L., Brunelle B.W., Bayles D.O., Alt D.P., Shore, S.M. Comparative Genomic and Transcriptional Analysis of Virulent and Non-virulent *Haemophilus parasuis* isolates. Abstract. ASM Microbe 2017 (American Society for Microbiology General Meeting) June 1-5, 2017. New Orleans, LA. USA.
- Sang, Y., Liu, Q., Miller, L.C., Lee, J., Ma, W., Blecha, F. (2017) Cross-Species Comparison Reveals Molecular and Functional Novelty of Porcine Interferon-Omega Subtype [abstract]. The American Association of Immunologists 2017, May 12-16, 2017, Washington DC.
- van Geelen, A.G.M., Buckley, A.C., Kulshreshtha, V., Brockmeier, S. A., Miller, L. C., Fleming, D., Loving, C., Faaberg, K. S., Lager, K. M. 2017. Live virus Immunization (LVI) with a recent 1-7-4 PRRSV isolate elicits broad protection against PRRSV challenge in finishing age swine. Abstract #53. 2017 Allen D. Leman Swine Conference, September 16-19, 2017, Saint Paul, MN, USA.
- Van Geelen, A.G.M., Faaberg, K.S., Phani, D., Montiel, N., Miller, L.C., Kulshreshtha, V., Buckley, A., and Lager, K.M. (2016). Comparative pathogenesis and characterization of contemporary 1-7-4 PRRSV isolates in weanling age piglets.[abstract]. American Association of Swine Veterinarians Annual Meeting February 25-28, 2017, Denver, Colorado.
- Wang, F., Otis, N., Chenge, J., Bester, S., Pegan, S. D., Faaberg, K. S. 2017. Nonstructural Protein 2 and Papain-like Protease 2 Chimeras between Highly-Pathogenic JXwn06 and Ingelvac PRRS® MLV Strains of Porcine Reproductive and Respiratory Syndrome Virus. Abstract S8. O-08. XIVth International Nidovirus Symposium, June 4-9, 2017, Kansas City, MO, USA.

3) Book Chapters or Monographs

D. FUNDING SOURCES

- Faaberg, Lager, Miller, Brockmeier, Nicholson, Vincent, Abente - USDA ARS Research Funds
- Sang, Rowland, Blecha, Miller – NIFA-AFRI - Antiviral regulation underlying the activation status of porcine monocytic innate immune cells
- Faaberg, Pegan – National Pork Board - Role of the viral ovarian domain protease in PRRSV pathogenesis
- Faaberg, Anderson, Lager – National Pork Board - United States Swine Pathogen Database
- Lager - Animal And Plant Health Inspection Service (APHIS), U.S. Department of Agriculture - Emerging Swine Disease Studies: Porcine Epidemic Diarrhea Virus (PEDV)
- Lager - Animal And Plant Health Inspection Service (APHIS), U.S. Department Of Agriculture - Identify Mechanisms of Viral Pathogenesis, Transmission, and Immunity of Porcine Epidemic Diarrhea Virus and Other Emerging Swine Coronaviruses
- Nicholson- Iowa Pork Producers Association (IPPA)-Comparative genomic and virulence analysis of *Streptococcus suis* isolates
- Vincent-NIAID-NIH CEIRS, USDA-APHIS

E. WORK PLANNED FOR NEXT YEAR

Miller:

- Establish that gene response pathways altered by PRRSV infection in monocytic cells provide a framework for identification of genes and gene products critical for anti-PRRSV regulation.
- Show that small non-coding RNAs (sncRNA) are a significant regulator of gene silencing when animals are faced with a pathogen that may modify their homeostatic status.
- Determine the diverse antiviral properties that IFN and ISG families have on swine viral pathogens.
- Maintain Surveillance for emerging swine diseases.

Faaberg:

- In vitro and vivo analysis of engineered PRRSV strains
- PEDV and PDCoV pathogenesis
- Swine Pathogen Database

Lager:

- Pathogenesis of Seneca virus A
- Pathogenesis of PEDV and PDCoV
- PEDV Immunology

Brockmeier:

- Use functional genomics to determine virulence mechanisms of *Streptococcus suis* and *Haemophilus parasuis*.
- Establish what effects antibiotic usage or infection with common pathogens has on the respiratory microbiome and carriage of common bacterial pathogens.

- Identify immunogenic, protective, and conserved proteins of *Streptococcus suis* and *Haemophilus parasuis* through immunoproteomics that will be cross protective against multiple serotypes.

Nicholson:

- Obtain complete whole-genome sequence of virulent and non-virulent *Streptococcus suis* isolates.
- Complete comparative genomic and transcriptional analysis of virulent and non-virulent *Streptococcus suis* isolates.
- Identify the genetic determinants that differentiate human and swine methicillin-resistant *Staphylococcus aureus* (MRSA) strains.
- Determine the role of biofilms in persistence of pathogens in the respiratory tract of swine.

Vincent:

- Perform routine sequence analysis of influenza A virus in swine surveillance sequence data to monitor for genetic and potential antigenic evolution. Select isolates for in vitro and in vivo studies.
- Test amino acid substitutions in H3 hemagglutinin genes of influenza A viruses to examine antigenic evolution.

Abente:

- Characterize swine innate and adaptive host immune gene profiles to wild type swine IAV infection.
- Test predicted antigenic targets in WIV, LAIV and vectored vaccine platforms against influenza A virus challenge in pigs.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

No specific progress – Plans to initiate studies in 2018

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Ongoing efforts involving swine influenza virus include utilizing contemporary isolates from North America, we are interrogating the zoonotic potential of these viruses as well as assessing virulence determinants. Studies include assessing antigenic relatedness of existing commercial vaccines with contemporary isolates.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

These studies primarily have value regarding public health impact – what is the zoonotic potential of circulating swine influenza viruses? However, this has ancillary impact for pork producers, informing risk and enabling de-risking of production. Also, analysis of potential efficacy of existing commercial vaccines through antigenic analysis can directly inform vaccination practices for producers.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Molecular epidemiology of swine influenza A viruses in the Southeastern United States, highlights regional differences in circulating strains. (2017) Kyriakis CS, Zhang M, Wolf S, Jones LP, Shim BS, Chocallo AH, Hanson JM, Jia M, Liu D, Tripp RA. *Vet Microbiol.* 211:174-179. doi: 10.1016/j.vetmic.2017.10.016. PMID: 29102115

Influenza D in Italy: towards a better understanding of an emerging viral infection in swine. (2017) Foni E, Chiapponi C, Baioni L, Zanni I, Merenda M, Rosignoli C, Kyriakis CS, Luini MV, Mandola ML, Bolzoni L, Nigrelli AD, Faccini S. *Sci Rep.* 7(1):11660. doi: 10.1038/s41598-017-12012-3. PMID: 28916759

2) Abstracts or Proceedings

Assessing of the zoonotic potential of swine influenza viruses in a primary respiratory cell culture model. Constantinos S. Kyriakis, Madelyn Krunkosky, and S. Mark Tompkins. CRWAD 2017 — The 98th Annual Conference of Research Workers in Animal Diseases December 1-5, 2017. Chicago Marriott, Downtown Magnificent Mile, Chicago, Illinois

3) Book Chapters or Monographs

none

D. FUNDING SOURCES

HHSN272201400004C

4/1/2014 – 3/31/2021

Emory University/NIH/NIAID

NIAID CENTERS OF EXCELLENCE FOR INFLUENZA RESEARCH AND SURVEILLANCE

The major goal of this project is to understand zoonotic potential of currently circulating swine influenza viruses.

E. WORK PLANNED FOR NEXT YEAR

We will continue work assessing zoonotic potential of swine influenza viruses, using primary human and swine cell culture systems. Ongoing studies include utilizing established murine models of infection to assess virulence, viral determinants of virulence, and mechanisms of severe disease (i.e. immune responses to infection). In addition, a subset of viruses will be assessed for virulence in swine. Moving forward we will be exploring evolutionary potential of viruses using in vivo and in vitro infection models, assessing reassortment of viruses. Of interest to stakeholders will be new collaborative studies exploring point of care sequence analysis of swine virus isolates, an approach to dramatically improve swine influenza surveillance. This will eventually expand beyond influenza.

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PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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Jens Kuhn, Virology Lead, NIH Integrated Research Facility at Fort Detrick (IRF-Frederick), kuhnjens@niaid.nih.gov

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

N/A

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

Research at the University of Wisconsin-Madison funded by NIH has focused on discovering and characterizing viruses of the family Arteriviridae. This work has focused on simian hemorrhagic fever virus and its relatives, which are related to PRRSV. This research is not part of any USDA-funded study but is relevant to the central biology of arteriviruses.

Specifically, we have deployed metagenomic methods for generating full-genome sequences of arteriviruses directly from infected host tissues. Using these methods, we have discovered and characterized 12 novel simian arteriviruses. These discoveries have helped inform a taxonomic reclassification that will soon be applied to the nidoviruses by the International Committee on the Taxonomy of Viruses. Research on the specific viruses is elucidating common determinants of arterivirus pathogenesis and immunity, which will inform the detection, prevention and control of PRRSV.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Understanding the PRRSV requires a comparative perspective. Our studies of the family Arteriviridae place PRRSV in a comparative perspective with its relatives. Our findings show that PRRSV is not the most diverse of the arteriviruses, and that it should probably be split into two species, corresponding to Type 1 and Type II PRRSV, and that patterns of evolution and host-switching that we have documented for the arteriviruses also apply to PRRSV, as well as to other RNA viruses of swine that may not yet have been discovered.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications (recent)

Moncla, L. H., A. M. Weiler, G. Barry, J. T. Weinfurter, J. M. Dinis, O. Charlier, M. Lauck, A. L. Bailey, V. Wahl-Jensen, C. W. Nelson, J. C. Johnson, Y. Cai, T. L. **Goldberg**, D. H. O'Connor, P. B. Jahrling, J. H. Kuhn and T. C. Friedrich (in press). Within-host evolution of simian arteriviruses in crab-eating macaques. *J Virol* 91(4).

Yu, S. Q., Y. Cai, C. Lyons, R. F. Johnson, E. Postnikova, S. Mazur, J. C. Johnson, S. R. Radoshitzky, A. L. Bailey, M. Lauck, T. L. **Goldberg**, D. H. O'Connor, P. B. Jahrling, T. C. Friedrich and J. H. Kuhn (2016). Specific detection of two divergent simian arteriviruses using RNAscope *in situ* hybridization. *PLoS One* 11(3): e0151313.

Bailey, A. L., M. Lauck, R. R. Ghai, C. W. Nelson, K. Heimbruch, A. L. Hughes, T. L. **Goldberg**, J. H. Kuhn, A. J. Jasinska, N. B. Freimer, C. Apetrei and D. H. O'Connor (2016). Arteriviruses, pegiviruses, and lentiviruses are common among wild African monkeys. *J Virol* 90(15): 6724-6737.

Wahl-Jensen, V., J. C. Johnson, M. Lauck, J. T. Weinfurter, L. H. Moncla, A. M. Weiler, O. Charlier, O. Rojas, R. Byrum, D. R. Ragland, L. Huzella, E. Zommer, M. Cohen, J. G. Bernbaum, Y. Cai, H. B. Sanford, S. Mazur, R. F. Johnson, J. Qin, G. F. Palacios, A. L. Bailey, Peter B. Jahrling, T. L. **Goldberg**, D. H. O'Connor, T. C. Friedrich and J. H. Kuhn (2016). Divergent simian arteriviruses cause simian hemorrhagic fever of differing severities in macaques. *mBio* 7: e02009-15.

Kuhn, J. H., M. Lauck, A. L. Bailey, A. M. Shchetinin, T. V. Vishnevskaya, Y. Bào, T. F. F. Ng, M. LeBreton, B. S. Schneider, A. Gillis, U. Tamoufe, J. L. D. Diffo, J. M. Takuo, N. O. Kondov, L. L. Coffey, N. D. Wolfe, E. Delwart, A. N. Clawson, E. Postnikova, L. Bollinger, M. G. Lackemeyer, S. R. Radoshitzky, G. Palacios, J. Wada, Z. V. Shevtsova, P. B. Jahrling, B. A. Lapin, P. G. Deriabin, M. Dunowska, S. V. Alkhovsky, J. Rogers, T. C. Friedrich, D. H. O'Connor and T. L. **Goldberg** (2015). Reorganization and expansion of the nidoviral family *Arteriviridae*. *Archives of Virology*: 161: 755-768.

2) Abstracts or Proceedings (recent)

Simons, N.D., Eick, G., Ruiz-Lopez, M. J., Chapman, C.A., Goldberg, T. L., Sterner, K.N., Ting, N., (2017). Host immune gene expression and viral infection status from whole blood transcriptomics in the Ugandan red colobus. American Association of Physical Anthropologists, New Orleans, Louisiana, USA.

Lester, J., Sibley, S. D., Hyeroba, D., Tumukunde, A., Weny, G., Dearlove, B., Jones, J. H., Switzer, W., Chapman, C. A., Ting, N., Frost, S. D., Goldberg, T. L. (2016). Patterns of infection and transmission within a wild non-human primate zoonotic reservoir. Wellcome Genome Conference: Exploring Human Host-Microbiome Interactions in Health and Disease, Hinxton, United Kingdom.

Simons, N.D., Christie, D. M., Eick, G., Ruiz-Lopez, M. J., Chapman, C.A., Goldberg, T. L., Ting, N., Sterner, K.N., (2016). Disease-associated genetic variation drives differential expression of MHC-DQA1 *in vitro*: A role for cis-regulatory variation in disease susceptibility in wild primates. American Association of Physical Anthropologists, Atlanta Georgia, USA.

Goldberg, T. L., Chapman, C. A., O'Connor, D. H., Friedrich, T., Lauck, M., Sibley, S., Bailey, A., Hyeroba, D., Tumukunde, A., Weny, G., Ting, N., Kuhn, J., Ghai, R., Simons, N., Dinis, J., Thurber, M. (2014). Cross-species transmission of taxonomically diverse pathogens in a community of wild primates. American Society for Tropical Medicine and Hygiene. New Orleans, Louisiana, USA.

3) Book Chapters or Monographs

[none]

D. FUNDING SOURCES

R01AI098420 (NIH-NIAID; *Biological and Human Dimensions of Primate Retroviral Transmission*) and related sources of internal and external support at NIH and UW-Madison and the Wisconsin National Primate Research Center.

E. WORK PLANNED FOR NEXT YEAR

Continue to characterize the diversity and pathogenesis of the arteriviruses in their natural hosts and in experimental systems.

