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, 12/03/2010

Brief Summary of Minutes of Annual Meeting. (Held on Friday, Dec. 3, at 1 PM -2:30 PM)
Dr. XJ Meng gave the opening remarks.
Dr. David Benfield gave a short history (started in 1999) and evolution of NC229 describing the "uniqueness" of this NC group being the first in a number of categories (eg. in submitting multi-investigator grants, formalizing an international meeting, promoting specialized editions of journals, to receive an excellence in multi-states award). It has continued to promote integrated approaches to research and may need to submit foundation or 501c3 organization grants to continue.
Dr. Peter Johnson and Dr. Margo Holland discussed funding opportunity changes in USDA (NIFA). Two handouts were received on categories of NIFA discretionary funding and areas funded in 2010 with the president's budget and senate committee action for 2011, along with a brief description of NIFA (see http://www.nifa.usda.gov).
Dr. Bob Rowland discussed post-PRRS CAP funding for NC229 with some discussion.
Closing remarks were made by Dr. Jane Christopher-Hennings.

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 (KSU-Sang/ Blecha/Rowland) performed a study identifying 39 type I interferon (IFN) genes. Recombinant IFN proteins show a wide range of activities, including some novel IFNs that are effective in controlling PRRSV replication. An analysis of cross-protection between diverse PRRSV strains is also being performed (Hesse, Rowland).

1.2. (UMD-Zhang/Zhu) PRRSV interferes with IFN signaling. Transcripts of IFN-stimulated genes, ISG15 and ISG56, and the STAT2 protein in PRRSV infected MARC cells were significantly lower than in mock-infected cells after IFNα treatment. PRRSV blocks STAT1/STAT2 nuclear translocation. IFN-induced phosphorylation of STAT1 and STAT2, and their heterodimer formation in the PRRSV-infected cells were not affected. Most STAT1/STAT2/IRF9 heterotrimers remained in the cytoplasm of infected cells, indicating that the nuclear translocation of the heterotrimers was blocked suggesting PRRSV interferes with activation and signaling of type I IFN by blocking the STAT1/STAT2 nuclear translocation. Overexpression of nsp1β inhibited expression of ISG15 and ISG56 and blocked nuclear

translocation of STAT1 suggesting that nsp1β might be responsible for IFN inhibition. PRRSV infection of primary porcine pulmonary alveolar macrophages (PAMs) also inhibited IFNα signaling. PRRS MLV activated expression of IFN-inducible genes including chemokines and antivirals in PAMs without the addition of external IFN, and had no detectable effect on IFN signaling.

1.3. (USDA-BARC), the PRRS Host Genetics Consortium (PHGC) was developed to determine the role of host genetics in PRRSV resistance. It is a multi-year project funded by NPB, USDA, universities and private companies. 8 sets of 200 nursery pigs were infected with PRRSV. Pigs became infected but some pigs clear virus from serum quicker with variable weight effects. DNA from all pigs is genotyped with PorcineSNP60 Genotyping BeadChip and data stored at www.animalgenome.org/lunney. Comparison of resistant/maximal growth pigs to susceptible/reduced growth pigs is performed. Support is from USDA, NRSP8 Swine Genome Coordinator and Genome Alberta. Initial results mapped viral load and weight gain during infection to multiple swine chromosomes.

The effect of PRRSV infection or vaccination on pigs was tested. Pigs were given a low or high virulent PRRSV, vaccine or were controls. Tissues were collected for innate immune response evaluation by swine oligo array pigoligoarray.org with statistical assessment of gene expression patterns (MSU). Cellular immune response, chemokine signaling and apoptosis were significantly activated in infected tonsils vs. those from vaccinated and control pigs.

1.4. (SHVRI) studied the 3'UTR of type 2 PRRSV using site-directed mutagenesis. At least 40 nt after the ORF7 stop codon were dispensable for the PRRSV viability. A chimeric PRRSV (type 2 with 3'UTR from type 1) was viable and was similar to the parental strain.

PRRSV expresses its genes via a set of nested subgenomic (sg) mRNAs. The utilization of TRS remains a puzzle, as many TRS-like sequences exist in viral genomes, yet only 6 or 7 sg mRNAs were transcribed in arterivirus infected cells. A PRRSV infectious cDNA clone pCPV expressing the capsid gene of PCV2 between PRRSV ORF1b and ORF2a was developed. The recombinant viruses contained a range of disparate deletions of the inserted PCV2 sequence, yet 2 stable recombinant viruses containing 41 and 275 nt of foreign sequences were generated. Further analysis of the sg RNA2 profile revealed that an array of novel sg RNA species was generated in infected cells. PRRSV can utilize foreign TRS-like sequences as transcriptional promoter and the insertion of foreign sequence provoked the generation of novel subgenomic RNAs utilizing cryptic TRS-like sequences that remain non-functional in native PRRSV.

1.5. (NADC-Faaberg/Brockmeier/Loving/Miller). Compared pathogenesis in pigs from challenge with 3 new Type 2 PRRSV.

Comparison in growth and disease after vaccination with nsp2 deletion mutant viruses and novel Type 2 challenge.

Faaberg/Lager compared virulence of a recombinant Chinese porcine high fever disease strain or VR-2332 in US swine.

Kehrli/Miller/Faaberg provided adenovirus expression of a region of PRRSV nsp 2.

Developed an infectious clone of Vietnamese porcine high fever disease strain and described cell culture phenotype (Faaberg, Guo).

Developed nsp9 and ORF6 mutants of MN184, an infectious clone of a new vaccine strain and examination of the interaction of nsp2 with host genes (Faaberg).

Developed an *in vitro* PRRSV infected tissue culture assay to screen for polyclonal B-cell activation (Miller/Kehrli).

Study of PRRSV strains with a reduced induction of polyclonal B-cell activation for possible vaccine (Miller/Kehrli/Faaberg).

Genomic DNA purification for SNP chip analysis to examine genotype in PCV2 host susceptibility (Miller/Rohrer-USMARC).

