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

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Minutes NC229 Meeting Chicago, IL. 12/08/2012

The meeting started at 1 PM. Reports were given by Dr. David Benfield (Administrative Advisor) and Dr. Peter Johnson (updates from USDA via teleconference). Dr. KJ Yoon (ISU) was elected the next Secretary for NC229. Additional presentations on the next 5 year grant were given by Dr. Fernando Osorio (UNL), Dr. Federico Zuckermann (UIUC) and Dr. Aradya Gourapura (OSU) for the 1st objective on PRRSV and for the 2nd objective on emerging viral diseases of swine presentations were given by Dr. KJ Yoon (ISU) (discussion on PED); Dr. Dan Rock (UIUC) on ASF and Dr. Amy Vincent (NADC). Meeting was adjourned by 5:30 PM.

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. (UCONN ,Risatti): We have identified swine macrophage proteins that interact with PRRSV NSP3 using a Yeast Two-Hybrid screening system. We focused on the interaction of NSP3 with host cell protein FKBP38 a FK506 binding protein associated with cellular processes. PRRSV has mechanisms to prevent host cell apoptosis likely mediated by PRRSV NSPs.

2. (UCONN: Garmendia) The aims of the study are to determine the sensitivity to and induction of IFNβ by PRRSV, to identify mechanisms of evasion of host's innate immune responses and determine correlations with virulence. We have shown significant differences among different PRRSV in sensitivity to IFNβ. Chimeric PRRSV induce variable synthesis bioactive IFNβ. Anti-swine IFNβ mAbs were developed and will now be used to test samples obtained from the swine experiment. Results from a recent vaccination and challenge showed that challenge exposure of pigs to PRRSV results in induction of IFN β in BAL fluids, regardless of their vaccination status.

3. (SDSU, X. Wang), Protein kinase R (PKR) is involved in anti-viral activities in response to many virus infections. Several recent studies, suggest the pro-viral properties of PKR, which may or may not be dependent on the catalytic activity of PKR. To reveal the role of PKR in the replication of PRRSV, we first examined the kinetics of PKR activation during infection. Results showed that PRRSV transiently activates PKR during 12- 24 h PI. eIF-2α, one of the downstream targets of PKR, was only significantly phosphorylated compared to mock-infected cells at late time points of infection. A reduced viral protein synthesis and virus titer were detected in cells transfected with PKR silencing RNA prior to infection, indicating the role of PKR in facilitating virus replication. This is further confirmed by the reduced virus titer in cells treated with a PKR specific inhibitor. Experiments are ongoing to verify these observations by using cells overexpressing PKR.

4. (Purdue + PHGC) The PRRS Host Genetics Consortium (PHGC) studies are aimed at identifying genes and pathways that are associated with pigs that clear PRRSV 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 four 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). All RNA samples were converted to cDNA and subjected to real time PCR using primers corresponding to markers

important for immune system activations involved in Th1, Th2, and immunological tolerance pathways.

5. (UMD) During the past year, we continued the studies to determine the mechanism of PRRSV interference with IFN-activated JAK/STAT pathway. We found that PRRSV nsp1β blocks STAT1/STAT2 nuclear translocation by interfering with their interaction with karyopherin-α1 (KPNA1 or importin-α5). KPNA1 is a key molecule in facilitating nuclear transportation of IFN-stimulated STAT1/STAT2/IRF9 heterotrimers. A nucleotide substitution resulting in an AA change of nsp1β at residue 19 from valine to isoleucine diminished its ability to induce KPNA1 degradation and to inhibit IFN-mediated signaling. Infection of MARC-145 cells by PRRSV also resulted in KPNA1 reduction, but an avirulent strain Ingelvac PRRS MLV did not. These results indicate that nsp1β blocks JAK/STAT pathway via inducing KPNA1 degradation and that the valine-19 in nsp1β correlates with the inhibition.

6. (UMD) We examined the interference of IFN-activated signaling by PRRSV viral proteins and compare the effects of several PRRSV strains. Among eleven non-structural proteins (nsps) and eight structural proteins of VR-2385, three nsps (1β, 7 and 12) and two structural proteins (GP3 and N) were found to significantly inhibit the expression of IFN-stimulated response element (ISRE) luciferase reporter. In MARC-145 cells, all the six PRRSV strains with the exception of MN184, blocked the activity of exogenous IFN-α. In primary porcine pulmonary alveolar macrophages (PAMs), all the six strains with the exception of MLV and NVSL inhibited the activity of IFN-α.

7. (UMD) Elevation of proinflammatory cytokines is thought to contribute to PRRSV pathogenesis. We found that PRRSV VR-2385 induces phosphorylation of signal transducer and activator of transcription 1 (STAT1) at serine 727 (pSTAT1-S727) in MARC-145 and PAM cells, which was interferon-independent. IngelVac PRRS MLV strain had a minimal effect on pSTAT1-S727. Compared to MLV-infected cells, VR-2385 infection caused significantly higher level of expression of proinflammatory cytokines, including interleukin 1 beta (IL-1beta) and IL-8.

8. (NADC, Lager) Conducted animal experiment to develop a PRRSV pathogenesis matrix

9. (KSU, Rowland, Sang) are performing a study characterizing the expression of interferon genes and cytokine proteins in the PRRSV-infected fetus.

10. (KSU, Wyatt, Ewen, Wilkerson, Rowland) are characterizing a newly discovered SCID pig as a model for understanding PRRSV immunity and pathogenesis.

11. (KSU, Sang) is performing an analysis of type 1 and type 2 macrophages in PRRSV immunity.

12. (KSU, Rowland and several outside collaborators) continue to work on marker on SSC4 linked to increased weight gain and reduced virus load during PRRSV infection.

13. (KSU, Rowland) performing an analysis of broadly neutralizing antibody.

14. (KSU, Hesse) investigated the response of pigs to PEDV infection

15. (KSU, Rowland and Prather (MU)) tested C169 knockout pigs for PRRSV infection.

16. (USDA-BARC) The PRRS Host Genetics Consortium (PHGC) was developed to determine the role of host genetics in resistance to PRRS and effects on pig health and growth. Pig resistance/susceptibility to PRRS was assessed. All pigs became PRRSV infected but some pigs cleared virus quicker with variable weight effects. Pig DNA was genotyped. Multivariate analyses of viral load and weight data identified PHGC pigs in different virus/weight groups. Ongoing serum cytokine and gene expression studies will compare PRRS resistant/maximal growth pigs to PRRS susceptible/reduced growth pigs.