ANNUAL STATION REPORT: PROJECT NC-229

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INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection: UMN continued work on mechanisms of immune protection and correlates of immunity, particularly in the area of neutralizing antibodies.
- Host genetic control of anti-PRRSV infection and vaccination responses:
- UMN, in collaboration with cooperating veterinarians and producers, characterized individual variation in anti-PRRSV antibody responses that may have a genetic basis.
- UMN characterized gene expression variation in that contributes to age-dependent immune variation in response to PRRSV.
- PRRSV Pathogenesis. UMN investigated highly pathogenic PRRSV from U.S. outbreaks.
- Association between PRRS incidence and epidemiological factors was quantified in sow farms
- Role of animal movement networks in PRRS epidemiology
- UMN assisted in epidemiological investigations of the introduction of PRRSV in Chile, 2013-2015.
- UMN developed methods to assess the efficacy of biosecurity methods to decrease the viability of airborne PRRSV

- UMN tested biosecurity methods to inactivate airborne PRRS virus.
- Characterized size of airborne particles associated with PRRSV under field conditions
- Developed a model to estimate PRRS virus introduction into filtered farms with negative-pressure

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

- Viral diseases of swine of recent origin. High-throughput nucleic acid sequencing and data analysis was applied to diagnostic lab cases from recent and novel rotavirus types.
- Analytical models were developed to estimate risk for transmission of porcine coronaviruses via contaminated feed and feed ingredients
- Epidemiological models to forecast the hypothetical transmission of FMDv within different types of swine farms were formulated and parameterized
- Investigated the seasonality of influenza A virus in breed to wean farms, and assessed the impact of climatic conditions on influenza infections at weaning
- Reported that multiple genome constellations of similar and distinct influenza A viruses co-circulate during epidemics in swine which may serve as a mechanism of virus persistence in growing pig populations
- Investigated the origin and persistence of influenza A virus in a live animal market in Minnesota
- Through complete genome sequencing of influenza A viruses isolated from farrow to wean farms, we revealed the emergence, persistence and subsidence of diverse viral genotypes and proposed mechanisms of virus introduction and persistence in pigs
- Evaluated biosecurity measures directed at preventing the indirect transmission of porcine epidemic diarrhea virus.
- Developed and assessed methods of air sampling and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms
- Established and validated novel sampling methods to conduct surveillance of influenza virus
- Developed a GMR biosensor chip to detect Influenza A virus
- Developed an in vivo passaged PEDV isolate for potential vaccine development
- Characterized the mucosal immune response to PEDV infection at the GI epithelium

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

- Quantifying the association between epidemiological factors and PRRSV incidence is pre-requisite for developing predictive models of PRRSV spread through systems and regions
- Advancement in the understanding of neutralizing antibody responses of swine to PRRSV and variation in individual animal responses is expected to provide new opportunities for genetic improvement of resistance to PRRSV as well as in the area of mechanisms of protective immunity.

- Molecular understanding of age-dependent resistance to PRRSV may lead to improved immunological tools for stimulation of immunological PRRSV resistance and improved vaccine prevention.
- Genetic analysis of rotavirus strain variation will aid in identification of conserved and variable regions associated with immune protection that is expected to improve prevention of rotaviral diarrhea.
- A risk analysis of transmission of PEDV was useful to qualitatively assessing virus transmission in important feed ingredients of porcine origin. These ingredients (meat and bone meal, spray dried porcine plasma) represent an important strategy to increase recycle of nutrients into animal feed that otherwise can increase environmental impact of food production. We used a combination of empirical evidence, expert advice, and mathematical models to answer these important questions. Therefore, these studies conducted at UMN are key to sustain food production.
- Epidemiological models of viral diseases exotic to the U.S. swine industry, such as the FMDV, help to develop preventive and control strategies to mitigate the impact of hypothetical epidemics
- Novel methods of sampling pigs may lead to more cost effective surveillance of influenza A virus
- Application of in depth sequencing of influenza viruses in farms evidences the high degree of co-circulation of genetically and antigenically distinct strains within farms Information on seasonality patterns observed for influenza infections may help target timing of vaccination strategies to decrease prevalence at weaning
- Modeling approaches to predict risk of PRRSV infections into filtered farms should help producers make biosecurity investment decisions
- Investigations into the transmission of influenza viruses within farms are providing new information in terms of dynamics, mechanisms and patterns of transmission in both, sow farms and growing pigs which should aid in the control of influenza virus
- Investigations into the influenza viruses isolated in winter and summer in an animal market in St. Paul, MN are indicating that viruses do not persist in the markets between seasons but that they originate from commercial pigs.
- Investigations into risk factors of influenza infections in piglets at weaning indicated that both, sow vaccination and gilt influenza status at entry are factors associated with influenza detection at weaning
- Prototype of a pen-side influenza virus test using GMR technology was developed in the laboratory

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

Alba A, Morrison R, Cheeran A, Rovira A, Alvarez J, Perez AM. Optisample™: Open web-based application to optimize sampling strategies for active surveillance activities at the herd level. Porcine Respiratory Reproductive Syndrome (PRRS) as a working example. Plos One

- Alkhamis M, Arruda A, Morrison R, Perez A. Novel approaches for Spatial and Molecular Surveillance of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) in the United States. *Nature Scientific Reports*.
- Alkhamis M, Arruda A, Vilalta C, Morrison RB, Perez AM. Surveillance of porcine reproductive and respiratory syndrome virus in the United States using risk mapping and species distribution modeling. *Preventive Veterinary Medicine*
- Alonso C, Raynor PC, Goyal S, Olson BA, Alba A, Davies PR, Torremorell M (2017). Assessment of air sampling methods and size distribution of virus-laden aerosols in outbreaks in swine and poultry farms. *J Vet Diagn Invest*, 29(3):298-304. doi: 10.1177/1040638717700221.
- Anderson BD, Lednicky JA, Torremorell M, and Gray GC. (2017). A Review of Bioaerosol Sampling in Swine Production Facilities. *Front Vet Sci*, <https://doi.org/10.3389/fvets.2017.00121>
- Arruda AG, Alkhamis MA, VanderWaal K, Morrison RB, Perez AM. Estimation of time-dependent reproduction numbers for porcine reproductive and respiratory syndrome (PRRS) across different regions and production systems of the United States. *Frontiers in Veterinary Science*.
- Arruda AG, Vilalta C, Perez A, Morrison R. Land Altitude, Slope, and Coverage as Risk Factors for Porcine Reproductive and Respiratory Syndrome (PRRS) Outbreaks in the United States. *Plos One*.
- Arruda P., Schwartz K., Arruda B., Rovira A., Vannucci F., Resende T., Nietfeld J., Sundberg P., Hause B. 2017 "Detection of a Novel Sapelovirus in Central Nervous Tissue of Pigs with Polioencephalomyelitis in the U.S". *Transboundary and Emerging Diseases*, 64:311-315.
- Chamba Pardo FO, Alba-Casals A, Nerem J, Morrison RB, Puig P, Torremorell M (2017). Influenza herd-level prevalence and seasonality in breed-to-wean pig farms in the Midwestern United States. *Front Vet Sci*. 4:167, <https://doi.org/10.3389/fvets.2017.00167>
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- Cottingim, K.M., L.J. Johnston, A.M. Hilbrands, P.E. Urriola, and G.C. Shurson. 2017 Ultraviolet irradiation of spray-dried porcine plasma does not affect the growth performance of nursery pigs when compared with nonirradiated bovine plasma. *J. Anim. Sci*. 95:3120-3128
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2) Abstracts or Proceedings

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- Kinsley, A. VanderWaal, K., Craft, M.E., Morrison, B., Perez, A. How can farm structure and demography influence pathogen persistence within a herd? Minnesota Veterinary Medical Association Annual Meeting. Minneapolis, Minnesota.
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- Kinsley, A., VanderWaal, K., R. Morrison, M. Craft, A. Perez. Managing complexity: simplifying assumptions of FMD models for swine. Invited oral presentation Allen D. Leman Conference. Sept 18, 2017, St. Paul, MN.
- Kinsley, A., VanderWaal, K. Craft, M., Morrison R., Perez, A. 2017. "Managing Complexity: Simplifying assumptions of foot-and-mouth disease models for swine. 15th Annual Ecology and Evolution of Infectious Disease meeting. Santa Barbara, CA.
- Kinsley, A.C., K. VanderWaal, M. Craft, R.B. Morrison, A.M. Perez. Managing complexity: simplifying assumptions of foot-and-mouth disease models for swine. Poster presentation at University of Minnesota College of Veterinary Medicine Points of Pride Research Day. October 4, 2017, St. Paul, MN.
- Krishna VD, Kim Y, Yang M, Vannucci F, Torremorell M, Cheeran MC-J (2017). Systemic and Mucosal Immune Response to Porcine Epidemic Diarrhea Virus (PEDV) in swine. Institute of Virology Symposium, Minneapolis MN.
- Krishna VD, Wu, K, Klien T, Perez A, Wang JP, Cheeran MC-J (2017). Influenza A virus detection using a giant magnetoresistance (GMR) biosensing portable handheld device Leman Swine Conference, St Paul, MN.
- Marthaler, D. and M.P. Murtaugh. 2016. Interpreting PRRS ORF5 sequencing, can we do better? Swine Health Monitoring Project. SHMP@umn.edu. 8/19/2016.
- Marthaler, D. and M.P. Murtaugh. 2016. Phylogenetic relationships and percent sequence identity in combination with RFLP to interpret virus relationships. Swine Health Monitoring Project. SHMP@umn.edu. 8/26/2016.
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- Neira Ramirez VM, Brito B, Saavedra M, Tapia R, Tapia K, Garcia V, Torremorell M, Medina R (2017). Genetic diversity of sine origin influenza A virus in Chile and the Latin American perspective. Proc Int Pig Vet Soc, PO-PT2-007, p 599.
- Payne B, Jacobs A, Dvorak C, Murtaugh M. 2016. PCV2 vaccine cross-protection: identification of sequences in successfully vaccinated field cases. 24th IPVS Abstract book. p 503
- Perez AM. Keynote presentation: Swine Health Monitoring Program in the US. GEOVET, Valdivia, Chile, November 2016
- Perez, A., & VanderWaal, K. Present and future of the Dr. Morrison Swine Health Monitoring Project (MSHMP). Invited oral presentation Allen D. Leman Conference. 2017 Sept 18, St. Paul, MN.
- Perez, A., & VanderWaal, K. Present and future of the Dr. Morrison Swine Health Monitoring Project (MSHMP). Invited oral presentation Allen D. Leman Conference. 2017 Sept 18, St. Paul, MN.
- Rahe M, Borgerding AJ, Wiseman B, Cenatti L, Martin D, Murtaugh MP. 2016. Establishment and characterization of a porcine lymphoma cell line. J Immunol 196 (1 Supplement) 216.18.
- Trudeau, MP, H Verma, F Sampedro, PE Urriola, GC Shurson, and SM Goyal. 2016. Environmental Persistence of Porcine Epidemic Diarrhea Virus (PEDV), Porcine Delta Corona Virus (PDCoV), and Transmissible Gastroenteritis (TGEV) in Feed Ingredients. iCOMOS - 2nd International Conference on One Medicine One Science. University of Minnesota – Minneapolis MN
- Valdes-Donoso P, VanderWaal K, Wayne S, Perez AM. Using machine learning to predict swine movements in a regional program (RCP) to control infectious diseases. GEOVET, Valdivia, Chile, November 2016
- VanderWaal, K. Data integration: Dynamic risk for swine diseases. Invited oral presentation at: Allen D. Leman Conference. 2017 Sept 16, St. Paul, MN.
- Yang Z, L. Galina, T. Knutson, A. Rovira, D. Marthaler. “Investigating porcine circovirus associated disease (PCVAD) in commercial swine herds by next generation sequencing.” AASV Annual Meeting, 2017. Denver, CO, USA.

3) Book Chapters or Monographs

- Torremorell M (2016). Immunity, diagnosis and intervention strategies of influenza infections in pigs. In: Animal Influenza. Ed. David Swayne, 2nd Edition. Wiley-Blackwell, pp: 452-458. Contribution: Wrote chapter 17.

D. FUNDING SOURCES

Dates	Title	Funding source	PI
2/15/16-2/14/19	Broadly neutralizing antibodies to PRRSV	USDA NIFA	Murtaugh

5/1/16-4/30/17	Pen-side respiratory pathogen identification	Boehringer Ingelheim Vetmedica	Murtaugh
5/1/16-4/30/17	Toward animal challenge-free prediction of vaccine efficacy	American Association of swine Veterinarians Foundation	Murtaugh
8/26/15-8/25/16	Energetics of B cell activation	Puretein Bioscience	Murtaugh
7/1/16-6/30/17	PRRS multistate project	UMN CVM Hatch	Murtaugh
7/1/17-30/17	In vitro vaccine testing model for evaluating the quality of humoral protection	UMN AES	Murtaugh
07/01/2015-06/30/2017	Management and analysis of big data for near real-time detection and early response to food animal health threats	Mn Drive GFV	Perez
07/01/2016-06/30/2018	Development of epidemiological tools for PRRS outbreak investigations	UMN Hatch funds	Perez
10/01/2017-09/30/2019	Developing multiplex Giant magnetoresistance (GMR) biosensors for the detection of swine respiratory pathogens.	CVM Emerging and Zoonotic Diseases	co-PI (Cheeran)
08/01/2017- 07/31/2018	A near-real time global surveillance system for swine diseases	Swine Health Information Center	Perez
05/01/2017- 06/30/2018	Using Swine Health Monitoring Project to Facilitate Business Continuity	MN Board of Animal Health	Perez
10/15/2017- 10/14/2018	Development and implementation of a domestic swine bio-surveillance monitoring and surveillance	Swine Health Information Center	Torrison

	system. Part 2: Multiple data streams integration and reporting		
10/15/2017- 10/14/2018	Enhancing Dr. Bob Morrison's Swine Health Monitoring Program (MSHMP) capacity and preparedness through the integration with outputs from a SHIC-led disease surveillance programs and research integration with US higher education institutions.	Swine Health Information Center	Corzo
11/01/2017 – 10/31/2018	Dynamic mapping of PRRS and PED infection risk across space and time	Swine Health Information Center	VanderWaal
07/17-06/19	A comprehensive surveillance system to control influenza in pigs	Rapid Agricultural Response Fund (renewal)	Torremorell, M
07/15-06/17	A comprehensive surveillance system to control influenza in pigs	Rapid Agricultural Response Fund	Torremorell, M
03/15-02/18	Characterization of influenza diversity in piglets and risk factors for diversity	USDA-AFRI-NIFA	Torremorell, M
01/01/17-04/30/17	Demonstration of airborne PRRSV inactivation by a non-thermal plasma	NPB (subcontract with Michigan)	Torremorell, M
09/30/16-09/20/21	Optimizing assessment of virus containing particles in animal agriculture	NIOSH/NIH	Raynor, P
09/30/16-09/20/21	Longitudinal study of infectious disease risks at the human-swine interface	NIOSH/NIH	Davies, P
07/15-06/17	Does prevalence of influenza A virus at weaning influence disease transmission rates, clinical manifestation of disease, and production performance?	NPB	Torremorell, M
09/14-08/17	Detection and control of PRRS virus and emerging viral diseases of swine	Minnesota Agricultural Experimental Station	Torremorell, M