Compared acute cytokine responses after infection with PRRSV, PRV, PCV2 and SIV (Miller).
Transcript expression analysis of TBLN of PRRSV/PRV/SIV/PCV-2 *in vivo* (Miller).
Conducted transcriptome and cytokine assays for PRV animal experiment (Miller/Lager/Zanella).
Developed type 1 IFN bioassay to examine PRRSV strain specific pathology (Faaberg/Miller/Guo).

1.6. (UIUC) PRRSV nsp1α has a suppressive activity for IFNβ production mediated through the RIG-I pathway. Nsp1α inhibited IκB phosphorylation and NF-κB translocation to the nucleus, causing inhibition of NFκB stimulated gene expression. Nsp1 blocked dsRNA-induced IRF3 and IFN promoter activities through nsp1 degradation of CBP, leading to the block of IFN response. Nsp1 may form a new class of viral antagonists for IFN. Myristoylation of PRRSV E protein is non-essential for PRRSV infectivity, but promotes the growth of the virus.

Enveloped viruses trigger secretion of IFNα by pDC but they remain quiescent when exposed to PRRSV, possibly due to virus-mediated suppression. An augmented phosphorylation of NFκB seen in activated pDC was not only unaffected by PRRSV but actually occurred in its presence. PRRSV may interact with a cell-surface protein(s) to impede signaling cascades involved in IFN-α production by stimulated pDC.

1.7. (UConn) studies on sensitivity to IFNβ and ability to induce type I IFN responses by PRRSV show significant differences in sensitivity to IFNβ among different PRRSV isolates and between MARC cells and PAMs. Field isolates and chimeric viruses were tested for induction of IFNβ in PAMs. Although variable, PRRSV induces IFNβ in PAMs. A flow cytometry assay, using anti-swine IFNβ Abs produced in this laboratory, was developed. swIFNβ was detectable in PAMs and chimeric and field strains of PRRSV induce IFNβ. Extracellular or secreted swIFNβ was detected by indirect ELISA in infected cells. Efforts are currently in identifying segments in the type I IFN pathway that may be blocked by the virus. Expression of TNFα and Mx was tested in PRRSV-infected PAMs. Results show that regardless of the IFNβ levels detected in ELISA, TNFα expression appeared to be blocked early after infection, whereas, Mx expression was detected early, but appeared variable suggesting that blocking of type I IFNβ activity may occur at the signaling phase.

1.8. (Purdue) How PRRSV controls host immune cell responses via Foxp3 expression and AKt pathway is being investigated. Goal: to determine putative gene sets and pathways that predict a pig's ability to clear infection and maintain weight gain. A 2nd study evaluates the host immune response to homologous and heterologous PRRSV challenge by validating the utility of gene sets and pathways for prediction of responsiveness to PRRSV infections in multiple populations.

1.9. (UMN) whole genome sequencing continued on field isolates indicating that recombination occurs in the field. Evolution of type 2 PRRSV was analyzed (UMN, Hong Kong Univ., Univ. of Guelph, Canada).

Research on homologous immune protection in reproductive disease was completed. Virulent virus infection prior to breeding provides solid protection but not complete prevention of reproductive losses and fails to prevent transmission of PRRSV to piglets.

At UMN/OSU, research was initiated on positive prognosticators of protection. Growing pigs were inoculated with a virulent field isolate in 2 studies. Unexpectedly, the virus did not cause clinical disease.

1.10. (UNL-Pattnaik/Osorio). Studied interactions of GP5, 2a, 3 and 4 and with the cellular receptor for PRRSV. Cloned each GP and CD163 receptor in expression vectors and examined their expression and interaction in transfected cells. A strong interaction with GP4 and 5 is seen, weak interactions with other minor envelope GPs and GP5. GP2a and 4 interacted, resulting in formation of multi-protein complex and

interactions with CD163. The carboxy-terminal of CD163 is not required for interactions with GP2a or 4, but is for susceptibility to PRRSV infection in cells. GP4 with GP2a, serves as the viral attachment protein for mediating interactions with CD163 for virus entry.

There is an anti-IFN effect of nsp1β. 4 of 10 nsps had inhibitory effects on βIFN promoter activation. The strongest inhibitory effect was by nsp1, then nsp 2, 11, and 4. Nsp1α, 1β and 11 had strong inhibitory activity, inhibiting dsRNA signaling. Nsp11 inhibited IRF3 and NFκB-dependent gene induction by dsRNA and Sendai virus. dsRNA-induced phosphorylation and nuclear translocation of IRF3 were strongly inhibited by nsp1.

Nsp 3-8 and ORF5 are important for virulence. Single and triple mutants of ORF5 were generated. A change in AA position 64 seemed to contribute most of the virulence. There are certain key AA in ORF5 contributing to PRRSV virulence.

1.11. (OSU), Evaluation of the efficacy of MLV-PRRSV vaccine administered IN to pigs with choleratoxin and OK432 to enhance the anti-PRRSV specific immunity was performed. OK-432 is a killed *Strep. pyogenes* product, is a DC maturation agent and promotes the production of inflammatory cytokines. OK432 upregulated the frequency of NK cells, CTLs, Th/memory and Th cells. The choleratoxin upregulated the frequency of PRRSV specific CTLs and Th/memory cells while both adjuvants upregulated IFNγ, IL-12, and IL-6 in lungs to MN184 challenge compared to mock or only MLV received and challenged pigs. This suggests that higher levels of Th1 and Th2 responses induced by choleratoxin and OK432 are beneficial.

PLGA microspheres were prepared and then killed PRRSV Ags were entrapped. Engulfment of nanoparticles by PAMs was observed and entrapped PRRSV antigens were co-localized in the early endosome compartment of PAMs, indicating these PRRSV antigens are targeted to APCs. *In vivo* studies are in progress.

1.12. (ISU) Findings: PRRSV can persist in pigs without significant viral genetic change.

It was demonstrated that IL-8, IL-1β and IFNγ levels are linked to PRRSV clearance.