17. (USDA-BARC) Genome wide association studies have identified genetic regions associated with resistance/susceptibility to primary PRRSV infection. Whole genome analyses focused on viral load (VL) and weight gain (WG). We identified a 38-SNP region on swine chromosome 4 (SSC4) that explained 14.6% and 9.1% of the genetic variance for VL and WG, respectively. The SSC4 marker may be useful for genetic selection of pigs for increased resistance or reduced susceptibility to PRRSV isolates that differ genetically and possibly pathogenically.

18. (USDA-BARC) Evaluation of differences in gene expression of whole blood RNA from PHGC pigs revealed a range of responses to PRRSV. RNA was extracted from blood from14 pigs at 7 time-points. An average of 58M high quality reads/sample was obtained and approx. 87% could be aligned to the pig reference genome (Sus scrofa 10.2). Additional analyses will decipher genetic mechanisms controlling host response to PRRSV infection.

19. (USDA-BARC) Swine genome studies have expanded our knowledge of genes involved in immune and disease responses. The Immune Response Annotation Group used computational curation and manual annotation of the swine genome assembly 10.2 (Sscrofa10.2) to refine the currently available automated annotation of 1,369 immunity-related genes through sequence-based comparison to genes in other species. Extensive annotation dramatically extends the genome-based knowledge of the molecular genetics and structure of a major portion of the porcine immunome. This phylogenetic analysis of the core immunome cluster confirms rapid evolutionary changes and such immune genes are important components of the pig's adaptation to pathogen challenge over time. Current efforts are aimed at using high-density SNP panels to infer MHC haplotypes to identify exact genetic alleles controlling anti-PRRS responses. These analyses should provide important tools for global analyses and data-mining of the porcine immune response.

20. (CAU) A series of full-length infectious cDNA clones with exchanged regions between highly virulent RvJXwn and phylogenetic close low-virulent RvHB-1/3.9 were constructed, and then the replication and pathogenicity of rescued chimeric virus were systematically compared. The results suggested that the Nsp9 and Nsp10 together contribute to the increased fatal virulence of HP-PRRSV emerging in China.

21. (CAU) The HP-PRRSV JXwn06 and low virulent HB-1/3.9 were confirmed to have distinct ability of TNF-α induction. By comparing the capability of all NSPs from these 2 different strains on inhibiting ERK signal pathway, we found that the HP-PRRSV could inhibit TNF-α through its Nsp1β and Nsp11, which may result in the increased virulence for piglets.

22. (UMN) Developed an experimental infectious disease model for the rapidly emerging new viral disease of swine, PED. 10-day-old pigs were used and Koch's postulates were fulfilled. The model is very sensitive to detect live virus and is currently used to assess infectivity of research samples.

23. (UMN) Whole genome sequencing of virulent field viruses was performed to evaluate potential genetic changes characteristic of novel strains associated with seasonal PRRS. Research was performed to analyze genetic variation in the population of PRRSV produced from permissive cells. MN developed a sequencing technique for PEDV based on the S gene, and applied it to farms. Variation among NA samples is very limited. The first whole genome of a PEDV detected in NA was sequenced. Several other whole-genome sequences are being generated.

24. (UMN) Studies investigated the NAb response in sows from herds exposed to virulent PRRSV. Research was performed to determine the role of plasmacytoid dendritic cells in anti-PRRSV host response. Effect of host age on macrophage permissiveness to PRRSV infection was examined.

25. (UIUC, Yoo Lab). Mutations that destroyed the PCPα activities (C76S, H146Y, and C76S/H146Y) in nsp1α did not affect the IFN suppressive activity of nsp1α, indicating that the cysteine protease activity did not participate in IFN suppression. The mutations of C70S, C76S, H146Y, and/or M180I, which coordinated the ZF2 motif, did not alter IFN suppression. The mutations of C8S, C10S, C25S, and/or C28S for the ZF1 motif impaired the IFN antagonism of nsp1α, showing that ZF1 was the essential element of nsp1α for IFN suppression. Wild-type nsp1α localized in the both nucleus and cytoplasm, but the ZF1 mutants that lost the IFN suppressive activity did not localize in the nucleus and remained in the cytoplasm.

26. (UIUC, Yoo Lab). Bayesian phylogeographic analyses of 7040 ORF5 sequences were used to reveal the recent geographical spread of Type2 PRRSV in NA.

27. (UIUC Yoo Lab). To discover the impact PRRSV infections on the cellular miRNAome, small RNA expression profiles were developed from PRRSV-infected swine alveolar macrophages *in vitro* using deep sequencing. A total of 40 cellular miRNAs were significantly differentially expressed within the first 48 hpi. Six miRNA, *miR-30a-3p, miR-132, miR-27b*, miR-29b, miR-146a* and *miR-9-2*, were altered at more than one time point. The most highly repressed miRNA at 24 hpi was *miR-147*. A *miR-147* mimic was utilized to maintain *miR-147* levels in PRRSV-infected SAMs.

28. (UIUC, Zuckermann/ Rock). A highly pathogenic PRRSV with a ORF5 1-22-2 RFLP was isolated in the porcine alveolar macrophage cell line ZMAC from a sow farm with 100% pre-wean mortality. A virus stock of the isolated virus (LTX1) was prepared after 1 passage in ZMAC cells. Inoculation of pigs with the LTX1 resulted in viremia with similar kinetics and viral load as those observed after inoculation with the "atypical PRRS" strain. The average viral load in the bronchoalveolar lavage collected at 14 DPC with LTX1 was 44-fold higher compared to that in pigs receiving the atypical virus. Analysis of the genome indicated that nsp2 of the LTX1 virus has the same three discontinuous deletions as the MN184, but also has a novel 5 AA deletion corresponding to positions 464-468 and numerous unique single mutations.

29. (UGA) We continued to explore the immune response to influenza virus and the contribution of the host tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) in primary and memory T cell responses. We established a primary normal swine bronchoepithelial cell culture system to evaluate the host cell response to influenza virus infection and replication, and are evaluating influenza reassortment. Studies continue on assessing the potential for reassortment of H1 and H3 human and swine influenza viruses in NSBE cells.

30. (UGA) We developed a PRRS-susceptible immortalized porcine stem cell line and are characterizing PRRS persistence in these iPSC cells and potential for vaccine production.