01/13-12/17	Genetically improving resistance of pigs to PRRSV infection	NIFA-USDA	Dekkers, J
04/17-03/18	Association of the Presence of Influenza A Virus in Pigs at Weaning with Post-Weaning Performance and Cost of Production	BOEHRINGER INGLEHEIM VETMEDICA, INC.	Culhane, M
07/2015 – 08/2017	Development of a live attenuated PEDV vaccine for weanling pigs	Emerging and Zoonotic Infectious disease signature program grant	Cheeran

E. WORK PLANNED FOR NEXT YEAR

- To investigate the role of neutralizing antibodies in PRRSV cross-protection.
- To investigate host factors associated with PRRSV susceptibility and resistance.
- To investigate host factors associated with PRRSV susceptibility and resistance.
- To determine infection incidence in growing pigs, specifically to identify when and how often new PRRSV infections happen in wean-to-finish pigs
- To evaluate risk factors associated to PRRSV infection in growing pigs.
- To associate production and economic impact of PRRSV infections in growing pigs. to investigate host factors associated with PCV2 susceptibility and resistance.
- To build a risk based model of porcine virus transmission in feed ingredients.
- To formulate models for forecasting risk for PRRSV spread
- To formulate models for between-farm transmission of exotic viruses
- To evaluate mechanisms of influenza virus transmission and persistence in piglets
- To evaluate the effect of maternally derived antibodies against influenza A virus on infection dynamics in growing pigs
- To investigate patterns and dynamics of influenza A virus transmission in growing pigs
- To investigate farm factors associated with influenza A virus detection in piglets at weaning
- To investigate the bi-directional transmission of influenza A virus between pigs and people
- To evaluate the impact of vaccination on influenza A virus genetic and antigenic diversity in piglets
- To evaluate strategies of vaccination to control influenza in piglets at weaning
- To investigate methodologies and approaches to inactivate airborne viruses
- To develop and optimize methods to assess virus containing particles in animal agriculture
- To develop antibody reagents that can distinguish *Mycoplasma hyopneumonia* in a standard ELISA test.
- To develop a diagnostic GMR biosensor array that can detect influenza, PRRSV and *Mycoplasma hyopneumoniae* in clinical samples.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Dr. Renukaradhya J Gourapura
Professor, Food Animal Health Research Program (FAHRP)
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2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

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Director, OARDC, The Ohio State University
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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

In a collaborative research project with Dr. Ying Fang, Kansas State University, we evaluated the efficacy of concurrent but consecutive vaccination of type 1 and type 2 PRRSV in pigs

In the US, both North American (Type 2) and European (Type 1) PRRSV are circulating in swine herds. Our collaborative study has evaluated the efficacy of consecutive and concurrent vaccination of pigs with modified live Type 1 and Type 2 PRRSV vaccine candidates. Results indicated that vaccination of pigs with both PRRSV genotypes at 3 days apart (type 1 MLV followed by type 2 MLV) provides better immune protection and clearance of both the viral infections than those pigs vaccinated simultaneously with both type 1 and type 2 MLVs.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

This study demonstrated that the consecutive vaccination with modified PRRSV Type 1 followed by Type 2 provides satisfactory protection against both the viruses.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

1. Dhakal, S, J. Hiremath, K. Bondra, Y.S. Lakshmanappa, D. Shyu, K. Oyuang, K. Kang, B. Binjawadagi, J. Goodman, K. Tabynov, S. Krakowka, B. Narasimhan, C.W. Lee and **G.J. Renukaradhya** (2017). Biodegradable nanoparticle delivery of inactivated swine influenza

virus vaccine provides heterologous cell-mediated immune response in pigs. J Control Release, 247:194-205. PMID: 28057521

2. Dhakal, S, J. Goodman, K. Bondra, Y.S. Lakshmanappa, J. Hiremath, D. Shyu, K. Oyuang, K. Kang, S. Krakowka, M.J. Wannemuehler, C.W. Lee, B. Narasimhan and **G.J. Renukaradhya** (2017). Polyanhydride nanovaccine against swine influenza virus in pigs. Vaccine, 35(8): 1124-1131. PMID: 28117173

2) Abstracts or Proceedings

1. Lunney J.K, M. Bailey, J. Manirarora, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang and L. Wooldridge. US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies. Abstract #263, 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
2. Dhakal, S and **G.J. Renukaradhya**. PLGA nanoparticle delivery of inactivated swine influenza virus vaccine provides heterologous protection through cell-mediated immunity in pigs. Abstract #102, AAVI mini-symposium and 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
3. Dhakal, S, J. Goodman, K. Bondra, Y.S. Lakshmanappa, J. Hiremath, D. Shyu, K. Oyuang, K. Kang, S. Krakowka, M.J. Wannemuehler, C.W. Lee, B. Narasimhan and **G.J. Renukaradhya** (2016). Polyanhydride nanovaccine against swine influenza virus in pigs. Abstract #117, AAVI mini-symposium and 97th Annual CWRAD meeting, December 4-6, 2016, Chicago, IL.
4. Francis, O, M. Bailey, L. Wooldridge, J. Manirarora, G.J. Renukaradhya, S. Kenney, J. Labresh, Y. Sang, J.K. Lunney. Development of new immune reagents for swine health, vaccine and disease studies. British Society for Immunology and Dutch Society for Immunology, Annual Congress meeting, Liverpool, UK, December 6-9, 2016.
5. Lee, C.W and **G.J. Renukaradhya**. Universal Influenza Vaccine. Represented OARDC, The Ohio State University to the US Capital Hill Washington DC meeting attended by US senators and USDA team, April 5, 2017.
6. Dhakal, S, K. Bondra, D. Shyu, K. Tabynov, C.W. Lee and **G.J. Renukaradhya**. Intramuscular route of delivery of PLGA-nanoFlu vaccine improves antibody response in pigs. OARDC Research Conference, The Ohio State University, Wooster, Ohio. April 20, 2017.
7. Dhakal, S, J. Hiremath, K. Bondra, Y.S. Lakshmanappa, D. Shyu, K. Oyuang, K. Kang, B. Binjawadagi, J. Goodman, K. Tabynov, S. Krakowka, B. Narasimhan, C.W. Lee and **G.J. Renukaradhya**. Biodegradable nanoparticle delivery of inactivated swine influenza virus vaccine provides heterologous cell-mediated immune response in pigs. Abstract # 263, Immunology 2017, AAI meeting, Washington Convention Center, Washington DC, May 12-16, 2017.
8. Manirarora, J, M. Bailey, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang, O. Francis, L. Wooldridge, and J.K. Lunney. US-UK Collaborative Swine Immune Toolkit Initiative: Development of new immune reagents for swine health, vaccine and disease studies. Immunology 2017, AAI meeting, Washington Convention Center, Washington DC, May 12-16, 2017.
9. Lakshmanappa, Y.S, P. Shang, S. Dhakal, S. Renu, B. Hogshead, P. Bernardo, X. Yan, Y. Fang and **G.J. Renukaradhya**. Concurrent but consecutive vaccination of modified live type 1

and type 2 PRRSV provides better protection in nursery pigs. Abstract #44, North American PRRS Symposium Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference, December 1-3, 2017, Chicago, IL.

10. Lunney J.K, M. Bailey, J. Manirarora, **G.J. Renukaradhya**, S. Kenney, J. Labresh, Y. Sang, O. Francis and L. Wooldridge. US-UK Collaborative Swine Immune Toolkit Initiative: Development and characterization of immune reagents for swine health, vaccine and disease studies. North American PRRS Symposium Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference, December 1-3, 2017, Chicago, IL.

3) Book Chapters or Monographs

1. **Book Chapter:** Garmendia, A.E, W. Mwangi and **G. J. Renukaradhya**. Chapter 29 – Porcine Reproductive and Respiratory Syndrome. Veterinary Vaccines for Livestock - 1st Edition, FAO. Editors - Samia Metwally, Ahmed Elldrissi and Gerrit Viljoen, Elsevier Publication 2017.

D. FUNDING SOURCES

1. **Funding agency:** USDA-AFRI, 2013-67015-20476 (MPI) (\$2,351,639) (RG share \$600,000)
Period: 11/01/2012 – 01/31/2018
Role: Multiple Principal Investigators; Chang-Won Lee (contact) and Renukaradhya Gourapura
Title of the project: Universal Flu Vaccine by a Norovirus P Particle Platform
2. **Funding agency:** USDA-AFRI US-UK grant (\$500,000) (RG share \$69,568), 2015-67015-23216 and BBSRC grant BB/M028232/1
Period: 04/1/2015 to 03/31/2018
Role: **PI:** Lunney, JK; **Co-PIs:** Bailey, M; Gourapura, RJ; LaBresh, JW; Sang, Y; Kenney, S.
Title of the project: Swine Immune Toolkit: Development of new immune reagents for swine health, vaccine and disease studies
3. **Funding agency:** USDA-AFRI, 2017-67015-26909, \$500,000 (RG share \$88,119)
Period: 08/15/2017 – 08/14/2020
Role: Co-Principal Investigator, PI: Diego Diel
Title of the project: A Multi-Species Vaccine Delivery Platform for Infectious Disease Prevention and Control in Livestock

E. WORK PLANNED FOR NEXT YEAR

1. Investigate the mechanisms involved in induction of protective mucosal response by nanoparticle based influenza virus vaccine candidates delivered intranasally in pigs.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: Kansas State University

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Raymond (Bob) Rowland, Professor

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Megan Niederwerder, assistant professor, mniederwerder@vet.k-state.edu

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

Role of nsp2 frameshifting (Fang). Our previous studies identified two novel PRRSV proteins, nsp2TF and nsp2N, which were expressed by novel -2/-1 programmed ribosomal frameshifting (PRF) mechanism. During the past year, we performed in depth analysis on the role of nsp2TF/nsp2N in suppressing host innate immune responses. We also assessed the potential application of nsp2TF-deficient mutants in MLV vaccine development. In a nursery pig model, the mutant virus-immunized pigs showed reduced lung lesion and also lower levels of viral loads in lung and tonsil at 14 days post challenge.

Novel PRRS vaccine (Fang). New PRRS vaccine construction strategies have been explored during the past two years. Collaborating with Dr. Biao He at University of Georgia, parainfluenza virus 5 (PIV5) vector-based PRRS vaccine is under development.

Knockout of maternal CD163 protects fetuses from infection (Rowland). CD163-positive fetuses, recovered between 109 days of gestation or 20 days after maternal infection, were completely protected from PRRSV in dams possessing a complete knockout of the CD163 receptor. The results demonstrate a practical means to eliminate PRRSV-associated reproductive disease, a major source of economic hardship to agriculture.

Peptide sequences in SRCR domain 5 of porcine CD163 involved in infection with PRRSV. HEK293T (HEK) cells transfected with domain-deleted constructs fused to enhanced green fluorescent protein (EGFP) were infected with a PRRSV-2 isolate expressing a red fluorescent protein (RFP). The results showed that cells expressing a deletion of the 101 amino acid SRCR5 or the 16 amino acid PSTII domain did not support infection. Insertion of proline-arginine (PR) dipeptides along the SRCR5 polypeptide was used to probe secondary and tertiary structures within SRCR5 involved in infection. The results from this study identify likely contact regions in

SRCR5 involved in forming the interaction between CD163 and the corresponding PRRSV protein.

Fecal microbiota transplantation improves outcome in nursery pigs (Niederwerder).

Previous work demonstrated an association between increased microbiome diversity and improved outcome characteristics following co-infection with PRRSV and PCV2, including reduced virus replication, improved weight gain, and decreased clinical disease. The current work focuses on modulating the microbiome composition through fecal microbiota transplantation (FMT). Morbidity and mortality due to PCVAD was reduced in pigs receiving FMT from a healthy high parity sow. The FMT pigs also possessed high antibody titers and reduced lung lesions. FMT represents a new strategy for improving outcomes following co-infections with PRRSV.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

E2 vaccine and companion ELISA for classical swine fever (Shi). We are developing and testing a novel E2-subunit vaccine for classical swine fever (CSF) that can be produced safely and cost-effectively in CSF free countries. We are also developing a unique ELISA that can differentiate CSF virus infected pigs from the pigs vaccinated with C-strain vaccines or E2 subunit vaccines.

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

Adenovirus-vectored novel African Swine Fever Virus multi-antigen cocktail elicit strong but non-protective immune responses in commercial pigs (Mwangi). Previous work focused on demonstrating the immunogenicity of seven adenovirus-vectored novel ASFV antigens formulated as a single vaccine. The cocktail primed strong ASFV antigen-specific IgG responses, which were recalled upon boosting. However, upon challenge with ASFV Georgia, vaccinated pigs had higher mean clinical scores, mean body temperatures, and decreased WBC counts as compared to the controls. Overall, the data suggest that the ASFV-antigen specific antibodies induced in the pigs enhanced ASF disease. The development of a protective ASFV subunit vaccine will require an immunization strategy that will elicit strong cytotoxic T lymphocyte response while limiting humoral immunity.

Risk of transboundary movement of ASFV via contaminated feed ingredients

(Niederwerder and Rowland). In collaboration with Scott Dee at Pipestone, we developed a model to study whether viruses, such as ASFV, CSFV and others, when mixed with feed ingredients could remain viable under the time and environmental conditions encountered during a trans-Atlantic shipment to the US. By using this model, we have shown that ASFV is capable of surviving the journey, suggesting that certain feed ingredients could serve as vehicles for infectious agents, thus posing a significant threat to the US swine industry.

Risk of African swine fever virus (ASFV) transmission in feed (Niederwerder and Rowland).

It is known that ASFV can be transmitted via the oral route through ingestion of swill or experimental inoculation. However, very little is known about the risk of ASFV Georgia 2007 transmission in contaminated feed. One important possibility is that the Georgia isolate may possess unique properties related to the stability of the virus in the environment. The goal of this work is to determine the median infectious dose (ID₅₀) for ASFV Georgia 2007 through oral exposure via natural drinking and eating behavior. Progress relates to the establishment of protocols for the propagation and detection of ASFV.