1.13. (VA Tech) The effect of an IFN-stimulated response element (ISRE) mutant of PCV2 on PCV2-induced pathological lesions in a PRRSV co-infection model was evaluated. An ISRE-mutant PCV2 was used to infect pigs with either ISRE mutant or wt PCV2 singly or in combination with PRRSV. The ISRE mutation reduced viral replication. Lesions were more severe in pigs coinfected with ISRE-mutant PCV2 and PRRSV than in pigs coinfected with wtPCV2 and PRRSV.

The hypothesis that current PRRS vaccines do not differ from pathogenic strains in the ability to induce Tregs was tested. PRRSV vaccine and parent strain are equally able to induce Tregs in pigs naturally infected with *M. hyopneumoniae* (LeRoith).

Tregs induction in DC from pigs co-infected with PRRSV and PCV2 were studied (LeRoith). DCs were split into 4 groups: PRRSV alone, PCV2 alone, PCV2 plus PRRSV, and controls. The PCV2 infected group induced more Tregs than controls or singly infected groups. In the PCV2 negative pigs, the coinfected group induced significantly more Tregs than controls or individually-infected groups, and the coinfected and PCV2 infected groups had greater Treg induction.

1.14. (SDSU-Wang) Investigation of the molecular mechanism of PI3K/Akt activation mediated by PRRSV and possible link between PI3K/Akt pathway and IFNα during infection of Mo-DC.

(Fang). Identification of 2 products of nsp1 in PRRSV infected cells function as IFN antagonists. Nsp1β significantly inhibited expression from an IFN-stimulated response element promoter after Sendai virus infection or IFN treatment and inhibited nuclear translocation of STAT1. Nsp1β inhibits IFN synthesis and signaling. Nsp1α inhibits IFN synthesis.

(Fang), the cysteine protease domain of PRRSV nsp 2 possesses deubiquitinating and IFN antagonism functions. To determine if the nsp2 protein antagonist function can be ablated from the virus,

point mutations in the OUT domain region were made. The mutations targeting a B-cell epitope in the OTU domain region generated viable recombinant viruses. Certain mutations lethal to virus replication impaired the ability of nsp2 to inhibit NFkB activation. Recombinant viruses didn't inhibit NFkB as effectively as wt virus.

(Fang) Immunodominant epitopes in PRRSV nsp2 are dispensable for replication, but play an important role in modulation of the host immune response.

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

2.1. (VA Tech) found PCV2 ORF3 is dispensable for virus infection but evidence of reduced pathogenicity is limited in pigs infected by an ORF3-null PCV2 mutant. ORF3 of PCV2 reportedly induces apoptosis and is associated with PCV2 pathogenicity. An ORF3-null PCV2 mutant (muPCV2) was created and demonstrated that the dimerized plasmid DNA of muPCV2 clone is infectious in pigs. The pathogenicity of the muPCV2 and the wt PCV2 was compared and pigs inoculated with muPCV2 had delayed seroconversion and lower serum viral load, but no significant differences were seen in the mean scores of histologic or gross lesions or the amount of PCV2-specific antigen in tissues.

2.2. (KSU/BARC & others) participated in the PHGC. Infection and sample collection of approximately 1600 pigs revealed the appearance of stratified subpopulations which possessed wide variations in weight, virus load and growth performance. The 60K SNP chip analysis is started on the first 600 pigs (Dekker).

2.3. (UMN/ISU/SDSU), the role of aerobiological mechanisms in PRRSV transmission was investigated. Biosecurity protocols to reduce airborne spread were validated, specifically, 2 air filtration options (antimicrobial and electrostatic filters).

2.4. (UMN) SIV transmission in naïve and vaccinated pigs was studied. Studies on spread of pH1N1, calculation of the basic reproduction ratio (Ro) based on the outcome of transmission experiments, effect of homologous or heterologous vaccination on transmission and protection was investigated. Vaccination reduced transmission from 100% to 37.5% with heterologous and to 0% with homologous vaccine. Ro was from 3.8-4.6 in controls to ~1 in heterologously vaccinated pigs, 0 in homologously vaccinated pigs.

1155 nasal swabs: 13% SIV PCR+; 46 pens were positive since at least 1 animal was PCR+. Of 105 ropes, 38/105 (36%) were positive by PCR. Nasal swabs and oral fluids were strongly correlated. Most negative oral fluids were from vaccinated pigs. Predicted probability to detect SIV in a pen was 69% if 9% of pigs were infected, 99.67% if 18% were infected, 99.99% if the % of infected pigs was 27-100%.

Disease ecology of SIV in breeding farms showed SIV was not detected in sows/gilts on the study farms, but was detected in neonatal pigs.

Sows were predominantly viremic, shed PCV2 in colostrum, oral fluids. High viral loads seen in the presence of high levels of anti-PCV2 Abs in serum and colostrum; PCV2 was observed on skin and in serum of pre-suckling piglets (~30%). PCV2 infection is persistent in the presence of Abs; piglet infection *in utero* is common.

2.5. (SHVRI-Yuan, Shishan) A molecular survey was conducted of PPV4 in China from 2006-2010. PPV4 is present in swine herds, 2.09% (12/573) in clinical samples and 0.76% (1/132) from healthy animals. None were detected in samples prior to 2009. The Chinese and American PPV4 sequences are closely related. Viral genomes in head-to-tail configuration of various lengths of the non-coding region were detected confirming that PPV4 is a unique, recently discovered virus in pigs. PPV4 is most closely related to bovine parvovirus 2 (BPV2); shares limited ORF1 and 2 identity. PPV4 encodes an ORF3 resembling the *Bocavirus* genus, but shares minimal AA identity with *Bocavirus* genus ORF3 proteins.

First evidence of infection by porcine bocavirus (PBoV) in Chinese swine; was more prevalent in weanling pigs with respiratory signs. Partial VP1/2 genes were highly conserved and only 5 frequent nt mutation positions exist in Chinese PBoV indicating it might be an emerging porcine respiratory virus.

