NC229: Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine

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NC229 Meeting Chicago, IL, 12/02/2010

Brief Summary of Minutes of Annual Meeting. (Held on Friday, Dec. 2, at 1:00 PM -3:00 PM)

1:00 XJ Meng, opening remarks

1:05 Update of NC229, Dr. David Benfield, Administrative Advisor ;

2012 appears to be "flat" for NC229; 2013 budget unknown due to election

For NC229 funding , current funding expires Sept.. 30th, 2014, so a new project would need to be written in 2013 for 2014. In 2012, there is a mid-year review.

The original NC229 project was written for PRRSV research primarily (with a push for the PRRSV CAP programs) and then expanded to PRRSV + emerging viral diseases of swine. With the CAP program ending, for the next written proposal, may want to consider emphasis on other viruses since some stations do not get any funding from their station directors (eg. to leverage other opportunities for funding).

1:15 Election of new NC-229 officer (Dr. Fernando Osorio was elected Secretary for NC229. Dr. Jane Christopher-Hennings is the Chair starting 2012, replacing Dr. XJ Meng who was chair for last 2 years)

1:20 Update from USDA-NIFA (Drs. Peter Johnson/Margo Holland):

Dr. Margo Holland:

FY 2012 funding is "flat". AFRI Indirect costs will increase to 30% for 2012 funding.

No less than 40% for applied research

No less than 2% for equipment grants

60% is for basic research (with 30% focused on multi-disciplinary teams)

2012 Foundation Programs to be released in early 2012 (need letter of intent)

Areas of interest include Animal Health and Production; Agricultural systems (may want to put emphasis on nanotechnology)

Grants generally $500,000 total

2011 Animal Health Foundation program had > 200 letters of intent with 160 proposals received; 12 proposals recommended for funding (8.3% success rate) with 4 awards for PRRSV research (3 standard research proposals and 1 seed grant) representing vaccine, health surveillance and host-pathogen interactions.

PRRSV CAP has been $1.2 million/year and is considered a "success story" of a multi-disciplinary group.

- $ 3 million has been leveraged (eg. NPB, University, Fed. Gov. private companies)

NIFA Fellowships:

Pre-docs $75,000 (for 2 years)

Post-docs $130,000 (for 2 years)

Dr. Peter Johnson:

NEW funding on Ecology and evolution of disease (NIFA, NSF, NIH) Due 1st Wed in Dec (this year Dec. 7th).—need to appeal to all agencies,

http://nsf.gov/pubs/2011/nsf11580/nsf11580.htm

Accomplishments: (30,000 characters limit for all accomplishments, 3,000 character limit per station for all accomplishments).

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. Elucidate the mechanisms of host-pathogen(s) interactions.

1.1 (NADC, Faaberg/Brockmeier/Loving/Miller) Compared pathogenesis in swine after challenge with 3 new Type 2 PRRSV field isolates *in vivo* and *in vitro*. Differences seen in viral growth kinetics and pathogenesis.

1.2 (NADC, Faaberg/Brockmeier/Loving/Miller) Compared growth and disease in swine after vaccination with nsp2 deletion mutant viruses and challenge with novel Type 2 isolate.

1.3 (NADC, Kehrli/Miller/Faaberg) Adenovirus expression of a region of PRRSV nsp 2.

1.4 (NADC Faaberg/Lager/Miller/Brockmeier/Kerhli/Nicholson) Compared high and low dose challenge of US swine with Chinese and Vietnamese HP-PRRSV.

1.5 (NADC, Faaberg)Development of infectious clone of a new vaccine strain.

1.6 (NADC, Faaberg) Examined the interaction of nsp2 with host genes.

1.7 (NADC, Miller/Kehrli) Developed PRRSV infected tissue culture assay to screen for polyclonal B-cell activation.

1.8 (NADC, Miller/Kehrli/Faaberg)Assessed PRRSV strains that have a reduced capacity for the induction of polyclonal B-cell activation for potential vaccine candidates.

1.9 (NADC, Miller; G Rohrer USMARC) Sample collection and genomic DNA purification for SNP chip analysis to examine genotype in host susceptibility to PCV2.

1.10 (NADC, Miller) Compared the transcript expression of tracheobronchial lymph nodes of pigs infected with PRV/PRRSV/SIV/PCV-2 *in vivo*.

1.11 (NADC, Miller) Determine the transcriptomic immune response to contemporary US and Asian PRRSV *in vivo*.

1.12 (NADC, Faaberg/Miller; B. Guo, Visiting Scientist) Developed type 1 IFN bioassay for examination of PRRSV strain specific pathology.

1.13 (NADC, Faaberg) Developing chimeric vaccine to Asian HP-PRRSV.

1.14 (UNL) Understanding the role of the nsp1 in PRRSV--Using nsp1β, a proteolytically processed functional product of nsp1 as bait, we have identified the cellular poly (C)-binding proteins 1 and 2 (PCBP1 and PCBP2) as 2 of its interaction partners. Interactions of PCBP1 and 2 with nsp1β was confirmed. In MARC cells, the cytoplasmic PCBP1 and 2 partially co-localize to the viral replication-transcription complexes. Recombinant purified PCBP1 and 2 bound to the viral 5' UTR. SiRNA-mediated silencing of PCBP1 and 2 in cells resulted in significantly reduced replication and transcription without effects on initial polyprotein synthesis. ID of cellular factors involved in PRRSV lifecycle give a better understanding of virus biology and has potential for development of anti-viral therapeutics.

1.15 (UMN) discovered a novel PRRSV protein expressed in infected cells that may have a role in cellular pathogenesis.

1.16 (UMN) characterized the molecular pathogenesis of PCV2 infection by transcriptome profiling. Discovered a pronounced gene expression induction profile characteristic of a classical IFN response to viral infection.

1.17 (PURDUE) Host immune responses to PRRSV infection may be correlated with genetic control. The PRRS Host Genetics Consortium (PHGC) studies are aimed at identifying genes and pathways associated with pigs that clear PRRS virus while continuing to gain weight. Analyses of data from each PHGC trial [viral load from 0-21 days post infection (dpi) and weight gain from 0-42 dpi] were used to statistical identify 4 groups of pigs: those with the best phenotype, low virus and high growth (LvHg), high virus and high growth (HvHg), high virus and low growth (HvLg), and, the worst, low virus and low growth (LvLg). Real time PCR was used with primers corresponding to markers important for immune system activation. Markers included: transcription factors TBX21 (T-bet), GATA3 (presumed Th1 and Th2 regulators, respectively), FOXP3, cytokines IL10, IFNg, CD163, the PRRSV receptor, and CD69, the early marker for T cell activation and proliferation. LvHg animals at 4-10 DPI exhibited increased ratio of TBX21/GATA3. LvHg animals had a robust increase in expression of IL10 and IFNG within 4-14 DPI, with high basal expression of CD69 relative to other groups at 0 DPI. In HvHg, TBX21/GATA3 was the highest during PRRSV infection, up regulation and expression of TBX21 and GATA3 was minimal, along with other markers. HvLg animals had high base line expression of GATA3 and FOXP3 transcription factors and delayed increase of TBX21/GATA3. In LvLg animals a high basal expression of GATA3 and FOXP3 was detected with delayed increased of TBX21/GATA3. In LvLg animals higher baseline expression for CD163 and cytokine IL10 markers was detected. The HvLg and LvLg animals exhibited up regulation of the markers responsible for Th2 immunity during infection. Our data correlated basal expression of CD69 as a factor leading to up regulation and activation of markers responsible for Th1 immune response. Basal expression of GATA3 and FOXP3 demonstrated activation of the markers responsible for Th2 pathway during infection.

1.18 (KSU) Sang, Blecha,Rowland characterized the expression 39 type I IFN genes and related receptors in the PRRSV-infected fetus.

1.19 (KSU) Hesse, Rowland performed an analysis of cross-protection between diverse PRRSV strains.

1.20 (KSU) Rowland identified a decoy epitope CP (169-180) in PCV2 capsid protein produced to immunization with capsid monomer. Immunization with whole capsid offered protection. This explains why production of large quantities of virus during infection are non-protective.