Mitigation of foreign animal disease introduction in feed (Niederwerder and Rowland). The goals of this project are to: 1) develop baseline data for the effectiveness of mitigants on the inactivation of ASFV, CSFV and Chinese PRV; 2) test candidate mitigants in a pig oral inoculation model via natural feeding behavior; and 3) evaluate the effectiveness of mitigants on inactivation of viruses in a transboundary model that simulates conditions when feed ingredients are shipped from another country. Progress to date includes the testing of medium chain fatty acids on their ability to inhibit ASFV infection *in vitro*.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Non-MLV CSF vaccines based on E2 that are effective create the opportunity to vaccinate pigs in CSF-free countries

The highly efficient -2/-1 programmed ribosomal frameshifting (PRF) mechanism, by which PRRSV efficiently produces novel proteins, nsp2TF and nsp2N can be applied to the development of new MLV vaccines for PRRS.

Diagnostic reagents and assays developed in our recent studies, including monoclonal antibodies, the pathogen array system, and diagnostic assays, provide important tools in emerging pathogen discovery, control and prevention.

Blocking PRRSV infection through the genetic modification of CD163 demonstrates a practical means to prevent PRRS.

The manipulation of the pig microbiome creates opportunities to improve animal health and provide alternatives to antibiotics and other growth promoters.

Understanding how pathogens are transmitted in feed and the development of interventions may prevent the introduction of the next “PEDV” –like outbreak.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

Refereed Publications:

Burakova, Y., Madera, R., McVey, S.; Schlup, J.R., Shi, J., (2017) Adjuvants for animal vaccines; *Viral Immunology*, available online 06/15/2017

Burakova, Y., Shi, J., Schlup, J.R. (2017) Impact of oil composition on formation and stability of emulsions produced by spontaneous emulsification; *Journal of Dispersion Science and Technology*, vol. 38 (12), 1749-1754

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- Guo R., D. Davis, Y. Fang, 2017. Intercellular transfer of mitochondria rescues virus-induced cell death but facilitates cell-to-cell spreading of porcine reproductive and respiratory syndrome virus. *Virology*. In press.
- Li Y., P. Shang, D. Shyu, A. C. Carrillo, P. Naraghi-Arani, C. J. Jaing, R. Gourapura, A. E. Firth, E. J. Snijder, Y. Fang, 2017. Nonstructural proteins nsp2TF and nsp2N of porcine reproductive and respiratory syndrome virus (PRRSV) play important roles in suppressing host innate immune responses. *Virology*. In press.
- Shang P, S. Misra, B. Hause, Y. Fang. 2017. A Naturally Occurring Recombinant Enterovirus Expresses a Torovirus Deubiquitinase. *Journal of Virology*, Jun 26;91(14). Spotlight Feature.
- Kimpston-Burkgren K, I. Correias, F. A. Osorio, D. Steffen, A. K. Pattnaik, Y. Fang, H. L. X. Vu. 2017. Relative contribution of porcine reproductive and respiratory syndrome virus open reading frames 2-4 to the induction of protective immunity. *Vaccine*. Aug 3;35(34):4408-4413.
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- Jaing, C, RRR Rowland, JE Allen, A Certoma, JB Thissen, J Bingham, B Rowe, JR White, J Wynne, D Johnson, N Gaudreault, DT Williams. 2017. Gene expression analysis of whole blood RNA from pigs infected with low and high pathogenic African swine fever viruses. *Nature Sci Rep*. in press.
- Heimerman, M, MV Murgia, P Wu, AD Lowe, W Jia, RRR Rowland. 2017. Linear epitopes in African swine fever virus (ASFV) p72 recognized by monoclonal antibodies prepared against baculovirus expressed antigen. *J Vet Diagn Invest*. In press.
- Popescu, LN, BR Tribble, N Chen, RRR Rowland. 2017. GP5 of porcine reproductive and respiratory syndrome virus (PRRSV) as a target for homologous and broadly neutralizing antibodies. *Vet Microbiol*. In press.
- Dekkers, J, Rowland RR, JK Lunney, G Plastow. 2017. Host genetics of response to porcine reproductive and respiratory syndrome in nursery pigs. *Vet Microbiol*. In press.
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Abstracts or Proceedings

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- Wang, L., R. Madera, Y. Burakova, S. Buist, Y. Sang, J. Nietfeld, J. Henningson, W. Gong, C. Tu, J. Shi. "Recombinant PRRSV P129 expressing CSFV E2 glycoprotein enhanced pathogenesis of CSFV in pigs"; 3rd International Conference on Vaccine Research and Development, Washington D.C., November 14th, 2017 (Oral presentation).
- Madera R, Gong W, Wang L, Burakova Y, Llellish K, Galliher-Beckley A, Nietfeld J, Henningson J, Jia K, Li P, Bai J, Schlup J, McVey S, Tu C, Shi J. Towards the development of a one-dose classical swine fever sub-unit vaccine; 3rd International Conference on Vaccine Research and Development, Washington D.C., November 14th, 2017 (Oral presentation).
- Rui Guo, Benjamin B. Katz, John M. Tomich, Duane Davis, Tom Gallagher and Ying Fang. 2017. Tunneling nanotubes provide a unique conduit for intercellular spread of PRRSV infection: A novel pathways for nidovirus transmission. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Ian Brierley, Andrew E. Firth, Jens Kuhn, Ying Fang. 2017. Characterization of a -2/-1 programmed ribosomal frameshifting in simian arteriviruses. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Duan-Liang Shyu, Pengcheng Shang, Jianfa Bai, Kang Ouyang, Santosh Dhakal, Jagadish Hireamt, Basavaraj Binjawadagi, Gourapura J. Renukaradhya, Ying Fang. 2017. Mutations in a highly conserved motif of nsp1beta protein attenuate the innate immune suppression function of porcine reproductive and respiratory syndrome virus (PRRSV). XIV International Nidovirus Symposium, Kansas City, KS.

- Xinyu Yan, Tao Wang, Ying Fang. 2017. Generation and characterization of monoclonal antibodies against simian hemorrhagic fever virus nonstructural protein 2. XIV International Nidovirus Symposium, Kansas City, KS.
- Pengcheng Shang, Saurav Misra, Ben Hause, Ying Fang. 2017. A naturally occurring recombinant enterovirus expresses torovirus protease. XIV International Nidovirus Symposium, June 1-6, 2017, Kansas City, KS; North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
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- Fangfeng Yuan, Rui Guo, Yanhua Li and Ying Fang. 2017. Potential role of porcine reproductive and respiratory syndrome virus structural protein nsp1alpha in mitochondrion dysfunction. XIV International Nidovirus Symposium, Kansas City, KS.
- Yanhua Li, Sawsan Naphthine, Emmely E. Treffers, Pengcheng Shang, Ali Tas, Andrew E. Firth, Ian Brierley, Eric J. Snijder and Ying Fang. 2017. A novel mechanism of protein-stimulated trans-activation of ribosomal frameshifting in porcine reproductive and respiratory syndrome virus: implication in improved vaccine development. XIV International Nidovirus Symposium, Kansas City, KS.
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- Rui Guo, Pengcheng Shang, Celena A. Carrillo, Xingyu Yan, Tao Wang, Crystal J. Jaing, Megan Niederwerder, Raymond R. Rowland, Ying Fang. 2017. Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV. North American PRRS Symposium, Dec 1-3, 2017; Conference of Research Workers in Animal Disease, Dec 3-5, 2017, Chicago, IL.
- Pengcheng Shang, Rui Guo, Celena A. Carrillo, Crystal J. Jaing, Ying Fang. 2017. Multiplexed digital mRNA profiling of immune responses in pigs persistently infected with porcine

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- Yin Wang, Wanglong Zheng, Elizabeth Poulsen, Xuming Liu, Jianqiang Zhang, Kyoung-Jin Yoon, Lalitha Peddireddi, Richard Hesse, Ying Fang, Gary Anderson, and Jianfa Bai. 2017. Development of a Luminex Multiplex Assay for the Detection and Differentiation of Type 2 PRRSV Field Strains and the Four US Vaccine Strains. 2017 North American PRRS Symposium on PRRS, Emerging and Foreign Animal Diseases and National Swine Improvement Federation NAPRRS-NSIF Joint Conference. December 1-3, 2017, and 98th Conference of Research Workers in Animal Disease. December 3-5, 2017, Chicago, IL.
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- Dong, J, J Lunney, E Fritz-Waters, Y Nguyen, RRR Rowland, A Hess, J Reecy, J Dekkers. 2016. The effect of PRRS viral level and isolate on tonsil gene expression 42 days after infection. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Popescu, L, NN Gaudreault, KM Whitworth, MV Murgial, JC Nietfeld, A Mileham, M Samuel, KD Wells, RS Prather, RRR Rowland. 2016. Genetically edited pigs lacking CD163 show no resistance following infection with the African Swine Fever Virus isolate, Georgia 2007/1. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Ke, H, M Kerrigan, M Han, RRR Rowland, D Yoo. 2016. SAP domain in nsp1-beta of porcine reproductive and respiratory syndrome virus (PRRSV) correlates with interferon suppression in cells and pathogenesis in pigs. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Liu, Q, J Lee, W Ma, RRR Rowland, F Blecha, Y Sang. 2016. Antiviral potency and functional novelty of porcine Interferon-Omega subtype. 2016 North American PRRSV Symposium, December 3-4, Chicago.
- Murgia, MV, NN Gaudreault, RRR Rowland. 2016. Identification of African swine fever virus p30 antigenic epitopes after experimental infection. 2016 North American PRRSV Symposium, December 3-4, Chicago.
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- Dee, S., F.V. Bauermann, M.C. Niederwerder, A. Singrey, T. Clement, M. de Lima, G. Patterson, M.A. Sheahan, Ana M.M. Stoian, Vlad Petrovan, C.K. Jones, J. DeJong, J. Ji, G.D. Spronk, J. Christopher-Hennings, J.J. Zimmerman, R.R.R. Rowland, E. Nelson, P. Sundberg, and D.G. Diel. 2017. Evaluation of the survival of viral pathogens in contaminated feed ingredients using transboundary shipment models. Oral presentation, 98th Conference of Research Workers in Animal Diseases, Chicago, IL. Abstract #78.
- Guo, R., P. Shang, C.A. Carrillo, X. Yan, T. Wang, C.J. Jaing, M. Niederwerder, R.R.R. Rowland, and Y. Fang. 2017. Double-stranded viral RNA persists *in vitro* and *in vivo* during prolonged infection of PRRSV. Oral and poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Dunkelberger, J.R., N.V.L. Serão, M. Niederwerder, M. Kerrigan, M. Schroyen, C.K. Tuggle, J. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Genomic prediction of a PRRS-vaccinated training population to predict host response to PRRS virus-only or PRRS virus/PCV2b co-infection. Poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Constance, L.A., J.B. Thissen, C.J. Jaing, K.S. McLoughlin, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Pre-challenge microbiome composition is associated with improved weight gain in pigs after vaccination with a porcine reproductive and respiratory syndrome (PRRS) modified live virus (MLV) vaccine followed by challenge with PRRSV

- and porcine circovirus type 2 (PCV2b). Poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C., R.R.R. Rowland, L.A. Constance, M.L. Potter, M.A. Kerrigan, R.A. Hesse, and A.G. Cino-Ozuna. 2017. Fecal microbiota transplantation improves outcome in nursery pigs following co-infection with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2d. Oral and poster presentation, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C., D. Diel and S. Dee. 2017. Novel approaches for assessing the risks of importing viruses from other countries through feed and feed ingredients. Invited program talk, North American PRRS Symposium and National Swine Improvement Federation Conference, Chicago, IL.
- Niederwerder, M.C. 2017. Role of the Microbiome in Porcine Respiratory Disease. Invited program talk, Swine Day, Animal Sciences and Industry, Kansas State University, Manhattan, KS.
- Niederwerder, M.C. 2017. Risk of Virus Introduction and Transmission in Feed. Invited program talk, Swine Day, Animal Sciences and Industry, Kansas State University, Manhattan, KS.
- Constance, L.A., J.B. Thissen, C.J. Jaing, K.S. McLoughlin, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Role of the gut microbiome in response to vaccination and viral respiratory infection in growing pigs. Poster presentation, Research and the State Forum, Kansas State University, Manhattan, KS. *Awarded as a Top 10 Presentation for showcase at the state capitol.
- Dunkelberger, J.R., N.V.L. Serão, M. Niederwerder, E. Waide, M. Kerrigan, M. Schroyen, C.K. Tuggle, J.K. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Pigs selected for increased natural resistance to PRRS are more resistant to PRRSV/PCV2b co-infection. Poster Presentation, Allen D. Leman Swine Conference, St Paul, MN.
- Niederwerder, M.C. 2017. Role of the microbiome in respiratory disease. Invited general session talk and Proceedings, 50th Annual Conference of the American Association of Bovine Practitioners, Omaha, NE.
- Ober, R.A., J.B. Thissen, C.J. Jaing, A.G. Cino-Ozuna, R.R.R. Rowland, and M.C. Niederwerder. 2017. Increased microbiome diversity is associated with improved growth rates of pigs after co-infection with PRRSV/PCV2. Poster presentation, XIVth International Nidovirus Symposium, Kansas City, MO.
- Jaing, C.J., J.B. Thissen, K.S. McLoughlin, M.C. Niederwerder, R.R.R. Rowland. 2017. Rapid disease diagnostics and surveillance using a broad-spectrum microbial detection array. Oral presentation, XIVth International Nidovirus Symposium, Kansas City, MO.
- Dunkelberger, J.R., N.V. Serão, Z.Q. Weng, M.C. Niederwerder, E.H. Waide, M.A. Kerrigan, J.K. Lunney, R.R.R. Rowland, and J.C.M. Dekkers. 2017. Biological evidence for genomic regions associated with host response to co-infection with PRRS virus and PCV2b in commercial nursery pigs. Oral presentation, Breeding and Genetics session, American Society of Animal Science Midwest Meeting, Omaha, NE. *J Anim Sci* 95: supplement 2: 16-16. doi:10.2527/asasmw.2017.034.
- Constance, L.A., B. Bloomberg, J.K. Lunney, J.C.M. Dekkers, R.R.R. Rowland, and M.C. Niederwerder. 2017. Comparison of morbidity and mortality after challenge with two North American PRRS virus isolates shows marked variation in time course and prevalence of clinical disease between isolates. Selected as top 15 poster submitted; presented in the

Veterinary Student Poster Competition, American Association of Swine Veterinarians Annual Meeting, Denver, CO.

Niederwerder, M.C., C.J. Jaing, J.B. Thissen, A.G. Cino-Ozuna, K.S. McLoughlin, and R.R. Rowland. 2017. Microbiome associations in pigs with the best and worst clinical outcomes following co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Poster presentation, Research Topics Session, American Association of Swine Veterinarians Annual Meeting, Denver, CO.