2.6. (UGA) pH1N1 contained the TRIG cassette that predominates in SIV and has infected both swine and avian populations. Exploration of the potential for swine, human and avian influenza viruses to reassort on the TRIG backbone in swine and primary swine epithelial cells, and primary human epithelial cells to elucidate the potential for reassortment in these species.

2.7. (Purdue) Study of TTV infection in commercial wean-to-finish populations concurrently infected with PRRSV, PCV2, and SIV is being investigated. Of 600 oral fluid samples, 120 have been tested, 25/120 (21%) were positive for TTV-1, 97 (80%) were positive for TTV-2; 23/25 TTV-1 positive samples were positive for TTV-2 suggesting TTV infection was common in the 10 commercial wean-to-finish cohorts.

2.8. (UNL/UWI/SDSU) PRRSV CAP2 project: Immunologic Consequences of PRRSV Diversity, understanding what defines PRRSV strains as immunologically homologous or heterologous is critical to the development of vaccines. 1) Sequence a core set of PRRSV representing the breadth of gene variation 2) associate relevant immunologic phenotypes with PRRSV genomic variation. Immunologic cross-protection among a subset of viruses from the core set will be quantified in pigs. Isolates in the core set will be characterized for cross-neutralization *in vitro*. Serum is used to evaluate inter-isolate variation for *in vivo* immune responses.

(UNL) Demonstration of potential T-cell epitopes present in nsp 9, 10 of type 2 PRRSV eliciting IFNγ responses. Characterization of the epitopes is important in modifying CMI. Nsp9 and 10 and were analyzed to determine their T-cell epitopes with proliferation assays and ELISPOT. In 4/78 nsp9 peptides and 2/54 nsp10 peptides were found to induce T-cell proliferation. Only 2 peptides of nsp9 and 2 peptides of nsp10 were detected using ELISPOT. Sequencing of 34 NA strains showed these epitopes were highly conserved, possible use for vaccines to provide cross-protection against PRRSV.

2.9. (ISU) Evaluation of duration of breeding herd PRRS virus-free status and its relationship with measured risk was performed.

Surveyed disease pressures in 26 Midwest herds.
Described reproductive failure associated with PPV and PCV2 coinfection.
Evaluated factors that influence the environmental stability of SIV and PRRSV.
Evaluated PRRSV transmission via ingestion of meat and exposure to aerosols from persistently-infected pigs.
Described ecology of influenza virus in non-porcine species.
Compared detection of endogenous retrovirus viremia in diseased vs healthy pigs by qtPCR.

2.10. (VA Tech) identified 4 distinct full-length genomic sequences of PTTV strains from a single pig. Results showed that these 4 prototype US strains of PTTV represent distinct genotypes or subtypes and a revised classification system for PPTV is proposed.

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

3.1. (KSU/others) are incorporating Luminex for the detection of PRRSV Abs and other pathogens for profiling multiple agents within a herd.

3.2. (SDSU/BARC) An FMIA to simultaneously quantify 8 porcine cytokines (IL-1β, IL-8, IFNα TNFα, IL-10, IL-12, IFNγ, IL-4) in serum using Luminex xMap™ technology was developed. Levels were evaluated in pigs vaccinated with a MLV vaccine or killed virus vaccine with adjuvant and then challenged with a non-identical PRRSV. Studies are ongoing to measure cytokines in PHGC sera to identify biomarkers that may predict which pigs are resistant to PRRSV. US Veterinary Immune Reagent Network produced additional mAbs for FMIAs. FMIA assays to detect Ags and Abs to multiple swine pathogens simultaneously (eg. PRRSV, PCV2, SIV) is ongoing.

3.3. Studies on viruses are hampered by lack of *in vitro* systems for propagation and amplification. SHVRI (Yuan) have devised an alternative culturing system by recombining the PRRSV infectious cDNA into a baculovirus vector for generation of infectious virus particles by expression of the full-length cloned genome from the modified baculovirus vector. The recombinant baculovirus, AcAPRRS, was used to infect sf9 cells and IFA demonstrated the presence of nsp2 and N protein. EM showed PRRSV particles. Infectious particles were produced in MARC cells inoculated with AcAPRRS, and growth characteristics were similar to the parental strain. Infectious PRRSV was generated following AcAPRRS transduction of BHK-21 cells and Vero cells that are not sensitive to PRRSV.

3.4. (NADC-Faaberg), developed and tested the ability to detect and sequence Asian porcine high fever disease strain transcript RNA.

Validation of a PRV rtPCR assay (Miller/Lager/Zanella).

3.5. (UIUC) Goal: to associate immunologic parameters (SN, T-cell responses) with specific genomic signatures in PRRSV. A novel method of sequencing library construction was developed which allows unbiased sequencing of full-length genomes using pyrosequencing. Full genome sequencing of 8 serologic groups was performed.

3.6. (UGA) the potential for prophylactic and therapeutic application of PRRSV-specific swine mAbs is studied along with its cost-effectiveness.

A human virus-like particle vaccine was tested in swine for induction of protective Abs. Both IM and aerosol delivery were tested. While the H1N1-specific vaccine was immunogenic, it failed to induce a protective response. A PIV5-vectored vaccine is being tested for immunogenicity with SIV antigens. If efficacy is observed with this vector against SIV, PRRSV antigens will be tested with future PIV5 constructs.

Cross-validating human and mouse Abs for reactivity to swine and ferret cells and cytokines using a multiplex assay is studied.

Establishing primary normal swine bronchoepithelial cell cultures has been done to directly measure innate responses to swine respiratory virus infection

3.7. (UMN) The sensitivity of PRRSv detection by PCR from serum and oral fluids embedded in FTA cards stored for 14 days was 101 $TCID_{50}$/ml. Sensitivity and specificity was equal in fresh serum and serum on FTA cards, independent of storage time (overnight or 2 weeks) or temperature (4ºC or 25ºC).

