The NC-229 (PRRS) Committee
presents the

2006
International PRRS Symposium

Research into Methods for the Integrated Control, Prevention, and Elimination of PRRS

Chicago, Illinois
December 1-2, 2006

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USDA NRI PRRS Coordinated Agricultural Project

PROGRAM and SCHEDULE OF ACTIVITIES
Theme: PRRSV eradication – Is it possible?

FRIDAY DECEMBER 1, 2006

14:00	Registrants check in (Pre-registration required. No walk-ins accepted)
	Poster set-up in rooms F, G, H (refer to poster numbers in proceedings)
	All lectures in Chicago Ballroom E
14:15	NC-229 Station Representatives meeting

OPENING SESSION
Co-Chairs: R. (Bob) Rowland and Fernando Osorio

16:00	Welcome. R. (Bob) Rowland, Chair, NC-229 Committee
16:10	Keynote Presentation: *The challenge of HIV global diversity for vaccine antigen design* (#1). Bette Korber. Los Alamos National Laboratory
17:00	Poster Session 1 (authors of odd-numbered posters at their posters until 18:00)
17:00	Reception and cash bar

SATURDAY DECEMBER 2, 2006

PRRS VIRUS HETEROGENEITY
Co-Chairs: Pamela Zaabel and Kay S. Faaberg

8:00	Keynote Presentation: *Infectious cDNA clones for PRRSV – past, present, future* (#9). Dongwan Yoo, University of Guelph
8:30	PRRSV glycoproteins: Mutation, expression, and functional characterization (#4). Kay S. Faaberg, University of Minnesota
8:45	Antibody response to the cysteine protease of PRRSV nsp2 (#16). Ying Fang, South Dakota State University (NPB PRRS Initiative grantee)
9:05	PRRSV surveillance, elimination, and immunity in boars and boar semen (#19). Travis Clement, South Dakota State University
9:20	Development and optimization of a blocking ELISA for Type 1 and Type 2 strains (#17). Steve Lawson, Rural Technologies Incorporated, Brookings, SD
9:35	Characterization of the North American (NA) and European (EU) PRRSVs found in a co-infected pig in Hong Kong (#5). Frederick Leung, University of Hong Kong, Hong Kong Special Administrative Region (HKSAR), China
9:50	Coffee break, Poster Session 2 (authors of even-numbered posters at their posters)

PRRSV PERSISTENCE AND GENETIC RESISTANCE
Co-Chairs: Joan Lunney and David Benfield

10:45	Keynote Presentation: *PRRSV persistence – information from the Big Pig experiment.* Jeff Zimmerman, Iowa State University
11:15	Genome-wide transcriptional map of innate immune responses to replication of PRRSV in alveolar macrophages of commercial breeds (#20). Tahar Ait-Ali, Roslin Institute, United Kingdom
11:30	The selected B cell population in PRRS has a naïve phenotype, undiversifed repertoire, and unusually hydrophobic HCDR3 (#33). John Butler, University of Iowa
11:45	Presence of interferon-alpha delays viral replication and reduces disease signs in pigs challenged with PRRSV (#27). Susan Brockmeier, USDA National Animal Disease Center
Noon	Luncheon - buffet available in poster area

PROTECTIVE IMMUNITY, PRRSV VACCINES
Co-Chairs: Federico Zuckermann and Hank Harris

13:00	Keynote Presentation: *Understanding mucosal immunity and disease resistance* (#40). Volker Gerdts, Vaccine and Infectious Disease Organization, University of Saskatchewan
13:30	Mechanisms of cross-protective immunity against PRRSV (#39). Michael Murtaugh, University of Minnesota
13:50	Deceptive imprinting and immune refocusing: The next generation of discovery and development for vaccines (#46). Peter Nara, Biological Mimetics, Inc. (NPB PRRS Initiative grantee)
14:10	Replicon particle co-expression of PRRSV GP5 and M proteins (#45). Matt Erdman, Iowa State University
14:25	ORF5 and ORF2 are the main structural genes carrying determinants of virulence of PRRSV (#50). Byungjoon Kwon, University of Nebraska
14:40	Serological marker candidates identified on structural and non-structural proteins of PRRSV (#51). Marcelo de Lima, University of Nebraska
14:55	Assessment of the efficacy of commercial PRRSV vaccines based on measurement of serologic response, frequency of gamma-IFN producing cells and virological parameters of protection upon challenge (#57). Federico Zuckermann, University of Illinois
15:10	Break – refreshments in poster area

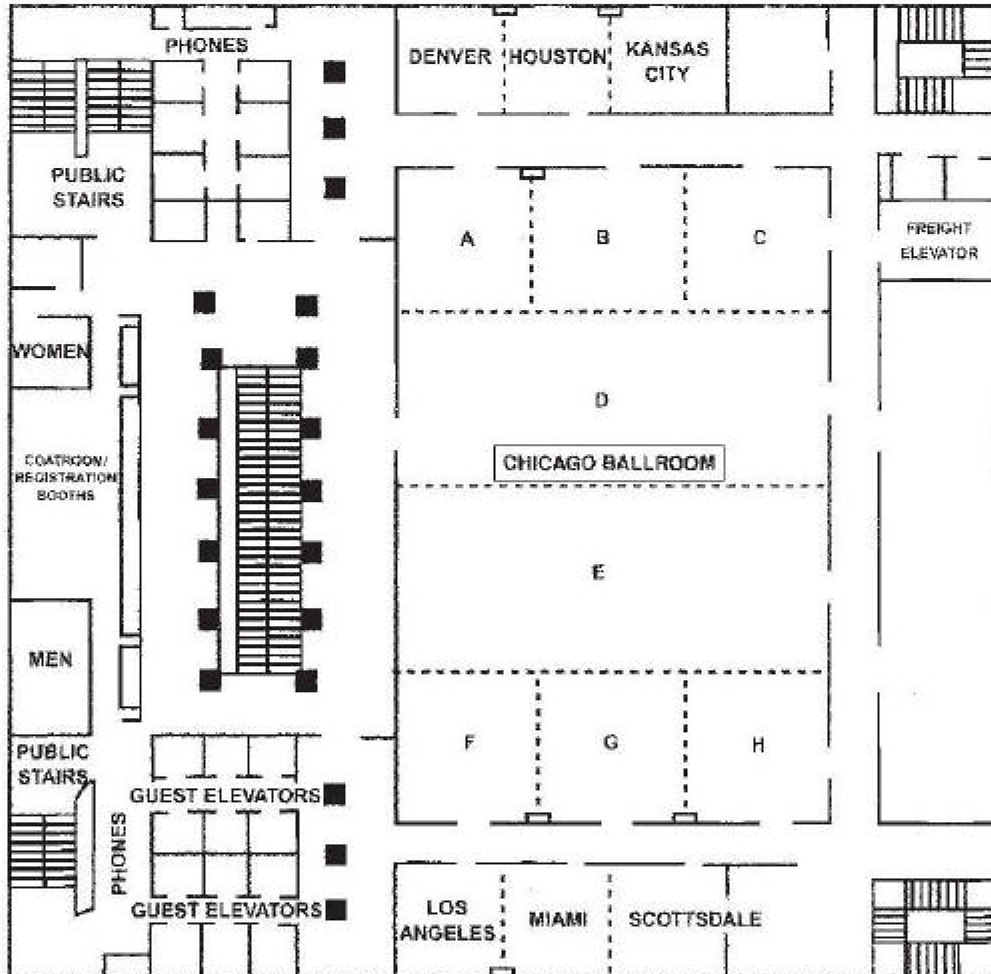
PRRSV ECOLOGY
Co-Chairs: Monte McCaw and Cate Dewey

15:30	Keynote Presentation: *Factors influencing viral disease transmission and elimination* (#68). Sten Mortensen, Danish Veterinary and Food Administration
16:00	Report from the American Association of Swine Veterinarians North American PRRSV Eradication Task Force Paul Yeske, Swine Vet Center
16:20	Regional eradication of PRRSV (#70). Bob Morrison, University of Minnesota (NPB PRRS Initiative grantee)

16:40	Regional Market Swine PRRSV Antibody Survey (#63). Jim McKean, Iowa State University
16:55	Susceptibility of young pigs to PRRSV infection by the aerosol route of exposure (#69). Joseph Hermann, Iowa State University
17:10	Aerosol transmission of PRRSV: an application to the field (preliminary data) (#58). Andrea Pitkin, University of Minnesota
17:25	Eradication of PRRSV In Chile (#66). Alejandra Estrada, Servicio Agricola y Ganadero
17:40	Adjourn. Please pick up your posters after the meeting.

LOCATION INFORMATION

Chicago Ballroom (5th Floor)
Chicago Marriott Downtown Magnificent Mile
540 North Michigan Avenue
Chicago, Illinois 60611



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SECTION 1: VIRUS HETEROGENEITY

THE CHALLENGE OF HIV GLOBAL DIVERSITY FOR VACCINE ANTIGEN DESIGN

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Los Alamos NM

Background: HIV evolves rapidly and there is a complex global map of HIV-1 genetic subtypes and their recombinants. Some regional epidemics are dominated by one subtype and vaccine strategies can be framed in the context of this more limited diversity, however even one subtype harbors extensive variation. Epidemics in other geographic regions result from a complex array of subtypes and inter-subtype recombinant forms.

Objectives: Our overarching objectives are to better understand the interplay between the host response and viral evolution, and the limitations and extent of HIV variation. We then incorporate this knowledge into rationally designing reagents for assessing immune responses to diverse HIV-1 strains, and to inform decisions regarding vaccine antigen design for subsequent testing.

Results and Conclusions: In terms of T-cell immunity, we have found that within-clade consensus sequences fail to detect many natural responses, and we can detect more responses using a strategy of peptide synthesis (toggled-peptides) that allows many variants to be included. The complexity of HIV diversity has driven us towards design and testing of vaccine antigens that have the theoretical potential for stimulating enhanced intra- and inter-subtype cross-reactivity. Our first attempts compare natural strains to artificial M group central antigens, and small animal vaccination studies show some promise. We have gone on to design sets of artificial HIV proteins (mosaics) that maximize the coverage of epitope variants in a given population.

IDENTIFICATION OF THE BINDING DOMAIN OF SIALOADHESIN ON MACROPHAGE CELLS TO PRRSV

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PRRS virus belongs to the family Arteriviridae, which shares a marked tropism for the monocyte/macrophage lineage *in vivo*. Heparan sulphate (HS) and Sialoadhesin (Sn) were considered to be the important receptors on porcine alveolar macrophages (PAM). HS can mediate virus attachment but no internalization; Sn is sufficient for both PRRS virus attachment and internalization. It was reported that the viral M protein on itself, or as a complex with GP5, was identified as a HS binding protein. But the viral proteins involved in the interaction with Sn are unknown. And the counterpart on the Sn, which attaches to the viral proteins, is also unclear. The aim of the present study was to investigate the binding domain of Sn with PRRS virus.

The gene coding for Sn was amplified by RT-PCR from PAM cells, and inserted into a eukaryotic expressional vector pcDNA3.1 (+) at the corresponding Sites to yield recombinant plasmid pSn. PK15 cell, a PRRS virus no-permissive cell line, was transfected by purified pSn. After 48hr post transfection, the cells were exposured to CH-1a isolate for the binding of virus, the cells were washed and fixed. In IFA test, the PRRS virus-attached cells could be detected by a monoclonal antibody against N protein. Subsequently, the full-length of Sn was divided into a series of fragments by PCR and transfected into PK15 cells. Finally, the sequence coded the amino acids from 17 to 150 of the Sn, in which the first domain contained only, was detected to be positive in attaching the virus. The attachment of virus to the transfected PK15 cells was inhibited by rabbit anti-Sn-polyclonal serum, and the similar inhibition on PAM cells was observed. By analysis with FAC, we found the inhibition of anti-serum was in a dose-dependent manner.

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ESSENTIAL AND NONESSENTIAL REGIONS OF PRRSV NSP2

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PRRS virus is an Arterivirus shown to have high sequence divergence. One region that has been identified of extreme divergence among strains is putative nonstructural protein (nsp2), located near the N-terminal end of the large replicase polyprotein. Nsp2 contains a very conserved N-terminal enzymatic domain, a conserved C terminal transmembrane region and a hypervariable middle region with unknown function. Nsp2 is responsible for the length difference between classical PRRS virus Type 1 (European; 15,111 bases) and Type 2 (North American; 15,411 bases) strains, and has been identified as containing B cell epitopes.

Utilizing an infectious clone of strain VR-2332 (pVR-FLV7), we studied virus growth with nsp2 deletions of various sizes. We found several 100aa regions were not critical for virus growth, all in the hypervariable middle region that encodes most of the putative B-cell epitopes. However, larger deletions were not as well tolerated, and resulted in initially slow growing virus, that became more competent over time. Nucleotide sequence analysis of this mutant virus is presently being completed. Nsp2 flexibility was also examined. Prior results showed that green fluorescence associated with PRRS virus growth was seen after in-frame insertion of the green fluorescent protein (GFP) into a 100aa deletion mutant, but GFP was only transiently expressed. Other GFP insertions did not yield viable virus. We also examined the capacity of the PRRS virus genome to accept other small immunogenic epitopes, and we found that c-myc, when inserted in place of GFP, produced mutant virus that was stable over several passages. Our work suggests protein conformation or other factors have importance in nsp2 function. Studies also showed that nsp2-GFP could be localized to the perinuclear region of viral infected MA-104 cells. No colocalization was seen using antibodies specific for cellular compartments, suggesting that PRRS virus nsp2 in MA-104 cells, when modified by GFP, does not localize to the ER as seen with a similar protein, equine arterivirus nsp2 (van der Meer et al, 1998).

PRRSV GLYCOPROTEINS: MUTATION, EXPRESSION AND FUNCTIONAL CHARACTERIZATION

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PRRS virus is a member of the Arterivirus family in the order Nidovirales. This RNA virus (15-15.5 kb) encodes 4 glycoproteins, GP2-5.

GP5, encoded by ORF5, has been shown to be the viral attachment protein and neutralizing antibodies have been shown to bind to this protein. In order to examine the role that GP5 N-glycosylation may play in viral neutralization, mutational analysis of an infectious clone of North American prototype strain VR-2332 (pVR-FLV7) was completed. There are four potential N-glycosylation sites in this PRRS virus strain. Mutation of the asparagine residues to serine was completed for each possible combination of residues, which totaled 15 mutant subclones. Each mutant subclone was placed back into pVR-FLV7 backbone and verified for mutation retention. Full length *in vitro* RNA transcripts were produced for all 15 of the mutants and these transcripts were then transfected into MARC-145 cells. Eleven of the 15 constructs yielded viral progeny. After 3 cell passages, we tested all 11 constructs for viral growth rate, plaque size and titer. Preliminary results suggest that all viral mutants produced smaller plaques and possessed a lower growth rate. Further analysis will include an examination of the subgenomic message display and the stability of the viral mutants after inoculation into swine.