1.21 (SHVRI) report viable chimeric viruses in which the envelope protein genes from ORF2a to ORF5 of vSHE (type 1) were swapped into the genetic backbone of vAPRRS (type 2). Found that the envelope proteins of type 1 were fully functional in type 2 PRRSV and the rescued chimeric progeny viruses showed robust genetic stability and similar replication properties to the parental strains *in vitro*. For PRRSV N protein, we found that type 1 N protein was functional in the type 2 PRRSV backbone. All cysteines in the N protein are non-essential for type 1 and 2 PRRSV viability. NEM treatment prevents disulfide-linked N dimerization in cells but not in extracellular virions.

1.22 (UCONN; Garmendia): Aims are to determine the sensitivity to and induction of IFNb by PRRSV isolates, to identify mechanisms of evasion of host's innate immune responses by PRRSV and to determine correlations with virulence. Field viruses were previously tested in their sensitivity to IFNb *in vitro*. Significant differences in sensitivity to IFNb among different PRRSV isolates and between MARC-145 cells and porcine alveolar macrophages (PAM) were demonstrated. The induction of IFNb by PRRSV was tested in PAMs using field isolates and a series of chimeric viruses derived from infectious clones of FL12 (virulent isolate) and a vaccine virus (attenuated), provided by Dr. Osorio, UNL. The

induction tests demonstrated that PRRSV isolates do induce IFNb in PAMs but such induction is variable. The IFNb induced was bioactive as shown by a dose-dependent reduction of VSV infectivity in cell culture. Mx and TNF were measured in PRRSV-infected PAMs. Mx is expressed at significant levels that generally correlate with the corresponding levels of IFNb produced. TNF production is detectable but levels are not significant and do not follow a pattern related to IFNb.

1.23 (ISU) Studies on enhanced pneumonia and disease in pigs vaccinated with an inactivated human-like (δ-cluster) H1N2 vaccine and challenged with 2009 pH1N1 influenza virus..

1.24 (ISU) Studies on cytokine and chemokine mRNA expression profiles in tracheobronchial LN from pigs singularly infected or coinfected with PCV2 and *Mycoplasma hyopneumoniae*.

1.25 (ISU) Studies on genetic and phenotypic characterization of a 2006 US PRRSV isolate associated with high morbidity and mortality in the field.

1.26 (ISU) Studies on the establishment of a DNA-launched infectious clone for a highly pneumovirulent type 2 PRRSV: Identification and *in vitro* and *in vivo* characterization of a large spontaneous deletion in the nsp2 region.

1.27 (OSU) To elucidate both cellular and innate cytokine response in growing pigs at very early stages of PRRSV infection in a commercial pig herd premises, 7 wk old pigs were infected. 1 in a pen of 25 was PRRSV infected and responses were assessed 2 DPI. All the infected and a few of the contact neighbor pigs were viremic. A majority of viremic pigs had more than 50% reduction in NK cell-cytotoxicity. At 2 DPI 1 fold increase in innate IFNa production was detected in plasma. Enhanced secretion of IL4, IL10 and IL12 (but not IFNg) in a majority of infected pigs was seen. A reduced frequency of myeloid cells, CD8+ and CD4+CD8+ T cells and increased T-regulatory cell population was detected in all the viremic pigs. Our results suggest that PRRSV modulate the innate and adaptive immune mediators from 2 DPI, resulting in subversion of host innate immunity from early stages post-infection.

1.28 (VA) We identified a large spontaneous deletion of 435-bp in the nsp2 gene of a highly pneumovirulent PRRSV, VR2385 . We established a DNA-launched infectious clone (passage 14) containing the 435-bp nsp2 deletion (pIR-VR2385-CA) and another DNA-launched infectious clone, pIR-VR2385-R, in which we restored the deleted 435-bp nsp2 sequence back to the pIR-VR2385-CA backbone. The growth characteristics of the 2 rescued viruses were compared, and results showed that the VR2385-CA virus with the nsp2 deletion replicated more efficiently *in vitro* than the VR2385-R virus with the restored nsp2 sequence but, VR2385-CA virus had a significantly reduced serum viral RNA load *in vivo*. In pigs, the nsp2 deletion had no effect on virulence. The spontaneous nsp2 deletion has a role for enhanced virus replication *in vitro* but has no effect on pathogenicity.

1.29 (VA) To determine whether cellular microRNAs (miRNAs) play a role in host response to PRRSV infection, we performed a global profiling of both cellular miRNA and mRNA in MARC cells infected with type 1 (SD01-08) or type 2 (VR2385) PRRSV. Results showed that the expressions of approximately 240 miRNAs were significantly altered with infection by PRRSV type 1 or 2 (114 for type 1, and 82 for type 2), and at least 15 specific miRNAs were shared by both types. Approximately 4,500 genes showed differential expression with infection by either virus type ($p<0.05$). We conducted a global human/bovine/porcine miRNA and porcine gene expression microarray analyses using a pool of lung homogenates of 10 SPF pigs at 14 DPI. Compared to the negative control, PRRSV infection resulted in significant changes of the expression level (>2-fold) of 17 miRNAs ($p<0.05$) and 3,713 mRNAs ($p<0.01$) including genes involved in host innate and adaptive immune responses. We found 270 unique miRNAs that were differentially up- (151 miRNAs) or down-regulated (119 miRNAs) in response to PRRSV infection. Deep sequencing data showed 1,892 novel putative porcine miRNAs that do not align to any

known *Sus scrofa* miRNAs. We have correlated inverse regulation between miRNAs and putative target genes to build a miRNA-gene network.

1.30 (SDSU: Z Sun, Y Li, Y Fang) investigated ISG15 and PRRSV nsp2 OTU domain mediated deisgylation function. IFN-stimulated gene 15 (ISG15) is an ubiquitin-like protein which is stimulated by type I IFN α/β or induced by viral or bacterial infection. Over-expression of ISG15 in cells significantly reduced the PRRSV titer and was confirmed using small interfering RNAs against ISG15-conjugating enzymes. IFN-induced antiviral activity is significantly alleviated by inhibiting ISG15 conjugation. *In vitro* deISGylation assay showed the N-terminal OTU domain of nsp2 has deconjugating activity towards ISGylated products. A 19 AA deletion plus a single AA mutation partially relieved the nsp2 de-ISGylation function. This showed that ISG15 conjugation has an important role in PRRSV infection and modifying certain regions of nsp2 could reduce the deISGylation ability of the virus.

1.31 (SDSU, KSU Lawson,Li, Patton, Langenhorst, Sun, Jiang, Hennings, Nelson, Knudsen, Fang, Chang). Constructed a recombinant PRRSV that encodes swine IL-1b as a separate subgenomic mRNA inserted between ORFlb and ORF2. MARC cells infected with recombinant virus secreted ILlb and had a similar growth rate to parental virus. No clinical signs were observed in the recombinant virus-infected nursery pigs and IL-lb, IL4 and IFNg were up-regulated in PBMCs.

1.32 (SDSU, LUMC, NL: Li, Tas, Sun, Snijder, Fang). MAbl and polyclonal antibodies were generated against PRRSV ORF1a-encoded nsps. Using these antibodies, we identified and characterized these ORFla-encoded nsps in infected MARC cells. This study confirmed the existence of proteolytic processing products of PRRSV ORFla encoded polyprotein in virus infected cells and provides a basis for both applied and basic research on the role of PRRSV nsps in viral replication and pathogenesis.

1.33 (BARC) The PRRS Host Genetics Consortium (PHGC) has expanded efforts to determine the role of host genetics in resistance to PRRS and in effects on pig health and related growth effects. The PHGC is a multi-year project that is funded by a US consortium representing the US National Pork Board (NPB), USDA ARS and NIFA, universities and private companies; it represents the first-of-its-kind approach to food animal infectious disease research. The project has used a Nursery Pig Model to assess pig resistance/susceptibility to primary PRRSV infection. Ten sets of 200 crossbred pigs from high health farms were donated by commercial sources and transported KSU. Pigs were infected with PRRSV and followed for 42 DPI. Blood was collected at 0,4,7,10,14,21,28,35 and 42 DPIi and weekly weights recorded. All 10 trials have been completed; each trial has affirmed that all pigs become PRRSV infected but some pigs clear virus from serum quicker with variable weight effects. Data is being stored in the PHGC database at ISU www.animalgenome.org/lunney.