Regional PRRSV eradication studies in Stevens Co, MN consists of 87 farms. The approx. number of sows is 17,844, with 16,700 sows owned by 5 entities. Surveillance has decreased since most, and possibly all farms have eliminated PRRSV. A risk-based surveillance system was developed. PRRS appears under control in the Co. and has now expanded.

3.8. (UNL-Ciobanu/Osorio/Johnson), studies found that host genetic variation influences the incidence of PCVAD. Obj 1) Detect regions of the genome that affect PCVAD severity and harbor key modulators of the changes in gene expression following infection. 2) Identify genes, pathways and combination of allelic variants that influence PCVAD severity. Hamps x Duroc pigs experimentally infected with PRRSV

gained less weight, had higher rectal temperature, viral loads, Ab titer, and incidence of lung lesions compared to NE Index Line. High pre-inoculation levels of IL8 and low post-inoculation levels of IFNγ were significantly associated with potential resistance to PRRSV. There was variation in magnitude and time of immune response after PCV2b challenge. Individuals displaying early or no immune response were less affected by inoculation. The lack of immune response was associated with reduced viremia, likely due to a mechanism that inhibits virus replication. There were moderate to high heritabilities for viremia and IgG in pigs naturally infected with PCV2. Viral load affected growth. In natural infection, viremia was highly genetically correlated with a PCVAD score.

3.9. (UMD-Zhang, Zhu), studies continued on developing anti-PRRSV PPMOs which are ssDNA analogs that exhibit highly specific binding to complementary RNA. A PPMO (5UP2) designed to complement sequence in the 5' region of the genome was effective in inhibiting PRRSV replication in cell culture. PPMO was given to 3 wk old piglets IN at 24 hrs before, and 2 and 24 hrs after PRRSV inoculation. Pigs given PPMO had significantly milder pneumonia, reduced viremia at 6 dpi; at 14 dpi, had a lower level of anti-PRRSV Abs than controls. All pigs had similar weight gain.

3.10. (CNB), animal studies using a TGEV vector expressing PRRSV M protein and GP5 mutant (altered glycosylation) showed all animals had high Abs against TGEV. After virulent PRRSV, there was a fast recall response; vaccinated animals had higher Abs, lung lesions in vaccinated animals were low. No full protection possibly due to low levels of neutralizing Abs prior to challenge.

A rTGEV vector was constructed, expressing PRRSV GP2a, GP3 and GP4. This rTGEV vector was not stable as GP3 gene was lost. rTGEV vectors expressing PRRSV Ags were not fully stable. Loss of PRRSV Ag expression could be the cause for the modest results obtained in protection experiments using live rTGEV vectors compared to killed vaccines. Therefore, as PRRSV M protein is fully stable when cloned in rTGEV vectors, it has been the base for vectors co-expressing this protein and different small GP5 domains containing the neutralizing epitope (GP5-NH_2). Eventually, this strategy would allow the elimination of GP5 domains providing T cell negative regulatory signals that may reduce the strength of the immune response to PRRSV antigenic domains involved in protection. The construction of rTGEV expressing GP5-NH_2 and M proteins (rTGEV-GP5-NH_2 -M) is planned.

Abs to detect GP5-NH_2 expression are not available, so an HA tag will be introduced. Recombinant TGEV viruses co-expressing GP5 fragments and M have been obtained.

Abs recognizing PRRSV GP2, GP3 and GP4 are not available. Each gene was cloned and high levels of each protein have been generated and purified. Polyclonal Ab generation is in progress.

3.11. (UWI) Novel approaches are used to identify a small number of representative viral genotypes from among a diversity of sequences. Techniques from network theory were adapted to rank PRRSV sequences in their importance among sequences (>10,000 in the literature). and these methods were applied to a highly-curated PRRSV database that combines high-quality, non-recombinant sequences from GenBank and PRRSVdb. Viruses represented by the top ranking sequences are valuable for study and can be incorporated into a polyvalent vaccine. Dr. Tavis Anderson, has performed analysis with support of an exchange program with the Univ, of Torino, Italy.

3.12. (ISU) evaluated Ab responses of nsps for detection and differentiation of Type 1 and 2 PRRSV.

Compared efficacy of PRRSV extraction and PCR for oral fluids showing PCR detection in oral fluids and serum over time is similar.

Evaluated PRRSV stability and anti-PRRSV Ab in oral fluids, determined samples should be chilled or frozen.

Described performance of a nucleoprotein ELISA for Ab detection and an ELISA in pigs using a commercial avian influenza epitope blocking ELISA.

Developed a matrix-gene based multiplex PCR for detection and differentiation of p H1N1 and other influenza A viruses in NA.

Showed that Mchip differentiates human H1N1, NA swine H1N1, and pH1N1.

Developed PCR for simultaneous detection of PCV2, PPV, PRV, and PRRSV.

Showed prolonged detection of PCV2 and anti-PCV2 Ab in oral fluids.

Developed an SN for Nipah Virus using pseudotype particles.

Compared efficacy of commercial PCV2 vaccines using a mixed PRRSV-PCV2-SIV infection.

Compared efficacy of passive vs active vaccination against PCV2 and impact of passive Abs on vaccination.

Compared PCV2 vaccines on challenge with PCV2, PRRSV and PPV.

Presented terminology for classifying swine herds by PRRSV status.

Reviewed veterinary vaccines for Henipaviruses in the OIE Manual.

3.13. (VA Tech) developed a SYBR green rtPCR and duplex nPCR assay for quantitation and differential detection of porcine TTV and 2 SYBR green-based PCR assays to quantify viral loads and differentiate 2 porcine TTV species (PTTV1 and PTTV2). A type-specific duplex nPCR was developed to simultaneously detect and distinguish between PTTV1a and 1b.

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

1. Genetic analysis of host response has revealed the diverse negative impacts of PRRSV on a population. Decreased performance is a loss to the producer's "bottom line". New IFN genes that possess potent anti-PRRSV can be incorporated into vaccines and other antiviral therapies.