Delineation of the roles GP2-4 play in PRRS virus infection and production of additional protein specific antibodies is another aim of our research. To begin these studies, ORFs 2a and 3 of three significantly different North American strains (VR-2332, MN184 and P921) were subcloned into pGEM-T. *In vitro* transcription and translation analysis in the presence and absence of canine pancreatic microsomal membranes was used to show how abundant expression of these ORFs could be completed. After bioinformatic analysis, the portions of GP2 and GP3 that were predicted to be soluble were inserted into the vector pEAK13cd5 (encoding the Fc domain of IgG1) for high level eukaryotic expression. The engineered proteins were expressed and secreted into the medium of transfected CHO-K1 cells. The secreted proteins will then be concentrated by centrifugation through a 20kDa membrane. Purified proteins will be used to immunize rabbits for production of polyclonal protein-specific antibody.

CHARACTERIZATION OF THE NORTH AMERICAN (NA) AND EUROPEAN (EU) PRRS VIRUSES FOUND IN A CO-INFECTED PIG IN HONG KONG

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In an epidemiological study of PRRS virus in Hong Kong during 2001 to 2006, we have identified the co-existence of both the North American (NA) and European (EU) genotypes in local pig farms, Therefore, it is worthwhile to evaluate the possibility of co-infection of both genotypes since it may give rise to the possibility of inter-type recombination. In this study, we investigated 35 pigs (4 sows and 31 piglets) for co-infection of the NA/EU PRRS virus between February to June 2006. Out of the four co-infected piglets identified, only one has been consistently detected with both genotypes for a period last up to 4 weeks. This piglet was sacrificed for further viral characterization in various organ tissues (lung, liver, lymph nodes, tonsils, spleen, kidney and heart) by RT-PCR, immuno-histochemistry and in situ hybridization. With the development of a tandem PRRS ELISA, serum antibodies against either genotype were differentially detected. Moreover, genotyping results revealed a shift of dominance from NA genotype infection to EU genotype during the monitoring period, suggesting no cross protection from either genotype. In addition, EU PRRS virus was identified in most of the tissues examined and NA PRRS virus was only detected in the lung tissue by RT-PCR. The IHC and ISH data further indicated that both NA and EU PRRS viruses were identified in the interstitial macrophages in the left cranial and left caudal lobes of the lung demonstrating co-infection by both PRRS virus genotypes. However, the low number of co-infection cases suggesting that a strong competition between the two genotypes for the interstitial macrophages as a host may occur. Although no inter-typed recombinant virus was identified, present study reveals the co-infection of both genotypes in lung tissue of a single pig, suggesting a possibility of inter-type recombination.

PRRSV: DIVERSITY OF STRAINS FOUND IN BRITAIN

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This research aims to identify and characterise strains of PRRS virus recently associated with disease in pigs in Britain. Reports of anomalies in serological testing have suggested increasing diversity and/or evolution of the virus.

Strains were characterised by analysing the genetic sequence of ORF7, ORF5 and ORF3 from PRRS viral RNA from tissue samples obtained from recent (2004-2006) clinical cases of the disease. These were then compared with corresponding data from strains isolated in Britain between 1991 and 1995, and with published data from other countries. A panel of twelve monoclonal antibodies was also used to evaluate the potential variation of particular epitopes found on the virus.

Phylogenetic analysis of the data reveals an increasing diversity among recent isolates from Britain. While all being of the European type, these remain distinct from most isolates of other countries. Several isolates were found to be remarkably similar to a modified live vaccine strain currently used in Britain. Clear differences in reactivity of the virus isolates against the panel of monoclonal antibodies were also observed.

These findings demonstrate a significant increase in the diversity of PRRSv strains circulating in British herds between the 1990s and 2005, and highlight potential problems with current vaccination methods used in Britain. We present these findings, along with some preliminary data on the evolutionary relationship among these British strains, and other European isolates.

EVOLUTIONARY BIOLOGY OF PRRS VIRUSES IN PIGS: MUTATIONS AND RECOMBINATION

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PRRS virus has demonstrated a high degree of genetic and antigenic variability, which imposes difficulties in controlling the disease. Sequential pig-to-pig passages of a virus which was previously conducted in our laboratory demonstrated that: a) continuous point mutations contributed to this variability even though the mutation rate was relatively low; and b) genetic changes of the virus over time was in a predicted and steady manner, supporting the "molecular clock" theory. However, it is not uncommon that multiple strains of PRRS virus co-circulate in a herd/site. Hence, recombination between 2 different strains can be expected besides random point mutation of each virus. More recently, we examined the recombination event and its impact on phylogenetic analyses of PRRS viruses by inoculating pigs, which were individually housed in HEPA-filtered isolation units, with two North American strains that were derived from the same parental virus and via sequential pig-to-pig passages of viruses in clinical specimens. A total of 760 plaque-cloned viruses (20 viruses per pig at each sampling) were obtained and subjected to sequence analyses for all structural protein genes (ORFs 2-7). Recombinants arose in all pigs inoculated with both viruses and were detected as early as 14 DPI. The dominant recombinant virus varied in each pig and over time, resulting in transfer of different recombinants to pigs in the subsequent passage. Crossover sites were random and varied among the observed recombinants. Phylogenetic analysis showed that the recombinants could be classified into different groups depending on ORF of choice due to random distribution of crossover sites throughout the genome, suggesting that recombination can contribute PRRS virus diversity and lead to inaccurate phylogenetic diagnostic results. In conclusion, both accumulation of point mutations and recombination contribute to the genetic diversity among PRRS viruses, which justifies further study of its impact on cross protective immunity.

EVOLUTIONARY DYNAMICS OF NORTH AMERICAN-TYPE PRRSV INTO THREE SUBTYPES CHARACTERIZED BY TWO DELETIONAL MUTATIONS IN NSP2 GENE

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Although North American-type (NA-type) PRRS virus shows high genetic variation, the evolutionary relationships among the strains remain to be understood. Here, complete genome analysis of the Japanese EDRD-1 strain identified the novel deletion of 117 bases, in addition to the insertion of 108 bases previously reported in the SP strain, in the nsp2 gene compared to that in the VR-2332 strain. Sequencing analysis of the partial nsp2 gene in another 30 Japanese isolates revealed that NA-type PRRS virus could be classified into 3 subtypes based on polymorphism of the nsp2 gene, which were tentatively termed subtypes E, V and S, as represented by the EDRD-1, VR-2332 and SP strains, respectively. Of 31 Japanese isolates, subtypes E, V and S accounted for 21, 9 and 1 isolates, respectively. The three subtypes formed distinct phylogenetic clusters, suggesting that they had diversified at an early stage of viral evolution. Furthermore, the SP strain contained both direct repeats flanking the 117-base deletion of subtype E and inverted repeats flanking the 108-base insertion of subtypes E and S, which were capable of inducing deletional mutations, suggesting that the 108 bases would not be an insertion in subtypes S and E but rather a deletion in subtype V. In terms of evolutionary dynamics, these observations led us to hypothesize that subtypes E and V individually evolved by different deletional mutations from a common ancestor of NA-type PRRS virus, while subtype S evolved simply by base substitutions from the common ancestor.

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INFECTIOUS CDNA CLONES FOR PRRSV - PAST, PRESENT, AND FUTURE

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The recent development of infectious cDNA clones is a major breakthrough in PRRS virus research and allows us to manipulate the viral genome and construct genetically-engineered mutant PRRS virus *in vitro*. The PRRS virus genome is compact, and is arranged in the way that the structural genes overlap from each other. The expression of individual genes requires a specific promoter sequence, and the expressed gene products function only in multimeric forms. Despite the complicated gene arrangements and expression schemes, a progress has been made to introduce specific modifications to the PRRS virus genome, including mutations, deletions, substitutions, and foreign gene expression. The reverse genetics system is the most powerful research tool to study structure-function relationship of PRRS virus proteins, to develop a molecularly attenuated marker vaccine, and to use PRRS virus as a foreign gene expression vector with a dual vaccine potential for swine diseases.

INCREASED VIREMIA, ALTERED IMMUNE RESPONSES, FEVER, AND PATHOLOGIC LESIONS FOLLOWING INTRANASAL HETEROLOGOUS WILD-TYPE PRRSV CHALLENGE: IDENTIFICATION OF MARKERS ASSOCIATED WITH INCOMPLETE CROSS-PROTECTION IN A RESPIRATORY DISEASE MODEL

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Objective: Investigate immune response differences associated with inadequate cross-protection against heterologous WT-PRRSv challenge in a respiratory disease model. ATTC 2332 was used to IN infect 10 Medicated Early Weaned pigs at 4 weeks of age and five pigs each were then IN challenged 6 weeks later with ATTC 2332 or heterologous WT NCPowell PRRS virus, respectively. Serum and temperatures were collected regularly, PMBCs at 21 days PC, and bronchial lymph nodes and lung tissue at 24 days PC. Lymphocytes and PBMC's were stimulated *in vitro* with homologous or heterologous LV or KV, supernatants and cell pellets collected for cytokine ELISA and quantitative RTPCR respectively. Significantly increased and prolonged group mean fever, viremia, percent pneumonia, and enlarged mediastinal LN were found in NCPowell WT PRRSv-challenged vs homologous virus challenged pigs. Differences were also noted in virus-specific SN titers and ELISA antibody response curves post-challenge. *In vitro* stimulation cytokine responses are currently being determined. Immune responses generated following VR2332 infection were inadequate to cross-protect against NCPowell WT PRRS virus challenge 6 weeks later. Post-challenge antibody responses were different.

LOCALIZATION PROPERTIES OF PRRSV NONSTRUCTURAL PROTEIN 2 (NSP2)

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Nonstructural protein 2 (nsp2) of PRRS virus contains a hypervariable domain within the C-terminal region. Using the pCMV-S-P129 full-length infectious PRRS virus cDNA clone, several foreign cDNAs were inserted into the most conserved area of the hypervariable domain of nsp2. The results showed that enhanced green fluorescent protein (EGFP)-containing genomes were viable, expressed EGFP, and could be packaged into virions. EGFP was expressed to high levels, but was eventually lost during passage of the recombinant virus. The nsp2 of the prototype arterivirus, equine arteritis virus, is involved in the formation of replication complexes associated with endoplasmic reticulum (ER) membranes in the perinuclear region of the cell. However, there are examples of arterivirus proteins that localize to other intracellular compartments, such as the nucleus. The purpose of this study was to characterize the intracellular localization properties of the nonstructural proteins of PRRS virus. The main replication complex scaffolding proteins, nsp2 and nsp3, did not co-localize with anti-Golgi or anti-calnexin (ER) antibodies. In addition nsp2, when expressed as an EGFP-tagged protein by a recombinant PRRS virus, also failed to co-localize with ER and Golgi markers, but did co-localize with the N protein. Confocal microscopy of individually expressed EGFP-labeled fusion proteins identified nsp2 and nsp3 as predominately cytoplasmic. Even though this study did not identify the membrane compartment associated with PRRS virus nsp2 and nsp3 localization, these results suggest that there may be some flexibility among the arteriviruses in the source of the membranes recruited in the formation of replication complexes.

SECTION 2: VIRAL DIAGNOSTICS

DEVELOPMENT OF AN OPTICAL-BASED NANOBIOSENSOR FOR DETECTION OF PRRSV

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Current methods for the detection of PRRS virus are often lengthy procedures that must be carried out in a laboratory setting or are meant to detect the antibodies produced by the infected animal, making detection difficult in animals that have been vaccinated against the virus. Because PRRS virus is a quickly spreading and easily adaptable virus, there is a need for expeditious detection in order to quarantine infected animals and halt the transmission of the virus. Therefore, a sensor that could quickly detect PRRS virus in a field application would be highly advantageous. The use of a nanosensor architecture applied to an optical biosensor system could carry out this function. The sensor architectures currently being developed utilize a PRRS virus monoclonal antibody conjugated with a fluorescent dye and bound to Protein A, which is conjugated to either a quantum dot or a gold nanoparticle. When exposed to the PRRS virus, a conformational change in the antibody causes a change in the fluorescent spectrum of the nanosensor. Preliminary results have shown that the PRRS virus can be detected with both the quantum dot- and gold nanoparticle-based architectures. Consequently, it can be concluded that the nanobiosensor method is a feasible technique for PRRS virus detection.

LOW VOLTAGE CAPILLARY ELECTROPHORESIS OF 750 BP PRRSV ISOLATES ON A MICROCHIP

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On-Chip DNA analysis has substantial field applications in crime investigation, viral disease detection, and genomic comparisons; however, the potential of such applications is limited by the time consumption and voltage requirement of the electrophoresis process. Though on-chip capillary electrophoresis is significantly less time consuming than traditional gel electrophoresis, on-chip capillary electrophoresis requires high voltage levels that limit field applicability. The development of capillary electrophoresce at lower, field-applicable voltages with gel matrices for faster and better-resolved DNA separation remains an important research endeavor. This study realizes such an endeavor by exploring the use of nanoplatinum doped agarose gel in on-chip capillary electrophoresis.

The on-chip electrophoresis system was composed of a PDMS micro-capillary with platinum electrodes. Using nanoplatinum doped agarose, electrophoresis of a 527 bp IBR isolate, a 750 bp PRRS isolate and a 100-1000 bp DNA ladder in these micron size capillaries was successful. Observations have concurred with earlier studies showing a two-fold enhancement of DNA mobility due to platinum doping of the gel. This mobility increase has been attributed to an increase in the dielectric constant of the gel medium caused by the nanoplatinum doping. As with standard electrophoresis, the on-chip, capillary electrophoresis of the PRRS and IBR isolates showed resolution based on DNA base pair number. Based on the use of platinum doped agarose in on-chip capillary electrophoresis, this study confirms a reduction of electrophoresis time observed in earlier studies and focuses on lowering voltage requirements.

DETECTION OF PRRSV INFECTION USING PEN-BASED ORAL FLUID SAMPLES: EXPERIMENTAL STUDY

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Objectives: The purpose of this study was to determine whether PRRS virus and/or anti-PRRS virus antibodies were present at diagnostically useful levels in the oral fluids of young pigs infected with PRRS virus under experimental conditions.