31. (UGA) We are exploring the potential for swine, human and avian influenza viruses to reassort on the TRIG backbone in primary swine epithelial cells, and primary human epithelial cells. A goal of these studies is to elucidate the potential for reassortment and determine the contribution of virus and host to reassortment. These studies are ongoing and in preparation for publication.

32. (UGA) Wee explored the potential for influenza viruses to infect bats. We assessed the potential for low pathogenic avian influenza to infect and transmit in the ferret model. We found that LPAI viruses readily infected and transmitted in in ferrets without adaptation and despite avian-specific receptor specificity. We assessed the potential for these viruses to infect and cause disease in domesticated cats. We assessed the potential for pH1N1 and swine H1N1to infect starlings and sparrows. We established the guinea pig transmission model.

33. (UGA) We continue to test the potential for a novel vaccine vector (PIV5) to serve as a vaccine against influenza virus. Using the mouse model of H5N1 highly pathogenic avian influenza virus (HPAIV) infection, we demonstrated that PIV5 expressing the HA of H5N1 provided robust protection from lethal challenge when vaccinated intranasally, intramuscularly, with live or killed vaccine. Similarly, vaccination with PIV5 expressing the internal, conserved NP protein also protectd from challenge. Finally, we demonstrated the utility of moving the transgene within the PIV5 genome to optimize expression and immunogenicity.

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

1. (NADC**,** Faaberg/Spear/Lager), Compared and contrasted the pathogenesis in swine after challenge with DIVA-tagged PRRSV MLV

2. (NADC) Faaberg) Virion purification; PRRSV nonstructural protein 2 studies

3. (NADC, Faaberg) Construction of chimeric PRRSV

4. (NADC, Lager/Vincent) Animal experiment to characterize H5N1 influenza infection in swine with recombinant viruses provided by Dr. Richard Webby.

5. (NADC, Lager) Animal experiments to evaluate potential infectivity of putative single-strand circular DNA viruses that could interact with PCV2.

6. (NADC, Miller) - Acute transcriptomic response in HP-PRRSV infected gnotobiotic pigs. Searchlight completed.

7. (NADC, Miller) Established globinRNA removal protocol from whole blood for transcriptomics

8. (NADC, Miller) Hi-Seq collaboration with Dr. Gary Rohrer

9. (NADC, Brockmeier) Continued analysis of bacterial enhancement of PRRSV strains

10. (NADC, Loving/Lager) Animal experiment evaluating protection against homologous PRRSV challenge following primary exposure challenge. Loving evaluated T cell responses and mucosal antibody responses.

11. (UW-Madison, Goldberg) Research (via NIH) has elucidated the ecology of SHFV, a relative of PRRSV. This research is mentioned because of its relevance to the ecology of arteriviruses.

12. (UNL, Pattnaik) We conducted the characterization of a serologic marker epitope, so-called epitope-M201, which can be a potential target for development of a live-attenuated DIVA vaccine against PRRSV. Epitope-M201 is located at the carboxyl terminus of the M protein. The epitope is highly immunodominant and well-conserved among type-2 isolates. Rabbit polyclonal antibodies prepared against this epitope are non-neutralizing; thus, the epitope does not seem to contribute to the protective immunity against PRRSV infection. The immunogenicity of epitope-M201 can be disrupted through the introduction of a single AA mutation which does not affect viral replication.

13. (CAU) A genotype 1 PRRSV GZ11-G1 was isolated. The genomic sequences analysis and phylogenetic trees showed it evolved from the vaccine Amervac PRRS. The further pathogenicity analysis indicated that GZ11-G1 could cause clinical signs and lung lesions. It is different from Amervac PRRS or genotype 2 isolate HB-1/3.9 at both the antigenic level and lesions. This is the first pathogenicity study of genotype 1 PRRSV wild isolate in Mainland China.

14. (UMN) MN continued research on seasonal PRRSV transmission dynamics. Airborne influenza transmission was characterized and modeled in acutely infected farms. Influenza virus was quantified in aerosols collected inside and outside swine facilities and in 2 live animal markets in MN. Environmental contamination of influenza virus was confirmed in high hand-contact surfaces easily accessible to personnel working or visiting farms. Indirect routes of influenza transmission via people acting as fomites was shown to be significant in the spread of influenza despite the adoption of biosecurity measures. Mathematical modeling of influenza virus transmission within swine farms and evaluation of the effects of vaccination on influenza dissemination was completed. MN is performing studies on the survivability of PEDV under different conditions of temperature, relative humidity and different matrices (feed, slurry).

15. (UMN) The role of the neonatal pig on influenza epidemiology has been studied at MN. Neonatal pigs were identified as a source of genetically diverse influenza viruses to growing pig populations. About 45% of farms (out of 52) monitored for 6 months weaned pigs positive with genetically distinct influenza viruses. Genetic mutations in influenza virus were detected in pigs with and without passive immunity. Persistence of influenza virus in wean-to-finish populations was shown to be prolonged despite the belief that influenza infections are short lived. Over 25% of pigs in a wean-to-finish population were shown to test positive more than once in non-consecutive weeks indicating that one possible mechanism for virus maintenance in populations includes re-infection despite the presence of immunity.

16. (UMN) Research was conducted on active surveillance for variant influenza viruses among swine, the environment, patrons and employees at live animal markets in Minnesota. The diversity of influenza viruses in live animal markets and the interspecies transmission between pigs and people was documented, indicating that live animal markets play an important role in the transmission of variant influenza viruses to people.

17. (UMN) Surveillance of influenza virus was also extended to the air and environment of 3 agricultural fairs.

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

1. (UNL Ciobanu) Pigs from various crossbred lines were experimentally infected with a PCV2b strain similar to a PCV2b strains known to induce clinical signs of PCVAD and high mortality. During challenge, weekly measurements of ADG, viremia, and PCV2 specific antibodies were profiled. Common sources of variation were evaluated by estimating pair-wise correlations between phenotypic and genomic prediction values and by genome-wide associations across traits. Viremia was the best indicator of decreased ADG following infection; moderate phenotypic correlations between viremia and ADG were observed starting with viremia at 14 DPI and ADG during the last 2 wks of challenge. A genome wide association study that included 56,433 SNPs uncovered two major SNPs that explain, 12.4% and 3.7% respectively, of the genetic variation for viral load. One SNP is located next to the SLA II complex of genes known for their role in immune response. These SNPs partially explained the negative correlations between viremia and growth.