2. 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 from 8 trials of 200 commercial pigs infected with PRRSV and followed for 42 days have affirmed that all pigs become PRRSV infected but pigs clear virus from serum at different rates and weight effects are variable. Genome wide association studies (GWAS) have mapped pig PRRS responses (viral load and weight gain during infection) to multiple swine chromosomes. Overall, the PHGC project will enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection.

3. Continuing development of Luminex system provides the means to 1) detect multiple antigens or Abs to multiple agents in a single small volume of sample, 2) increase sensitivity and specificity, 4) reduce the cost of testing, and 5) semi-quantitative output without need for serial dilution of a sample to an endpoint, and 5) test for agents in non-serum samples, such as oral fluids and meat juice. A fluorescent microsphere immunoassay (FMIA) has been developed to simultaneously quantify 8 porcine cytokines in serum using Luminex xMap™ technology. It has been optimized to detect innate (IL-1β, IL-8, IFNα, TNFα); regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN-g) and Th2 (IL-4) cytokines. The assay has been tested for comparative vaccine studies and shown that higher serum levels of IL-12 are not predictors of effective vaccine responses. This assay will be a useful tool to determine cytokine involved in genetic resistance to PRRSV and immune responses to other swine pathogens.

4, Gene expression in lymph nodes of PRRSV-infected pigs (USDA-ARS-NADC, Laura Miller, Greg Harhay, Kelly Lager) provides identification of 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. The proposed research is part of an overall plan to evaluate the pig's response to PRRSV

infection at the gene expression level. This research is part of two concurrent lines of research at NADC designed to understand how the virus functions, and how the pig responds to a PRRSV infection. Gaining insight into how the virus causes disease may aid development of more cross-protective vaccines that would certainly lead to the production of healthier swine. If more efficacious vaccines were available, then they may lead to strategies to eliminate PRRSV from U.S. swine, a feat that would provide long-term economic impact.

5. Evaluation of viral, bacteriological and host responses after infection of swine with different strains of PRRSV (USDA-ARS-NADC, Faaberg, Miller, Lager, Brockmeier, Loving, Kehrli) provides examination of several PRRSV strains including the growth of nsp2 deletion mutants in swine, whether the nsp2 deletions can serve as vaccines to improve responses to challenge strains, comparative virulence properties of newer Type 2 U.S. isolates, and high-dose inoculation of recombinant Asian porcine high fever disease strains of PRRSV in comparison with U.S. strain VR-2332. These studies 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 can then better understand what factors are at play during high virulence infections vs. low virulence infections. With this knowledge, we can then develop better vaccines and vaccination strategies.

6. Identification of Type I Interferon Antagonists of PRRSV (National Pork Board, Faaberg, Miller, Visiting Scientist Baoqing Guo) developed a cell culture bioassay to examine the ability of PRRSV to inhibit type 1 interferon, enabling analyses related directly to PRRSV and not to an engineered expression system. He has also cloned all genes of strain MN184 using a vector that allows abundant expression of each protein in transfected MARC-145 cells, to understand the virulence of that strain. This work will enable a better understanding of the inhibitory actions of different field isolates, chimeric and deletion mutant viruses.

7. Sequencing, Cloning and Characterization of a 2007 Vietnam PRRSV isolate (National Pork Board, Faaberg). Provided purified RNA from a Vietnamese isolate which was transferred to the Faaberg laboratory for characterization. They cloned ORF4-3'end into bacteria, and produced in vitro derived RNA transcripts. We then forwarded blinded serial dilutions of the Vietnamese RNA along with VR-2332 RNA for analyses in major U.S. veterinary diagnostic laboratories. Each laboratory was able to detect both viruses at 100 copies/μl, some were able to detect both viruses at 10 copies/μl. All laboratories were able to derive the sequence of ORF5 and the correct RFLP pattern was reported.

8. At Univ. of IL, 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 suggest that PRRSV Nsp1α is a multifunctional nuclear protein participating in the modulation of the host IFN system.

9. PRRSV is the first arterivirus demonstrated to repress the ability of pDC to mount an type I IFN and pro-inflammatory cytokine response to itself as well as to a known stimulatory virus, TGEV, and a TLR9 agonist, ODN D19. As this functional impediment could have important ramifications regarding the host's innate immune responses to infection (Univ. of IL).

10. Blocking against virus-mediated inhibition of the innate immune response may lead to the future development of effective vaccines (Univ. of IL).

11. Analysis of full-length PRRSV genomes sequences will help identify regions of immunologic significance in the PRRSV genome (Univ. of IL).

12. 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 them directly to PRRSV projects (UGA).

13. Using freshly isolated PBMC infected with PRRSV p129 strain it was determined that an imbalanced host immune responses was associated with down regulation of AKt pathway and induction of Foxp3 T regulatory cells which contributed in part by weak and/or premature termination of T cell receptor (TCR) signaling (Purdue).

14. Effectiveness of homologous PRRSV vaccination was demonstrated; however for heterologous PRRSV challenge, early activation of T-reg and Th2 type immune responses may be a mechanism for increased PRRSV pathogenicity (Purdue).

15. For a TTV virus study: TT virus was detected in commercial herds and detected the virus in oral fluids by PCR and sequence (Purdue).

16. Through collaborative research accomplishments in the study of aerobiology, UMN now understands the risk factors involved in the area spread of PRRSV that give stakeholders positive information to help prevent PRRS (UMN)

17. UMN documented the climactic conditions that impact the aerobiology of PRRSV and *M. hyopneumoniae*, giving producers information to modulate biosecurity procedures based on likelihood of airborne transmission (UMN).

18. UMN now has the ability to develop science-based biosecurity plans for preventing the spread of PRRSV between herds, an obvious high value contribution to the swine industry (UMN).

19. Aerobiology and biosecurity advancements provide a scientific foundation for economical prevention of airborne spread of respiratory disease in swine herds (UMN).