Methods: The presence, level, and duration of PRRS virus and anti-PRRS virus antibodies in serum and oral fluids was evaluated in 3 age groups of pigs (4, 8, and 12 weeks of age). Each age group consisted of 16 pigs (12 PRRS virus-inoculated, 4 negative controls). The pigs were intramuscularly inoculated on DPI 0 with 2 ml of a preparation containing 1 x $10^{1.69}$ $TCID_{50}$ of PRRS virus per ml. Thereafter, serum, buccal swabs, and oral fluids were collected at regular intervals for 63 days. Samples were assayed for the presence of PRRS virus by qRT-PCR and for the presence of specific anti-PRRS virus antibodies by ELISA and IFA.

Summary of results: Real time PCR results showed a positive correlation between PRRS virus titers in serum and oral fluid samples. Oral fluid samples were positive in over 75% of the PRRS virus-inoculated pens through day 28 post inoculation. Likewise, PRRS virus-specific antibody was detected in oral fluid samples.

Conclusions: Oral fluid samples contain PCR-detectable PRRS virus levels adequate for surveillance. PRRS virus-specific antibodies are present in oral fluids, but further research will be required to optimize antibody assays for routine use.

DETECTION OF PRRSV INFECTION USING PEN-BASED ORAL FLUID SAMPLES: FIELD STUDY

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Objectives: The purpose of this study was to determine whether PRRS virus and/or anti-PRRS virus antibodies were present at diagnostically useful levels in the oral fluids of young pigs from farms known to have circulating PRRS virus.

Methods: On 3 finishing sites, pens of approximately 25 pigs were monitored over time for PRRS virus infection using oral fluid and serum samples. Samples were collected when pigs entered the facilities at 3 weeks of age, then at 5, 8, 12, and 16 weeks of age. Oral fluids were collected by suspending cotton rope in each pen for approximately 20 minutes. The oral fluids were then extracted from the rope. Serum samples were collected from a convenience sample of 5 pigs per pen. Samples were assayed for the presence of PRRS virus by qRT-PCR and for the presence of specific anti-PRRS virus antibodies by ELISA and IFA.

Summary of results: Estimated virus titers in serum and oral fluids were positively correlated. On a pen basis, qualitative results of serum and oral fluid samples resulted in a high level of agreement in regards to the infection status of pens. Likewise, PRRS virus-specific antibody was detected in oral fluid samples.

Conclusions: Oral fluid samples may offer a cost effective, efficient, and practical method for the surveillance of PRRS virus in the production setting.

EVALUATION OF THE CYSTEINE PROTEASE DOMAIN OF PRRSV NSP2 AS A POTENTIAL NEW ANTIGEN FOR ELISA DEVELOPMENT

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The movement of the swine industry towards finding strategies to eliminate PRRS virus will require an adequate serological diagnostic assay that can detect various strains of PRRS virus and also have the capacity to differentiate between type1 and type2 PRRS virus isolates. The objective of this research was to evaluate the cysteine protease domain (CP) of PRRS virus nsp2 as a potential new antigen for the development of a sensitive, specific and differential ELISA test. The CP regions of Type 1 and Type 2 PRRS virus were expressed as recombinant proteins. Three hundred and fifty three serum samples from a group of twenty individual pigs experimentally infected with Type 1 or Type 2 PRRS virus were tested using CP-based ELISAs. Antibody specific to the CP domain can be detected as early as 14 dpi, and the antibody response lasted to 202 dpi. The capability of the CP-based ELISA for detecting serum antibody response from pigs infected with various genetically different field strains was determined. Four hundred and thirty-six serum samples submitted to the SDSU diagnostic laboratory were also tested. The CP-based ELISA possesses 93% agreement with the IDEXX ELISA. To differentiate Type 1 and Type 2 PRRS virus, we developed an epitope-based ELISA using a conserved epitope, ES2 in the CP region of Type 1 PRRS virus. The results showed that the ES2 epitope-based ELISAs are specific for identifying Type 1 PRRS virus. Receiver operating characteristic analysis based on the 100 known positive and 107 known negative samples showed good specificity (94.4%) and sensitivity (94.5%) for the Type 1 PRRS virus. This project addresses the "proof of concept" phase for new diagnostic assay development and more detailed "full validation" studies will be pursued based on the preliminary data generated from this project.

DEVELOPMENT AND OPTIMIZATION OF A BLOCKING ELISA FOR TYPE 1 AND TYPE 2 STRAINS OF PRRSV

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The PRRS virus continues to be a significant economic concern for swine producers in the U.S. and throughout the world. Recently, the presence of an emerging European-like strain that is genetically and antigenically distinct from the original European Type 1 and U.S. Type 2 strains has impacted the sensitivity of current diagnostic techniques and consequently has complicated the detection of PRRS virus in swine herds. The IDEXX HerdChek® PRRS assay, a commercially available enzyme-linked immunosorbant assay (ELISA) has become the industry standard for the detection of antibodies against PRRS virus. The need to accurately determine the PRRS virus serostatus of herds and individual animals has prompted the development of confirmatory tests that enable differentiation of true positive samples from presumed false positives. A highly specific and repeatable blocking ELISA (bELISA) was developed with the use of both U.S. Type 1 and 2 nucleocapsid (N) proteins as the antigen and two competitive monoclonal antibodies specific for highly conserved regions within the N protein. Validation of the bELISA was performed by using 537 serum samples from 42 individual animals that were experimentally infected with either U.S. Type 1 or U.S. Type 2 PRRS virus. Receiver operating characteristic analysis determined a diagnostic sensitivity and specificity of 99.3% and 99.1%, respectively. Further analysis of the data enabled us to establish a definitive cutoff point of 39.6 and to identify times of seroconversion for both PRRS virus genotypes. Furthermore, the bELISA was able to resolve 72% of unexpected positive IDEXX ELISA results obtained from a collection of nearly 196 diagnostic field samples. Our results show that the bELISA may be useful as a follow up test to evaluate suspect results obtained with the IDEXX ELISA, due to its increased sensitivity and detection capabilities.

PREPARATION OF MONOCLONAL ANTIBODIES AGAINST THE NON-STRUCTURAL PROTEINS OF PRRSV

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The PRRS virus non-structural proteins (nsps) are predicted to be cleaved into 13 polypeptides, which mediate both genome replication and transcription of a nested set of subgenomic mRNAs that encode structural proteins of the virus. Little is known about the structure and function of PRRS virus nsps. One of the key reagents to study protein structure-function and design antiviral intervention strategies is the monoclonal antibody (mAb). The objective of this project was to generate a full panel of mAbs against PRRS virus nsps. Recombinant proteins encoding the nsp1, 7, 8, 12, and functional domains of nsp2, 4, 9-11 were *in vitro* expressed in *E. coli.* Synthetic peptides were synthesized as immunogens for nsp3, 5, 6 and 10 were synthesized. BALB/C mice were immunized with recombinant proteins or synthetic peptide antigens. Hybridoma clones secreting specific MAbs were initially screened by indirect immunofluorescent assays against selected PRRS virus isolates. Specificity of the MAbs was further analyzed by Western blotting or immunoprecipitation. A panel of mAbs against nsp1, 2, 3, 4, 7 and 8 were produced. MAbs against the remaining nsps are at different stages of production. This project provides basic key reagents for study of the fundamental biology of the PRRS virus nsps. The proteins and mAbs produced from this project will also be good candidates for future development of diagnostic assays to specifically identify and differentiate Type 1 and Type 2 PRRS virus.

PRRSV SURVEILLANCE, ELIMINATION, AND IMMUNITY IN BOARS AND BOAR SEMEN

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The long-term overall goal of this project is to: "provide a PRRS virus-free semen supply". The specific immediate goals of this study were to: 1) Evaluate surveillance methods for PRRS virus detection in boars by comparing samples currently submitted to diagnostic laboratories to determine whether "pooling" of blood swabs, serum, raw semen and extended semen can consistently detect acute infection;. 2) Evaluate the immune response to PRRS virus in the boar, reproductive tract and semen;. 3) Evaluate a PRRS virus elimination technique from semen which separates seminal white blood cells from sperm to determine whether this separation might consistently "purify" the semen of PRRS virus. Twenty-nine PRRS virus naïve boars were obtained and inoculated with a low virulent PRRS virus strain. Blood swabs, serum and raw and extended semen were collected 3 times weekly for 15 days. A quantitative real-time PCR assay was performed using undiluted, 1:3 and 1:5 dilutions of all samples. With the low virulent PRRS virus strain, only 15 of 29 boars became viremic by 3 days post inoculation (DPI). For these 15 boars, the sensitivity of serum and blood swab was very similar, being very low at 1 DPI (7%) and high thereafter ranging between 87-100%. Sensitivity estimates for pooled serum and blood swabs ranged between 71-100%. Sensitivity for semen samples was very low, ranging from 14 to 29%. The first positive semen samples were detected at 5 DPI and 9 boars did not test positive in semen at any collection time. Results indicated that using blood swabs or serum undiluted or with 1:3 or 1:5 dilutions have similar sensitivities and are useful in detecting early infection in adult boars. Studies involving the gradient method of semen purification and the immunology of the reproductive tract are on-going.

SECTION 3: IMMUNE AND GENETIC RESPONSE

GENOME-WIDE TRANSCRIPTIONAL MAP OF INNATE IMMUNE RESPONSES TO REPLICATION OF PRRSV IN ALVEOLAR MACROPHAGES OF COMMERCIAL BREEDS

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It is increasingly recognized that genetic components involved in host susceptibility represent an important step forward for the development of a disease control programs for PRRS. The present study was undertaken to evaluate the innate immune response of the alveolar macrophage of commercial pigs to PRRS virus infection. Here we report a comparison of the *in vitro* replication of PRRS virus and the genome wide transcriptional host response in alveolar macrophages derived from five genetic lines including Large white, Pietrain, Landrace and two synthetic lines selected for dam line robustness and efficient lean growth. First, we established that the bronchoalveolar lung fluid preparations of each line were essentially composed of macrophages/monocytes cells and derived from healthy piglets. We next showed that, *in vitro*, PRRS virus kinetics of replication and growth in the Landrace alveolar macrophage animals were significantly and reproducibly reduced or delayed when compared with the other four lines. As no secondary pathogens were detected, this crucial information indicated the possibility that either intrinsic or apparent genetic factors are likely to be responsible for the *in vitro* attenuation of PRRS virus replication. While no change in sialoadhesin PRRS virus co-receptor accumulation and localization were detected genome-wide transcript analysis using the Affymetrix platform (24,123 genes monitored) was investigated. Groups of transcripts associated with this PRRS virus susceptibility and reduced-susceptibility were identified and mapped, in silico, on the porcine genome. This effort will be discussed in conjunction with kinetics of cytokine productions. Taken together, this work reveals for the first time the transcriptional map of the innate immune response in alveolar macrophage of two breeds showing different level of susceptibility to PRRS virus infection and indicates signalling pathways likely to operate during PRRS virus infection.

USP18 CONTRIBUTES TO PRRSV ANTIVIRAL RESPONSE BY REPRESSING JAK-STAT SIGNALING

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The signalling pathways operating during early PRRS virus infection in swine alveolar macrophage are poorly understood. In the course to characterize the early innate immune response of PRRS virus-infected alveolar macrophages, Zhang et al (1999, Virology 262:152-162) have identified a transcript encoding an ubiquitin-specific protease (UBP) that is specifically up-regulated upon viral infection. Conjugation and de-conjugation of ubiquitin to intracellular proteins has emerged as a mechanism of fundamental importance in the regulation of the innate and the adaptive immune system. It is therefore crucial to understand the function of UBP during the response to PRRS virus in alveolar macrophages as it could highlight key mechanisms important for the establishment of viral growth. We found that UBP gene is an ortholog of the human ubiquitin specific protease 18 (USP18) gene and demonstrated that it encodes a biochemically functional de-ubiquination enzyme. UBP should now be called USP18. USP18 transcripts accumulation is part of the type-I interferon response as it is concomitantly up-regulated together with interferon-? ?mR? ? in polyIC-treated alveolar macrophages. In PRRS virus-infected alveolar macrophages as well as in Marc-145 cells, transient USP18 transcript accumulation was always found to precede PRRS virus RNA replication and interferon-? transcript accumulation. We found that overexpression of a functional USP18 was inhibiting PRRS virus growth via the specific inhibition of signalling pathways that lead to ISRE regulation. This work suggests the possibility that PRRS virus requires a functional JAK-STAT pathway for its early growth. Furthermore these data put forward that PRRS virus may have a differential control on type I-interferon response as it uncouples transcript accumulations of USP18 and interferon-? . This report will discuss the possibility that PRRS virus may operate via modulation of ubiquitin-de-conjugating pathways by means of inhibition of either USP18 transcript accumulation or protein destabilization, to promote its own growth.

RECOMBINANT ADENOVIRUS EXPRESSING PRRSV MINOR ENVELOPE PROTEIN GP3 INDUCES CELL-MEDIATED AND HUMORAL IMMUNE RESPONSES IN MICE

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PRRS is still a major problem in the swine industry worldwide. Both live attenuated and killed vaccines are commercial vaccines against PRRSV have been widely used, and they play an extent effect in prevent and control PRRSV. However, they cannot provide effective protection against PRRSV infection due to their inherent character. Thus, a lot of alternative engineering vaccines have been studied and tested in the last few years. The antigens in their studies were focused on the main structure proteins, such as GP5, M protein. However, very little has been known about the minor envelope proteins of PRRSV. GP3 is one of the important minor envelope proteins of PRRSV. It plays a role in clearing the virus infection and may be involved in viral neutralization alone with the GP5 and M protein. In order to known the immunogenicity of GP3 protein, 2 recombinant adenoviruses expressing GP3 and tGP3 (ORF3, nt193-765) of PRRSV were first constructed, designated as rAd-GP3 and rAd-tGP3, respectively. And the expressed GP3 and tGP3 protein in infected 293 cells were confirmed by indirect immunofluorescence assay and Western blot analysis. Then 96 6-8 weeks old female BALB/c mice were randomly divided into 4 groups, including 2 groups were immunized subcutaneously (s.c.) at day 0 and boosted at days 14 with the adenovirus recombinants rAd-GP3, or rAd-tGP3, respectively. At each immunization, the mice received 10^5 $TCID_{50}$ of each recombinant. Two control groups received the Ad5 vector alone (wtAd) or PBS following the same immunization protocol. At days 14, 28, 42 and 56 days post immunization (dpi), the mice of each group were euthanized and the serum samples (n=6) were obtained from mice for detection of specific antibody responses. The results showed that the antibody responses in the recombinants-inoculated groups were increased at 14 days post-primary immunization (DPI). During the period of 28-56 DPI, the PRRSV-specific ELISA antibody level induced by rAd-tGP3 was significantly higher than that of rAd-GP3 ($P<0.05$). Meanwhile mice inoculated with rAd-GP3 and rAd-tGP3 produced neutralizing antibodies to PRRSV at 42 DPI. At 56 DPI, mice immunized with rAd-tGP3 developed neutralizing antibodies with titers of 1:14 that was significantly higher than mice immunized with rAd-GP3 with 1:10 ($P<0.05$). Furthermore, lymphocyte proliferation and CTL responses were measured for evaluation of cellular immune responses. At 14, 28, 42 and 56 DPI, splenocytes from the mice were harvested and tested. The results showed that a proliferative response was detected as early as 14 DPI. The response of the group receiving the rAd-tGP3 at 42 dpi was significantly higher than that of rAd-GP3 group ($P<0.05$). Splenocytes from mice immunized with rAd-GP3 had PRRSV GP3-specific CTL activity, as did splenocytes from mice immunized with rAd-tGP3. At 28 DPI, strong CTL responses of experimental groups immunized with rAd-GP3 and rAd-tGP3 were elicited, but CTL activity induced by immunization with rAd-GP3 was significantly lower than that of rAd-tGP3 ($P<0.05$). It indicated that GP3 expressed in recombinant adenoviruses could induce not only humoral immunity, but also cellular immunity in mice, which including T-cell proliferation responses and cytotoxic T responses. The cellular immunity might play an important role in protective immunity against PRRSV.