2. (SDSU) Substantial progress has been made in PED diagnostic development. PEDV was isolated from intestinal contents of diagnostic cases using Vero-76 cells with 2.5µg/ml TPCK-treated trypsin. The PEDV-CO isolate at passage 5 was also received from NVSL and further adapted to cell culture through 15+ passages. Consistent high-titer virus stocks approaching 7 logs/ml are produced. These virus stocks are being used in studies of PEDV environmental stability and sanitation efforts.

3. (SDSU) The mAbs, monospecific hyperimmune serum and related reagents produced in this project should prove of substantial value in the detection of PEDV following VI attempts and in a variety of diagnostic methods such as IHC, antigen capture assays and fluorescent antibody technologies. They are currently being utilized in PEDV environmental stability studies and in fluorescent focus neutralization (FFN) assays for assessment of neutralizing antibodies produced following PEDV infection.

4. (SDSU) Cell culture adapted PEDV was used to develop an indirect fluorescent antibody (IFA) test for PEDV serology. A serological ELISA using expressed and purified PEDV nucleoprotein (NP) was developed optimized and is in the final stages of validation. It has demonstrated good correlation with IFA results from known PEDV seropositive and naïve populations.

5. (UW) Work on genetic and antigenic diversity within PRRSV was completed. We developed a novel analytical approach to identify a small number of representative viral genotypes from among the diversity of viral sequences available in GenBank and PRRSVdb. Viruses represented by the top ranking sequences are valuable targets for future study and a polyvalent vaccine development.

6. (UW-Madison). A post-doctoral researcher, Dr. Tavis Anderson, was employed for these analyses, and we secured additional personnel support through an international exchange program with the University of Torino, Italy. Dr. Anderson is now an Assistant Professor at Georgia Southern University and he will continue work on bioinformatics and polyvalent vaccine development at USDA.

7. (OSU) We developed a biodegradable PLGA nanoparticle-entrapped killed PRRSV vaccine (Nano-KAg) and given IN to evaluate immune correlates. In Nano-KAg vaccinated homologous virus challenged pigs, complete clearance of viremia was observed associated with a significant increase in virus neutralizing titers in the lungs. Nano-KAg vaccinated pigs had increased levels of IFN-γ and decreased levels of TGF-β. Restimulation of mononuclear cells of vaccinates secreted significantly increased IFNγ and IL12. Higher frequencies of $CD3^+CD8^+$, $CD4^+CD8^+$, and γδ T cells and reduced frequency of $Foxp3^+$ T-reg cells were observed in vaccinates. In vaccinated but heterologous PRRSV challenged pigs, reduction in pathology, reduced viremia and viral load in the lungs was seen. Enhanced frequency of $CD4^+$ cells, increased IFN-α and IFN-γ, reduction in Tregs population, and decreased secretion of IL-10 and TGF-β was detected. Increased virus specific IgG and IgA, and Nabs were detected in vaccinates. We showed benefits of IN delivery of a nanoparticle-based killed PRRSV vaccine in inducing cross protective immune response.

8. (OSU). We standardized PRRSV NA assay using oral fluid collected over a period of 3 months from modified live vaccinated pigs, and oral fluid and serum samples collected from individual boars vaccinated (PRRS-MLV) or infected with a virulent PRRSV strain. Our results suggested that PRRSV NA titer of greater than 8 in oral fluid samples is virus specific, and it is detected from 4 weeks after vaccination or infection. Our results also showed that PRRSV NA titers in oral fluid samples are correlated with serum titers, and maternally derived PRRSV specific NA titers are detectable in the litters at the time of weaning. We have standardized and validated pen-based oral fluid PRRSV NA assay, which has 94.3% specificity and 90.5% repeatability.

9. (PURDUE). A relatively new method has been implemented allowing the detection of a wide variety of PRRSV strains by utilizing multiple primer sets and rt PCR. We utilized a single primer set designed from the conserved region of the PRRSV genome using a rt PCR to establish a more cost effective alternative. All the cases submitted to the ADDL and identified positive for PRRSV by the PRRSV kit from Tetracore® during the 2010-2011 fiscal year were analyzed. The diversity of the PRRSV genome among the submitted cases was determined by phylogenetic analysis ranging around 40% difference from type 1 and 2. All cases which were positive by the Tetracore® method were identified as positive using a single primer set designed from the PRRSV conserved region by PCR. We demonstrated that by using a single primer set in, PRRSV was detected across a wide diversity of the viral genome and produced comparable CT to a similar commercial assay.

10. (UMD) We identified an atypical PRRSV strain, A2MC2, which is able to induce type I IFNs. A2MC2 induction of neutralizing antibodies *in vivo* was compared with the Ingelvac PRRS MLV and VR-2385. A2MC2 resulted in earlier onset and significantly higher levels of PRRSV NAbs than the MLV. The A2MC2-induced NAbs were capable of neutralizing VR-2385. Pulmonary alveolar macrophages collected during the necropsy in the A2MC2 group had higher level expression of IFN-γ than the MLV group. A2MC2 can be further explored for development of an improved vaccine against PRRS.

11. (CNB-CSIC) focus was in the improvement of rTGEV vectors stability and the generation of new antigenic structures that may confer protection against PRRSV. Several rTGEV vectors were generated, stably expressing different PRRSV antigenic structures: rTGEV-M, expressing M protein; rTGEV-GP5fr-M, co-expressing a 33 aa GP5 ectodomain fragment, containing the epitope recognized by NAbs, and full-length M protein; rTGEV-GP3fr, expressing a 54 AA fragment from GP3 ectodomain, containing the epitope recognized by NAbs: rTGEV-GP4fr, expressing a fragment GP4 ectodomain, containing the epitope recognized by NAbs; rTGEV-

GP3fr-MNH_2, expressing a chimeric protein, consisting in a GP3 fragment containing the epitope recognized by NAbs fused to the amino-terminus of M protein.

12. (CNB-CSIC) The protection induced by rTGEV vectors was evaluated. 45 piglets were divided in 3 groups and were oral and IN vaccinated with each rTGEV vector described above (Group A), or empty rTGEV vector (Groups B and C). 2 wks later, animals were boosted. 2 wks after boost, animals from Grps. A and B were IN challenged with virulent PRRSV. 20% of the vaccinates (A) had clinical respiratory symptoms vs 60% from non-vaccinatess (B). A decrease in lung lesions was seen in vaccinates. There was a 6-fold reduction in virus titers in vaccinates. NAbs in the vaccinates were lower than non-vaccinates. This data were indicative of a limited protection conferred by rTGEV vectors expressing PRRSV antigens. Pigs were seropositive for TGEV after vaccination. Humoral responses demonstrated no significant differences between Group A and B. After challenge, vaccinated animals showed a faster and stronger induction of antibodies recognizing GP5, indicating a recall response in vaccinated piglets that was not fully protective.