1.34 (BARC) Genome wide association studies have identified genomic regions that determine PRRS resistance/susceptibility. DNA from PHGC pigs has been genotyped with the PorcineSNP60 Genotyping BeadChip (> 60K single nt polymorphisms or SNPs). (Data stored at ISU www.animalgenome.org/lunney. Multivariate analyses of viral load and weight data have identified PHGC pigs in different virus/weight categories, so that ongoing serum cytokine and gene expression studies can compare data from PRRS resistant/maximal growth pigs to susceptible/reduced growth pigs. PRRS CAP, NRSP8 Swine Genome Coordinator and Genome Alberta have supported SNP chip analyses and PRRS CAP the state-of-the-art genome wide association studies (GWAS) to identify genetic determinants of PRRS resistance/susceptibility. Based on evaluation of phenotypic traits, viral load [VL, area under the curve 0-21 dpi], and 0-42dpi weight gain (WG), response to PRRSV challenge was shown to be moderately heritable at 0.30 each. Regions on swine chromosomes 4 (SSC4) and SSCX appear to be associated with VL, and on SSC1, 2, 3, 4, 7, and 17 with WG. Regions on SSC4 for VL and WG are almost perfectly negatively correlated. Investigations revealed that the BB genotype for this region of

SSC4 is the desirable genotype and has a low frequency (0.02), suggesting that genetic progress can be made by selective breeding. Functional gene and protein transcriptomic analyses are ongoing to ID gene networks and resistance associated biomarkers that differ in high versus low VL pigs.

1.35 (BARC) Identifying host gene expression changes that are involved in regulating responses to PRRSV infection**.** Grants from USDA NIFA and Genome Alberta are supporting whole blood gene expression analyses using microarrays and RNAseq (next generation transciptomics), respectively. The goal is to assess differential expression of individual genes and to discover networks and pathways enriched for those genes, in pigs showing different responses to PRRSV infection. RNA from 3 pigs per group was hybridized to the 20K 70-mer oligonucleotide Pigoligoarray following a blocked reference design with time 0 of each individual animal as a reference sample. Expression levels for 491 genes showed significant viral level-growth interaction for all time-points. Differentially expressed (DE) genes at early time-points (4, 7, 14 dpi) were evaluated by enrichment analysis with Ingenuity Pathways Analysis software www.ingenuity.com; for comparisons of viral level, 308 genes were DE with 16 significant gene networks ($p \leq 0.0001$); for growth level, 367 genes were DE with 17 significant gene networks. Confirmatory qPCR work will explore these DE genes and their roles in PRRS control. The more significant biological functions identified (FDR$\leq$ 5%) were those related to cell death, cellular function, maintenance and compromise, and inflammatory disease. For the growth comparison at 4 dpi in Lv animals, the antigen presentation pathway was over-represented (FDR$\leq$5%).

1.36 (UMD) found that PRRSV inhibits IFN downstream signaling and continued the studies to identify the interference mechanism. We found that NSP1β of PRRSV VR2385 interferes with IFN signaling pathway. VR2385 is a virulent strain whereas NSP1β of Ingelvac PRRS MLV does not affect IFN signaling. NSP1β of VR2385 blocks nuclear translocation of the IFN-induced STAT1/STAT2/p48 heterotrimer. IFN-induced phosphorylation of both STAT1 and STAT2 and their heterodimer formation were not affected. The NSP1β of VR2385 interferes with the interaction of STAT1 and importin. NSP1β may be the viral protein that interferes with the activation and signaling of type IFN.

1.37 (UMD) During studies of IFN signaling, we noticed 1 lab mutant induces synthesis of type I IFNs in cultured cells and has no effect on IFN downstream signaling. This mutant was plaque purified and named A2MC2. A2MC2-infected MARC cells resulted in strong inhibition of replication of an IFN-sensitive virus, Newcastle disease virus (NDV). Analysis of A2MC2-infected MARC cells showed that the transcripts of IFN-stimulated genes were elevated. Inhibition of A2MC2 replication abolished its capability to induce IFNs. A2MC2 does not affect IFN downstream signaling and induces IFN in PAMS. Infection of PAMs leads to little cytopathic effect.

1.38 (UIUC D Yoo) found that the suppression of NF-kB activation and sumoylation of PRRSV Nsp1α is mediated by protein inhibitor of activated STAT1 (PAIS1). We examined the role of PRRSV Nsp11, and endoribonuclease, on virus replication and IFN regulation.

1.39 (UIUC, Zuckermann) found that the influence of PRSV on the IFNα response of macrophages to infection with PRRSV is strain-dependent. Macrophages appeared non-responsive in the presence of either of 2 wild-type PRRSV, copious amounts of this cytokine was released by ZMAC cells exposed to a 3rd individual. This “unconventional” isolate was unique since it induced type I gene transcription and stimulated the phosphorylation of IRF3 to a greater extent than ZMAC cells infected with either “conventional” virus. Since the phosphorylation of a 2nd transcriptional factor, NFκß, also involved in the initiation of IFNα/ß gene transcription was comparatively unaffected in any of the virus-infected cells, the “conventional” viruses may be blocking IFNα production by interfering with a step(s) in the pathway leading to IRF3 activation.

Objective 2. Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine.

2.1 (UGA) Studied ability of swine, human and avian influenza viruses to reassort on the TRIG backbone in swine and primary swine and human epithelial cells with the goal to elucidate the potential for reassortment. We also explored the evolution of AIVs in poultry and waterfowl.

2.2 (UGA) Explored the potential for avian and swine origin influenza viruses to infect and transmit in mice and ferrets.

2.3 (UGA) Explored receptor specificity of avian, human and swine influenza viruses.

2.4 (UGA) Explored the potential for avian influenza viruses to infect felines and tested for exposure of feral cats to AIVs to test their potential as an alternate reservoir/vector.

2.5 (UMN, Guelph, Hong Kong). PRRSV diversity based on sequencing/RFLP typing was described for type 2 PRRSV.

2.6 (UMN). Influenza

Transmission dynamics and transmission parameters were evaluated in pigs with passive immunity induced by influenza vaccination. Homologous passive immunity decreased transmission but did not prevent it. Transmission was similar in pigs without passive immunity and in pigs with heterologous immunity.

Influenza ecology studies showed that influenza infections in closed grow-finish populations were prolonged (70 DPI).

Oral fluids were a sensitive method to detect influenza infections in populations.

Weaned pigs are a source of virus introduction for grow-finish populations.

Methods to study aerosol transmission of influenza virus in pigs were validated to detect influenza virus from aerosols generated from infected pigs. Detection of infectious aerosols may be related to the number of pigs shedding virus in a population. Positive aerosols could be detected when just a few pigs were known to be shedding. Detection of infectious aerosols has been shown in the field, inside pig barns and exhaust fans.

2.7 (UMN, ISU, SDSU) showed long-distance airborne spread of PRRSV and identified climactic conditions which support it. Multiple air filtration interventions were validated to prevent airborne spread of PRRSV and *M hyopneumoniae*.

2.8 (UMN, Pipestone) PRRSV shedding was decreased in pigs that had been vaccinated with a MLV vaccine compared to non-vaccinates. Decrease shedding was seen in oral fluids at 36 DPI and in air samples collected in exhaust fans. PRRSV was detected in air samples from non-vaccinated pigs for up to 70 days and 45 days for vaccinates.

2.9 (KSU, BARC, others) participated in the PHGC. Results include a genome wide association study of the first 600 pigs. The results show the ID of genomic markers that are linked to virus load and weight gain. To date, 2000 pigs are in the study.

2.10 (SHVRI) Viral RNA synthesis regulatory elements identification. The 5' untranslated region (UTR) of the genomic RNA is believed to be vital for the replication of PRRSV yet its functional mechanism remains largely unknown. Using a full-length cDNA clone we found SL1 was essential for infectivity of PRRSV. SL2 was a key regulatory structural element for PRRSV replication, particularly sg mRNA synthesis.

2.11 (ISU) Studies on the systematic review of factors that influence the persistence of influenza in environmental matrices.

2.12 (ISU) PCV2:

Studies on commercially produced spray dried porcineplasma contains high levels of PCV2 DNA but did not transmit PCV2 when fed to naïve pigs.