A PORCINE MYELOMONOCYTIC CELL LINE, 3D4/31 IS SUSCEPTIBLE TO PRRSV INFECTION *IN VITRO*

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PRRS virus has been characterized into two genotypes, North American (NA) and European (EU). It has been reported that MA-104 and Marc-145 cells were the only permissive continuous cell lines to PRRS virus infection where the EU PRRS virus required further adaptation for propagation. Thus, primary culture of porcine alveolar macrophage (PAM) isolated from SPF piglet has been used for EU PRRS virus isolation. In this study, we have examined a continuous porcine alveolar macrophage cell line, 3D4/31, which previously reported to support several porcine virus replication, for its permissiveness to both NA and EU typed PRRS virus infection. Results showed that 3D4/31 is permissive to PRRS virus infection as evidence by positive findings from indirect immunofluorescence staining for intracellular viral antigens; electron microscopy for intracellular viral particles and plaque assay to confirm the infectivity of viral particles. In addition, both NA and EU PRRS virus produced a significant cytopathic effect on 3D4/31 cells at 72hr post-infection and cell death analysis was confirmed by DNA fragmentation and cell titre luminescence assay. As 3D4/31 is a myelomonocytic cell line, our data also demonstrated that the immunological indicators, e.g. IFN-? and IL-6 as analyzed by RT-PCR, were enhanced during infection. In summary, 3D4/31 cells line is a useful *in vitro* model for PRRS virus isolation, particularly the EU genotype and for studying the biology and persistent infection of PRRS virus. It has been reported that PAM susceptibility to PRRS virus varies from different genetically diverse lines of pigs, therefore, the continuity advantage of 3D4/31 cells line overcome the problems of instability of PRRS virus isolation due to the discontinuous nature of PAM.

ANTIBODY RESPONSE TO THE CYSTEINE PROTEASE OF PRRSV NON-STRUCTURAL PROTEIN 2

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The PRRS virus nsp2 is predicted to be the largest protein of PRRS virus. Its N-terminal end encodes a putative cysteine protease responsible for the nsp2/3 cleavage. The cysteine protease is also predicted to function as a co-factor with the nsp4 serine protease to process the other non-structural protein cleavage products. Besides its role in virus replication, nsp2 was determined to be highly immunogenic. In this study, using a reverse genetic system, we deleted the immunogenic epitopes on the nsp2 of a North American Type 1 PRRS virus. The results showed that all of the epitope deletion mutants were viable except for a mutant containing a deleted ES2 epitope, which is located in the cysteine protease domain region. Antibody response to the ES2 epitope was evaluated using a panel of sera samples from pigs experimentally infected with Type 1 PRRS virus. The antibody response to the ES2 epitope was higher than the response to epitopes located in other regions of nsp2. The anti-ES2 antibody response was detectable by 14 dpi, and still can be detected at 85 dpi. At the same time, the antibody response to the predicted cysteine protease region of Type 2 PRRS virus was evaluated using serum samples from a group of pigs experimentally infected with Type 2 PRRS virus from 0 dpi to 202 dpi. Similar to results of Type 1 PRRS virus, anti-cysteine protease response could be detected from 14 dpi to 202 dpi. These results suggest the PRRS virus nsp2 cysteine protease not only plays a key role in virus replication but may also be involved in the modulation of host immunity.

IgY NEUTRALIZING ANTIBODIES AGAINST PRRSV REDUCE VIRAL MOVEMENT IN A POSITIVE FARM UNIT

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Introduction. The immune response against PPRSV is mainly mediated by cellular immune response and the humoral response only plays a minor role. Nowadays neutralizing antibodies function is controversial. Neutralizing antibodies appear until at least 4 weeks post-inoculation furthermore these antibodies do enhance PRRS virus replication in macrophages. Nevertheless the passive immunity transfer of neutralizing antibodies confers protection and could lead to sterilizing immunity. The use of specific avian immunoglobulins (IgY) against PRRS virus represents an alternative PRRS virus infection treatment. The aim of this trial was to determine the effect of specific IgY administration on naïve gilts when introduced into a positive herd.

Material and Methods. Three PRRS virus strains isolated in Tehuacan Puebla were used to hyperimmunize a SPF flock. The specific antibodies against PRRS virus were purified from the egg yolk and quantified by MNT and employed as treatment in susceptible gilts. A group of 120 gilts was introduced in a positive farm unit with an average 30% positive sows; a second group of 104 gilts was introduced a month later in the same reproductive herd. The naïve gilts received 5 mL purified neutralizing antibodies i.m. 15 days before introducing them in site I, and the second treatment was given one day after the introduction. The gilts and sows were monitored for a period of six months. Performing ELISA, RT-PCR, and virus isolation were done every three weeks and all reproductive parameters were recorded.

Results and discussion. The gilts remained seronegative for three months, after which all naïve gilts seroconverted, even though PRRS virus was detected only in 10% of the gilts. The introduction of naïve gilts in a positive herd lead to an increment of abortions, stillbirths, and return of estrus in gilts and sows, as well as mortality increases in nursery. In this trial, the reproductive parameters did not show significant changes. Additionally, PRRS virus was not detected in 90% of susceptible gilts during the observation period, the administration of specific avian immunoglobulins diminished the virus replication in a positive herd, up to levels that did not affect reproductive parameter rates whilst avoiding economic losses due to virus outbreaks in herds.

PROTECTION OF ALVEOLAR MACROPHAGES AND MARC 145 CELLS FROM PRRSV CHALLENGE BY SWINE INTERFERON-BETA

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Interferon beta, a type I IFN, is crucial in initiating the innate immune response and in the generation of the adaptive response. This study demonstrated the capacity of swine interferon beta (swIFNβ) to protect porcine alveolar macrophages (PAM) and MARC145 cells from infection with PRRS virus. The swIFNβ used in this study was produced in HEK293 cells via a recombinant replication-defective human adenovirus 5 (Ad5) encoding the swIFNβ gene (Ad5 swIFNβ). At various times after infection of HEK293 cells infected with either Ad5-swIFNβ or Ad5-Blue (control) supernatants were harvested, acid treated (pH 2.0) and neutralized. To test antiviral activities, target cells, MARC145 or PAMs, were incubated for 18-20 hours with dilutions of either of the treated supernatants. The cells were then infected with PRRS virus, and the presence of cytopathic effects (CPE) recorded. The results showed that cells treated with Ad5-swIFNβ supernatants had no CPE while the cells treated with control supernatant had CPE. As expected uninfected control cell monolayers remained intact throughout the test period, whereas untreated, PRRS virus-infected cells exhibited marked CPE. Culture supernatants of IFN-primed PAMs or MARC145 cells were collected at various time-points post-infection for determination of viral RNA loads using real time RT-PCR. The PCR analysis of these culture supernatants supported the findings of the protective effects of swIFNβ recorded as CPE, and demonstrated that protection occurred in a dose dependent manner. Furthermore, supernatants of HEK293 cells infected with Ad5-swIFNβ were affinity purified on a Sepharose-anti-swIFNβ column. Priming with the IFNβ affinity fractions protected MARC145 cells from infection with PRRS virus, thus confirming earlier results obtained with crude supernatant preparations. These findings demonstrate that swIFNβ is capable of protecting both PAMs and MARC145 cells from subsequent infection with PRRS virus.

PRESENCE OF INTERFERON-ALPHA DELAYS VIRAL REPLICATION AND REDUCES DISEASE SIGNS IN PIGS CHALLENGED WITH PRRSV

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Compared to other viruses that infect the respiratory system, PRRS virus appears to induce only modest levels of interferon-alpha (IFNA). However, IFNA has been shown to inhibit PRRS virus replication *in vitro*, and indirectly to inhibit replication *in vivo* by decreasing replication of PRRS virus in pigs coinfected with viruses that induce high levels of IFNA. In this study, pigs were injected with an advanced generation, nonreplicating adenovirus vector expressing porcine IFNA (AdIFNA) and then challenged with PRRS virus to determine more directly whether the presence of increased levels of IFNA would decrease viral replication and/or disease. Groups of 10 pigs each were inoculated with AdIFNA and not challenged, AdIFNA and challenged with PRRS virus 1 day later, or inoculated with the same adenovirus that does not express IFNA (Adnull) and challenged 1 day later with PRRS virus. IFNA levels in all pigs inoculated with the AdIFNA were elevated the day of challenge (1 day after inoculation) but were undetectable by 3 days after inoculation in the pigs that were not challenged with PRRS virus. Pigs inoculated with AdIFNA and challenged with PRRS virus had lower febrile responses, decreased percentage of lung involvement at 10 days post infection, decreased hyperplasia of the lymph nodes at 10 days post infection, delayed viremia and antibody response, and higher serum IFNA levels as a result of PRRS virus infection, as compared to pigs inoculated with Adnull and challenged with PRRS virus. These results indicate that interferon-alpha can have protective effects if present during the time of infection with PRRS virus.

DO IMMUNE GENES INDICATE WHICH PIGS WILL HAVE PERSISTENT PRRSV INFECTIONS?

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The "Big Pig" project is a multi-disciplinary, multi-institutional approach to 1) establish a better estimate of the proportion of pigs with persistent PRRS virus infections and 2) identify virological or immunological correlates of persistent infection or immunity for diagnostic use. This study highlights immune response differences between pigs that have evidence of long term persistent PRRS virus infection at >/= 112 days post inoculation (dpi) and compare those responses to control pigs and pigs that apparently cleared the infection in the first 28 dpi. Two-week old pigs (n=109) were inoculated with PRRS virus ATCC VR-2332 and 56 age-matched animals served as uninoculated controls. Sets of PRRS virus infected and control pigs were euthanized at 2 week intervals through 203 dpi and tissues collected. We prepared RNA from respiratory immune and regional mucosal tissues [lung, tracheobronchial lymph nodes and tonsil]. A panel of 23 immune markers, representing innate, T helper and regulatory genes, were assayed on tissue cDNA from pigs with persistent versus non-persistent PRRS virus infections and controls. Low levels of interferon-gamma and certain innate immune genes were expressed by all infected pigs. Final statistical analyses for all tissues are continuing. We hope to determine if there is a pattern of cytokine expression that might help predict which pigs will clear virus, and distinguish them from those that remain persistently infected. A parallel test of serologic cytokine levels is underway. We expect this data will reveal differential gene and protein expression associated with PRRS virus clearance and help identify novel regulatory pathways that would stimulate PRRS virus immunity. Supported by USDA ARS and NRI PRRS CAP1 funds.

PASSIVE IMMUNIZATION OF YOUNG PIGLETS WITH HYPERIMMUNE PLASMA CONTAINING VIRUS NEUTRALIZING ANTIBODIES TO PRRSV

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The goal of our research was to produce polyvalent hyperimmune plasma with high-titers of virus neutralizing (VN) antibody to both homologous and heterologous PRRS virus strains, and to evaluate protection provided by passive immunization of 3-week old piglets with the hyperimmune plasma in a live PRRS virus challenge model. The hyperimmunization process was conducted via infection of gilts with 3 strains of live PRRS virus. Hyperimmune plasma, as well as normal swine plasma, was harvested at necropsy and used in subcutaneous injections of 3-week old piglets. This plasma had VN titers ranging from 1:64 to 1:1024 by fluorescent focus neutralization when tested against strains used in hyperimmunization. VN titers for heterologous strains ranged from <1:4 to 1:512. Passively treated pigs and control pigs were challenged with a PRRS virus strain 24 hours after receiving plasma. Only 1 out of 4 pigs immunized with hyperimmune plasma became viremic at 14 days post infection (dpi), while 4 out of 4 pigs receiving normal swine plasma were viremic at 3 dpi through 14 dpi. These results confirm the previous report by Osorio et al regarding the protective role of neutralizing antibody in reduction and prevention of viremia due to PRRS virus.

PRODUCTION OF EQUINE PLASMA CONTAINING HIGH NEUTRALIZING ANTIBODY TITERS TO PRRSV

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Previous studies have indicated that passive immunization of pigs with hyperimmune swine plasma can confer complete or partial protection to virulent PRRS virus challenge. While horses historically have been used to raise large volumes of antibody to different organisms and toxins, there is currently no published data regarding the inoculation of horses with live PRRS virus. The objective of our research was to produce high-titer antiserum in horses which could be used to passively immunize pigs for prevention of PRRS. Two horses were inoculated with four different live PRRS virus strains by intramuscular injection. Serum was taken at various times after each inoculation. Antibody response was determined by fluorescent focus neutralization (FFN) against one of the strains used. Horses did not become febrile or develop other clinical signs of disease during the study, and viremia was not detected. FFN titers increased from <1:4 initially to 1:256. These results indicate that horses are able to produce high neutralizing antibody titers to PRRS virus following inoculation with live virus. The results of passive immunization of pigs with equine plasma and subsequent challenge with homologous PRRS virus will be presented at the meeting.

STRUCTURAL STUDIES OF ARTERIVIRUS PROTEINS

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Very little structural information is currently available for PRRS virus or any other arterivirus. The overall objective of our study is to provide a structural framework for understanding PRRS virus infection, immunity, assembly and pathogenesis by a combination of cryo-electron microscopy (cryo-EM) and X-ray crystallographic structure determination.