13. (VA-TECH) We utilized DNA shuffling, to attenuate PRRSV by DNA shuffling of the viral envelope genes from multiple strains. The GP5 genes of 7 genetically divergent PRRSV and the GP5-M genes of 6 different PRRSV were shuffled. 2 representative chimeric viruses, DS722 with shuffled GP5 genes and DS5M3 with shuffled GP5-M genes, were rescued. A comparative pathogenicity study in pigs revealed that pigs infected with the 2 chimeric viruses had significant reductions in viral-RNA loads and in lung lesions, indicating attenuation of the chimeric viruses. Pigs vaccinated with the chimeric virus DS722, but not pigs vaccinated with DS5M3 acquired protection against PRRSV challenge at a level similar to the parental virus. DNA shuffling of envelope genes rapidly attenuated the virus.

14. (NADC, Nicholson, Spear and Faaberg) Tested new diagnostic nucleotide array.

15. (NADC, Faaberg and Spear) Development of additional DIVA vaccines.

16. (NADC, Spear, Faaberg) Developed ELISA for analysis of animal samples with DIVA Tag

17. (VA-TECH) We molecularly bred PRRSV through DNA shuffling of the GP4 and M genes, separately, from 6 genetically different strains of PRRSV to ID chimeras with improved heterologous cross-neutralizing capability

18. (KSU, Rowland, Fang, Opriessnig (ISU)) developed a Luminex platform for the detection of antibodies against PRRSV, PCV2 and SIV.

19. (KSU, Gabler and Rowland) are performing a study to determine the effect of PRRSV infection on feed digestibility.

20. (USDA-BARC, SDSU) A multiplex FMIA was developed to quantify serum cytokines using Luminex xMap™ (IL-1b, IL-8, IFN-a, IL-10, IL-12, IL-4, CCL2). Pigs were defined to 4 groups; high viremia-high growth (HvHg), high viremia-low growth (HvLg), low viremia-high growth (LvHg), low viremia-low growth (LvLg). After PRRSV, all cytokine levels except IL-4 were altered .

21. (UMN) MN developed several quantitative RT-PCR protocols to detect PEDV; a protocol based on detection of the S gene performed the best. A multiplex real-time RT-PCR for clinical samples

to detect PEDV and TGEV in the same sample. MN is in the process of comparing this newly developed multiplex assay to other commercially available PCRs for PEDV. MN developed an immunohistochemistry technique to detect PEDV antigen in formalin-fixed paraffin-embedded samples. MN adapted a protocol for the isolation of PEDV in Vero cells.

22. (UMN) Studies were completed on the efficacy and cost-effectiveness of air filtration of large sow farms in hog dense regions. Methods were developed to evaluate filter performance against PRRS.

23. (UMN) Efforts on controlling aerosol dissemination centered on evaluating the electromagnetic particle ionization system to decrease infectious aerosols of PRRSV and influenza virus were studied.

24. (UGA) We explored host gene requirements for influenza virus replication, and have addressed how microRNAs govern their expression. These studies have identified miRNAs and multiple cellular targets for influenza viruses.

25. (UGA) We are currently using historical swine influenza sequence data to assess the evolution rates of SIV in swine herds in the United States as compared other global locations and as compared to human influenza viruses of the same subtype. This work is extremely preliminary and no results are available at this time.

26. (UGA) Test the potential for a vaccine vector (PIV5) to serve as a vaccine against influenza virus. Using the mouse model of H5N1 (HPAIV) infection, we demonstrated that PIV5 expressing the HA of H5N1 provided robust protection from lethal challenge when vaccinated IN, IM with live or killed vaccine. Vaccination with PIV5 expressing the internal, conserved NP protein also protected. We are developing a novel bivalent, adjuvanted vaccine using the F protein of RSV to enhance HA-specific immunity while priming and F-specific immune response.

27. (UGA) We are developing a surface enhanced Raman spectroscopic assay for detection of influenza virus and PRRSV.

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

1. (VA-TECH) Molecular breeding via DNA shuffling has important implications for future development of a broadly protective vaccine against PRRSV and will generate broad general interest in the scientific community in rapidly attenuating other important human and veterinary viruses.

2. (UCONN: Risatti): Detecting PRRSV genetic determinants associated with disease caused by the virus may contribute with information needed for rational engineering of PRRS live attenuated viruses.

3. (UCONN: Garmendia): Investigating IFN beta will contribute to gain a better understanding of the innate response to PRRSV which in turn will be useful to the overall knowledge of mechanisms of general pathogenesis, immune evasion and protection or lack thereof.

4. (USDA-ARS-NADC, Faaberg, Spear, Lager, Brockmeier, Miller, Loving, Butler): Constructs

of Ingelvac® PRRS MLV were evaluated for their growth in swine and to determine if they induce antibodies to an inserted foreign tag. We analyzed animal samples infected with several PRRSV strains with and without bacteria, using common pathogenesis indicators. We investigated the effects of different PRRSV strains in gnotobiotic pigs, to seek a reliable index of pathogenicity. This allows us to survey viral growth properties, disease pathogenesis in swine, secondary bacterial pathogens that may arise during infection, the immune responses and host gene expression patterns that differ between PRRSV strains to understand what factors determine high vs. low virulence infections for development of better vaccines and vaccine strategies.

5. (SDSU) Current PRRSV vaccines are not highly effective in preventing PRRSV infections. A better understanding of virus-host interaction will facilitate the development of novel vaccine candidates against PRRSV.

6. (SDSU) Availability of high-titer cell culture adapted PEDV is particularly valuable for virus stability and disinfectant studies as simple virus re-isolation can be used to assess presence of viable PEDV, rather than relying on time-consuming and expensive swine bioassay systems. These virus stocks and associated re-isolation procedures are being used to develop appropriate biosecurity protocols specific to PEDV.

7. (SDSU) MAbs and related reagents will prove very valuable in the confirmation of PEDV antigen in cell culture and tissue samples associated with diagnostic cases and research studies.

8. (SDSU) Diagnostic serology tests such as IFA, virus neutralization and ELISA will be of substantial value in the control of PEDV. Specialized adaptations of PEDV neutralization assays may also provide good indicators of which animals may be immune or protected against PEDV associated disease.