20. Studies on protection against high consequence influenza virus infections, shows that current vaccines can provide heterologous protection that reduces the impact of new biotypes (UMN).

21. PCV2 transmission in utero and after farrowing help producers understand that PCV2 vaccination occurs in piglets that already are infected with PCV2 (UMN).

22. FTA cards are a valuable tool for the transport of PRRSV infected samples, however sensitivity of PCR in field samples is lower than sensitivity obtained from testing fresh samples (UMN).

23. Preliminary results of this study highlight the role of the neonatal pig in the circulation of influenza virus in swine breeding farms, thus may be critical for the continued circulation of virus in breeding farms and for the spread of influenza virus through the movement of weaned pigs (UMN).

24. 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. This patent is being explored now by a veterinary biologics company

25. A new provisional claim for invention was filed:"Method to Develop a Differential Live Vaccine Against PRRSV " (inventors Osorio, FA, Pattnaik, AK, De lima, M and Vu Hiep) claim filed on Oct 27 2010, EFS ID:8715785, Application Number:61407278

26. Studies on PRRSV inhibition of interferon signaling showed that virulent VR2385 and vaccine strain MLV have variable effects, which has a biological relevance on PRRS vaccine design. Our studies of PPMOs have demonstrated that a PPMO inhibits PRRSV replication and reduces disease development in piglets. Specific antiviral PPMOs can complement other approaches for PRRS prevention and control (UMD).

27. 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 (UWI).

28. Our study confirmed the protective immune responses against PRRSV challenge induced by adjuvants, Choleratoxin and OK-432. *In vitro* analyses of killed PRRSV antigen entrapped nanoparticles indicated that *in vivo* delivery of this inactivated vaccine has the potential to elicit protective anti-PRRSV immunity. We have made significant progress in the area of mucosal vaccine development. NPB funded our study for the second year on development of mucosal vaccine to PRRSV (OSU).

29. Research advances over the last year continue to expand our understanding of PRRSV and emerging viral diseases of swine e.g., influenza virus, Nipah virus, PCV2, porcine parvovirus, pseudorabies virus, epidemiology, pathobiology and provide new ideas for preventing, countering and/or eliminating these infections. Extensive work has been done regarding the emergence of genetic and antigenic variation during replication in pigs and its role in persistence. Continued assessment and research in diagnostic technology contributes to the improvement and refinement of our ability to detect and diagnose PRRSV infection. 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 eventual elimination and eradication of PRRSV and other viral infections from individual farms and regions (ISU).

30. The establishment of an improved reverse genetic system and the identification of a porcine monocytic cell line supporting PRRSV replication will aid future studies of host-virus interaction of PRRSV. The demonstration of a potential role of ISRE element in PCV2 in viral pathogenesis and the exchangeable replication factors between PCV1 and PCV2 will aid the future vaccine development efforts. The identification of multiple novel PTTV strains with distinct genotypes or subtypes in a single pig in the U.S. pave the way for future disease characterization (VA Tech).

31. The PRRSV genes and mechanisms that modulate the host immune responses are important to investigate for the development of 2nd generation vaccines and/or anti-virals that could inhibit PRRSV replication. In our studies, it was determined that two auto-cleavage products of PRRSV nonstructural protein 1 (nsp1) in PRRSV infected cells function as interferon antagonists. In addition, the cysteine protease domain of PRRSV nonstructural protein 2 (nsp2) possesses deubiquitinating and interferon antagonism functions. Mechanistically, no detectable IFN-α was found in the supernatants of PRRSV-infected Mo-DC. PRRSV activates PI3K dependent Akt (PI3K/Akt) during early infection and inhibits PI3K/Akt during late infection. Currently, the molecular mechanism of PI3K/Akt activation mediated by PRRSV and the possible link between PI3K/Akt pathway and interferon-α during PRRSV infection of Mo-DC are being investigated (SDSU).

32. To evaluate the immune responses of PRRSV infection and for vaccine design, a swine specific multiplex assay to detect 8 cytokines simultaneously was developed (SDSU).

D. PRRS PUBLICATIONS ISSUED OR "IN PRESS"

1) Refereed publications [50000 w spaces = limit]

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Zimmerman J, Holtkamp DJ, O'Connor A. 08/01/09 to 05/31/11. Identifying ecologic and epidemiologic factors in the control of PRRS: A field-based approach. 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) - $240,232.

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F. WORK PLANNED FOR NEXT YEAR

1. Continue to perform work in the areas of innate immunity, diagnostics and host genetic resistance (KSU).

2. New assays (cytokine FMIA, microarray and RNAseq analyses) will be used 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. Further evaluation and collaborative utilization of swine cytokine multiplex assays will be performed for detection of cytokines in oral fluids and other substrates. Additionally, 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 (USDA-BARC).

3. Develop and test vaccination strategies based on recombinant virus

- Develop and test adenovirus constructs expressing novel PRRSV genes
- Assess viral and immune response characteristics to rPRRSV *in vivo*
- Assess viral and immune response of PRRSV vaccine candidates that have a reduced capacity for the induction of polyclonal B-cell activation in vivo.
- Transcript expression analysis of tracheobronchial lymph nodes of PRRSV/PRV/SIV/PCV-2 infected pigs.
- Investigate clinical and pathological repercussions of infection with Asian porcine high fever disease strains of PRRSV
- Investigate bacterial component of Asian porcine high fever disease strains of PRRSV (NADC)

4. Continue the research on the influence of PRRS virus on the innate immune response of swine as well as the immunological consequences of PRRSV diversity. However due to the very limited and restrictive availability of competitive funds to perform PRRS virus research these activities are likely to stop fairly soon once the funds are exhausted. (UIUC)

5. Efforts to test novel vaccine vectors for induction of PRRSV-specific immunity are still planned, currently constructs are tested for immunogenicity in other models. Work continues with primary cell lines and testing of swine reagents for measuring innate immune responses to viral infection (UGA).