The PRRS virus virion consists of a lipid envelope that contains several envelope proteins, GP2—GP5, E and M, surrounding a nucleocapsid core composed of protein N that encapsidates the RNA genome. The most abundant envelope proteins GP5 and M are the main constituents of the viral envelope while GP2, GP4, E(2b) and possibly GP3 are minor components that may be important in host interactions and tissue tropism. Several non-structural proteins (NSPs) are involved in the replication of the virus, including the three viral proteases NSP1, NSP2 and NSP4.

We have solved the X-ray crystal structure of the C-terminal shell-forming domain of PRRS virus-N as well as that of EAV-N. The proteins form dimers with a four-stranded ? -sheet floor, superposed and flanked by N- and C-terminal ? -helices. The same motif was recently also found in the C-terminal part of the much larger N protein from the coronaviruses SARS-CoV and IBV.

An unusual aspect of the arteriviral envelope proteins is their large endodomains. We have expressed the endodomains of GP5 and M, but both proteins are flexible and show a tendency to aggregate over time. Current work is focused on GP2 and the non-structural proteins.

PRESENCE OF HAPTOGLOBIN ?1S SUBUNIT IN SERUM IS ASSOCIATED WITH ACUTE PRRS V INFECTION

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In PRRS, the current diagnosis is based on screening for antibodies by ELISA and confirming the presence of the organism by PCR. However, detection of antibodies is not possible before seven days after infection and PCR is not feasible as an on-site field test. An early warning biomarker of infection would improve diagnosis and facilitate better preventive strategies. Here, we hypothesized that PRRS virus infection produces a serum protein profile characteristic of early infection. The objective was to obtain a serum profile of low molecular weight proteins in PRRS virus-infected and non-infected porcine sera by mass spectrometry. The sera from pigs infected with various isolates of PRRS virus (n=25) and controls (n=34) were processed to remove high molecular weight proteins using organic solvent precipitation. The extracted low molecular weight proteins were desalted in a C4 resin column, mixed with sinapic acid and analyzed on MALDI-TOF. Comparative analysis of serum protein profiles revealed that a protein with an m/z value of 9244±2 appeared in PRRS sera within one day of infection, with sensitivity of 0.92 and specificity of 0.94 at seven days of infection. When sera from pigs infected with non-PRRS virus pathogens (n=24) were included in the analysis the specificity dropped to 0.83. The 9244±2 was identified through SDS-PAGE and LC/MS/MS as the a1S subunit of porcine haptoglobin. This was further confirmed by immunoblotting the organic solvent-extracted serum proteins with anti-porcine haptoglobin antibody. The results suggest that the a1S subunit of haptoglobin, a well known acute phase protein, is a potential protein biomarker for acute PRRS. The presence of free a1S subunit in serum of PRRS virus infected pigs may provide new insights into biochemical processing of haptoglobin and its role in PRRS virus pathogenesis.

THE SELECTED B CELL POPULATION IN PRRS HAS A NAÏVE PHENOTYPE, UNDIVERSIFIED REPERTOIRE AND UNUSUALLY HYDROPHOBIC HCDR3

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Isolator piglets infected with PRRS virus develop lymphoid hyperplasia, hypergammaglobulinemia and autoimmunity (Lemke et al 2004). Preliminary characterization of the expanded B cell population in these animals reveals a naïve population that continues to express CD2. Spectratypic analyses (CDR3 length analysis) of B cells in PRRS animals revealed the same profile in every tissue which contrasts with the pattern in SIV-infected littermates. While the spectratype was similar for different PRRS virus-infected piglets, the profile was animal-specific. The CDR3 region of the antibody heavy chain (HCDR3) is the major contributor to antibody specificity and diversity, especially in swine because of their limited combinatorial diversity (Sun et al 1998;Butler et al 2000;2006). Sequence analysis of the major HCDR3 lengths in PRRS indicated a highly skewed hydropathicity profile favoring hydrophobic sequences encoded by reading frame 3 of D_HA and a near absence of HCDR3s with neutral charge that normally dominate hydropathicity profiles in non-PRRS animals. We propose that B cells with hydrophobic HCDR3s in PRRS virus-infected piglets are targeted for T cell-independent proliferation without repertoire diversification. The ligand responsible may co-ligate the BCR to an innate immune receptor in the manner of LPS to promote B cell proliferation without T cell help.

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IN VITRO INHIBITION AND ENHANCEMENT OF PRRSV INFECTION BY PORCINE SEMINAL PLASMA IS NOT DUE TO THE EFFECTS OF COMPLEMENT

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Semen is an important route of PRRS virus transmission. This appears to be particularly true for artificial insemination (AI). During AI, semen is diluted to increase breeding coverage and to improve the survival of healthy sperm. How this dilution of semen for AI impacts infection of sows and fetal tissues by PRRS virus has not been studied in detail. We previously showed that boar seminal plasma, and several of its fractions, inhibit PRRS virus infection of MARC-145 cells at high concentration, but enhance the infection at lower concentrations. Further, the heparin-binding fraction of seminal plasma was primarily responsible for these effects. It has been recognized that antibody and complement can facilitate PRRS virus entry into macrophages at sub-neutralizing concentration (ADE). To rule out an ADE-like effect, we tested seminal plasma for complement activity that would in inhibition and/or enhancement PRRS virus infection of MARC-145 cells. Boar seminal plasma was heat inactivated for 30 min at 56°C, or for 10 min at 100°C to neutralize complement and heat denature most proteins, respectively. The precipitated materials following heating were removed by centrifugation, and the clear supernatant was subjected for the further testing. The effect of heat inactivating seminal plasma at 56°C moderately altered its ability to inhibit or enhance PRRS infection. However, heating seminal plasma to100^{o}C strongly reduced both the inhibition and enhancement effects on PRRS virus infection. These results indicate that a heat labile substance, but not complement, in boar seminal plasma plays a major role in inhibition and enhancement of PRRS virus infection.

ABSENCE OF IRF-3 ACTIVATION IN PRRSV INFECTION

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PRRS virus may persist in infected pigs for up to 6 months after initial exposure, indicating a possible suppression of the host immune system. The objectives of this study were to examine and elucidate the mechanism of Type I interferon down-regulation by PRRS virus *in vitro*. By RT-PCR and quantitative real-time RT-PCR, the relative mRNA level of IFN-α was found to be lower in PRRS virus-infected Marc-145 cells compared to cells induced by double-stranded RNA analogue poly I:C. The effect of PRRS virus infection on interferon regulatory factor 3 (IRF-3), an essential IFN transcription factor, was explored. By a luciferase reporter assay, it was found the virus consistently down-regulated expression of IRF-3 as compared to poly I:C treated cells. When Marc-145 cells were transfected with a GFP-IRF3 expression plasmid and infected with PRRS virus, GFP-IRF3 did not localize to the nucleus as is typical once this protein is activated. When HeLa cells were co-transfected with GFP-IRF3 and the nucleocapsid (N) protein of PRRS virus, cells expressing both GFP-IRF3 and N protein did not show any nuclear localization of GFP-IRF3 despite the stimulation by poly I:C. These findings suggest not only that PRRS virus suppresses IFN but also that it may do so by interfering with IRF3 activation. Our study implicates that the N protein is involved in the immune evasion tactic for PRRS virus.

ROLE OF STRUCTURAL PROTEINS IN CROSS NEUTRALIZATION AMONG PRRS VIRUSES

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The following study was conducted to assess the role of each structural protein in cross neutralization among PRRS viruses. As GP5 is believed to be the most important component in virus neutralization (VN), sequence elements within ORF5 associated with VN were determined by genetically comparing field isolates (n=69) classified into different antigenic groups based on their susceptibility to neutral PRRS virus envelope glycoprotein (GP5) and membrane protein (M) are type I membrane proteins which form a disulfide bond-linked heterodimer on the PRRS virus virion envelope. The complex is responsible for cell attachment, and antibodies directed against GP5 neutralize viral infectivity. While the role of GP5, in particular, has been extensively studied in PRRS virus immunity and pathogenesis, little is known about the role of the heterodimeric structure in anti-viral immunity, even though it appears to be essential to the infectious process. We hypothesized that antibodies are directed to conformational epitopes on the ectodomains of GP5 and M proteins, and that these antibodies may be involved in virus neutralization. To test the hypothesis, we expressed in bacteria a recombinant single-chain protein encoding the four predicted ectodomains. Pigs infected with PRRS virus developed anti-GP5-M antibody response that was long-lasting, while the neutralizing antibody titers were low when determined at 193 days after challenge. There was no apparent correlation between anti-GP5-M antibody response and the serum total neutralization titers or to resistance to challenge. Pigs immunized with GP5-M developed high antibody response and demonstrated partial protection against challenge. However, there was no neutralizing activity detected. Additional experiments were performed to determine if GP5-M added to MA104 cell culture blocked viral infection. The protein had no effect on PRRS virus infection. The data from these studies suggest that antibodies to GP5-M may play a role in protection against PRRS virus that is not dependent on viral neutralization.

PORCINE HUMORAL IMMUNE RESPONSE TO PRRSV GP5-M

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PRRS virus envelope glycoprotein (GP5) and membrane protein (M) are type I membrane proteins which form a disulfide bond-linked heterodimer on the PRRS virus virion envelope. The complex is responsible for cell attachment, and antibodies directed against GP5 neutralize viral infectivity. While the role of GP5, in particular, has been extensively studied in PRRS virus immunity and pathogenesis, little is known about the role of the heterodimeric structure in anti-viral immunity, even though it appears to be essential to the infectious process. We hypothesized that antibodies are directed to conformational epitopes on the ectodomains of GP5 and M proteins, and that these antibodies may be involved in virus neutralization. To test the hypothesis, we expressed in bacteria a recombinant single-chain protein encoding the four predicted ectodomains. Pigs infected with PRRS virus developed anti-GP5-M antibody response that was long-lasting, while the neutralizing antibody titers were low when determined at 193 days after challenge. There was no apparent correlation between anti-GP5-M antibody response and the serum total neutralization titers or to resistance to challenge. Pigs immunized with GP5-M developed high antibody response and demonstrated partial protection against challenge. However, there was no neutralizing activity detected. Additional experiments were performed to determine if GP5-M added to MA104 cell culture blocked viral infection. The protein had no effect on PRRS virus infection. The data from these studies suggest that antibodies to GP5-M may play a role in protection against PRRS virus that is not dependent on viral neutralization.

PORCINE MONOCYTE- AND SKIN-DERIVED DENDRITIC CELLS ARE SUSCEPTIBLE TO PRRSV INFECTION, BUT CYTOKINE PRODUCTION IS DIFFERENTLY MODULATED

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Previous reports from our group described that monocyte-derived dendritic cells are infected with PRRS virus, and the expression of MHC-II, CD80/86 and IL-10 production are down regulated by viral infection. In this report, we compared the susceptibility of monocyte- and skin-derived dendritic cells to PRRS virus infection. In previous experiments, we used mature dendritic cells, but in this work we used immature dendritic cells, which were obtained after culturing adherent cells in presence of IL-4 and GM-CSF during 5 days. Skin-derived dendritic cells were obtained after density centrifugation of over night cultures of skin explants. Expression of CD80/86, MHC-II, CD14, CD1, and CD172 was used to confirm the phenotype of these cells. To evaluate the susceptibility cells were infected with PRRS virus at an m.o.i. of 0.1 for 1 h, and were incubated for 24 h. Supernatant was collected and kept at -70 °C for virus titration. Total RNA was extracted from the cells for PRRSv and cytokine quantification by real time PCR. Our results showed that PRRS virus infected and replicated in both, immature monocyte- and skin-derived dendritic. However, the profile of cytokines expression in both cells was different. Immature monocyte-derived dendritic cells expressed more IL-10 in comparison with mock-infected cells. In contrast, skin-derived dendritic cells did not induce the expression of IL-10, but induced the expression of IFN-a. These results suggest that (1) ability of PRSS virus to modulate immune response depends on the type of dendritic cell, and (2) IFN-a did not inhibit the infection of skin-derived dendritic cells.

MECHANISMS OF CROSS-PROTECTIVE IMMUNITY AGAINST PRRSV

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Exposure to PRRS virus establishes a form of immunological memory that restricts or prevents a second infection by the same or different PRRS virus. Protection is characterized by absence or severe reduction in viremia, little or no disease, little or no anamnestic antibody response, and no change in cell-mediated immunity measures. However, episodic field observations of chronic and endemic PRRS and of "vaccine failure" suggest that cross-protective immunity may be a variable feature of the response to heterologous PRRS virus challenge. The mechanisms of protection against PRRS virus are assumed to operate according to accepted immunological principles through T_H1 and T_H2 pathways to elicit cytotoxic T cell and neutralizing antibody functions, but the tools to assess these functions in pigs are not well developed. Primary infection can occur in the presence of neutralizing antibody and low levels may even enhance infection. PRRS virus-specific memory T cells also would be expected to provide immune surveillance and protection against reinfection, but there is no correlation between T cell responses and viral clearance. Thus, inferences about protection against PRRS virus infection based on direct challenge studies are more reliable than are measures of immune responsiveness. Three issues to consider in cross-protection against PRRS virus are 1) attenuated vaccine viruses are immunologically the same as virulent field viruses, so immune responses also are fundamentally the same; 2) there is little correlation between degree of cross-protection and neutralizing antibody level; and 3) PRRS disease occurs in three different developmental stages, fetus, young and growing pig, and adult gilts and sows. Age-associated immunological differences, in addition to host genetic and epigenetic factors, may contribute significantly to the induction of anti-PRRS virus immunity. Since there is no significant antigenic variation in a vaccine strain of virus and protection is produced against heterologous viruses, host animal differences that affect the potency of vaccinal immunity may be an important factor contributing to the apparent efficacy of cross-protection.

UNDERSTANDING MUCOSAL IMMUNITY AND DISEASE RESISTANCE

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The induction of mucosal immunity is an essential component of disease resistance and thus represents an important goal of modern vaccine development. Immunity at the mucosal surfaces consists of both innate and acquired immunity. Both are largely interconnected with antigen presenting cells, such as dendritic cells, representing the link between innate and acquired immunity. In the present talk, an overview will be given about the mechanisms by which the immune system recognizes pathogens at the mucosal surfaces, and how this knowledge will serve in the development of modern mucosal vaccines for swine.