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

10. (ISU) Research has expanded our understanding of PRRSV, PCV2, influenza A, and other emerging viral diseases of swine and provide new ideas for preventing, countering and/or eliminating these infections. New work on the ecology and epidemiology of these agents provide insight into the mechanisms by which they maintain endemnicity. Research in diagnostic technology is contributing to the improvement and refinement of our ability to surveil, detect, and diagnose respiratory viral infections to provide highly cost-effective methods of tracking infection and implementing area elimination/eradication programs. This research will make possible the eventual elimination and eradication of viral infections from individual farms and regions.

11. (OSU) Intranasal delivery of nanotechnology based inactivated PRRSV vaccine may be a suitable strategy to elicit anti-PRRSV immune response and to clear viremia in pigs.

12. (OSU) Conventional ELISA results help only in PRRS survey. In contrast, pen-based PRRSV NA assay could provide information on PRRS herd immune status in vaccinated and/or infected recovered pigs and could be used to evaluate the levels of cross protective immune response against variant PRRSV strains.

13. (UMD) PRRSV A2MC2 inducing interferons in cultured cells may be beneficial for vaccine development to induce protective immunity against PRRS. This isolate induces higher titer of neutralizing antibody in pigs than MLV.
14. (UMD). Nsp1β of virulent VR-2385 inhibits interferon signaling by interfering with STAT1 nuclear translocation, while nsp1β of Ingelvac MLV has no effect. This result has a biological relevance on PRRS vaccine design.

15. (UMD) PRRSV VR-2385 induces pSTAT1-S727 and the expression of proinflammatory cytokines contributes to the insight of PRRSV pathogenesis.

16. (UMD) IFN signaling showed that several PRRSV proteins are involved in the interference with IFN signaling and that some PRRSV strains, such as NVSL and MN184, have variable effects on IFN-activated signaling in MARC-145 and PAM cells. These results may benefit vaccine development.

17. (UNL) Provision of an important starting point for the development of a live-attenuated DIVA vaccine against type-II PRRSV.

18. (UNL) The influence of host genetics on PCVAD susceptibility could lead to increase knowledge of swine immune system, and identification of genes involved in PCVAD susceptibility. Selection based on DNA markers associated with PCVAD susceptibility has the potential to reduce economic losses, improve animal welfare and provide alternatives to vaccination.

19. (KSU) The SCID is model will identify components of innate and adaptive immune protection that will be incorporated into the next generation of vaccines.

20. (KSU) The genomic marker on SSC4 is in the process of being tested by the industry for the development of marker-assisted selection.

21. (KSU) The Luminex multiplex serological assay technology is being transferred to a company for the development of a commercial kit.

22. (KSU) Understanding the effect of PRRSV infection on digestibility will be incorporated into the formulation of nutritional regimens that optimize growth during PRRSV infection.

23. (KSU) Reagents developed from the PEDV study are distributed to other labs for the purpose of assay development.

24. (KSU) CD169 is not a receptor for PRRSV

25. (USDA-BARC) Studies continue on the role of host genetics in resistance to PRRS and in effects on pig health and growth. A genome-wide association study revealed regions on SSC4 and X for VL and on SSC1, 4, 7, and 17 for WG. Pig response to PRRSV has a strong genetic component with a major QTL on SSC4 explaining a substantial proportion of the genetic variance. These results could have a major impact in the swine industry by enabling geneticists to develop plans for marker-assisted selection of pigs with improved response to PRRS.

26. (USDA-BARC, SDSU) An FMIA was developed to simultaneously quantify porcine cytokines in serum and oral fluids. It detects IL-1b, IL-8, IFN-a, L-10, IL-12, IL-4 and CCL2. Serum IL-8, IFN-a and CCL2 are significantly altered after PRRSV infection. Changes in cytokine and

chemokine levels reflect potentially different viral control mechanisms. Correlations of cytokine profiles with serum viral levels, growth performance and genetic background are continuing in hopes of revealing candidate biomarkers of PRRS responses.

27. (CAU) The works on HP-PRRSV pathogenicity (Objective 1-1, above) is not only the first unambiguous illumination about the key virulence determinant of Chinese HP-PRRSV, but it also provides an opportunity to better understand the pathogenic mechanism of this virus.

28. (CAU) The works on pathogenicity of genotype 1 PRRSV (Objective 2-1, above) is the first pathogenicity study on wild isolate in Mainland China.

29. (CAU) The works (Objective 1-4 above) revealed one of the important mechanisms of how HP-PRRSV significantly suppresses innate immune responses.

30. (UMN) Air filtration research provides producers with technical knowledge and economic data to facilitate implementation of effective methods for reduction of airborne viral infection, including PRRSV and influenza virus, in swine herds.

31. (UMN) Economic cost-benefit analyses demonstrates the advantage of air filtration technologies for disease reduction and prevention in sow herds.

32. (UMN) After taking into account the production improvement and the PRRS status of the weaned piglets from both types of farms, the pay-back period of air filtration was calculated to be between 2 and 3 years depending on the initial investment.

33. (UMN) Analysis of risks of influenza transmission within and between farms will facilitate development of effective methods to reduce transmission and identify factors that influence transmission between pigs and between humans and pigs.

34. (UMN) Whole genome sequencing is expected to reveal candidate elements associated with virulence and cross-protective immunity that will facilitate development of improved tools for treatment and prevention of PRRS.

35. (UMN) Elucidation of mechanisms of induction of cross-protective antibody production is expected to provide a rational basis for development of improved vaccines.

36. (UMN) Identification of live animal markets as a source of influenza virus diversity and transmission of variant influenza raises awareness of multiple transmission opportunities.

37. (UMN) Characterization of routes of influenza exposure to people in commercial farms, live animal markets and agricultural animal fairs identified aerosols and hand contact surfaces as possible routes of influenza infection in people.

38. (UMN) Indirect transmission of influenza viruses via fomites was possible despite the implementation of moderate biosecurity measures.
39. (UMN) Approximately 45% of breeding farms weaned influenza-positive pigs, increasing the awareness of influenza virus transmission dynamics in swine operations.

40. (UMN) The effectiveness of electromagnetic particle ionization in reducing influenza and PRRSV aerosols under experimental and field conditions provides producers with another tool for disease control.

41. (UIUC) Our results indicate that the ZF1 motif of nsp1α plays an important role for IFN regulation and further demonstrate that the CBP degradation is likely the key mechanism for IFN suppression mediated by the nsp1α subunit protein of PRRS virus.