Book Chapters or Monographs- none to report

D. FUNDING SOURCES

Fang, Y. A novel arterivirus protein and expression mechanism: implication in vaccine and companion diagnostic assay development (USDA-NIFA, 01/01/2015 – 12/31/2019; \$472,179).

Biao, H., Y. Fang. Developing a Parainfluenza Virus 5 (PIV5)-based PRRS Vaccine (USDA-NIFA, 1/1/2016 – 12/31/2018, \$450,000).

Fang, Y., G. Anderson. Generation of reagents for differentiation of swine pathogens (private company, 04/01/2015-03/31/2018, \$250,000).

Fang, Y., S. Baker. The XIV International Nidovirus Symposium (USDA-NIFA conference grant, 06/01/2017-05/31/2018, \$15,000).

Baker, S., Y. Fang. International Nidovirus Symposium (NIH R13 AI129358, 12/01/2016-12/30/2017, \$5,000).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li, M. Potter and G. Anderson. Development and evaluation of antibody detection assay for PCV3 virus (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$71,600).

Marthaler, D., Y. Fang, M. Murtaugh. Development and validation of a rapid ELISA against porcine teschovirus in serum and oral fluid (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$66,033).

Marthaler, D., Y. Fang, M. Murtaugh. Development and validation of ELISA against porcine sapelovirus in serum and oral fluid (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$66,033).

Liu, X., J. Bai, Y. Fang, W. Ma, L. Peddireddi, Y. Li and G. Anderson. Development of antibody detection assays for swine influenza B, C, and D viruses. (Swine Health Information Center, 11/15/2017 – 11/14/2018; \$106,100).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li and G. Anderson. Detection and differentiation of Seneca Valley virus (SVV) from foot-and-mouth disease virus (FMDV). (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$65,700).

Bai, J., Y. Fang, L. Peddireddi, X. Liu, Y. Li and G. Anderson. Detection and differentiation of PCV3 from PCV2a, PCV2b and the highly prevalent PCV2d mutant strains. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$56,700).

Peddireddi, L., J. Bai, Y. Fang, X. Liu, R. Hesse, B. Hause, G. Anderson, B. Arruda and P. Arruda. Development of sensitive and reliable diagnostic assay to detect atypical porcine pestivirus (APPV) in swine. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$55,267).

Liu, X., J. Bai, Y. Fang, L. Peddireddi, Y. Li and G. Anderson. Multiplex real-time RT-PCR assay for simultaneous detection and differentiation of swine influenza C, D, and B viruses. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$58,500).

Li, Y., Y. Fang, J. Bai, X. Liu, L. Peddireddi, G. Anderson and C. Stahl. Development of a TaqMan quantitative RT-PCR test for porcine parainfluenza virus 1. (Swine Health Information Center, 11/15/2016 – 11/14/2017; \$56,500).

Bai, J., Y. Fang, X. Liu, J. Zhang, KJ. Yoon. Detection and Differentiation of Field Strains and Commonly used Vaccine Strains of Type 2 PRRSV in the U.S. (National Pork Board, 11/15/2016 – 11/14/2017, \$79,080).

Niederwerder. 2017 – 2018. “Fecal microbiota transplantation as an alternative tool for increasing porcine reproductive and respiratory syndrome (PRRS) vaccine efficacy and reducing the effects of PRRS.” College of Veterinary Medicine Success For Young Investigators Grant Program. Total Awarded Funding Amount: \$15,000.

Jones, Niederwerder, Rowland et al., 2017 – August 2018. “Validation of a low-cost tool for Senecavirus A detection, and surveillance of viral prevalence in United States feed mills.” Swine Health Information Center. Total Awarded Funding Amount: \$21,500.

Jones, Niederwerder et al., 2017 – April 30, 2018. “Assessing the role of medium chain fatty acids as an alternative to medically important antibiotics.” National Pork Board. Total Awarded Funding Amount: \$73,597.

Dee, Niederwerder, Rowland et al., Cassie Jones, and Steve Dritz. April 5, 2017 – April 4, 2018. “Evaluation of chemical mitigants for neutralizing the risk of foreign animal diseases in contaminated feed ingredients.” Swine Health Information Center. Total Awarded Funding Amount: \$120,000.

Niederwerder, Hesse. 2016 –2017. “Comprehensive Literature Review on the current knowledge for Porcine Epidemic Diarrhea Virus (PEDV) and Porcine Deltacoronavirus (PDCoV).” National Pork Board. Total Awarded Funding Amount: \$10,000.

Niederwerder. 2016 –2017. “Assessing microbiome diversity as a tool for the mitigation of viral disease in nursery pigs.” Kansas State University College of Veterinary Medicine Intramural Success for Young Investigators Grant. Total Awarded Funding Amount: \$14,995.

Niederwerder, Rowland, et al., Swine health Information Center (SHIC) and Kansas NBAF matching funds, 2017-2018, Assessing tools for the mitigation of foreign animal disease introduction and transmission in feed \$275,000.

Niederwerder, Rowland, et al., National Pork Board, NPB#17-057, 2017-2018 and Kansas NBAF matching funds, Assessing the risk of African swine fever virus (ASFV) transmission in feed. \$290,000

Dee (Niederwerder, Rowland) et al., Swine Health Information Center (SHIC) and Kansas NBAF matching funds, 2017-2018, Evaluation of the risk of transboundary movement of ASFV via contaminated feed ingredients. \$140,000.

Rowland, National Pork Board, NPB Project No. 17-160, 2017-2018, Adaptation of PRRSV to genetic modifications in CD163, \$66,000.

Rowland, Fang and Prather, USDA AFRI 2016-09462, 2017-2020, Preventing porcine reproductive and respiratory syndrome (PRRS) through modifications in the virus receptor, CD163, \$330,000.

Rowland and Prather, National Pork Board, NPB, 2016-2018, Genetic modifications in CD163 that confer complete resistance of pigs to infection with PRRSV, \$128,000.

Mwangi and Rowland, National Pork Board, 2017, Efficacy of prototype live-vectored polyvalent African swine fever virus vaccines, approximately \$200,000.

Rowland, NPPC, 2016-2017, Risk if SVA transmission by pig meat. \$40,000.
Mwangi and Rowland, USDA NIFA, 2016-2019 Protective efficacy of an adenovirus-vectored ASFV multi-antigen cocktail, Rowland budget = \$80,000
Shi. Evaluation of a plant-made CSFV vaccine during a challenge study in swine. iBio CMO, LLC. Bryan, TX 77807.
Shi. Characterization of mammalian inflammatory and innate immune responses to *Culicoides* *Sonorensis* cellular lipids and evaluate use of adjuvants. USDA ARS, AR9865

E. WORK PLANNED FOR NEXT YEAR

Continue to develop E2 CSF vaccines
To test PRF manipulation in highly pathogenic PRRSV field strains
Explore the new vector platform(s) for PRRS vaccine development
Develop diagnostic reagents and assays for emerging swine pathogens
Continue to work on the interaction between CD163 and PRRSV-1 and PRRSV-2 isolates
Seek additional resources and funding to evaluate the effect of microbiome manipulations on pig health following infection with PRRSV
Understand the risk and mitigation of ASFV and other transboundary diseases in pigs

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION:

A. Personnel

1) NC-229 STATION REPRESENTATIVE (*name, position, email*):

Zhang, Yanjin; University of Maryland; zhangyj@umd.edu

2) Other PRINCIPAL LEADERS associated with the projects (*name, position, email*):

Zhu, Xiaoping, UMD

Xiao, Zhengguo, UMD

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

1. We continued studying the atypical PRRSV strain, A2MC2, which is able to induce type I interferons in cultured cells. A2MC2 was found to induce higher level of neutralizing antibodies *in vivo* compared with the Ingelvac PRRS MLV and VR-2385. We discovered that the middle half of the A2MC2 genome is needed for triggering the IFN synthesis. First, a cDNA infectious clone of this atypical strain was constructed as a DNA-launched version. Virus recovery was achieved from the infectious clone and the recovered virus, rA2MC2, was characterized. The rA2MC2 retained the feature of interferon induction in cultured cells. Infection of pigs with the rA2MC2 virus caused viremia similar to that of the wild type virus. Chimeric infectious clones were constructed by swapping genomic fragments with a cDNA clone of a moderately virulent strain VR-2385 that antagonizes IFN induction. Analysis of the rescued chimeric viruses demonstrated that the middle two fragments, ranging from nt4545 to nt12709 of the A2MC2 genome, were needed for the IFN induction, whereas the chimeric viruses containing any one of the two A2MC2 fragments failed to do so. The results and the cDNA infectious clone of the IFN-inducing A2MC2 will facilitate further study of its biology, ultimately leading towards the development of an improved vaccine against PRRS.
- We have also continued our study on PRRSV interaction with the JAK/STAT pathway. We studied PRRSV effect on signal transducer and activator of transcription 3 (STAT3). STAT3 is known to play critical roles in cell growth, proliferation, differentiation, immunity and inflammatory responses. We discovered that PRRSV infection led to significant reduction of STAT3 protein level but had minimum effect on its transcripts. Further study showed that non-structural protein 5 (nsp5) of PRRSV induced the STAT3 degradation by increasing its polyubiquitination level and shortening its half-life from 24 h to approximately 3.5 h. The C-terminal domain of nsp5 was shown to be required for the STAT3 degradation. Moreover, the STAT3 signaling in the cells transfected with nsp5 plasmid was significantly inhibited. This study provides insight into the PRRSV

interference with the JAK/STAT signaling, leading to perturbation of the host innate and adaptive immune responses.

Objective 2. Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Our studies on the interferon-inducing PRRSV A2MC2 and construction of infectious cDNA clone are beneficial for vaccine development and biology study of this strain. Better protective immunity against PRRS is expected from an optimized A2MC2.

Our studies on STAT3 may contribute to our understanding of PRRSV interference of host immune response.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed Publications

1. Eve Fontanella, Zexu Ma, **Yan-Jin Zhang**, Alessandra Castro, Huigang Shen, Patrick G Halbur, Tanja Opriessnig: An interferon inducing porcine reproductive and respiratory syndrome virus vaccine candidate elicits protection against challenge with a heterologous virulent type 2 strain in pigs. *Vaccine*. 2017 Jan 3;35(1):125-131.
2. L. Yang, R. Wang, Z. Ma, Y. Xiao, Y. Nan, Y. Wang, S. Lin, and **Y. Zhang**: Porcine Reproductive and Respiratory Syndrome Virus Antagonizes JAK/STAT3 Signaling via Inducing STAT3 Degradation. *Journal of Virology* 2017, 91:e02087-16.
3. L. Yang and **Y. Zhang**. Antagonizing Cytokine-Mediated JAK-STAT Signaling by Porcine Reproductive and Respiratory Syndrome Virus. *Veterinary Microbiology* 209C (2017) pp. 57-65. *Review*
4. Z. Ma, Y. Yu, Y. Xiao, T. Opriessnig, R. Wang, L. Yang, Y. Nan, S. Samal, P. Halbur, and **Y. Zhang**: The Middle Half Genome of Interferon-Inducing Porcine Reproductive and Respiratory Syndrome Virus Strain A2MC2 Is Indispensable for Host Recognition. *Journal of General Virology* 2017 Jul;98(7):1720-1729.
5. Yuchen Nan, Chunyan Wu, Guoqian Gu, Weiyao Sun, **Yanjin Zhang** and En-Min Zhou Improved Vaccine against PRRSV: Current progress and future perspective. *Front. Microbiol.* 8:1635. *Review*

2) Abstracts or Proceedings

1. L. Yang, Z. Ma, and **Y. Zhang**: PRRSV Interference with the Cytokine-mediated JAK/STAT Signaling. *2016 PRRS Symp.*
2. E. Fontanella, Z. Ma, **Y. Zhang**, A. Castro, H. Shen, P. Halbur, T. Opriessnig: An interferon inducing PRRSV vaccine candidate protects against challenge with a heterologous virulent type 2 strain in a conventional pig model. *2016 PRRS Symp.*

3) Book Chapters or Monographs

Zexu Ma, Liping Yang, and **Yan-Jin Zhang**. Porcine Reproductive and Respiratory Syndrome Virus: Propagation and Quantification. *Current Protocols in Microbiology*. Book chapter. *In press*.

D. FUNDING SOURCES

Maryland Agricultural Experiment Station

E. WORK PLANNED FOR NEXT YEAR

We will continue to characterize the mechanism of PRRSV A2MC2 in inducing production of type I interferons and explore passaged A2MC2 for vaccine development. We will also continue to study the mechanism of PRRSV interference with innate immune response and examine PRRSV-host interactions.

Project Initiation

Title: Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine			
Accession No.	1006533	Sponsoring Institution	National Institute of Food and Agriculture
Project No.	VA-136308	Project Status	ACTIVE
Funding Source	Hatch/Multi State	Multistate No.	NC229
Start Date	07/01/2015	DUNS Number	003137015
Submitted By	Robin Williams	End Date	09/30/2019
		Date Submitted to NIFA	11/13/2017

Project Director

Kevin Lahmers

Clinical Associate Professor

540-231-7632

klahmers@vt.edu

Performing Organization/Institution

SAES - VIRGINIA POLYTECHNIC INSTITUTE AND
1880 PRATT DR STE 2006
BLACKSBURG, VIRGINIA 24060-3580

Performing Department

College of Vet Medicine

Co-Project Directors

Meng, X. J.

Collaborating Departments

College of Vet Medicine

Collaborating/Partnering States

CONNECTICUT

ILLINOIS

INDIANA

IOWA

KANSAS

MARYLAND

MINNESOTA

NEBRASKA

NORTH DAKOTA

OHIO

SOUTH DAKOTA

VIRGINIA

WISCONSIN

WYOMING

Collaborating/Partnering Countries

{NO DATA ENTERED}

Collaborating/Partnering Organizations

{NO DATA ENTERED}

Non-Technical Summary

Currently singular vaccines against either PRRSV or PCV2 are available but a bi-valent vaccine against both PRRSV and PCV2 are lacking. The objective of this project is to evaluate the use of non-pahtogenic PCV1 and the vaccine virus PCV1-2 as potential vaccine delivery vectors for the development of a bi-valent vaccine against both PRRSV and PCV2. We expect that the project will validate the use of PCV1 as a useful vaccine delivery vector for other swien pathogens, and we also expect that we will demonstarte that the vaccine virus PCV1-2 can serve as a vaccine delivery vector for creating bi-valent vaccines against other swine viruses.