DETECTION OF T CELL RESPONSE AFTER *IN VITRO* STIMULATION WITH GP5 PEPTIDES FROM PRRSV VR-2332 ISOLATE

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The objective of this research was to use the techniques that we had adapted in our laboratory to identify T cell epitopes to determine whether or not chemically synthesized peptides representing the GP5 protein, as determined from the published amino acid sequence of the VR-2332 isolate, would stimulate IFN-gamma synthesis and $CD4^{+}$ T cell proliferation in previously infected pigs. An ELISPOT assay was used to enumerate the cells secreting IFN-gamma after stimulation with individual peptides. A flow cytometric proliferation assay using PKH67 labeled cells was developed to detect $CD4^{+}$ T cells responding to individual peptides. Our data suggest that one of the peptides representing a small portion of the GP5 molecule can induce $CD4^{+}$ T cell proliferation and IFN-gamma secretion from mesenteric lymph node cells from pigs infected with VR-2332 between 5-6 months prior to analysis.

WILD-TYPE, AS WELL AS ATTENUATED VERSIONS OF PRRSV, SELECTIVELY INHIBIT THE ABILITY OF PORCINE PLASMACYTOID DENDRITIC CELLS TO PRODUCE INTERFERON ALPHA

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Plasmacytoid dendritic cells (PDC) are the most potent source of interferon (IFN)-α and thus are primarily responsible for the initial protective response elicited during a virus infection. However, the limited amounts of this cytokine detected in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSv) indicates that this pathogen somehow circumvents this innate immune reaction. We evaluated the behavior of porcine PDC exposed to PRRSv both *in vitro* and *in vivo*. When freshly purified PDCv were incubated with wild type PRRSv, the resultant IFN-α response was meager and at least 100-fold less intense than that registered in the presence of transmissible gastroenteritis virus (TGEv). Moreover, while the PDC underwent physical changes in the presence of TGEv, contact with PRRSv failed to cause a similar type of maturation morphogenesis. That PRRSv was actually affecting PDC function was established by the demonstrated ability of this entity to repress the otherwise vigorous IFN-α responsiveness to TGEv of an isolated porcine peripheral blood mononuclear cell (PBMC) population that was partially comprised of PDC. A similar impact of PRRSv on the performance of PDC was observed during the temporal monitoring of the TGEv response of PBMC obtained from pigs either infected with wild type PRRSv or vaccinated with attenuated strains. In this case, a significant reduction in the amount of IFN-α secreted by the PBMC in the presence of TGEv was noted when the cells were obtained within seven days after the introduction of PRRSv into the animals, and was no longer detected 2 weeks later. This phenomenon could not be attributed to the disappearance of circulating PDC since the proportion of these cells in the PBMC population was approximately five-fold greater than that measured in the pigs prior to PRRSv infection. This inhibitory ability of PRRSv may be rather unique since a similar type of impairment was not noticed when swine were infected with Pseudorabies virus. In addition the inhibitory activity was selective since PRRSv did not affect the ability of PDCs to secrete IL-8 in response to PRRSv or other stimuli.

SECTION 4: VACCINES

BLOCKING OF PRRSV REPLICATION BY STEALTH RNAi IN MARC-145 CELLS

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PRRS virus is a contagious disease characterized by reproductive failure in sows, as well as respiratory distress in young pigs and an influenza-like disease in grower-finisher swine. The disease is now endemic in the swine population in many countries and is one of the most economically important swine diseases in US. RNA interference (RNAi) has been used increasingly for reverse genetics in invertebrate and mammalian cells. In this study pegylated liposomal formulation (stealth) RNAi was used to block PRRS virus viral RNA genome in cell culture. Five target genes were selected including PRRS virus RNA genome at 5'UTR, ORF-7, and cellular receptors; Sn, CD168 responsible for virus entry as well as downstream cellular target Rab9. M145 cells were used for PRRS virus virus propagation, viral titration. Stealth antisense oligos designed from specific viral RNA genome 5'UTR and ORF-7 showed reduction of viral yield 4 logs at 48 hours post infection, scavenger receptor CD168 (PRRS virus specific receptor) stealth oligos showed reduction in viral titer up to several logs at 72 hours post infection. Reduction of viral genome material was confirmed by quantitative RT-PCR on individual samples. These findings showed the potential of anti-sense antiviral oligos with pegylated liposome formulation known as stealth for control of PRRS virus infection *in vitro.*

COMPARATIVE SEROLOGICAL INVESTIGATION OF EARLY PIGLET VACCINATION WITH INGELVAC® PRRS MLV TO VACCINATION AT WEANING

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The objective of this field study was to compare the serologic response in pigs vaccinated with Ingelvac® PRRS MLV at 10-12 days of age to animals vaccinated at weaning. Piglets were born to PRRS positive sows and the study was carried out in a commercial farrow-to-finish farm. A total of 40 animals were allocated to either vaccination at 10-12 days of age or vaccination at weaning (16-22 days). Blood samples were taken immediately before vaccination and at 14, 28, 42 and 56 days following vaccination with either vaccination scheme. The serum was analysed with the IDEXX ELISA HerdChek® PRRS 2XR test kit.

The null hypothesis was that vaccination of young piglets at 10-12 days of age would have a different serologic response pattern as compared to pigs vaccinated at weaning. As it was expected that age had a significant effect on S/P ratios and as the exact age of the animals varied within the experimental groups, a statistical model was established for fixed effects (proc glm, SAS version 8.02) that accounted for effects of the "group" as well as age effects (exact age in days) as a covariate. Simultaneous 95% confidence limits were used to compare the two vaccination regimes. As individual animal S/P ratios may vary the differences between the means of the subsequent time points were used to compare the two groups. Animals of both groups were seronegative at the time of vaccination and titers increased until day 42 and then subsequently declined in both groups in the same manner. The pattern of serological response was similar in the two groups as evident from the Table:

Table 1: Simultaneous 95% confidence limits for the titer difference between the two groups

Difference between timepoints	Difference between LS means	Simultaneous 95% Confidence limits for the LS means of the two groups		p value
Day14 – Day 0	0301	-0581	1183	0493
Day28 – Day14	252	0528	4513	00147
Day42 – Day28	-0695	-1999	0608	0286
Day56 – Day42	-0456	-199	1077	055

The statistical significant difference between the two groups between day 28 and day 14 was caused by 8 animals of the group vaccinated at weaning that had significantly higher S/P ratios than animals of the group vaccinated at 10 days of age. However, this effect disappeared by day 42. As of day 42 until the end of the study the pattern of serological response was not significantly dofferent in the two groups. The pattern of response for serum neutralizing antibodies for both groups was similar, however, with a delayed onset. Nevertheless, the were no significant differences with regard to SN titers at day 56 which also confirm that vaccination at the two time points yielded similar responses. Based on the post vaccination serological response it can be concluded that vaccination around day 10 of age and vaccination at weaning (around 21 days of age) yield comparable serological responses.

REPLICON PARTICLE CO-EXPRESSION OF PRRSV GP5 AND M PROTEINS

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The importance of the immune response to a heterodimer similar to PRRS virus GP5-M has already been established for equine arteritis virus (EAV), another member of the *Arteriviridae* family. However there has been little investigation into PRRS virus GP5 and M protein co-expression in recombinant vaccine studies. We have recently shown that an alphavirus-based virus-like replicon particle (VRP) is a highly effective, non-propagating vector for immunizing pigs against foreign antigens. The purpose of this study was to determine the ability of a replicon vector to co-express GP5 and M. The PRRS virus GP5 and M genes were first cloned into replicon vectors individually. These replicon vectors produced subgenomic transcripts of the correct size and the replicons that expressed the highest level of each individual gene were selected to generate double sub-genomic replicons co-expressing both. These double promoter replicons were analyzed by IFA, western blot and northern blot. Analysis indicated that double promoter constructs were producing the correct sized subgenomic transcripts and correct PRRS virus proteins detected by PRRS virus convalescent pig serum. Each replicon was then packaged into a VRP vaccine by supplying alphavirus structural proteins *in trans*.. These VRP were incubated for 18 hours on Vero cell monolayers followed by cell lysis. Western blot analysis indicated that the VRP co-expressed PRRS virus GP5 and M monomers as well as the GP5-M heterodimer. Vaccination-challenge trials in pigs using GP5, M, and GP5-M VRP are in progress.

DECEPTIVE IMPRINTING AND IMMUNE REFOCUSING—THE NEXT GENERATION OF DISCOVERY AND DEVELOPMENT FOR VACCINES

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The list of disease-causing microbial pathogens is significantly longer than the list of microbes currently controlled or eliminated by vaccine development. It appears that current research and development directed at antigen delivery, vectors, presentation, expression systems and cytokine steering approaches, although important, have not fully addressed the problem with developing broadly efficacious vaccines. <u>As such, it stands that selected genetic instability of the pathogen leading to antigenic variation, coupled with non-protective, strain-restricted immunodominance stands as one of the major obstacles in vaccine design today.</u> The immune defense system of the host operates by surveying the "antigenic space" through conformational shapes and linear sequences of chemical information. It appears that the certain microbial pathogens have continued the evolution of selecting for and presenting chemical shapes and sequences on their surfaces (epitopes), which in some cases are more immunodominant and immunoregulatory relative to other epitopes or antigenic determinants. It appears that these immunodominant non-protective epitopes have evolved to take a immunologic hit without fully compromising the infectivity or virulence of the microbial organism. These immunodominant epitopes appear act to decoy, misdirect and dysregulate the host's immune system, termed Deceptive Imprinting. The technology of Immune Dampening/Refocusing maps, identifies and through site-directed mutagenesis techniques selectively immune dampens these immunodominant and regulatory T and B cell epitopes. Vaccines made by this technology are purposely lacking these epitopes which when used as immunogens induce a specific serologic pattern and induce broader cross-strain neutralizing antibodies and cell-mediated immune responses and protection. The presentation will focus on reviewing current studies in this area and recent pre-clinical studies in which the phenomena and technology are being exploited.

FUNCTIONAL MAPPING OF THE PRRS V CAPSID PROTEIN NUCLEAR LOCALIZATION SIGNAL AND ITS PATHOGENIC ASSOCIATION

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PRRS virus nucleocapsid (N) protein is the most abundant viral structural protein. In addition to its normal cytoplasmic distribution, a portion of N protein is specifically localized to the nucleus and nucleolus during infection. A nuclear localization signal (NLS; 41-PGKKNKKKN-49) has been identified and shown to be sufficient for translocation of N into the nucleus. Previously, an NLS-knockout mutant virus (PGGGNKKKN) was used to show that the N protein nuclear localization is non-essential for PRRS virus replication *in vitro* and *in vivo*. The virus demonstrated reduced viremia and increased induction of neutralizing antibodies but was subject to strong selection pressure in the pig, resulting in reversion of the point mutations and reacquisition of NLS function. To further define the role of NLS in virulence, a series of "reversion resistant" mutations were generated by amino acid deletions and substitutions. Some mutations were non-viable, but two specific mutants (PG--SKKKS and PG--S-KKS) produced mutant viruses that were genotypically stable up to 20 passages in cell culture. Infection of piglets with these mutants induced faster and higher levels of neutralizing antibodies. Both mutants showed a shorter duration and a lower titer viremia than those of wild type virus. RT-PCR from tonsils at 28 days post-infection showed that both mutants persisted in the tonsils but at a lower level than the wild type virus. No reversion to NLS function was detected in either mutant virus, from any pig. The data show that N protein nuclear localization is non-essential for *in vitro* replication, but nevertheless important for PRRS virus pathogenesis.

RECOMBINANT PRRSV EXPRESSING THE PORCINE CIRCOVIRUS TYPE 2 CAPSID PROTEIN - IMMUNOGENICITY IN PIGS

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Development of infectious cDNA clones allows engineering of the PRRS virus genome, and the use of PRRS virus as a vaccine vector. Previously, we constructed several recombinant viruses expressing GFP, and showed the potential of PRRS virus as a vector for foreign gene expression. Here we extend those findings by showing that PRRS-based vectors can induce antibody responses to transgenes in the pig. GFP and porcine circovirus Type 2 (PCV2) capsid protein were used as test antigens. PCV2 -associated diseases (PCVAD) includes post-weaning multisystemic wasting syndrome (PMWS) and Porcine Dermatitis and Nephropathy Syndrome (PDNS). Like PRRS virus, PCV2 infects alveolar macrophages. As was previously described for the GPF construct, the PCV2 capsid protein gene was cloned and inserted into the PRRS virus isolate P129 genome between the non-structural and structural protein coding sequences, as an additional subgenomic RNA. Transfection of MARC-145 cells with the plasmid resulted in the generation of recombinant PRRS virus designated P129-PCV2. The growth of P129-PCV2 was slightly slower compared to the parental P129. Transcription of the PCV2 capsid mRNA was confirmed by RT-PCR and capsid protein was detected by immunostaining of infected cells. Three groups of piglets were immunized twice, intramuscularly, with P129-PCV2, P129-GFP, or parental P129. Induction of specific antibodies to the foreign gene products was examined. Our results demonstrate that PRRS virus-based expression vectors are capable of inducing humoral immune responses to foreign antigens in pigs, and point towards their use as multivalent vaccines.

IMMUNOGENICITY AND PROTECTION EFFICACY OF A HERPES SIMPLEX VIRUS-BASED AMPLICON VECTOR EXPRESSING CODON-OPTIMIZED GLYCOPROTEIN 5 OF PRRSV

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In previous studies, we have shown that plasmid DNA priming followed by boosting with a herpes simplex virus (HSV) -1 amplicon vectored human immunodeficiency virus (HIV) -1 gp120 antigen induced potent T cell-mediated immunity and antibody response in immunized mice. To evaluate the potential application of this immunotherapeutic approach for PRRS virus vaccine development, we generated plasmid DNA and a HSV1 amplicon vector encoding the codon-optimized glycoprotein 5 (sGP5) gene of PRRS virus. The immunogenicity and protection efficacy of these constructs were evaluated in pigs. Results showed that sGp5-specific, interferon-gamma secreting CD4 T cells and antibody responses were induced via the DNA prime/amplicon boost regimen. Increases in CD4 T cell immunity and antibody responses were observed following challenge of animals with PRRS virus, indicative of a memory immune response elicited by the vaccination. However, no PRRS virus-specific neutralization (VN) antibody was detected after the boosting immunization, although a subset of animals developed VN antibody following virus challenge with titers ranging from 1:4 to 1:16. Viral RNA was detected in the sera of all animals at 10 days after challenge. Overall, HSV-1 amplicon-vectored PRRS virus sGp5 induced PRRS virus-specific T cell and antibody responses in immunized animals, but such immune responses were insufficient to provide protection against viremia in animals after PRRS virus challenge.