Establishment and maintenance of a PCV2-free breeding herd on a site that experienced a natural outbreak of PCV2-associated reproductive disease.

Studies on high prevalence of PCV viremia in newborn piglets in 5 clinically normal swine breeding herds in North America.

PRRSV influences infection dynamics of PCV2 subtypes PCV2a and PCV2b by prolonging PCV2 viremia and shedding.

Shedding and infection dynamics of PCV2 after experimental infection and after natural exposure.

2.13 (ISU) Median infectious dose (ID_{50}) of PRRSV isolate MN-184 via aerosol exposure.

2.14 (OSU) To potentiate the effect of killed PRRSV vaccine, poly(lactide-co-glycolide) (PLGA)-nanoparticles were prepared to encapsulate killed-PPRSV antigens. In nanoparticle-killed-PRRSV vaccinated pigs a reduction in viremia with complete viral clearance by day 15 was seen. In the lungs of nanoparticle- PRRSV vaccinated MN184 challenged pigs a significant reduction in PRRSV antigen load and a reduction in inflammatory cells infiltration was observed. Immunologically, increased frequency of immune cells which initiate Th1response associated with production of IFNα, IL12, IFNγ and IL6 cytokines was detected. Enhanced titers of PRRSV specific total and virus neutralizing antibodies were detected in nanoparticle-killed-PRRSV vaccinates.

2.15 (UW) developed novel analytical approaches to identify a small number of representative viral genotypes from among the diversity of viral sequences available in GenBank and PRRSVdb. We adapted techniques from network theory to rank PRRSV sequences in terms of their importance and applied these methods to a highly-curated PRRSV database that combines high-quality, non-recombinant sequences from both GenBank and PRRSVdb. Viruses represented by the top ranking sequences are valuable targets for future study and can be eventually incorporated into a polyvalent vaccine.

Objective 3. Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine.

3.1 (NADC, Faaberg/Nicholson) Analyzed efficacy of using a diagnostic microarray to detect different PRRSV isolates.

3.2 (NADC, Lager/Miller; E. Zanella, Visiting Scientist) Validation of a real-time PCR for PRV.

3.3 (UGA) Studied prophylactic and therapeutic application of PRRSV-specific swine mAbs.

3.4 (UGA) Explored aerosol vaccination for SIV vaccines in mice and ferrets.

3.5 (UGA) Established primary normal swine bronchoepithelial cell cultures to measure innate responses to swine respiratory viruses.

3.6 (UGA) Tested a variety of PIV5-based live-attenuated vaccines against influenza virus.

3.7 (UGA) Developed new method for rapid detection of influenza virus:

3.8 (UGA) Developed assay for simultaneous serological detection of PRRSV and PCV2.

3.9 (UNL) previously identified immunodominant B-cell linear epitopes in the proteins of a type 2 PRRSV strain FL12 (infectious cDNA clone-derived PRRSV pathogenic strain) with the epitope number 201 (EP-201) of the M protein as a marker candidate (conserved epitope). A triple mutant (TM) carrying 3 AA substitutions in the epitope 201 of PRRSV FL12 was generated. The TM was no longer recognized by anti-201 mAb, was stable *in vivo,* did not elicit antibodies to peptide 201 and can be used as a DIVA marker vaccine strain (with current development of a companion ELISA). We are pursuing the development of DIVA vaccines using PRRSV mutants devoid of the only dispensable structural gene of PRRSV (ORF5a) and by developing mutants devoid of antigenic reactivity in conserved epitopes of structural genes.

3.10 (UMN, ISU) Development of methods for viral and serological monitoring of PCV2 in oral fluids using an experimental model. Prevalence of PCV2 in US finishing herds was characterized. MN participated with IA, IN, KA, NC, Canada and WV to compare PCV2 serological detection methods for diagnostics.

3.11 (UMN) PCR for detection of cytomegalovirus and lymphotrophic herpesvirus.

3.12 (UMN, OH) Novel vaccine development against PRRSV.

3.13 (UMN, ISU) Multiplex methods for serological detection of PCV2 and PRRSV.

3.14 (UMN, IA, SD, Guelph, Newport Labs) identified new PRRSV RFLP types in D-lab submissions.

3.15 (UMN) discovered a novel PRRSV protein, ORF5a, that is immunogenic and induces antibody responses in pigs.

3.16 (KSU, ISU, SDSU) Rowland, Zimmerman, Fang, Opriessnig developed a multiplex Luminex assay for the detection of antibodies against PRRSV, PCV2 and SIV and initiated a multi-lab validation of Luminex assays for serum and oral fluids.

3.17 (KSU Wyatt) characterized a T cell epitope in the PCV2 capsid protein.

3.18 (SHVRI) DIVA vaccine development used JX143 (parental virus HP-PRRSV). After serial psg., 88 extra AA deletions were found in the Nsp2 region and was genetically stable up to the 100th psg. (JXM100). An Nsp2-88aa epitope-based ELISA was developed. 9 pigs were divided into 3 groups and inoculated with parental JX143 (A), JXM100 (B) or were mock-infected (C). Grp. A were immunized with JXM100 virus from which antibodies could not be detected against the corresponding 88 AA deleted epitope until JX143 challenge. This demonstrated that the recombinant marker virus with the diagnostic test, enables serological differentiation between marker virus-infected and wild-type infected pigs.

3.19 (ISU, USDA, AASV, NPB) produced an instructional video and poster on collection and processing of swine oral fluids for disease monitoring and was distributed to AASV members by NPB.

3.20 (ISU) Multiple publications on PCV2 vaccines.

3.21 (ISU) Disinfection protocols reduce the amount of PCV2-contaminated livestock transport vehicles.

3.22 (ISU) Prolonged detection of PCV2 and anti-PCV2 antibody in oral fluids following experimental inoculation.

3.23 (ISU) Comparison of RNA extraction and PCR methods for the detection of PRRSV in oral fluid.

3.24 (ISU) Inhibition of PRRSV infection in piglets by a peptide-conjugated morpholino oligomer.

3.25 (ISU) Kinetics of UV_{254} inactivation of selected viral pathogens in a static system.

3.26 (ISU) Multiplex method for the simultaneous serological detection of PRRSV and PCV2.

3.27 (ISU) Terminology for classifying swine herds by PRRSV status.

3.28 (SDSU: Langenhorst, Lawson, Sun, Li, Hennings, Nelson, Fang) developed an FMIA for detection of PRRSV antibodies in oral fluid and serum. Recombinant nucleocapsid and nsp7 from genotypes 1 and 2 were used as antigens.

3.29 (SDSU: Li, Chen, Sun, Fang). NSP1b is a strong IFN antagonist. Site-specific mutations were introduced into nsp1b and these had reduced ability to inhibit IFNb activation. Recombinant viruses with these mutations were produced for vaccine development.

3.30 (BARC) A multiplex FMIA was developed to simultaneously quantify porcine cytokines in serum or oral fluids (eg. IL1b, IL6, IL8, IFNa, TNF-a, IL-10, IL-12, IFNg, IL4, CCL2). The assay will be of value in immunity, vaccine, challenge studies, determining genetic resistance to PRRSV and responses to other swine pathogens.