United States Department of Agriculture
Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Goals / Objectives

(1)

The overall objective for this five-year NC-229 project is to reduce the impact PRRS has on producers, and to assess the feasibility and financial acceptability of PRRS area control and/or elimination for producers. To that end, we focus on the following major points, which faithfully represent the current research priorities of the US swine industry (Pork Check off NPB) :
1.1) PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection, 1.2) PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA), 1.3). Economic Impact of Interventions: determining the economic benefit of vaccination in positive herds

(2)

Develop effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence, which includes the following: 2.1) Porcine Epidemic Diarrhea Virus, 2.2) Swine Influenza Virus, 2.3) African Swine Fever, 2.4) Emerging serotypes of swine rotaviruses

Methods

We plan to evaluate the potential use of the non-pathogenic porcine circovirus type 1 (PCV1) as a vaccine delivery vector against other swine pathogens such as PRRSV. Immunogenic epitopes from swine pathogens such as PRRSV will be cloned into the infectious clone of PCV1, and viable chimeric viruses will be generated and their immunogenicity and potential use as a vectored vaccine will be tested in pigs.

We also plan to determine if the commercial vaccine against PCV2, the chimeric PCV1-2 virus, can be used as a vector to develop a bi-valent vaccine against both PCV2 and PRRSV. PRRSV antigenic epitopes will be cloned into the backbone of the vaccine virus PCV1-2, and viable chimeric viruses will be recovered and characterized for the ability to induce protective immunity in pigs against both PCV2 and PRRSV.

The project will be evaluated based on the outcomes such as potential vaccine candidates, journal publications as well as scientific meeting presentations.

Target Audience

The target audiences are swine veterinarians, and research scientists through scientific meeting presentations of the research results as well as scientific journal publications of the research data.

Products

- *Publications in peer-reviewed journals
- *Graduate PhD students in agricultural sciences
- *Scientific presentations in national and international conferences

Expected Outcomes

- *Increase in the knowledge regarding our understanding the mechanisms of pathogenesis of PRRSV and PCV2.
- *Increase in the knowledge of understanding the protective immunity and vaccine design against PRRSV and PCV2.

Keywords

Porcine circovirus type 1 ~Porcine circovirus type 2 ~Porcine reproductive and respiratory syndrome virus ~Vaccine vector

Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.3	0.0	0.1	0.0	0.4
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.1	0.0	0.0	0.0	0.1
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.4	0.0	0.1	0.0	0.5

Animal Health Component 100 %

Is this an AREERA Section 204 Integrated Activity? No

Activities

Research 100 %
 Extension 0 %
 Education 0 %

Research Effort Categories

Basic 50 %
 Applied 50 %
 Developmental 0 %

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
311	3510	1101	34
311	1030	1101	33
722	3510	1090	33

Knowledge Area

311 - Animal Diseases; 722 - Zoonotic Diseases and Parasites Affecting Humans

Subject Of Investigation

1030 - Papaya; 3510 - Swine, live animal

Field Of Science

1090 - Immunology; 1101 - Virology

Associated Planned Programs

Plan Year	Program Name	Percentage
2015	Agriculture Profitability and Sustainability	80
2015	Food, Nutrition, and Health	20

Project Initiation

Accession No. 1006533

Project No. VA-136308

Multistate No. NC229

Assurance Statements

1. Are Human Subjects Involved? ☒ No ☐ Yes

If YES to Human Subjects

Is the Project Exempt from Federal regulations?

☐ Yes

If yes, select the appropriate exemption number.

☐ No

If no, is the IRB review Pending?

☐ Yes☐ No IRB Approval Date

Human Subject Assurance Number

2. Are Vertebrate Animals Used? ☐ No ☒ Yes

If YES to Vertebrate Animals

Is the IACUC review Pending?

☐ Yes☒ No IACUC Approval Date July 07, 2016

Animal Welfare Assurance Number 16-097 (CVM)

Project Signature Panel

Dr. Saied Mostaghimi

Director

Virginia Agricultural Experiment Station

Assurance Statement Panel

Dr. Saied Mostaghimi

Director

Virginia Agricultural Experiment Station

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: November 30 2016 to December 1, 2017

INSTITUTION OR STATION: University of Connecticut

A. NC-229 REPRESENTATIVE:

Guillermo Risatti, University of Connecticut, guillermo.risatti@uconn.edu

Other PRINCIPAL LEADERS associated with the projects

Antonio Garmendia, University of Connecticut, Antonio.garmendia@uconn.edu

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV (Risatti; Garmendia).

We plan to test whether IFN β levels correlate with protection from PRRS. For this purpose, ongoing vaccination/challenge studies in swine will be a source of samples to examine IFN β and downstream ISGs responses *in vivo*. It is expected that bioactive IFN β will be produced by PAMs of swine infected with PRRSV in a strain-dependent manner. These studies will include evaluation of IFN β expression, Mx and ISG15 expression as a measure IFNAR-mediated signaling and overall anti-viral bioactivity in BAL fluids, virus-stimulated PAMs, serum. A series of anti-swine IFN β monoclonal antibodies (mAbs) were developed to be utilized to assess IFN β in immunoassays and bioassays (Garmendia). Emily Morse an honor's student tested whether envelope proteins devoid of viral nucleic acid extracted from CsCl purified PRRSV induced IFN β in normal porcine alveolar macrophages (PAMs). At two concentrations of envelope proteins tested to stimulate PAMs there were relatively low but significant increases in IFN β mRNA expression when compared to baseline levels ($p < 0.05$) as measured by quantitative RT-PCR. The data suggest that replication of virus may not be strictly necessary for induction of IFN β . In fact virus replication may result in inhibition of IFN β induction with some strains of virus as some NS proteins known to inhibit such induction will be produced. Research conducted to test IFN β and downstream ISGs responses of PAMs to infection with PRRSV showed that bioactive IFN β was produced although this was variable. The study also showed that Mx1 protein was expressed and indicated as IFNAR-mediated signaling and roughly followed the IFN β responses. In conclusion, IFN β induction/signaling do occur variably upon infection of natural host cells with PRRSV. Interestingly, Mx-1 expression by infected PAMs generally correlated with IFN β production (The activation of the IFN β induction/signaling pathway in porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus is variable Overend C., J. Cui, M. Grubman, A.E. Garmendia Vet Res Commun 41(1):15-22. 2017 (Garmendia).

We are developing an ELISA DIVA test for differentiating animals vaccinated from Classical Swine Fever Virus (CSFV) infected animals as a companion assay for a modified live marker vaccine that our group have designed (*Development of an improved live attenuated antigenic*

marker CSF vaccine strain candidate with an increased genetic stability. Holinka LG, Fernandez-Sainz I, Sanford B, O'Donnell V, Gladue DP, Carlson J, Lu Z, Risatti GR, Borca MV. 2014. Virology. Dec; 471-473:13-8). The test is based on the use of a CSFV E2 modified glycoprotein expressed in baculovirus/insect cell system. When added to a commercially available CSFV antibody ELISA detection test together with swine sera, the E2 modified protein competes with those antibodies elicited by the marker vaccine. However, the modified protein is unable to compete with antibodies elicited by a natural infection with wild-type viruses. We have been able to confirm the working hypothesis. A MS student Yuxiang Wang has been mentored under this project. (Risatti).

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

The objective of the study is to examine the role of IFN beta in protective immunity against PRRS. Investigating IFN beta will contribute to gain a better understanding of the innate response to PRRSV which in turn will be useful to the overall knowledge of mechanisms of general pathogenesis, immune evasion and protection or lack thereof. (The activation of the IFN β induction/signaling pathway in porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus is variable Overend C., J. Cui, M. Grubman, A.E. Garmendia Vet Res Commun 41(1):15-22. 2017 (Garmendia).

The project is aimed to improve/produce biological control tools against CSF. A DIVA companion test is needed for the vaccine candidate develop in collaboration with Plum Island Animal Disease Center, ARS, USDA (*Development of an improved live attenuated antigenic marker CSF vaccine strain candidate with an increased genetic stability. Holinka LG, Fernandez-Sainz I, Sanford B, O'Donnell V, Gladue DP, Carlson J, Lu Z, Risatti GR, Borca MV. 2014. Virology. Dec; 471-473:13-8).* (Risatti).

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1. Publications in press
2. Abstracts or Proceedings

E. FUNDING SOURCES FOR PRRSV RESEARCH

Hatch Multistate Project, NC229. Storrs Agricultural Experiment Station (Risatti).

Hatch Project, Storrs Agricultural Experiment Station (Garmendia).

Polyvalent T cell Mosaic Vaccine to Cross-Protect Swine against Heterologous PRRSV Strains. USDA/NIFA Grant Number 2011 67015-30176 (Garmendia).

F. WORK PLANNED FOR NEXT YEAR

- 1) This year we plan to retest levels and bioactivity of IFN β in representative archival samples from a recent vaccine study. Additionally samples collected in an ongoing vaccine/challenge study are included in the testing for IFN β levels and bioactivity to determine how these correlate with protection outcomes. Measurements of IFN β will be made during vaccination, after challenge and at necropsy in serum, culture fluids of PBMNC stimulated *in vitro* with virus or viral antigens, BAL fluids and PAMs stimulated as the PBMNC. ELISAs, flow cytometry and bioassays will be utilized to do the

evaluation. In addition, the induction of IFN β by stimulation of PAMs with detergent extracts of viral proteins will be extended to proteins extracted from different strains of virus and will be compared with induction outcomes resulting from infection with the corresponding infectious viruses. (Garmendia).

- 2) Studies on ASFV virulence and protection, CSFV DIVA ELISA as companion test for an experimental modified-live marker vaccine (Risatti).

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence. (Risatti)

We are engaged in a collaboration with Plum Island Animal Disease Center (PIADC), ARS, USDA, in a project entitled “Development of recombinant African Swine Fever Virus (ASFV) attenuated viruses containing multiple deletions for use as vaccine candidates.” (Risatti).

We are in the process of developing collaborative work (e.g. Uganda) for establishing ASF surveys among domestic pigs and for assessing features of circulating viruses in the that country.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDER

Basic research on the role of specific ASFV genes in virus virulence is under investigation. The purpose is to identify virus targets that once modified render attenuated virus that might be used as vaccine candidates.

A communicable disease surveillance system such as for ASF is aimed to detect early presence of the disease and to estimate risks associated with disease spread. Active surveillance refers to the systematic collection, analysis, and interpretation of disease data (i.e.: ASF) for use in planning, implementing and evaluating animal disease control measures.

Improving biologicals tools for better control of CSFV. We will continue working on developing of an ELISA test that can be used as companion assay for an experimentally developed modified-live marker vaccine.

D. ASF PUBLICATIONS ISSUED OR “IN PRESS”

1) Publications

Holinka LG, O'Donnell V, **Risatti GR**, Azzinaro P, Arzt J, Stenfeldt C, Velazquez-Salinas L, Carlson J, Gladue DP, Borca MV. Early protection events in swine immunized with an experimental live attenuated classical swine fever marker vaccine, FlagT4G. *PLoS One*. 2017 May 24; 12(5):e0177433. doi: 10.1371/journal.pone.0177433. eCollection 201.

Borca MV, O'Donnell V, Holinka LG, Sanford B, Azzinaro PA, **Risatti GR**, Gladue DP. Development of a fluorescent ASFV strain that retains the ability to cause disease in swine. *Sci Rep*. 2017 Apr 24; 7:46747. doi: 10.1038/srep46747.

O'Donnell V, **Risatti GR**, Holinka LG, Krug PW, Carlson J, Velazquez-Salinas L, Azzinaro PA, Gladue DP, Borca MV. Simultaneous Deletion of the 9GL and UK Genes from the African Swine Fever Virus Georgia 2007 Isolate Offers Increased Safety and Protection against Homologous Challenge. *J Virol*. 2016 Dec 16; 91(1).

2) Abstracts or Proceedings

University of Connecticut, College of Agriculture, Health and Natural Resources, **Graduate Research Forum**, March 25th 2017, Storrs, CT. **“Development of ELISA-based test for serological differentiation of vaccinated from Classical Swine Fever Virus infected animals”**. Yuxiang Wang¹, Manuel V. Borca² and Guillermo R. Risatti¹. (1) Department of Pathobiology and Veterinary Science, College of Agriculture Health and Natural Resources, University of Connecticut. (2) Plum Island Animal Disease Center, Agricultural Research Service, US Department of Agriculture.

Conference of Research Workers in Animal Diseases, Chicago, IL, USA, December 1-5, 2017. **“Understanding the diverse roles of viroporin activity of classical swine fever virus protein p7”**. M. Borca¹, E. Largo², N. Huarte², L. Holinka¹, K. Berggren¹, E. Ramirez-Medina^{1,3}, G. Risatti³, J. Nieva², D.P. Gladue¹. (1) PIADC, ARS, USDA, USA; (2) University of the Basque Country, Bilbao, Spain; (3) University of Connecticut, USA.

E. FUNDING SOURCES FOR ASF and CSF RESEARCH

Plum Island Animal Disease Center, ARS, USDA.

F. WORK PLANNED FOR NEXT YEAR

Active surveillance of ASF is planned to continue next year in both countries.

Collaborative research with PIADC on development of recombinant African Swine Fever Virus (ASFV) attenuated viruses containing multiple deletions for use as vaccine candidates.

ANNUAL STATION REPORT: PROJECT NC-229

PERIOD COVERED: November 30, 2016 to December 1, 2017

INSTITUTION OR STATION: South Dakota State University

A. Personnel:

1) NC-229 STATION REPRESENTATIVE: Eric A. Nelson; SDSU; eric.nelson@sdstate.edu

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Control of PRRSV

- **PRRSV Immunity and Vaccinology: understanding correlates of immunity and mechanisms to broaden protection**

Research efforts directed toward PRRSV control primarily focused on innate immunity in PRRSV pathogenesis, virus host interactions, and a virus-like particle (VLP) approach for PRRSV vaccine development. Studies lead by the X. Wang lab, suggested that PRRSV may have evolved strategies to overcome the formation and anti-viral activity of stress granules (SGs) during viral infection. One possible mechanism mediated by PRRSV may be to modulate the expression of G3BP1, a key component of SGs. The efficacy of PRRSV VLPs together with the use of a novel 2', 3'-cGAMP VacciGrade™ adjuvant in an animal challenge model was also explored. PRRSV nucleocapsid protein specific antibody was detected in all animals at day 10 after challenge, but no significant difference was observed among vaccinated and control groups. Surprisingly, a significantly higher viremia was observed in the VLPs and VLPs plus adjuvant groups compared to the control group. The increased viremia correlated with a higher interferon- α induction in the serum of the VLPs and the VLPs plus adjuvant groups. PRRSV VLPs and PRRSV VLPs plus adjuvant failed to provide protection against PRRSV challenge.