ORF5 AND ORF2 ARE THE MAIN STRUCTURAL GENES CARRYING DETERMINANTS OF VIRULENCE OF PRRSV

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As a novel approach to uncover the virulence determinants in PRRS virus, we generated a series of chimeric viruses containing specific genomic regions of an attenuated PRRS virus vaccine strain (Prime Pac) within the genomic context of a highly virulent infectious clone (FL12). By studying *in vitro* growth kinetics in both MARC 145 cells and porcine alveolar macrophages (PAM), as well as *in vivo* pathogenicity using a sow reproductive failure model, we confirmed that some of the non-structural (NSP3-8) and structural (ORF2-7) regions of the PRRS virus genome contain major virulence determinants. While some non-structural regions (NSP1-3 and NSP10-12) showed an intermediate attenuation phenotype, NSP9 could be ruled out as an important determinant of virulence. We further dissected the structural gene area and generated 6 chimeras representing each individual structural ORF (2 through 7). We examined the *in vitro* growth and *in vivo* virulence characteristics of these chimeras. The results show that ORF5 encoding glycoprotein (GP) 5 is the most important structural gene for PRRS virus virulence, with ORF2 encoding GP2 and protein E, also contributing, to a lesser extent, to virulence. In order to confirm the role of these two structural genes in virulence, we performed a reciprocal complementary (gain-of-function) experiment in which the ORF2 and ORF5 genes of FL12 were replaced, either alone or simultaneously, by the corresponding genes of the infectious clone (PP18), derived from the Prime Pac vaccine strain. These chimeric viruses were not distinguishable by a full conclusive re-gaining of the virulence based on reproductive failure model. However, some parameters of infection (viremia and kinetics of antibody response) mimic those of the virulent donor strain (FL12) and differ from those of the attenuated recipient strain (PP18). The ORF5 structural gene plays a major role in determining virulence of PRRS virus, and to a lesser extent, ORF2.

SEROLOGICAL MARKER CANDIDATES IDENTIFIED ON STRUCTURAL AND NON-STRUCTURAL PROTEINS OF PRRSV

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This study reports a detailed investigation of the B-cell linear epitopes contained in a US-type PRRS virus strain, as consistently recognized by the antibody response mounted by infected pigs. To this purpose, 213 overlapping 15-mer synthetic peptides spanning the entire sequence of a non-structural (nsp2) and all of the structural proteins of a North American strain (NVSL97-7895) were used individually as antigen in a peptide-based indirect ELISA. Antisera raised in 15 piglets infected with $10^{5.0}$ $TCID_{50}$ of FL-12 (PRRS virus derived from a full-length cDNA infectious clone prepared from NVSL 97-7895 strain) were used for screening of the peptide-specific antibody response. Interestingly, nsp2 was found to contain the highest frequency of immunogenic B-cell linear epitopes when compared to the structural proteins. Among the 97 peptides corresponding to the sequence of nsp2, 18 were localized in predicted hydrophilic domains and were immunoreactive with more than 50% of the sera tested. Ten of these peptides were reactive with 80-100% of the examined sera. In the structural proteins, epitopes consistently recognized by the immune sera were located at gp2 (n=2), gp3 (n=4), gp5 (n=3), M (n=2) and N (n=2). Additionally, the two epitopes identified in the C-terminal end of the M protein (ORF6) exhibited a unique combination of immunogenicity and sequence conservation among isolates from both PRRS virus genotypes. Seroconversion kinetics demonstrated that the antibodies recognizing the immunodominant epitopes appeared regularly between days 7 and 15 pi, remaining detectable until the end of the experiment (day 90 pi). Current efforts center on the construction of mutant PRRS virus viruses lacking the selected immunogenic and conserved B-cell linear epitopes by using site-directed mutagenesis. If proved dispensable for viral replication without affecting the immunogenicity of the viral strain, the identified epitopes may be considered serological markers for differential (infection vs. vaccination) replicating vaccines derived from infectious cDNA clones.

DEVELOPMENT OF A BROADLY PROTECTIVE PRRS VACCINE

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Current PRRS vaccines provide limited protection against heterologous strains of viruses. The sub-optimum level of protection is likely caused by 1) a high degree of sequence variation in structural proteins and 2) deceptive imprinting due to the presence of immunodominant non-protective epitopes (IDNPEs). We hypothesize that variable IDNPEs act as decoys to mislead the host from mounting humoral or cellular immune responses against more highly conserved epitopes that may otherwise induce cross-strain protection. We have proposed to apply immune refocusing technology to the development of a broadly protective PRRS vaccine. Our review of available sequence databases, epitope discovery data, and published literature has led us to focus on the design of new vaccines containing immune refocused GP5 glycoproteins. We have identified two GP5 domains that appear to contain IDNPEs. By site-directed mutagenesis, we have introduced a series of mutations in the N-terminal ectodomain and the C-terminal endodomain of GP5 for expression in recombinant vaccinia virus vectors. A subset of the GP5 mutants have been co-expressed with the viral M protein to determine whether the formation of GP5-M heterodimers result in improved immunity. A preliminary antigenicity study in mice is in progress. The vaccine candidates which induce broadened protection, as assessed by increased neutralization titers against heterologous viruses as compared to unmodified glycoprotein, will be selected for vaccine challenge studies in swine.

EXPRESSION OF RECOMBINANT GP5 IN TRANSGENIC CORN CALLUS FOR PRRS V VACCINE DEVELOPMENT

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Edible plant vaccines are an exciting new alternative in prevention of infectious disease in livestock. Preliminary work has been undertaken to create lines of transgenic maize suitable for use as an edible vaccine for PRRS virus. A codon optimized ORF 5 sequence for protein production in maize was designed from cDNA of US Isolate ATCC VR 2385 and synthesized. This sequence was inserted into a DNA plasmid construct including appropriate promoters, terminators, and leading sequences for maize. Maize callus was transformed with this construct via particle bombardment. Recombinant GP5 was identified in maize callus extract by Western Blot, and the protein migrated in the same pattern as the GP5 derived from PRRS virus. The recombinant GP5 is subjected to further analysis for the confirmation of its identity and glycosylation patterns.

INVOLVEMENT OF CYSTEINE RESIDUE(S) WITHIN THE M PROTEIN OF PRRSV IN INFECTIOUS VIRION PRODUCTION

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The PRRS virus M is one of the major unglycosylated virion envelope proteins. It contains four cysteine residues at amino acid positions 9, 53, 99 and 102. It has been postulated that formation of disulfide-linked heterodimer between the M and the GP5, the major glycoprotein on the virion envelope is important for virus assembly and infectivity. To identify the cysteine residue involved in heterodimer formation, we individually mutated each of the four cysteine residues of M in context of the full length PRRS virus infectious cDNA clone and examined recovery of infectious PRRS virus. Our results demonstrate that mutation of cysteine residue at position 9 to alanine (C9A) abrogated production of infectious progeny virus, indicating C9 is critical in infectious virion production. Infectious viruses carrying mutations at positions 53, 99, and 102 could be readily recovered and grew to titers similar to WT PRRS virus. In transient transfection studies, we have observed that C9A mutant M protein did not interact well with the viral GP5 protein. In contrast, the other mutants (C53A, C99A and C102A) of M protein interacted well with the GP5 protein. These results indicate that C9 of M protein is involved in direct interaction with GP5, and that is an important event in the production of infectious PRRS virus virion.

DELETIONS AND HETEROLOGOUS GENE EXRESSION IN NSP2 OF PRRSV

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Vaccines that can differentiate infected from vaccinated animals (DIVA) are a new development in PRRS vaccine design. Using reverse genetics and a PRRS virus infectious cDNA clone, we constructed a viable PRRS virus that contains a 132 amino acid deletion in nsp2. The deleted region is relatively conserved among known isolates and is predicted to be immunogenic. The replacement of the deleted region with GFP resulted in normal virus yields on MARC-145 cells and porcine alveolar macrophages. However, replacement with a much smaller HA peptide (YPYDVPDYA) resulted in an approximate 10 fold decrease in viral yield. Virus yields increased after further passage. The 132 amino acid nsp2 peptide, when expressed as a recombinant protein in bacteria and coated onto ELISA plates, was recognized by sera from pigs infected with a heterologous virus, VR-2332, but not by sera from naïve pigs. The results from this study can be directly applied to the development of tagged modified live virus (MLV) vaccines that can 1) identify vaccinated pigs, 2) distinguish vaccinated from naturally infected pigs, and 3) detect the loss of immune protection following vaccination.

IMMUNIZATION OF SWINE WITH REPLICON PARTICLES: PROOF OF CONCEPT

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Virus-like replicon particle (VRP) vaccines derived from Venezuelan equine encephalitis virus have previously been used to show that co-expression of the G_L and M proteins of equine arteritis virus are required for protection. We have recently developed VRP co-expressing GP5 and M proteins of PRRS virus, however there are no previous reports of immunizing swine with VRP vaccines. The purpose of this study was to determine the ability of VRP vaccines to induce an immune response in pigs using the hemagglutinin (HA) protein of A/Wyoming/03/2003 (H3N2) influenza virus. Pigs were obtained at 3 weeks of age and divided into 3 groups of 4. On Day 0 and again on Day 14, pigs were vaccinated intramuscularly with 10^8 IU/ml of VRP expressing the HA protein (groups 1 and 2) of or a control VRP (group 3). The VRP in group 1 were derived from VEE 3014 (wt strain) and the VRP in group 2 were derived from VEE TC-83 (vaccine strain). The antibody response was determined by hemagglutinin-inhibition (HI) assay. Prior to vaccination all pigs were HI negative (GMT=12) and pigs in group 3 remained HI negative throughout the study (GMT=20). An HI response was detected in groups 1 (GMT=67) and 2 (GMT=56) following the priming dose. Following the booster vaccination a strong HI response was detected in groups 1 (GMT=2985) and 2 (GMT=2985) with maximum titers reaching 1:5120. No difference in response was seen between groups 1 and 2 indicating that VRP from the non-select TC-83 vaccine strain can be used in future trials. These results indicate that VRP can successfully express a foreign antigen *in vivo* in the pig and induce an immune response. This proof of concept work supports the *in vivo* evaluation of VRP co-expressing PRRS virus GP5 and M proteins as a novel vaccine for PRRS virus.

ASSESSMENT OF THE EFFICACY OF COMMERCIAL PRRSV VACCINES BASED ON MEASUREMENT OF SEROLOGIC RESPONSE, FREQUENCY OF GAMMA-IFN PRODUCING CELLS AND VIROLOGICAL PARAMETERS OF PROTECTION UPON CHALLENGE

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The efficacy of two different types of commercial vaccines against PRRS virus (Euro-type), was evaluated based on clinical parameters upon challenge as well as post-challenge virological profiles (viremia and viral load in tissues upon necropsy, measured in both cases by quantitative real time PCR). In an attempt to establish correlates of protective immunity, two commonly proposed parameters predictive of immunity were measured: 1) serologic responses (ELISA and neutralizing antibodies), 2) frequency of gamma interferon-producing cells in peripheral blood mononuclear cell fraction. The vaccines compared consisted of two commercially available products that are regularly marketed in Spain: one modified live virus and one killed vaccine. The efficacy assay was carried out by vaccinating homogeneous groups of female swine 5 and-a-half months of age and then challenging them with a EU-1 PRRS virus strain (Lelystad). The results obtained show that the modified live virus vaccine was the only type of vaccine capable of establishing effective protective homologous immunity, as measured by viral load in blood and tissues. The killed vaccine, in spite of this product evoking a strong spontaneous gamma interferon response and sizable post-challenge titers of virus-neutralizing antibody, evoked no measurable protective immunity. In the case of the modified live vaccine, the protection exhibited did not appear to be based on humoral but rather on cell-mediated immunity.

SECTION 5: ECOLOGY, EPIDEMIOLOGY, ELIMINATION

AEROSOL TRANSMISSION OF PRRSV: AN APPLICATION TO THE FIELD (PRELIMINARY DATA)

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The objective of this study is to develop a model of a swine production region that is endemically infected with PRRS virus and to evaluate routes of transmission and protocols of biosecurity. It is hypothesized that controlling aerosol spread of PRRS virus is essential for sustainable eradication and that aerosol spread of PRRS virus is influenced by season. The project will be run for one year utilizing over two thousand pigs. There are four buildings with different biosecurity protocols to monitor how the virus spreads in the absence or presence of intervention. Routes of transmission that are monitored are transport, aerosols, insects, fomites, pigs, personnel and facilities. The virus used for experimental infection was the MN-184 PRRS virus. All samples collected are tested by Taq Man qualitative PCR. Six replicates have been completed thus far. Preliminary results have shown that two infections have been due to aerosols and two by insects. Current and more detailed results will be presented at the time of the conference. Based on preliminary data, aerosol and insect transmission of PRRS virus may be potential routes of spread in this model. Furthermore, the use of air filtration may reduce the risk of aerosol transmission.

SUSCEPTIBILITY OF YOUNG PIGS TO PRRSV INFECTION BY AEROSOL ROUTE OF EXPOSURE

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Data on the specific exposure dose necessary for infection via the aerosol route is needed to assess the likelihood of aerosol transmission in the field. Therefore, the general objective of this experiment was to evaluate the susceptibility of young pigs to PRRS virus via aerosol exposure. In each of seven replicates, 9 of 10 individually-housed pigs were administered a specific dose of PRRS virus isolate VR-2332 by aerosol exposure. The 10th pig (negative control) served as a sentinel, i.e., was exposed to ambient room air during pig exposure. Serum samples were collected on 0, 5, and 10 days post exposure to establish whether exposure resulted in infection. Infection status post exposure was based on the detection of virus in serum by virus isolation and reverse transcription-polymerase chain reaction (RT-PCR). The number of pigs infected among the total number exposed by dose was used to derive the dose-response curve for aerosol exposure. Infectivity data indicated pigs were highly susceptible to infection via aerosol exposure.