3.31 (UIUC, Laegreid; UNL, Osorio, Pattnaik) confirmed that N-glycan moieties in GP5 of type 2 PRRSV are important for the virus to escape neutralizing antibodies and that the N-glycan in GP3 is important in protecting the virus from antibody neutralization.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS: [<500/statement]

1. Evaluation of PRRSV strains in vivo. USDA-ARS. Examination of several PRRSV strains allow us to survey the viral growth properties, the disease in swine, the commensal bacteria that may arise during infection, the innate response, the adaptive immune response and the host gene expression patterns that

differ between PRRSV strains. We use the gained knowledge to better understand PRRSV pathogenesis. With this knowledge, we can then develop better vaccines and vaccination strategies.
2. Construction of a chimeric vaccine to protect against Asian HP-PRRSV. USDA-ARS.
HP-PRRSV strains have proven to be a serious threat to our nations pork industry. Utilizing an infectious clone to a vaccine strain in combination with several structural genes from the Asian lineage, along with an identifiable foreign marker, we are constructing a vaccine to be used to protect swine in case such HP-PRRSV strains appear in the U.S.
3. Gene expression in lymph nodes of PRRSV-infected pigs. USDA-ARS. The goal of this discovery project is to identify changes that occur in gene expression in porcine lung lymph nodes following PRRSV infection. Knowledge derived from this study will more clearly define the negative effect of PRRSV on the pig immune system, and it may be used to design better cross-protective vaccines.
4. Progress in this reporting period continues to focus on non-PRRS viruses (e.g. influenza). While not directly related to PRRSV, this work is contributing directly to development of swine reagents and resources (e.g. primary swine epithelial cell lines). As we develop these tools for other (re-)emerging swine diseases, we plan to apply many of these approaches directly to PRRSV projects (UGA).
5. In regard in influenza as an emerging (re-emerging) disease of swine, we have made extensive advances in understanding features of the virus and host that influence infection, tropism, and potentially reassortment. We have also explored a number of vaccine and anti-viral therapies for influenza and developed a novel approach for rapid and sensitive detection of influenza virus; all of which may directly impact swine and/or human health (UGA).
6. Four PRRSV-related refereed papers involving our laboratories have been published in refereed journals during the period covered in this report (UNL).
7. US and European Patent Title: Methods and Compositions for Vaccination of Animals with PRRSV Antigens with Improved Immunogenicity. Inventors: Ansari, I, Osorio FA, and Pattnaik, AK Serial No. 12/064, Issued: October 27, 2009. During 2011 this patent is being explored by a veterinary biologics company (UNL)
8. Provisional claim for invention: "A reverse genetics method to develop a PRRSV attenuated live vaccine strain with DIVA differential capacity that would permit distinguishing naturally infected from non-infected, just vaccinated animals" (Osorio, FA, Pattnaik, AK, Kwon, BJ, and Vu H.) filed on March 4, 2011, EFS ID: 9585692 Application No. 61449138 (UNL)
9. Elucidation of neonatal infection with minimal impact of maternal immunity illuminated the critical need to control and eliminate early influenza virus infection (UMN).
10. Commercial vaccines are not likely to benefit influenza control since homologous protection is required to prevent transmission.(UMN)
11. PRRSv MLV vaccines and air filtration interventions can aid in regional elimination of PRRSv (UMN).
12. New protein identification provides novel targets for immune protection (UMN).
13. PCV2 pathogenesis characterization increases knowledge of key pathogenic features.
14. Genomic markers for improved response to PRRS creates the opportunity to conduct marker-selected breeding of pigs (KSU).
15. Identification of a decoy epitope in PCV2 capsid is being used for assays that assess protective immunity following infection or vaccination (KSU).
16. Luminex is being developed as a substitute for standard ELISA approaches (KSU)
17. PRRS is a more complicated swine disease in China. A lot of work for completely controlling PRRS needs to be done in China. Vaccination is the primary choice for the majority of pig products for preventing and controlling PRRS. The viral replication and transcription mechanism and related foundation research should be conducted and a new generation of DIVA vaccines should be developed (SHVRI)
18. Shishan Yuan, Jian Lv, Xiangjian Li, Jianwu Zhang, Dandan Yu, Zhi Sun, Fei Gao, Zuzhang Wei, Jinshan Zhuang, Tao Tan, Haihong Zheng, Feifei Tan, Changlong Liu, Jiaqi Lu, Yanfang Cong,

Xiaoming Wang, Hao Zheng. The construction of highly pathogenic PRRSV recombinant plasmid and genetic engineering vaccine. Patent NO. 200710172364.8; Announcement No.: CN101205539A; Publication No.: 2010062500313230)

19. Output for project: "Assessment of Virulence of PRRSV Isolates Based Both on their Sensitivity to IFNb and Ability to Induce Type I IFN Responses". Mr. Christopher Overend a doctoral candidate in Pathobiology and Veterinary Science working on the project has successfully completed his degree. Data obtained in this project are routinely discussed with our collaborator Dr. Marvin Grubman, a scientist from Plum Island Animal Disease, Center who shares interests in the area of type I IFN (UCONN).

20. Epidemiology/pathobiology/diagnostic studies provide ideas for detecting, preventing and eliminating viruses. Work has been done on genetic/antigenic variation during replication and persistence and new methods of surveillance for cost-effective methods of tracking infection and use in elimination/eradication. Advances in these areas linked with research in viral ecology/epidemiology and improvements in vaccines leads to possible elimination/eradication of viruses from farms and regions (ISU).

21. Presence of replicating PRRSV in pigs from very early days post-infection modulates the innate immune function resulting in subversion of immunity. As this study was performed in pigs maintained in natural commercial environmental settings, the outcome of this study has more practical implications in development of new generation protective vaccines. Our results on nanoparticles-based PRRSV-killed vaccine suggested that mucosal immunization has the potential to induce protective immunity to PRRS (OSU).

22. Understanding the role nsp2 in PRRSV virulence has important implication in developing better vaccines against PRRSV (VA).

23. Understanding the PRRSV-host miRNAs interaction provides new insight into the role of miRNAs in PRRSV pathogenesis. (VA)

24. A panel of monoclonal and polyclonal antibodies to non-structural proteins of PRRSV will be important tools in studies of PRRSV replication and pathogenesis. (SDSU)

25. Recombinant PRRSV can be produced whereby Interleukin 1 B expression or modifications in nsp2 can enhance viral specific immunity. These are important modifications to further vaccine design. (SDSU)

26. A multiplex FMIA for antibodies against PRRSV differentiates types I and II and can be used for multiplexing for serological profiling (SDSU).

27. The PRRS Host Genetics Consortium (PHGC) is helping to dissect the role of host genetics in resistance to PRRS and in effects on pig health and related growth effects. Results using a Nursery Pig Model of commercial pigs infected with PRRSV and followed for 42 days have affirmed that all pigs become PRRSV infected but they clear virus from serum at different rates with variable weight effects. Genome wide association studies (GWAS) have mapped pig PRRS responses (viral load and weight gain during infection) to multiple swine chromosomes. This result suggests that genetic progress can be made by selective breeding.(BARC)

28. Functional gene and protein transcriptomic analyses are underway with PHGC samples to identify gene networks and resistance associated biomarkers that differ in high versus low VL or WG PHGC pigs. Pathways have been identified and are being validated. Overall, the PHGC project will enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection.(BARC).

29. An FMIA has been developed to simultaneously quantify multiple porcine cytokines in serum using Luminex xMap™ technology. It has been optimized to detect innate (IL-1b, IL-6, IL-8, IFN-a, TNF-a); regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN-g) and Th2 (IL-4) cytokines. The assay has been tested for levels of porcine cytokines in oral fluids with positive results. This assay will be a useful tool to determine cytokine involved in genetic resistance to PRRSV using PHGC samples (BARC).

30. Antigenic/genetic variation in PRRSV is a major impediment to vaccine development. By "distilling" this diversity down to a manageable unit, we are hoping to provide guidance for the development of next-generation polyvalent vaccines that have maximum broad efficacy (BARC).

31. Our studies on PRRSV inhibition of interferon signaling showed that NSP1β of virulent VR2385 may be the viral protein that interferes with IFN signaling, while NSP1β of Ingelvac MLV has no effect. This result has a biological relevance on PRRS vaccine design (UMD).

32. Our finding of a lab mutant A2MC2 inducing interferons in cultured cells may be beneficial for vaccine development to induce protective immunity against PRRS. This isolate is expected to induce higher titer of neutralizing antibody in pigs (UMD).

33. PRRSV expresses proteins that circumvent the type I IFN response and other cellular processes and to compensate the small coding capacity of PRRSV, these proteins are multifunctional. Studies for Dr. Yoo's laboratory suggest that PRRSV Nsp1α and NSP11 are a multifunctional nuclear protein participating in the modulation of the host IFN system (UIUC).

34. Studies from Dr. Zuckermann's lab suggest that PRRSV is inhibiting the ability of porcine macrophages to produce IFNa in response to infection by interfering with the activation of the transcription factor IRF-3 but not NFkB (UIUC).