42. (UIUC). The directions and intensities in our inferred virus traffic network closely mirror the hog transportation. Most notably, we reveal multiple viral introductions from Canada in to the United States causing a major shift in virus genetic composition in the Midwest USA that went unnoticed by the regular surveillance and field epidemiological studies. Overall, these findings provide important insights into the dynamics of Type 2 PRRSV evolution and spread that will facilitate programs for control and prevention.

43. (UIUC) The miRNA study revealed a subset of a large number of miRNAs that is being altered in PRRSV infected macrophages. Virus replication was negatively impacted by high levels of *miR-147*. Target gene identification suggests that these miRNAs are involved in regulating immune signaling pathways, cytokine and transcription factor production. Whether down-regulation of *miR-147* is directly induced by PRRSV, or if it is part of the cellular response and PRRSV indirectly benefits remains to be determined. No evidence could be found of PRRSV-encoded miRNAs.

44. (UIUC) The appearance of similar deletions in nsp2 in field PRRS viruses of different lineages and levels of virulence suggests a role for this protein in pathogenicity. Contagion might be increased by a higher virus load in the airways.

45. (UGA) The majority of studies during this reporting period have been on emerging viral diseases of swine, i.e. influenza. However, we are now applying these platforms to PRRSV and other swine disease control.

46. (UGA) In regard to influenza as an emerging (re-emerging) disease of swine, we continue to make extensive advances in understanding features of the virus-host interface that influence infection, tropism, and 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. These studies directly impact swine and/or human health, and address the One Health paradigm.

D. PUBLICATIONS ISSUED OR "IN PRESS"

1) Refereed publications [50000 w spaces = limit]

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4) Videos

Porcine reproductive and respiratory syndrome (PRRS) specific videos are at these links:

Solving Swine Diseases: http://www.youtube.com/watch?v=4lAX8FSzgfY

USDA researchers are working to find vaccines to help pigs resist deadly and costly diseases.
Pig immune research: http://www.youtube.com/watch?v=08GAWDiSh6Y

E. FUNDING SOURCES FOR RESEARCH:

BOEHRINGER INGELHEIM VETMEDICA (UMN)

BOEHRINGER-INGELHEIM VETMEDICA - Characterization and Modification of PRRSV and PRRS Disease (NADC, Faaberg and Kehrli)

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NATIONAL PORK BOARD - Efficacy trial of PRRSV modified live DIVA vaccines augmented with heteroclite-vectored cytokines (NADC, Faaberg).

NATIONAL PORK BOARD - PRRSV Identification by Virochip (NADC, Nicholson and Faaberg).

NATIONAL PORK BOARD - Comparison of porcine high fever disease isolates of PRRSV to US isolates for their ability to cause secondary bacterial infection in swine (NADC, Brockmeier, Faaberg, Loving, Nicholson, Baker).

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NIH HHSN266200700006C; 03/30/07 – 03/29/14, UGA NIAID Center of Excellence for Influenza Research and Surveillance (contract). 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.

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The major goal of this contract is to develop a SERS-based detection to identify influenza virus and specifically genetic features associated with.

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Presidential Initiative for Interdisciplinary Research, Cho M, Roth J, Yoon K. Integrated, interdisciplinary vaccine research against antigenically diverse viruses. (ISU)

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STATE OF MINNESOTA RAPID AGRICULTURAL RESPONSE FUND (UMN)

UNIVERSITY OF MINNESOTA SWINE DISEASE ERADICATION CENTER (UMN)

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UNL Life Sciences Competitive Grants Program (FY2011) (Facilitating UNL and Industry Partnerships) Optimization of Invention: A DIVA marker for PRRSV vaccination. 07/01/2011-06/30/2013 Osorio FA and Pattnaik AK

USDA AFRI NIFA Determining Virus-Like Particle (VLP) Feasibility For New Porcine Reproductive And Respiratory Syndrome Virus (PRRSV) Vaccines.. May 2012- April 2014. (SDSU, Wang)

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

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

USDA, PRRSV PRRS CAP 2. Michael Murtaugh and Renukaradhya J. Gourapura. Positive Prognosticators of Immune Protection and Prophylaxis against PRRSV in Swine Herds, August 2009 to July 2013.
USDA. Hatch Multistate Project, NC229. Storrs Agricultural Experiment Station. (UCONN Risatti)

USDA. Hatch Project, Storrs Agricultural Experiment Station (UCONN Garmendia)

USDA/NIFA (UMN)

USDA:NIFA:AFRI Animal Health and Disease Zimmerman J, Frana T, Imerman P, Wang C, Yoon KJ. Safeguarding the viability of U.S. swine producers through a comprehensive and integrated swine health surveillance system. Part 1. Optimization of diagnostic assays for oral fluid specimens.

USDA-AFRI. Ying Fang and Renukaradhya J Gourapura. Innovative strategies to enhance PRRSV specific innate and mucosal immunity: implication for development of broadly protective PRRSV vaccine. February 2012 – February 2017.

USDA-AFRI-NIFA 2011-02917 (Animal Health and Production and Animal Products: Animal Health and Disease) Dec 15 2011- Dec 14 2014 Pattnaik AK (PI) and Osorio FA (co-PI) Porcine Reproductive and Respiratory Syndrome Virus: Modulation of Innate and Acquired Immune Response

USDA-AFRI-NIFA 2013-01035 (Animal Health and Production and Animal Products: Animal Health and Disease)Molecular Structures of Porcine Reproductive and Respiratory Virus (PRRSV) that Contribute to Protective Immunity 10/102013-09/30/2016 Osorio FA (PI), Pattnaik Ak and Kauvar L (co-PIs)

USDA-ARS - Parallel Monitoring Porcine Disease of Economic Importance in the United States and China (NADC, Faaberg, Brockmeier)

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

USDA-NIFA, A multi-component virus vectored vaccine for PRRSV and other swine pathogens. Elankumaran Subbiah, XJ Meng. 2012-2016.

USDA-NIFA, Engineering PRRSV vaccines that confer heterologous protection. XJ Meng, YW Huang, Tanja Opriessnig. 2011-2015.

USDA-NIFA, PRRSV T-cell immunity. Meng SJ, Sakthivel Subramaniam, Tanja Opriessnig. 2013-2017.

USDA-NIFA, Role of PRRSV nucleocapsid protein in PRRSV pathogeneisis, XJ Meng, Kenney SP. 2011-2013.