- **Host genetic control of anti-PRRSV infection and vaccination responses**
- **PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA)**
- **Economic Impact of Interventions: determining the economic benefit of vaccination in positive herds**

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

- **PEDV Diagnostics, immunity and vaccinology**

Neutralizing monoclonal antibodies (mAbs) against the spike protein of porcine epidemic diarrhea virus (PEDV) were used to map neutralizing epitopes. Epitope mapping by peptide ELISAs revealed that seven of these mAbs recognized linear neutralizing epitopes located in the N-terminus of the S2 glycoprotein subunit. Additionally, one mAb recognized a neutralizing epitope located in the C-terminus of S2, while only one neutralizing mAb reacted against a region of the S1 glycoprotein subunit. The mAbs that recognized epitopes within the S2 subunit presented the highest neutralizing activity, suggesting the S2 glycoprotein subunit contains immunodominant neutralizing epitopes of PEDV.

Additional mAbs were developed against the PLP2 region of PEDV in support of research efforts led by scientists at USDA-NADC. These reagents will be valuable for studying the interaction of non-structural proteins to better understand how they contribute to PEDV pathogenesis.

A recombinant ORFV-based vaccine candidate for PEDV was developed and its immunogenicity and protective efficacy was evaluated in pregnant gilts. Animals were immunized with the ORFV-based recombinant alone or immunized and exposed orally to live PEDV. Immunization with ORFV-PEDV-S alone or with ORFV-PEDV-S + live PEDV elicited the development of PEDV specific antibodies in serum, colostrum and milk of immunized sows. Upon challenge, reduced mortality was observed in animals born to immunized gilts, when compared to sham-immunized controls.

Another approach under investigation involves development of a nano-particle based vaccine platform for PEDV. Codon-optimized PEDV spike gene expression constructs were generated and fused into a ferritin nanoparticle scaffold plasmid. Expression and antigenicity of these nanoparticle constructs is being assessed *in vitro* prior to producing a Newcastle Disease Virus (NDV) vector expressing the PEDV spike-ferritin nanoparticles and conducting *in vivo* mouse experiments.

- **Senecavirus A epidemiology, diagnostics and pathogenesis**

Senecavirus A (SVA) is a re-emerging pathogen of swine that causes vesicular disease that is indistinguishable from Foot and Mouth Disease (FMD) in affected animals. Since its re-emergence in the US in July 2015, over 250 outbreaks have been confirmed. Our group has been actively working in different aspects of SVA epidemiology, infection immunity and pathogenesis, and on diagnostic assay development and validation. To date, we have obtained over 40 complete genome sequences of contemporary US and Brazilian SVA isolates and prepared a manuscript to assess the evolution and genetic diversity of these isolates in comparison with historical isolates. We have also conducted comprehensive studies to characterize the pathogenesis and immunity to SVA infection.

Additionally, diagnostic assays and reagents are currently under development and some in final stages of validation.

- **Swine influenza virus(SIV) evolution and detection**

Influenza is another significant pathogen of swine. PCR assays for influenza A are well established, but pigs can also be infected with influenza B, C and D. Therefore, we are developing assays designed to provide cost efficient testing, promoting the continued surveillance for all swine influenza viruses. Prototype assays have been developed for influenza B, C and D. These assays are being combined in panels and more fully validated. Well validated and rapid diagnostic tools such as these new multiplex real-time PCR assays will be vital for continued swine health and production while enhancing the One Health Initiative.

- **SIV Control by vaccination or other interventions**
- **SIV at the human-animal interface**
- **African Swine Fever (ASF): Vaccine Design and Development**
- **CSFV vaccination, diagnosis epidemiology**
- **Assessing pathogen survival in feed**

Since the emergence of PEDV in the US in 2013, the team at SDSU has been working closely with Pipestone Applied Research to assess potential risk factors that may have contributed to emergence of the virus in the US. Results from the initial study, demonstrating that PEDV survives in different feed matrices under transportation conditions simulating a trip from Asia to the US led to an expansion of this study. Our group, together with Pipestone Applied Research and collaborators from Kansas State University assessed the survival of 11 additional pathogens in feed ingredients. Results from this study showed that several other pathogens of importance to swine and/or surrogate viruses also survive the journey in the feed matrix. A report of the results from this study has recently been submitted for publication and is currently under review.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Innate immunity is the first line of defense against virus infections. A better understanding of innate immunity against PRRSV and PEDV will allow us to better understand viral pathogenesis, which in turn may facilitate the development of novel prophylactic strategies against these devastating swine diseases.

New monoclonal antibody-based reagents for Senecavirus A and a fluorescence-based virus neutralization assay for the detection of neutralizing antibodies are now available to researchers and diagnosticians throughout the US. Availability of these tools should provide substantial benefit to the swine industry in the control of Senecavirus A.

New knowledge generated from our studies on Senecavirus A has directly impacted the swine industry by providing critical information on the pathogenesis and immune responses of this important pathogen. We expect that this information will have an even broader impact in the future by allowing the design of improved prevention and control strategies.

Research on novel vector platforms and vaccine candidates for livestock species has had a significant impact on our understanding of novel approaches to vaccine design. Preliminary data generated as a part of this project was used to obtain two large grants from NIFA-USDA (Standard-Foundational) and from the South Dakota Governor's Office of Economic Development (Established the South Dakota Center for Biologics Research and Commercialization, SD-CBRC).

The transboundary risk of feed ingredients contaminated with high consequence pathogens and surrogate viruses representing foreign animal diseases was evaluated in a model simulating shipment from China to the US. Results demonstrate the ability of multiple viral pathogens to survive in certain feed ingredients, including soybean meal. This study suggests that contaminated feed ingredients could present transboundary risk factors for high consequence pathogens.

D. PERTINENT PUBLICATIONS ISSUED OR "IN PRESS"

1) Refereed publications

Maggioli, M.F., Lawson, S., de Lima, M., Joshi, L.R., Faccin, T.C., Bauermann, F.V., Diel, D.G. Adaptive immune responses following Senecavirus A infection in pigs. Accepted. *Journal of Virology*. November 2, 2017.

Martins, M., Joshi, L.J., Rodrigues, F.S., Anziliero, D., Frandoloso, R., Kutish, G.F., Rock, D.L., Weiblen, R., Flores, E.F., Diel, D.G. 2017. Immunogenicity of ORFV-based vectors expressing the rabies virus glycoprotein in livestock species. *Virology*, 511:229-239. doi:10.1016/j.virol.2017.08.027.

Okda, F.A., Lawson, S., Singrey, A., Nelson, J., Hain, K.S., Joshi, L.R., Christopher-Hennings, J., Nelson, E.A., Diel, D.G. 2017. The S2 glycoprotein subunit of porcine epidemic diarrhea virus contains immunodominant neutralizing epitopes. *Virology*, 509:185-194. doi:10.1016/j.virol.2017.06.013.

Van Noort, A., Nelsen, A., Pillatzki, A.E., Diel, D.G., Li, F., Nelson, E., Wang, X. 2017. Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2', 3'-cGAMP VacciGrade™ adjuvant exacerbates viremia after virus challenge. *Virology Journal*, 14(1):76. doi: 10.1186/s12985-017-0746-0.

Wang, X., M. Ohnstad, A. Nelsen, E. Nelson. 2017. Porcine Epidemic Diarrhea Virus Does Not Replicate in Porcine Monocyte-derived Dendritic Cells, but Activates the Transcription of Type I interferon and Chemokine. *Veterinary Microbiology*. 208:77-81.

Wang, X., Nelson, E. A. 2016. Ultrastructure and morphogenesis of PEDV in PEDV-infected Vero-76 cells. *Current Topics in Virology*, 13, 41-46.

Zhai SL, Zhou X, Zhang H, Hause BM, Lin T, Liu R, Chen QL, Wei WK, Lv DH, Wen XH, Li F, Wang D. 2017. Comparative epidemiology of porcine circovirus type 3 in pigs with different clinical presentations. *Virol J.* 14(1):222. doi: 10.1186/s12985-017-0892-4. PMID: 29132394.

Zhai SL, Zhang H, Chen SN, Zhou X, Lin T, Liu R, Lv DH, Wen XH, Wei WK, Wang D, Li F. 2017. Influenza D Virus in Animal Species in Guangdong Province, Southern China. *Emerg Infect Dis.* (8):1392-1396. doi: 10.3201/eid2308.170059. PMID: 28726609.

Chen Z, Liu S, Sun W, Chen L, Yoo D, Li F, Ren S, Guo L, Cong X, Li J, Zhou S, Wu J, Du Y, Wang J. 2016. Nuclear export signal of PRRSV NSP1 α is necessary for type I IFN inhibition. *Virology.* 499:278-287. doi: 10.1016/j.virol.2016.07.008

Zhai SL, Wei WK, Li XP, Wen XH, Zhou X, Zhang H, Lv DH, Li F, Wang D. 2016. Occurrence and sequence analysis of porcine deltacoronaviruses in southern China. *Virol J.* 13:136. doi: 10.1186/s12985-016-0591-6. PMID: 27496131.

Hu Y, Cong X, Chen L, Qi J, Wu X, Zhou M, Yoo D, Li F, Sun W, Wu J, Zhao X, Chen Z, Yu J, Du Y, Wang J. 2016. Synergy of TLR3 and 7 ligands significantly enhances function of DCs to present inactivated PRRSV antigen through TRIF/MyD88-NF- κ B signaling pathway. *Sci Rep.* 6:23977. doi: 10.1038/srep23977. PMID: 27046485.

2) Abstracts or Proceedings

Dee S., Niederwerder M., Diel D.G. 2017. Modeling the transboundary survival of foreign animal and endemic disease pathogens via contaminated feed ingredients. United States Animal Health Association (USHA) Annual 121st Meeting. Subcommittee on Global Health and Trade, Oct 12-18, 2017, San Diego, CA.

Diel D.G., 2017. Emerging pathogens of swine: Implications for diagnostics and lessons learned from Senecavirus A. American Association of Swine Veterinarians (AASV) Annual Meeting. Feb 25-27, 2017, Denver, CO.

Joshi, L.R., Fernandes, M.V.H., Clement, T., Lawson, S., Resende, T.P., Vannucci, F.A., Nelson, E.A., Diel D.G. 2016. Pathogenesis and infection dynamics of Senecavirus A in pigs. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Fernandes, M.H.V., Okda, F., Joshi, L.R., Hain, K.S. Nelson, E.A., Christopher-Hennings, J., Osorio, F.A., Vu, H., Diel, D.G. 2016. Evaluation of immunodominant B- and T-cell epitopes as inducers of protective immunity against porcine reproductive and respiratory syndrome virus. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Lawson, S., Maggioli, M.F., Joshi, L.R., Fernandes, M.H.V., Christopher-Hennings, J., Nelson, E.A., Diel, D.G. 2016. Immune Responses to Senecavirus A in Pigs. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Hain, K.S., Joshi, L.R., Okda, F., Nelson, J., Singrey, A., Lawson, S., Martins, M., Pillatzki, A., Kutish, G.F., Nelson, E.A., Flores, E.F., Diel, D.G. 2016. Development of a recombinant parapoxvirus expressing the spike protein of porcine epidemic diarrhea virus. Conference for Research Workers in Animal Diseases Meeting. Dec 4-6, 2016. Chicago, IL.

Joshi, L.R., Mohr, K.A., Gava, D., Kutish, G., Piñeyro, P., Zhang, J., Caron, L., Schaefer, R., Diel, D.G. 2016. Genetic characterization and phylogenetic analysis of Senecavirus A. North American PRRSV and other emerging viruses Symposium. Dec 3-4, 2016. Chicago, IL.

Lawson, S., Singrey, A., Joshi, L.R., Leat, J., Nelson, J., Diel, D.G., Christopher-Hennings, J., Nelson, E.A. 2016. Development of antibody reagents and assays for Senecavirus A serodiagnosis. North American PRRSV and other emerging viruses Symposium. Dec 3-4, 2016. Chicago, IL.

Tignon, M., I Christiaens, H. Nauwynck, D. Ojkic, E. Nelson, A.B. Cay. 2017. European Symposium of Porcine Health Management. Comparative study of serological methods for diagnosis of porcine epidemic diarrhea virus (PEDV) infection. May 3-5, 2017. Prague, Czech Republic.

Kraft, J., K. Woodard, L.G. Gimenez-Lirola, B. Setness, J. Ji, P. Lasley, E.A. Nelson, J. Zhang, D. Baum, P. Gauger, J. Zimmerman, R. Main. 2017. Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination. American Association of Swine Veterinarians. Feb 25-28, 2017. Denver, CO.

Singh, P., J. Karsky, E. Nelson, S. Ramamoorthy. 2016. Quantification of the porcine epidemic diarrhea virus (PEDV) by a colorimetric assay. Conference for Research Workers in Animal Diseases. Dec 6, 2016. Chicago, IL.

Pandey, K., Zhong, S., Wang, X. 2017. Role of GTPase-activating protein-binding protein 1 (G3BP1) in porcine epidemic diarrhea virus replication. Nebraska Center for Virology Inter-Campus Annual Retreat. March 19, 2017. Nebraska City, NE.

Van Noort, A. M., Wang, X., A. V. N., Pillatzki, A. E., Diel, D. G., Li, F., Nelson, E. A., Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2'3'-cGAMP VacciGrade adjuvant exacerbates viremia after virus challenge. 2016 PRRSV Symposium. Dec 4, 2016. Chicago, IL.

3) Book chapters or monographs

Fernandes, M.H.V., Diel, D.G. 2017. Emerging viral diseases: Porcine epidemic diarrhea virus. *In*: Flores, E.F. Veterinary Virology. 3 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 36, p.571.

Canal, C.V., Diel, D.G. 2017. Poxviridae. *In*: Flores, E.F. Veterinary Virology. 3 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 19, p.571.

Delhon, G., Diel, D. G. 2017. Asfarviridae. *In*: Flores, E. F. Veterinary Virology. 3 ed., Editora UFSM (Ed), Santa Maria, Brazil; Part II. Chapter 20, p.605.

4) Theses/Dissertations Published

Okda, F.A. 2017. PhD Dissertation, Surveillance of emerging livestock viruses. South Dakota State University, Brookings, SD.

Hain, K.S. 2017. M.S. Thesis. Development and characterization of a recombinant Orf virus expressing the spike protein of porcine epidemic diarrhea virus. South Dakota State University, Brookings, SD.

Joshi, L.R. 2017. M.S. Thesis. Senecavirus A: Epidemiology, pathogenesis and infection dynamics. South Dakota State University, Brookings, SD.