35. The data presented by Dr. Laegreid's lab firmly confirms the important notion that GP3 may be involved in inducing neutralizing antibodies. Collectively, our work aimed at deciphering the transcriptional and cytokine response of cells as we as porcine alveolar macrophages to PRRSV infection will likely lead to the development of strategies to developed better vaccine against this costly disease.

D. PUBLICATIONS ISSUED OR "IN PRESS"

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3) Abstracts or proceedings

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Proceedings, 6th International symposium on emerging and re-emerging pig diseases, Barcelona, Spain, June 2011 = Proc Emerg Dis 2011

Proceedings, 30th Annual Meeting, American Society for Virology, Minneapolis Minnesota = Proc ASV 2011

Proceedings, 54th Annual Conference, American Association of Veterinary Laboratory Diagnosticians. Buffalo, New York = Proc. AAVLD 2011.

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Zimmerman JJ. 2011. Swine diagnostics based `on non-invasive sampling: Oral fluids. Anais do XV Congreso Brasileiro de Veterinários Especialista em Suinos (ABRAVES). Fortalez, CE, Brasil, pp. 213-214.

E. FUNDING SOURCES FOR RESEARCH:

American Protein Corporation, Inc. Opriessnig T, Halbur PG. Studies to investigate the role of spray-dried plasma in PCV2 transmission. $123,666. 4/1/10-3/31/12 (ISU)

Boehringer-Ingelheim PRRS Research Initiative. Dee SA. A re-evaluation of slurry as a risk factor for the survival and transmission of PRRSV. $25,000 April 1, 2011-March 31, 2012. (UMN)

EU FP7 project：“PoRRSCon-New tools and approaches to control Porcine Reproductive and Respiratory Syndrome (PRRS) in the EU and Asia”（FP7-KBBF-2009-3）（2010-2014）(SHVRI)

Genome Alberta Applied Livestock Genomics Program (ALGP) #29. Moore S, Lunney JK, Kemp B. “Canadian Component of the PRRS Host Genetics Consortium (PHGC).” CA$465,871; 12/10-11/13.

Genome Canada 2010 Large Scale Applied Research Project Competition. Plastow et al., 2011-2014., Application of genomics to improving swine health and welfare, CA$4,999,091 overall; Rowland lab $800,000. (KSU); Lunney lab $165,000 (BARC)

Industry (Private), Yoo, D. (2010-2011), $63,339, Interferon phenotypes of PRRSV and viral determinants (UIUC).

Industry (Private), Yoo, D. (2010-2011), $80,122, Molecular bases for interferon modulation by PRRSV. (UIUC).

Iowa Attorney General's Office Innovative Swine Industry Enhancement Grant Program, - Zimmerman J, Wang C. 05/01/11 to 04/30/12. Sample size recommendations for the detection of PRRSV infection using pen-based oral fluid samples. $50,538. (ISU).

Iowa Livestock Health Advisory Council Opriessnig T. Effect of porcine Torque teno virus (TTV) status on PRRSV vaccine efficacy.. $25,000. 1yr. (ISU)

Iowa Pork Producer Association. Opriessnig T, Halbur PG. Effect of PCV2 vaccination on chronic PCV2 infection and determination of infectivity of PCV2 present in chronically infected pigs. $25,000. 1 yr. (ISU)

Iowa Pork Producer Association. Opriessnig T, Halbur P, Johnson J. Comparison of PCR and serological assays for reliable, early and fast detection of PRRSV in boar studs. $66,432. 1 yr. (ISU)

Iowa Pork Producers Association, ISU Vice Provost for Research and Economic Development, and Healthy Livestock for Iowa Yoon KJ, Baker R, Janke B, Halbur P, Main R. Genetic assessment of VDL SIV isolate pool for evidence of the swine flu strain reported to be infecting people and development of a high-throughput differential test for the novel strain. $72,000 ($24,000 each from Iowa Pork Producers Association, ISU Vice Provost for Research and Economic Development, and Healthy Livestock for Iowa). May 1, 2009-April 30, 2010. (ISU)

MN Ag Exp Sta. Murtaugh MP, Rossow K, Dee S. PRRS: integrated control and elimination of PRRS in the US. $53,000. 10/1/09-9/20/11. (UMN)

National Pork Board. Dee SA. An evaluation of back-drafting of non-filtered air as a source of PRRSV infection to pigs housed in filtered facilities and whether selected intervention strategies can reduce this risk. $41,916. September 1-August 31, 2011. (UMN)

National Pork Board 2007-2011. Rowland and Lunney, et al., PRRS host genetics consortium: A proposal to develop a consortium to study the role of host genetics and resistance to PRRSV. $800,000. (KSU, BARC)

National Pork Board - Lunney J, Christopher-Hennings J, Nelson E, Fang Y, Steibel JP, Zimmerman J. 01/01/10 to 12/31/11. Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay. $103,929. (BARC, SDSU, ISU)

National Pork Board - Rowland R, Zimmerman JJ, Opriessnig T, Fang Y, Green J. 07/1/2011 to 06/30/2012. Multi-institutional development and validation of a multiplex fluorescent microsphere immunoassay for the diagnosis of multiple agents in serum and oral fluid. $152,267. (KSU, ISU, SDSU)

National Pork Board (#11-037) - Fang Y, Rowland R, Zimmerman J, Irwin CA, Christopher-Hennings J, Nelson E. 01/01/11 to 06/30/12. Novel multiplex diagnostic assays development for diagnosis of porcine respiratory disease complex. $74,999. (SDSU, KSU, ISU)

National Pork Board (#11-113) - Zimmerman J, Irwin CK, Yoon KJ. 07/1/2011 to 06/30/2012. Preweaning surveillance: Finger on the pulse of PRRSV epidemiology, transmission and spread. $49,477. (ISU)

National Pork Board (#11-165) - Wang C, Zimmerman JJ, Kim J-K, Ramirez A, Holtkamp DJ. 10/1/2011 to 09/30/2012. Design and analysis of PRRSV surveillance: Temporal and spatial sampling, mapping, monitoring and automated rapid detection of outbreaks. $24,378. (ISU)

National Pork Board Molecular Structures of PRRSV that Contribute to PRRSV Protective Immunity; P.I.: A.K. Pattnaik; Total Costs: $ 138,600; Grant Period: 12/01/2009 - 11/30/2010, Total Amount: $138,600 . Principal Investigator: A. Pattnaik, co-PI: FA Osorio. (UNL)

National Pork Board Sandbulte M, Roth J. Immune correlates of clinical outcomes in maternal antibody-positive piglets vaccinated with attenuated or killed SIV and challenged with an antigenic variant. $75,000. 9-1-11 to 8-30-12. (ISU)

National Pork Board. Dee SA. Supplemental funding for an assessment of air filtration for reducing the risk of Airborne spread of PRRSV to in large commercial sow herds in swine dense regions. $64,600, November 1, 2009-October 31, 2010. (UMN)

National Pork Board. Evaluation of pathogenesis of concurrent SIV and PCV2 infection in CD/CD pigs" (PURDUE)

National Pork Board. Fang Y., W. Zhang, J. Christopher-Hennings, E.A. Nelson and R.B. Baker. Development of an epitope-based vaccine against swine influenza A using a non-toxic enterotoxin as the carrier-adjuvant.

National Pork Board. Identification of conserved T-cell epitopes contained in the non-structural genes of PRRSV which contribute to broad protective immunity Grant Period: 10/01/2010 - 10/01/2011. Total Amount: $100,000 Principal Investigator: F A Osorio, co-PI: A. Pattnaik (UNL)

National Pork Board. Opriessnig T, Halbur PG. The prevalence of PCV2 viremia in conventional piglets born to PCV2- vaccinated and non-vaccinated sows and effect of PCV2 viremia on pig performance. $48,170. 5/1/10-4/30/11. (ISU)

National Pork Board. Rowland, 2010-2011. NPB. Serological approach for diagnosis and surveillance of multiple agents in serum and oral fluid samples, $45,000. (KSU)

National Pork Board. Rowland, Zimmerman, Opriessnig, Fang. 2011-2012, Multi-institutional development and validation of a multiplex fluorescent microsphere immunoassay for the diagnosis of multiple agents in serum and oral fluid samples. $152,000 (KSU, ISU, SDSU)

National Pork Board. Zhang Y, Opriessnig T. An interferon-inducible porcine reproductive and respiratory syndrome virus isolate. $106,751 1 yr. (ISU)

National Pork Board. Murtaugh MP. Structural characterization of the PRRSv glycan shield. $73,312. 11/1/09-10/31/11. (UMN)

National Pork Board. Renukaradhya J. Gourapura. Development of novel mucosal vaccines for the control of PRRSV outbreaks. Dec. 2009 to Nov. 2011. $144,957 for two years (OSU).