6. The work is now concentrated on investigating the signaling phase of type I IFN pathway in the context of PRRSV infection. This is planned in order to identify potential mechanisms used by the virus to block or evade the innate immune response. (Objective 1) (UConn).

7. Host-virus interaction is being studied for PRRSV using PMBC samples and microarray/RT-PCR analysis (Purdue).

8. Air filtration will be evaluated as a means to reduce PRRSV infection in large breeding herds in swine dense regions. This project has expanded from 5 filtered (treatment) herds and 26 non-filtered (control) herds in year 1 to 21 treatments and 16 controls in year 3. In conjunction with this study, 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 will be conducted.

Evaluation of the ability of UV light to inactivate PRRSV on common farm surfaces and fomites and re-evaluate the shedding of virus in feces and the associated biosecurity measures for manure handling procedures on farms will be conducted.

Studies to elucidate influenza ecology, to further understand transmission of influenza including aerosol transmission in commercial pigs, and to evaluate the effect of vaccination on flu virus change will be continued.

Evolution of PRRSV will be expanded to include additional swine growing regions of Canada.

Immunology of PRRSV and basic investigations of PRRSV structure relevant to pathogenesis and immunity will be continued (UMN).

9.

a) Continue research on virulence markers of PRRSV (collaborating agencies: Nebraska, Illinois)
b) 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, and ISU)
c) Continue research on induction of protective immunity of PRRSV(collaborating agencies: Nebraska, Illinois, South Dakota, and ISU)
d) Seek funding (NIFA AFRI-USDA) and continue research on characterization of the action of viral proteins that influence innate immunity X PRRSV (collaborating agencies: Nebraska, Illinois, Cleveland Clinic)
e) Seek funding (NIFA AFRI-USDA) and continue research on characterization of the action of viral proteins that influence innate immunity X PRRSV (collaborating agencies: Nebraska, Illinois, Cleveland Clinic)
f) Seek funding (NPB, AFRI-USDA) and continue research on genetic control of PCAVD in pigs (collaborating agencies: Nebraska, ISU)
g) 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)

10. The PRRS virus-cell interactions will be investigated. (UMD).

11. Analysis of the stability and expression levels of rTGEVs expressing GP5-NH_2 fragments and M protein. To analyze the rTGEV improvement, the stability of GP5 domain expression, and GP5 fragment-M heterodimer formation will be monitored. If GP5 fragment is still toxic for rTGEV system, a systematic mutation approach will be developed. A collection of point mutants in each aa of GP5 fragment will be generated synthetically. These mutant fragments will be subsequently introduced in the rTGEV system and analysis of stability of GP5 expression will be performed. (CNB)

12. Over the next year, the analysis of PRRSV diversity will be completed, and final sequence rankings will be generated to inform future studies on PRRSV, and especially the development of effective polyvalent vaccines. The curated database will be made publically available, which should be of high value for any researchers wishing to examine PRRSV genetic diversity and evolution. (UWI)

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

1. Functional genomics of PRRSV
    a. Continue to study establishment of broader cross protection for PRRSV using chimeric virus technology.
    b. Continue to study PRRS viral virulence and corresponding genetic markers in search of a way to develop next-generation MLV vaccines.

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

1. Evolutionary biology of PRRSV
    a. Recombination and impact on the pathogenesis and immunobiology

2. Survival analysis for PRRS seroconversion – within herd transmission

3. Influenza ecology
    a. Study the role of small mammals in transmission of influenza virus at interface between domestic and wildlife animals
    b. Study cross-species transmission

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

1. Detection
    a. Optimization and evaluation of the HerdCheck* PRRS X3 antibody test kit for use with oral fluids
    b. Development of nanoliter PCR-based panel test for simultaneous detection of swine pathogens in various clinical specimens including oral fluid
    c. Compare commercial PCR assays for the detection of PRRSV.
    d. Evaluate swine influenza virus strain-specific antigenic epitopes that might enable development of serological assays for pandemic H1N1 surveillance in swine; and the evaluation of selected antigenic epitopes and genetic factors of swine influenza virus that may lead to heterologous immunity elicited by influenza vaccines to enable development of improved vaccines with broader protective immunity against novel merging influenza strains.

2. Prevention
    a. Develop specific vaccination strategies for FMDV and CSFV
    b. Develop (CFSPH, NPB, AASV) a set of educational materials related to vesicular diseases in swine to distribute to swine producers and veterinarians

3. Control
    a. Develop a repeatable model for area PRRS virus control and elimination projects in areas with significant pig movements, enhancing reporting capabilities in Production Animal Risk Assessment Program (PADRAP) Online.
    b. Develop (CFSPH), produce and distribute: a DVD (with printed resources) on oral fluids in swine; educational pieces for producers and veterinarians on SIV surveillance; technical educational pieces with in-depth information on SIV and SIV surveillance; PowerPoints for program promotion; and web-based for NVAP. (ISU).

14. The extended adjuvanticity of Choleratoxin and OK432 in PRRSV challenge studies will be evaluated. Detailed immune cells and cytokine analysis will be performed and the viral load in nanoparticles based vaccine immunized and PRRSV challenged pigs will be determined. In collaboration with Dr. Murtaugh (PI, PRRS CAP2), the effect of PRRSV vaccine intervention on innate immune parameters in naïve finishing herd to PRRSV outbreaks will be investigated. The field based work will continue to determine the immune correlates of protection in the pig herd. (OSU)

15. Elucidate the mechanisms of host-pathogen(s) interactions**:** Strategy to appropriately manipulate PRRSV-encoded antagonists of the IFN system and virulence factors for the development of a new generation of modified live vaccines are being developed.

Diagnostic test development: our research is directed applying our technology to other swine pathogens, including PCV2, SIV, M. hyopneumoniae. Multiplex antigen and antibody detection assays will be developed using various clinical samples (serum, oral fluids).

Investigate the molecular mechanism of PI3K/Akt activation mediated by PRRSV and the possible link between PI3K/Akt pathway and interferon-α during PRRSV infection of Mo-DC. (SDSU)