E. FUNDING SOURCES FOR SWINE VIROLOGY RESEARCH

Diel D.G., Maggioli M.F., Joshi L.R., Bauermann, F.V., Nelson E.A., Hennings. J. Investigating the effect of stressors on Senecavirus A pathogenesis and the potential occurrence of the “carrier state” in sows. National Pork Board. 12/17-11/18.

Diel D.G. Maggioli M.F., Bauermann F.V., Nelson E. Young, A., Gourapura, R. A multi-species vaccine delivery platform for infectious disease prevention and control in livestock species. USDA-NIFA. 9/2017-3/2021.

Diel D.G., Bauermann, F.V., Singrey A., Nelson E. Evaluating survival of viral pathogens in porcine plasma. Sonac-Darling. 9/2017-8/2018.

Diel D.G., Dee, S., Bauermann F.V., Singrey A., Nelson E. Evaluation of chemical mitigants for neutralizing the risk of foreign animal diseases in contaminated feed ingredients. Swine Health Information Center. 8/2017-7/2018.

Niederwerder, M., Rowland R.R., Dee, S., Diel D.G. Evaluation of the risk of transboundary movement of ASFV via contaminated feed ingredients. Swine Health Information Center. 3/2017-2/2018.

Ma, W., D. Wang. Study influenza B in pigs with PRRSV infection. NIH R21. 12/2016 - 11/2018.

Christopher-Hennings, J., Nelson, E., Diel, D. G., Li, F., Scaria, J., Wang, D., Chaussee, M., Herrmann, S. South Dakota Center for Biologics Research (SD-CBRC), South Dakota Governor’s Office of Economic Development. 6/2017-6/2022.

Diel D., Clement. T., Bauermann, F.V., Nelson E.A., Christopher-Hennings, J. Development of rapid diagnostic capability for encephalomyocarditis virus - (EMCV). Swine Health Information Center. 11/2016 - 10/2017.

Rauh, R., Diel D., Christopher-Hennings, J. Development of a high throughput real-time RT-PCR assay (dry, room temperature stable and fluid formats) for the detection and discrimination of Senecavirus A (SVA), Foot-and-mouth disease virus (FMDV) and Swine Vesicular Disease virus (SVDV). Swine Health Information Center. 11/2016 - 10/2017.

Dee, S., E.A. Nelson, D. Diel, C, Neill. Evaluating the survival of FAD viral surrogate pathogens in ingredients shipped from China to the US. Swine Health Information Center. 4/2016 - 4/2017.

Dee, S., E. Nelson, D. Diel, T. Clement, A. Singrey, J. Hennings. An evaluation of a shipping model to investigate foreign animal disease introduction into the USA. American Association of Swine Veterinarians. 3/2016-2/2017.

Dilberger-Lawson, S. R., Nelson, E. A., Singrey, A. R., Development of monoclonal and polyclonal antibodies against PEDV PLP2, USDA NADC, 03/2017-12/2017.

Dilberger-Lawson, S. R., Diel, D. G., Singrey, A. R., Clement, T. J., Hennings, J., Nelson, E. A. Development of a bELISA for serological diagnostics and surveillance of SVA infection, National Pork Board, 06/2017-06/2018.

Li, F., Clement, T. J., Hennings, J., Nelson, E. A., Rauh, R., Hause, B., Detection and differentiation of influenza Types A, B, C and D in swine, Swine Health Information Center, 11/16-11/2017.

Voronov, A., S. Ramamoorthy, S. Stafslie, E. Nelson. Polymeric adjuvants for peptide vaccines. ND-APUC 6/2015-6/2017.

Wang, D., E.A. Nelson, R. Kaushik, F. Li. Novel porcine epidemic diarrhea virus vaccine pipeline. USDA AFRI. 12/2015-12/2017.

F. WORK PLANNED FOR NEXT YEAR

Objective 1: Control of PRRSV.

We will continue to investigate the role of stress granules (SGs) in PRRSV and PEDV replication and host innate immunity. We will primarily focus on the kinetics and mechanistic basis of viruses and SGs interaction.

Objective 2: Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

One goal for the next year will be to utilize new, recently developed expressed protein antigens and monoclonal antibodies to formulate improved competitive ELISA-based assays for Senecavirus A serology. FMIA-based assays will also be evaluated.

We will continue our efforts to develop and fully validate new real-time PCR assays for high impact viral diseases of swine. With funding from the Swine Health Information Center and industry partners, we will continue focus on full validation of real-time PCR assays for rapid diagnosis of encephalomyocarditis virus (EMCV) and detection and differentiation of influenza Types A, B, C and D in swine.

We will continue efforts related to the development and evaluation of recombinant vaccine candidates for endemic and emerging viral pathogens of swine. Further study will focus on understanding basic aspects of SVA innate immune evasion and pathogenesis; along with development of vaccine candidates for Senecavirus A. Additional efforts will focus on improved vaccine strategies for swine influenza.

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: December 1 2016 to December 30, 2017

INSTITUTION OR STATION:
China Agricultural University (CAU)

A. NC-229 REPRESENTATIVE:

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Other PRINCIPAL LEADERS associated with the projects

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

*Per station for ALL Accomplishment = Maximum 3,000 characters including spaces;
Full NC229 report for ALL Accomplishment = Maximum 30,000 characters):*

This section focuses on intended activities, outputs, and short-term outcomes. The report should also reflect on the items that stakeholders want to know, or want to see. The accomplishments should cover only the current year of the project.

Objective 1. Control of PRRSV

Indicate progress in any the following areas, as appropriate in each case/station

*PRRSV Immunity and Vaccinology : understanding correlates of immunity and mechanisms to broaden protection,
Host genetic control of anti-PRRSV infection and vaccination responses*

(1) We have proved DDX18, which is a member of DEAD-box RNA helicases (DDXs) family, participated in viral replication. Previously, we found the DDX18 interacts with both nsp2 and nsp10 of PRRSV by Co-Immunoprecipitation (Co-IP). In the present study, we demonstrated the interactions of DDX18 with nsp2 and nsp10, and located DDX18's binding regions as the N-terminus of nsp2 and both the N-terminus and C-terminus of nsp10. The expression of the nsp2 or nsp10 in MARC-145 cells and primary PAM cells redistributed DDX18 from the nucleus to the cytoplasm, and promoted the viral replication, but silencing of the DDX18 gene in MARC-145 cells down-regulated the replication of PRRSV.

(2) The interaction of interleukin-2 enhancer binding factor 2 (ILF2) with nsp9 or nsp2 was first demonstrated in 293FT cells co-transfected with ILF2-expressing plasmid and nsp9-expressing plasmid or nsp2-expressing plasmid. The interaction of endogenous ILF2 with the nsp9 or nsp2 of PRRSV was further confirmed in MARC-145 cells transduced with GFP-nsp9-expressing lentiviruses or infected with PRRSV JXwn06. The RdRp domain of nsp9 was shown to be responsible for its interaction with ILF2, while three truncated nsp2 were shown to interact with ILF2. Moreover, we observed that ILF2 partly translocated from the nucleus to the cytoplasm and co-localized with nsp9 and nsp2 in PRRSV-infected MARC-145 cells and PAMs.

(3) In our researches, we first predicted by software that the multiple proteins of porcine reproductive and respiratory syndrome virus (PRRSV) could be sumoylated. Next, we confirmed that Nsp1 β , Nsp4, Nsp9, Nsp10 and nucleocapsid (N) protein of PRRSV could interact with the sole SUMO E2 conjugating enzyme Ubc9, and Ubc9 could be co-localized with Nsp1 β , Nsp4, Nsp9 and Nsp10 in the cytoplasm, while with N protein in both the cytoplasm and nucleus. Finally, we demonstrated that N protein could be sumoylated by either SUMO1 or SUMO2/3. In addition, the overexpression of Ubc9 could inhibit viral genomic replication at early period of PRRSV infection and the knockdown of Ubc9 by siRNA could promote the virus replication.

(4) In the present study, the pathogenicity of a NADC30-like strain CHsx1401 for piglets was analyzed, and the potential cross-protective efficacy of three MLV vaccines including two commercial MLV vaccines and an attenuated low pathogenic PRRSV against this virus was further evaluated in piglets. The NADC30-like CHsx1401 was shown to cause fever, respiratory clinical signs, and lung gross and microscopic lesions of the inoculated piglets, suggesting that this virus is moderate virulent for piglets. Vaccination of piglets with the MLV vaccines could not reduce the clinical signs and lung lesions, and was partially efficacious in the reduction of viral loads in sera upon NADC30-like CHsx1401 challenge, indicating that these three MLV vaccines provide extremely limited cross-protection efficacy against the NADC30-like virus infection. Additionally, Ingelvac PRRS MLV appeared to exert some beneficial efficiency in shortening the period of clinical fever and in improving the growth performance of the challenged pigs.

PRRSV Epidemiology and Surveillance: understanding virus transmission and differential testing of animals (DIVA)

(1) In the present study, the genetic characterization of a recombinant type 2 PRRSV (designated TJnh1501) was analyzed and its pathogenicity for piglets was examined. Our study showed that each region of TJnh1501 genome had 96.67–100% nucleotide and 96.5–100% amino acid identities with a Chinese highly pathogenic PRRSV-derived modified-live virus (MLV)-like except for its nonstructural protein 2 (nsp2)-coding region; while its nsp2-coding region shared higher nucleotide (84.44–85.85%) and amino acid (82.44–84.79%) identities with NADC30 and NADC30-like CHsx1401, and in particular, the highly variable region of nsp2 exhibited characteristic 131-aa deletion identical to NADC30 and NADC30-like CHsx1401. Meanwhile, we identified two recombination breakpoints located in the nt1737 and nt3506 of nsp2-coding region, which had higher nucleotide homology with NADC30 and NADC30-like CHsx1401. Moreover, TJnh1501 infection could cause persistent fever, moderate respiratory clinical signs, higher viremia, and obvious gross and microscopic lung lesions in piglets. The virus was shown to have lower pathogenicity than HP-PRRSV JXwn06, but higher than NADC30-like CHsx1401 for piglets.

Economic Impact of Interventions: determining the economic benefit of vaccination in positive

Objective 2 Developing effective and efficient approaches for detection, prevention and control of pressing viral diseases of swine of recent emergence.

Indicate progress in the following areas: as appropriate in each case/station:

- PEDV Diagnostics
- PEDV immunity and vaccinology. .
- Swine influenza virus(SIV) evolution and detection
- SIV Control by vaccination or other interventions
- SIV at the human-animal interface
- African Swine Fever (ASF): Vaccine Design and Development
- CSFV vaccination, diagnosis epidemiology

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Impact statements (500 characters per statement)

This section focuses on actual or intended potential long-term outcomes and impacts, covering only the current year of the project. The report should also reflect on the items that stakeholders want to know, or want to see. List any grants, contracts, and/or other resources obtained by one

or more project members as a result of the project's activities. Include the recipients, funding source, amount awarded and term if applicable.

- (1) Our findings proved that the cellular RNA helicase DDX18 plays a role in the replication of PRRSV, and provides insights into the understanding of PRRSV replication.
- (2) Our analysis indicated that knockdown of ILF2 favored the replication of PRRSV, while over-expression of ILF2 impaired the viral replication in MARC-145 cells. It also gives us another insight into the understanding of PRRSV replication.
- (3) These findings revealed the SUMOylation property of PRRSV N protein and the involvement of Ubc9 in PRRSV replication through interaction with multiple proteins of PRRSV. To our knowledge, this is the first study indicating the interplay between SUMO modification system and PRRSV.
- (4) Our findings gave valuable guidance for the choice and use of PRRSV MLV vaccines to control NADC30-like virus infection in the field.
- (5) Our findings revealed that TJnh1501 is a recombinant type 2 PRRSV from the recombinant event between NADC30-like and MLV-like derived from the Chinese highly pathogenic PRRSV, and it exhibits intermediate virulence for pigs. This study adds valuable evidence for understanding the role of genomic recombination in the evolution of PRRSV.
- (6) In the review “Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus”, we summarized the recent advances in our understanding of the pathogenesis, evolution and ongoing field practices on the control of this troubling virus in China.

D. PERTINENT (SWINE VIROLOGY) PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

- a) Jin H, Zhou L, Ge X, et al. *Cellular DEAD-box RNA helicase 18 (DDX18) promotes the PRRSV replication via interaction with virus nsp2 and nsp10*. Virus research, 2017, 238: 204-212.
- b) Wen X, Bian T, Zhang Z, et al. *Interleukin-2 enhancer binding factor 2 interacts with the nsp9 or nsp2 of porcine reproductive and respiratory syndrome virus and exerts negatively regulatory effect on the viral replication*. Virology journal, 2017, 14(1): 125.

- c) Wang C, Zeng N, Liu S, et al. *Interaction of porcine reproductive and respiratory syndrome virus proteins with SUMO-conjugating enzyme reveals the SUMOylation of nucleocapsid protein*. PloS one, 2017, 12(12): e0189191.
- d) Zhou L, Yang B, Xu L, et al. *Efficacy evaluation of three modified-live virus vaccines against a strain of porcine reproductive and respiratory syndrome virus NADC30-like*. Veterinary Microbiology, 2017.
- e) Bian T, Sun Y, Hao M, et al. *A recombinant type 2 porcine reproductive and respiratory syndrome virus between NADC30-like and a MLV-like: Genetic characterization and pathogenicity for piglets*. Infection, Genetics and Evolution, 2017, 54: 279-286.
- f) Han J, Zhou L, Ge X, et al. *Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus*. Veterinary Microbiology, 2017.

2) Abstracts or Proceedings

Cite authors, year, title, meeting (use abbreviations, e.g., Proc., CRWAD, AASV, 2008 PRRS Symp., etc.) Do not give full dates.

- a) Identification Critical Amino Acids in Nsp9 and Nsp10 Determining the Fatal Virulence of the Chinese Highly Pathogenic PRRSV. **Lei Xu**, Lei Zhou, Weifeng Sun, Pingping Zhang, Xinna Ge, Xin Guo, Jun Han, Hanchun Yang. XIVth International Nidovirus Symposium, 2017, Kansas City, USA.

3) Book chapters or monographs

Give full citation

E. FUNDING SOURCES FOR SWINE VIROLOGY RESEARCH

1) Current

- a. Major Program of National Natural Science Foundation of China (31490603)
- b. The earmarked fund for Modern Agro-industry Technology Research System of China (CARS-36) from the Ministry of Agriculture of the People's Republic of China.
- c. National Basic Research Program of China 481 (2014CB542700) from the Chinese Ministry of Science and Technology
- d. Key Program of National Natural Science Foundation of China (31330077)

F. WORK PLANNED FOR NEXT YEAR