Natural Science Foundation of China " The research for PRRSV pathogenic mechanism" (30972204) (2010-2013)(SHVRI)

NIAID 1 R01 AI070847-01A2; 6/25/08 – 06/14/13. Title: "Developing a Paramyxovirus-based H5N1 Vaccine" The major goal of this grant is to develop a PIV5-based influenza virus vectored vaccine.

NIAID 1 U01 AI083005-01. 04/01/09 – 03/31/14.Title: "Manipulating natural host immunoregulation via IDO during viral infection. "The major goal of this grant is to investigate the mechanisms of natural immunoregulation during influenza viral infection centered around the immunosuppressive enzyme indoleamine-2,3-dioxygenase (IDO). Total Direct costs: $2,388,917. (UGA)

NIH HHSN266200700006C; 03/30/07 – 03/29/14 Title: NIAID Center of Excellence for Influenza Research and Surveillance (contract, UGA)The major goal of this contract is to understand how AIV viruses mutate after passages through susceptible species and development of therapies to prevent/treat AIV infection. Total Direct costs: $37,000,000

NIH R01AI074667. Meng XJ, Halbur PG, Huang YW. Mechanism of hepatitis E virus replication and pathogenesis. $1,561,797. 03/01/2008 to 02/30/2012.(VA, ISU)

NIH R15. Rowland and Blecha, 2010-2013, A model for developmental IFN gene regulation in the virus-infected fetus. $300,000 (direct costs) (KSU)

Pfizer Animal Health Inc. Opriessnig T, Halbur PG. Effect of PCV2 vaccination on emergence of new PCV2 subtypes. $102,511. 8/1/10-7/31/11. (ISU)

Pfizer Inc. Opriessnig T, Meng XJ, Halbur PG. Characterization of potentially unknown viral or bacterial pathogens in cases of high morbidity and mortality in pigs. $865,830. 7/7/08-7/6/11. (ISU, VA)

Total Direct costs: $854,829 (UGA)

PRRSV PRRS CAP 2. Murtaugh and Renukaradhya J. Gourapura. Positive Prognosticators of Immune Protection and Prophylaxis against PRRSV in Swine Herds. Aug. 2009 to July 2013. $165,000 per year (RJG share $34,864)(OSU)

Univ Minn. Torremorell M, Gramer M, Murtaugh MP, Rovira A. Genetic and phenotypic characterization of endemic SIV isolates and evaluation of alternative but relevant routes for SIV transmission. $166,000. 1/1/10-6/30/11 (UMN)

UNL Life Sciences Competitive Grants Program (FY2011) (Facilitating UNL and Industry Partnerships) Optimization of Invention: A DIVA marker for PRRSV vaccination Granting Agency: Total Amount $30,000 Grant Period: 07/01/2011-06/30/2013 Roles: PI FA Osorio, co-PI: AK Pattnaik (UNL)

USDA - Irwin C, Zimmerman J, Main R. 06/15/10 to 06/14/12. Optimization and validation of detection of influenza virus in oral fluids for NAHLN laboratories. $379,610 Gold sheet #111452 (ISU)

USDA AFRI/NIFA Animal Genome, Genetics, and Breeding Program. Lunney JK,C Ernst, V. Honavar, Z Jiang, R Pogranichniy, JP Steibel, C Tuggle. Identifying porcine genes and gene networks involved in effective response to PRRS virus using functional genomics and systems biology. $750,000. 2010-2012.

USDA APHIS Roth J, Zaabel P. Swine influenza surveillance as part of a comprehensive and integrated swine surveillance: Outreach and education for swine producers and swine veterinarians. $149,600. 8/10/10 – 3/31/12 Extended (ISU)

USDA ARS NADC Roth J. Identification of epitopes and genetic factors of swine influenza virus that lead to heterologous immunity elicited by influenza vaccines to support development of vaccines with broader protective immunity against novel emerging influenza strains. $500,000. 3/1/10 - 2/28/13. (ISU)

USDA CSREES NRI, Yoo, D. (2008-2011), $375,000, Evasion strategies of PRRSV from the host defense (UIUC).

USDA HATCH Grant, Yoo, D. (2010-2011), $28,500, Development of PRRSV as a multivalent vaccine vector (UIUC).

USDA Hatch. Nelson, E.A., J. Christopher-Hennings, Y. Fang. Improved methods for the diagnosis of Porcine Reproductive and Respiratory Syndrome and other important viral diseases of swine.

USDA Multi-State Research (NC-229) Program, Laegreid, W. (2010-2011), $10,000, PRRSV and emerging diseases in swine (UIUC).

USDA Multi-State Research (NC-229) Program, Yoo, D. Interaction of PRRSV Nsp1a and protein inhibitor of activated STAT1 (PIAS1) m Yoo, D. (2010-2011), $9,800, PRRSV and emerging diseases in swine.

USDA Multi-State Research (NC-229) Program, Zuckermann, F. (2010-2011), $18,000, PRRSV and emerging diseases in swine (UIUC).

USDA NIFA Functional Genomic grant #2010-65205-20433 (PURDUE).

USDA NIFA PRRS CAP2: Objective 3. Lunney JK, J Dekkers, R Fernando, Z Jiang, H-C Liu, R Pogranichniy, JM Reecy, R Rekaya, M Rothschild, D Smith, JP Steibel, C Tuggle. PRRS CAP Host genetics: Characterization of host factors that contribute to PRRS disease resistance and susceptibility. Host Genetics. $560,000. 2009-2012.

USDA NIFA. Patience et al., 2010-2015. Improving biological nutrient use and thus feed efficiency in the U.S. pork industry through innovative scientific and extension approaches for a more sustainable production. $65,000 (KSU)

USDA NRI 2009-35204-05290. Rowland and Hesse, 2009-2111., Mapping host protective immunity in the PCV2 capsid protein, $240,000. (KSU)

USDA NRI Coordinated Agricultural Program (CAP), 2008-2012. Rowland et al., Integrated strategies to control and reduce the impact of PRRS virus control, $4.9 million. (KSU)

USDA NRI PRRS CAP 2. Zimmerman JJ, Dee SA**,** Davies PD, Holtkamp D, O'Connor A, Pohl S. Identifying ecological and epidemiological factors in the control of PRRS: A field-based approach. $180,000, March, 2010-February, 2011. (UMN)

USDA NRI PRRS CAP 2. Murtaugh MP, Gourapura A. Positive prognosticators of PRRSV. $236,000. (UMN)

USDA NRI PRRSV Diversity CAP2 (Year 2) Grant Period: 11/01/2010 - 10/30/2011, Total Amount: $98,418 Granting Agency Name: /KSU, Principal Investigator: Osorio, FA (UNL)

USDA NRI. Wang, X, PI, Interaction between PRRSV and interferon alpha/beta induction signaling pathways.

USDA NRICGP Project No. No.2008-00903Porcine Reproductive and Respiratory Virus: role of viral genes in virulence/attenuation. Grant Period: 09/01/2008 - 08/31/2011Total Amount: $374900 Principal Investigator: Osorio, FA, co-PI: A. Pattnaik. (UNL)

USDA –PRRSV CAP/Kansas State University (Multi-university collaboration and research funding program) "PRRSV CAP Host genetics: characterization of host factors that contribute to PRRS disease resistance and susceptibility" (PURDUE)

USDA PRRSV CAP2. Laegreid, W.W., F.A. Osorio, T. Goldberg, J. Christopher-Hennings and E.A. Nelson. Immunologic consequences of PRRSV diversity. 1/09 to 1/13 (UIUC, UNL, UW, SDSU).