USDA-NIFA-AFRI - Antiviral regulation underlying the activation status of porcine monocytic innate immune cells (NADC, KSU Sang, Rowland, Blecha, Miller)
USDA-NIFA-AFRI. Yoo, D. (2013-2018), Immune evasion of PRRS virus and a novel approach to vaccine design (UIUC)

ZOETIS INC., PRRSV vaccine development. XJ Meng, YW Huang. 2010-2014.

ZOETIS. Gauger P, et al. Evaluation of cross-protection in experimental PRRSV vaccinated conventional swine challenged with a contemporary, heterologous PRRSV. (ISU)

ZOETIS. Gauger P, et al. Evaluation of Fostera™ PRRSV serum neutralizing antibodies against contemporary PRRSV and challenge of conventional, naïve pigs with contemporary PRRSV. (ISU)

ZOETIS. Gauger P, et al. Evaluation of shedding in experimental PRRS-vaccinated grower pigs challenged with a heterologous PRRSV. (ISU)

F. WORK PLANNED FOR NEXT YEAR

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

1. "Mapping Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Genetic Determinants of Macrophage Host Range and Immune Modulation". For the next year we are planning to continue with the studies described above that are aimed to assess the role of NSP3-FKBP38 interaction in apoptosis of PRRSV infected cells. Different approaches, established in the laboratory during last year, including production of mutant PRRSV by site directed mutagenesis, confocal microscopy studies on co-transfected cells, GST pull-down assays transfected and infected cells, and flow cytometry to measure apoptosis in transfected and infected cells will be combined to reach the proposed aim. (UCONN: Risatti)

2. "Assessment of Virulence of PRRSV Isolates Based Both on their Sensitivity to IFNβ 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 as it relates with viral diseases of swine. Data has been recently presented at a Graduate Research Forum and in a seminar in our department. A refereed publication (He et al, 2011) and one manuscript submitted (Overend et al) summarize the data produced in the study. Manuscript (Overend et al) will be re-submitted. Samples collected from a recent vaccine study are being processed and tested for virus loads, antibody and cellular responses and IFN levels. (UCONN: Garmendia)

3. Assessment of host response to different strains of PRRSV (NADC)

4. In vivo analysis of engineered PRRSV (NADC)

5. In vivo analysis of heteroclite vector cytokines (NADC)

6. We will continue to work on PRRSV host interaction with a special focus on the role of innate immunity such as PKR against PRRSV infection (SDSU, Wang).

7. We will also continue to study the mechanism of PRRSV interference with IFN-activated JAK/STAT pathway. (UMD)

8. To write the new NC229 proposal (2014-2019) (UNL)

9. Continue to make progress on the characteristics of PRRSV immunity, including the role of genetics (KSU).

10. Continue to study the role of macrophages and dendritic cells in PRRSV immunity (KSU).

11. Develop a SCID model for investigating PRRSV immunity and pathogenesis (KSU).

12. Work on the roles of virus-host proteins interaction played on the viral replication and host anti virus strategies. (CAU)

13. to continue investigations of mechanisms of PCV2 pathogenesis (UMN).

14. to continue investigations of PRRSV evolution, structure and immunity (UMN).

15. We will continue studies focused on swine influenza, expanding platforms to PRRSV were appropriate. Also, we hope to receive the renewal for the NIAID Center of Excellence for Influenza Research and Surveillance contract including an expanded focus on SIV surveillance and evolution in the United States (UGA).

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

1. To evaluate filter materials for cost-effective reduction and prevention of airborne viral spread between farms (UMN).

2. To investigate the role of contaminated slurry as a means of pathogen dissemination between farms (UMN).

3. To collaborate with USDA APHIS on the NAHMS swine health survey to assess PCV2 status (UMN).

4. To continue investigations into the impact and magnitude of the electromagnetic particle ionization system to decrease infections within and between farms (UMN).

5. To continue to elucidate mechanisms of influenza transmission within and between farms (UMN)

6. To evaluate the frequency and diversity of influenza aerosol infections (UMN)

7. To continue the genetic characterization of influenza viruses in commercial pigs, agricultural animal farms and live animal markets (UMN)

8. Continue write-up and presentation of completed work on arteriviruses (UW-Madison).

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

1. Test new PRRSV diagnostic microarray against several PRRSV strains (NADC)

2. We will continue to explore the potential utility of virus-like particles approach for PRRSV vaccine development. (SDSU, Wang)

3. We will also continue our efforts to develop improved diagnostic and control methods to help reduce the impact of PEDV on the US swine industry (SDSU).

4. We will extend our work on nanoparticle and virus-like-particles based killed PRRSV vaccine to induce cross-protective immunity against PRRSV in pigs. (OSU)

5. Continue to identify potent cost-effective mucosal adjuvants to use with PRRSV vaccines in pigs. (OSU)

6. Detection of the new viral infection in animals and PRRSV, influenza disease prevention. (PURDUE)

7. We will continue to characterize the mechanism of PRRSV A2MC2 in inducing production of type I interferons and explore A2MC2 for vaccine development (UMD).

8. To approach cross-protective immunity against PRRSv by : 1) subunit vaccines to induce broadly neutralizing antibodies,2) vectored platforms containing conserved epitopes, or 3) synthetic PRRSV designed as a consensus antigenic sequence (UNL)

9. Improvement of heterologous protein expression levels. PRRSV M protein was fully stable in rTGEV vectors. Therefore, it will be used as a model for further improvement of heterologous genes expression levels. Different rTGEV vectors, expressing M protein under the control of a set of TRSs, previously identified by our group, will be engineered. Both mRNA and protein expression levels will be analyzed by quantitative RT-PCR and Western blot, respectively. In addition, the correlation between protein expression increase and vector stability will also be analyzed. (CNB, Enjuanes)

10. Improvement of heterologous gene expression by reduction of the rTGEV recombination rate. Most likely, the modest protection results obtained with the rTGEV vectors stably expressing PRRSV antigens could be due to their small size. Reducing size antigen an increase in rTGEV vector was achieved, maybe interfering with proper antigen folding or immunogenicity. Therefore, the identification of viral genes involved in recombination is an essential goal for further rTGEV vector development. The elimination of these viral genes activity would allow the design of recombination-defective rTGEV vectors, most likely improving heterologous genes stability. We will focus our attention in a viral endonuclease, encoded by nsp14, that has been previously involved in coronavirus proofreading system. We will engineer rTGEV mutants in nsp14 that will be characterized and their recombination rates will be also analyzed. (CNB, Enjuanes)

11. 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 as well as insight into new mechanisms of PRRS resistance. 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, Lunney)