USDA. PRRSV PRRS CAP 2. Murtaugh and Renukaradhya J. Gourapura. Positive Prognosticators of Immune Protection and Prophylaxis against PRRSV in Swine Herds. Aug. 2009 to July 2013. $165,000 per year (RJG share $34,864) (OSU, UMN)

USDA/AFRI Immune Response to PRRSV Glycoproteins. Grant Period: 09/01/2009 - 08/31/2012. Total Amount: $371,230 Principal investigator: A. Pattanik, co-PI: FA Osorio (UNL)

USDA:CSREES National Research Initiative, Competitive Grants Program 230.1 Animal and Plant Biosecurity (USDA NIFA Award 2008-55620-19132 subcontracted through Kansas State University) - Zimmerman J, Holtkamp DJ, O'Connor A. 08/01/09 to 05/31/12. Identifying ecologic and epidemiologic factors in the control of PRRS: A field-based approach. $363,473. Gold sheet #96044. (ISU)

USDA-NIFA (Multi-institutional collaboration and USDA) "Identifying porcine genes and gene networks involved in effective response to PRRS virus using functional genomics and systems biology" (PURDUE)

USDA-NIFA-2010-03437. Meng XJ. Engineering PRRSV Vaccines that confer heterologous protection. 12/15/2011- 12/14/2014. (VA).

F. WORK PLANNED FOR NEXT YEAR

Objective 1. Elucidate the mechanisms of host-pathogen(s) interactions.

Immunity (PRRSV):

1. Assess viral and immune response characteristics to rPRRSV in vivo (NADC)
2. Transcript expression analysis of tracheobronchial lymph nodes of PRV/PRRSV/SIV/PCV-2 infected pigs (NADC).
3. Transcript expression analysis of tracheobronchial lymph nodes of Asian porcine high fever disease strains of PRRSV infected pigs (NADC).
4. Continue research on characterization of the action of viral proteins that influence innate immunity X PRRSV (collaborating agencies: Nebraska, Illinois) (UNL)
5. Seek funding (NIFA AFRI-USDA) and continue research on the ability of different PRRS glycoproteins to evoke cross-neutralizing antibody response against PRRSV (collaborating agencies: Nebraska, Illinois, Universidad Complutense Madrid)(UNL)
6. Seek funding (NIFA AFRI-USDA) and continue research on characterization on conserved T cell epitopes of PRRSV proteins and aspects of the NSP proteins that influence innate immunity X PRRSV (collaborating agencies: Nebraska, Illinois, Cleveland Clinic) (UNL)
7. Continue to study characterizing the expression 39 type I IFN genes and related receptors in the PRRSV infected fetus. (KSU)
8. Lunney and collaborators. Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay. (BARC, ISU)
9. Test IFNβ responses in swine challenged with PRRSV (UCONN).
10. To further investigate the post-transcriptional mechanism of type I interferon regulation by PRRSV in Mo-DC. (SDSU)
11. Continue investigations of PRRSV evolution, structure and immunity (UMN).

12. Study virus and host interaction during PRRSV infection in pigs (PURDUE).
13. Determine role of certain swine chromosomal regions or genes on swine responses to PRRSV infection and related growth effects. (BARC)
14. Use new assays (cytokine FMIA, microarray and RNAseq analyses) to produce more data on PHGC pig samples. These should result in deeper phenotypes for comparing pigs with high/low PRRS burden and high/low weight gain (BARC).
15. Further evaluation and collaborative utilization of swine cytokine multiplex assays will be performed for detection of cytokines in oral fluids and other substrates (BARC).
16. As part of the US Veterinary Immune Reagents Network (US VIRN www.vetimm.org) we will continue to develop immune reagents for the research community, including cloned cytokines and chemokines, and monoclonal antibodies to them and cell surface receptors (BARC).
17. We will continue studies on the mechanism of PRRSV interference of IFN downstream signaling and identify the viral protein(s) that is responsible for the inhibition. Studies on the IFN-inducing mutant will be conducted to determine its capability in inducing neutralizing antibody in pigs. We will also identify its difference from other strains and determine the sequence variations that might be responsible for the IFN induction (UMD).
18. We plan to continue the research on the influence of PRRSV on the innate immune response of swine as well as the immunological consequences of PRRSV diversity (UIUC).

Virus structure (PRRSV):

1. Structural studies of PRRSV nonstructural protein 2 (NADC)
2. Based on the reverse genetic manipulation, we will continue to progress in 1) structure–function relationship of PRRSV proteins; 2) Viral RNA synthesis regulatory elements identification. 3) DIVA vaccine development (SHVRI)

Other emerging viral diseases:

1. Mechanism of hepatitis E virus replication and pathogenesis. (ISU, VA)
2. Influenza studies (UGA)
3. Seek funding (NPB, AFRI-USDA) and continue research on genetic control of PCAVD in pigs (collaborating agencies: Nebraska, ISU) (UNL)
4. Continue investigations of mechanisms of PCV2 pathogenesis (UMN).

Objective 2. Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine.

PRRSV

1. Seek renewal of funds (CAP2) and continue research on sero-typing of PRRSV strains and characterization of PRRSV strain diversity (collaborating agencies : Nebraska, Illinois, Wisconsin, South Dakota, ISU, and Universidad Complutense Madrid) (UNL)
2. Assess the sustainability and cost-benefit of filtration as a means to reduce the risk of airborne spread between farms (UMN).
3. Re-investigate the role of PRRSV-contaminated slurry as a means of viral spread between farms (UMN).
4. PRRS Host Genetics Consortium. Identify markers and genes related to resistance to PRRS (KSU) (BARC)
5. Study innate immune pathways that relate to PRRS resistance (KSU)

Other emerging viral diseases:

1. Continue investigations of influenza transmission dynamics (UMN).
2. Several research groups plan ecological studies involving influenza virus, PCV2, and/or PRRSV

Objective 3. Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine.

Detection:

1. Develop and test PRRSV diagnostic microarray (NADC)
2. Validate Luminex assays for respiratory pathogens (KSU).
3. Several research groups will be involved in the development of improved diagnostic methods and assays for agents in the porcine respiratory disease complex (ISU)
4. We plan to develop an array of new veterinary diagnostic tests, using enzyme-linked immunosorbent assay (ELISA), multiplex fluorescent microsphere immunoassay (FMIA) and immunochromatographic strip or lateral flow technologies. New assays will be optimized and validated according to recognized quality assurance standards. (SDSU)

Prevention (PRRSV):

1. Assess viral and immune response of PRRSV vaccine candidates that have a reduced capacity for the induction of polyclonal B-cell activation in vivo (NADC).
2. Develop a broadly-protective vaccine against PRRSV (VA).
3. We will analyze the adjuvanticity of Mycobacterium tuberculosis whole cell lysate on nanoparticles based killed PRRSVvaccine in PRRSV MN184 challenged pigs (OSU).
4. Based on the reverse genetic manipulation, we will continue progress in the following aspects:
    a. structure–function relationship of PRRSV proteins.
    b. Viral RNA synthesis regulatory elements identification.
    c. DIVA vaccine development.
5. Develop and test efficacy of chimeric vaccine constructs (NADC)
6. Develop and test adenovirus constructs expressing novel PRRSV genes (NADC)
7. To continue development of a marker system to convert a regular PRRSV modified live vaccine into a DIVA vaccine (Nebraska, Illinois, South Dakota State U) (UNL)
8. Continue to perform an analysis of cross-protection between diverse PRRSV strains (KSU).
9. Continue to understand immune responses in pigs infected with PRRSV in the presence or absence of vaccination in the field conditions. (OSU, UMN)
10. Over the next year, we will complete our analysis of PRRSV diversity and generate final sequence rankings to inform future studies on PRRSV, and especially the development of effective polyvalent vaccines. We will also make publicly available our curated database, which should be of high value for any researchers wishing to examine PRRSV genetic diversity and evolution (UW).

Control (PRRSV):

1. Initiate the therapeutic antibody project for PRRSV in swine (UGA).
2. Use new assays (cytokine FMIA, microarray and RNAseq analyses) to produce more data on PHGC pig samples. These should result in deeper phenotypes for comparing pigs with high/low PRRS burden and high/low weight gain. (BARC)

Other emerging viral diseases:

1. Several research groups will be involved in the continued development and evaluation of vaccines against PCV2, Nipah Virus, PRRSV, influenza virus (ISU).