EFFECT OF DIFFERENCES IN ROUTE AND TIME OF EXPOSURE TO PRRSV ON THE REPRODUCTIVE OUTCOME OF SOWS

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This longitudinal prospective cohort study was designed to compare the reproductive performance of randomly assigned pigs inoculated with a farm strain of PRRS virus to that of naturally infected pigs on an operational swine farm in the Midwestern United States. Eighty PRRS naïve replacement gilts were randomly assigned to three different cohorts. Cohort-1 consisted of 20 pigs exposed to an attenuated farm strain of PRRS virus by intramuscular injection at 4 weeks of age and maintained in an isolation facility. Cohort-2 consisted of 20 pigs "contact exposed" by cohabitation with cohort-1 pigs at 4 weeks of age. Cohort-3 consisted of 40 animals co-mingled with pigs of cohorts 1 and 2 when all were moved in to a grow-finish facility at 8 weeks of age (delayed contact exposure). Serial samples (blood, serum and/or tonsil biopsies) were collected until 85 days of the first gestation. Reproductive outcomes were later compared. Serum samples were banked for analysis of humoral immunity using FFN test (data not available yet) and PBMCs from blood were used to analyze cell mediated immune response using the ELISPOT assay. Tonsil biopsies were used for reverse transcription-PCR (RT-PCR) testing for viral RNA and genetic characterization of PRRSv (ORF 5 sequences). RT-PCR on tonsillar RNA was positive on 18 out of 20 pigs in cohort-1, 17 out of 20 pigs in cohort-2, and 20 out of 40 pigs in cohort-3 at 10 weeks of age. Viral RNA could not be detected in tonsil of any pig by RT-PCR at 34 weeks of age (pre-breeding). Phylogenetic evaluation of ORF sequences indicated an overall mean pairwise nucleotide diversity of 0.06 %. At 17 weeks of age (half way between entry into grower finisher and pre-breeding), the ELISPOT responses to VR2332 of pigs in cohorts 1, 2 and 3 were 96 ± 48, 97 ± 54, and 83 ± 49 respectively. The total number of piglets weaned averaged 9.9 ± 2.8, 8.9 ± 3.6 and 9.7 ± 3.3 in cohorts 1, 2, and 3 respectively. These results indicate that pigs in all three cohorts were infected and were able to mount a nearly similar cell mediated immune response to PRRS virus. Lack of genetic variation in ORF 5 amplicons indicated absence of of recombination events and possibly absence of other PRRSv strains. Cellular immune response was a poor predictor of reproductive performance. For unclear reasons, sows in cohort-2 (contact exposed) had the lowest reproductive performance. Intentionally inoculated (acclimatized) pigs performed no better than contact exposed pigs. We conclude that acclimatization via inoculation offered no ultimate production-related advantages in this setting over natural infection.

NOVEL ANTIVIRALS AGAINST PRRSV

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In spite of vigorous tries, the current strategies and vaccines to control porcine reproductive and respiratory syndrome (PRRS) are incapable to accomplish the goal. Hence alternative strategies are needed. The object of the present study is to evaluate a group of antisense phosphorodiamidate morpholino oligomers (PMOs) for their ability to suppress PRRS virus replication in cell culture. PMOs are analogs of single-stranded DNA oligomers that contain a modified backbone conferring highly specific binding to RNA and resistance to nucleases. Twelve PMOs were design to bind to PRRS virus RNA sequences and were synthesized as peptide conjugates (P-PMO) to facilitate efficient delivery in cell culture. P-PMOs, 5UP2 and 5HP with sequences complementary to the 5'-end and one combination of two P-PMOs, 6P1 and 7P1 with sequences complementary to ORF6 and ORF7, respectively, of PRRS virus genome were found to be highly effective in inhibiting PRRS virus replication in CRL11171 cells. Treatment of CRL11171 cells with 5UP2 resulted in 4.5 log reduction in PRRS virus yield, while a control P-PMO had no detectable effect on the virus yield. These four P-PMOs reduced PRRS virus replication in a dose-dependant and sequence-specific manner. Treatment of cells with combination of 6P1 and 7P1 led to 3.5 log reduction in virus yield, while 6P1 or 7P1 alone had much less effect. The inhibitory effect of P-PMOs on PRRS virus replication was also observed in swine alveolar macrophages (SAM). Immunofluorescence assay with a PRRS virus specific monoclonal antibody confirmed the P-PMOs inhibitory effect in both CRL11171 and SAM. Real-time RT-PCR was used to detect PRRS virus RNA level. Results demonstrated that P-PMO treatment led to reduction of PRRS virus RNA after 5UP2 and 5HP were added to cells at up to 6 h post-virus inoculation. The combination of 6P1 and 7P1 had no detectable effect on PRRS virus RNA level, indicating they did not inhibit viral RNA synthesis but inhibited viral translation of ORFs 6 and 7, respectively, as expected. Cell viability assays detected no cytotoxicity of these P-PMOs within the concentration-range of this study for either CRL1171 cells or SAM. These results suggest potential applications for the novel P-PMOs as effective antiviral compounds to control PRRS virus infection.

PRODUCTION OF PRRSV NEGATIVE PIGS FROM A PRRSV POSITIVE HERD DOES NOT DEPEND ON SEROSTATUS OF SOWS

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Majority of Thai swine herds are PRRS virus positive and negative replacement gilts are not available commercially resulting on PRRS virus eradication through management strategies including total depopulation and repopulation with negative replacement, and herd closure not possible. The objective of the study was to investigate the potential of producing PRRS virus negative pigs from a PRRS virus positive herd using a combinatorial testing of both serological and RT-PCR assays and to determine the correlation of S/P ratios of sows pre-farrowing and production of PRRS virus negative pigs. Negative pigs were defined by pigs tested negative for PRRS virus by both serological and RT-PCR assays at the end of nursery period. The investigation was conducted in a 1,200-sow herd operating traditional two-site production system. In the study, sows were tested serologically for PRRS virus pre-farrowing. Piglets were tested for PRRS virus by both serological and RT-PCR assays prior to weaning and at the end of nursery period. To date, over a thousand of negative pigs have been produced from sows with S/P ratios ranging from 0.00 to 2.80 and the production of negative pigs was independent of sow parity. Less than 20 positive pigs were produced from sows with S/P ratios ranging between 0.40 to 1.20 and these pigs were PCR positive prior to weaning, which could be infected transplacentally. High correlation between S/P ratios of sows and of pigs was demonstrated. While sows with low antibody level can readily produce negative pigs prior to weaning, pigs with higher S/P ratios required more time for antibody level to decline. The results of the study suggested that PRRS virus positive herds can produce PRRS virus negative pigs. The production of PRRS virus negative pigs does not depend on antibody levels of sows, but might depend on transplacental infection.

REGIONAL MARKET SWINE PRRS V ANTIBODY SURVEY

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Objectives for this survey of Midwestern market swine, designed to provide PRRS epidemiologic information in two time periods (December-February, July-September), were 1) to enumerate PRRS antibodies in market swine presented at eight Iowa abattoirs (25% national capacity) by utilizing meat extracts and 2) to benchmark PRRS antibody levels.

The test population was sampled daily by USDA-APHIS-VS employees in conjunction with PRV surveillance. Diaphragm (50-75 gms.) was harvested from five carcasses per lot, placed in whirl-pak bags and identified with lot number, date and plant. Lots were classified "positive" if a single sample was found within the 5 samples/lot. Test samples were submitted to the ISU-VDL for analysis by the IDEXX HerdChek PRRS ELISA test. Based upon earlier studies a 1:10 dilution for meat juice was utilized for equivalency with the standard serum 1:40 dilution. Results are reported at a 0.2 cut-off value. In the December – February period individual samples tested were 74.8% (81,728/109,260) positive, 85.8% (18,864/21,986) of lots, and 89.7% (3,474/3,866) of producers were determined to be "positive." Results from a second iteration July - September 2006 will be presented. This project demonstrates flexibility of a market-based surveillance system to answer short-term questions during routine disease monitoring and generated useful benchmark values. Increasing herd size and location in "hog-dense" areas were judged significant risk factors. Evaluation of a known negative weaned pig population indicated that exposures occurred during the grow-finish stage, but were reduced from the general population. Seasonal differences will be presented. Data supports a major role for inter-herd infection, but does not elucidate causation.

A BAYESIAN APPROACH TO EVALUATING TEST PERFORMANCE BY COMBINING INFORMATION FROM MULTIPLE EXPERTS AND DATA FROM A COMMERCIAL ELISA

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PRRS virus costs the swine industry over $560 million every year, so it is important to accurately assess diagnostic tests for PRRS. Standard Bayesian statistical methods for evaluating the performance of diagnostic tests in the absence of a gold standard test require the input of expert information, in the form of prior distributions elicited from the expert, for inference about test sensitivities, specificities and disease prevalence [1]. Current methodology permits information from only one expert to be included in the inferences about the test. However, some researchers and practitioners may not be comfortable with inferences that depend, in part, on a single expert.

The aim of this research was to extend the current Bayesian methodology to include information from multiple experts to provide inferences for the sensitivities and specificities of diagnostic tests and disease prevalence. A unique feature of the method is that an expert who is not in agreement with the collection of other experts can have their influence on inference automatically down-weighted.

The data consists of IDEXX ELISA results from 48 swine that were periodically tested for PRRS virus during an experimental infection. The software "BetaBuster" was used to elicit prior distributions for sensitivity, specificity and prevalence from three swine experts. The expert information generally agreed but provided slightly larger probability interval due to differences in expert information. Posterior estimates for the diagnostic test were: se=0.99 and sp=0.98. These decreased when one expert provided lower prior values than the other experts, but then recovered when the expert became an "outlier," that is, deviated dramatically from the other experts. Conclusion: the new method provides robust inference for test performance characteristics and protects against undue influence by a single expert.

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SEROLOGICAL PREVALENCE OF PRRSV IN THE SHANDONG AREA IN CHINA

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The seroprevalence of PRRS was evaluated in pig farms in the Shandong area in China. A total of 560 swine serum samples were collected from pigs that were not vaccinated with a PRRS vaccine and tested for PRRS virus antibody using a commercial ELISA antibody detection kit. Fifty seven % (321/560) were positive. To our surprise, only 31.3% (94/300) of the examined serum samples were positive in small-scale pig farms (less than 100 pigs per farm) in the remote countryside. However, in large-scale pig farms (more than 100 pigs per farm), 87.3% (224/260) sera were found to be positive. This study confirms the existence of PRRS in the pig farms in Shandong area and indicates that the prevalence of PRRS was associated with the scale of pig farms and breeding density of pig populations in this area.

ERADICATION OF PRRSV IN CHILE

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PRRS virus was first described in the USA in 1987 and was rapidly recognized elsewhere, being reported in Europe in 1990 and the across the world.

The first official diagnosis of PRRS in Chile was made in 2000. Random serological test showed 30% of the infected herds formed clusters, within the farm the prevalence was 12%. Due to this situation, the country began an eradication project which was conducted by the SAG with collaboration of the pig Chilean industry represented by the Pork Producers Trade Association of Chile (ASPROCER).

The sanitary measures implemented were basically two-fold: depopulating-repopulating in mono sites and at multisites (two or three-site production), a specific procedure depending on the individual herd's characteristic.

Eradication has been carried on without using any vaccination and based on a plan specific for each infected herd. This plan consists in a partial depopulation in multisites and total depopulation in monosites. For the rest of the herds there is National Surveillance Program.

This paper describes sanitary measures for production systems in monosites and multisites herds and also describes its results.

COMPARISON OF SERUM AND MEAT JUICE ANTIBODY LEVELS IN EXPERIMENTAL AND FIELD-DERIVED SWINE

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Evaluation of paired samples of serum and meat juice experimentally and field-derived infected swine are reported. Serum and meat juice were obtained from each animal contemporaneously at necropsy/harvest. Experimental animals were acutely convalescent and field-derived animals were market weight swine obtained from sources with both known and unknown prior PRRS status. Serum and meat juice samples were examined at Iowa State University Veterinary Diagnostic Laboratory using the IDEXX Herdchek PRRS ELISA according to label directions. The sera and meat juice from 8 control and 46 PRRS infected experimental animals were analyzed and reported at the 0.40 cut-off and a dilution of 1:40. All control (negative) animals were accurately denoted with both serum and meat juice. Four (4) of the serologic positive animals were negative on meat juice at 1:40 dilutions. Three of these had serum values approximating the cut-off value. There was no statistical difference in predictive values for dilutions at 1:10, 1:20, and 1:40. A subsequent dataset which represented field-based exposures was obtained from animals submitted for the Austin Barrow Show progeny test and from the ISU Swine Nutrition herd. A total of 168 paired meat juice and sera were obtained at a cooperating commercial abattoir. Forty-four (44) tested positive and 124 negative in serum. Sera were analyzed at 1:40 dilutions and meat juice at dilutions of 1:2, 1:5, 1:10, 1:20, and 1:40. All serum negative animals were negative on meat juice at all dilutions. Of the 44 positive sera there were 4 negative at the 0.4 cut-off. The best correlations between serum and meat juice values were obtained at 1:2, 1:5 and 1:10 dilutions without modifying the 0.4 cut-off. Therefore we propose to use 1:10 dilution of the current IDEXX test when using meat juice for PRRS antibody detection in field-derived samples. Meat juice represents an easily obtained surveillance sample and can be expected to generate reliably comparable data to serum samples from the same animals, providing another tool for identification of PRRS infected animals.

FACTORS INFLUENCING VIRAL DISEASE TRANSMISSION AND ELIMINATION

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Studies of viral disease transmission among herds in disease-free animal populations may help to understand the relative importance of disease transmission routes and the mehods required to regain a disease free status. The recent European veterinary history is rich in examples of classical swine fever and foot and mouth disease outbreaks. Knowledge gained from these examples and the methods of disease control will be presented to facilitate a clearer perspective on PRRS transmission and PRRS elimination.

ENVIRONMENTAL STABILITY OF INFECTIOUS PRRSV

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The effect of temperature on the inactivation of infectious PRRS virus was evaluated. Four North American (Type 1) isolates (VR-2332, JA142, MN-184, and Ingelvac® PRRS ATP vaccine virus) in cell culture maintenance medium were maintained at four temperatures (4, 10, 20, and 30°C) and sampled repeatedly over time. Tissue culture infectious dose 50% ($TCID_{50}$) and quantitative RT-PCR assays were performed to determine the concentration of infectious virus and total viral RNA for each isolate at each time point. Titers of infectious virus were plotted by time and the half-life (T1/2) calculated for each virus isolate at each temperature. A comparison of virus T1/2 by temperature found no significant differences among the isolates tested. Therefore, a single non-linear regression model was derived with which it is possible to calculate PRRS virus T½ for temperatures between 0°C and 50°C. This information is relevant to field and laboratory settings in which PRRS virus decontamination is an issue.

REGIONAL ERADICATION OF PRRSV – A PILOT PROJECT

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Eradication of PRRS virus from a region would involve having the knowledge to stop new herd infections and eliminate the virus from infected farms. Much has been learned on both these fronts in recent years leading us to pose the hypothesis that we know enough today to eradicate PRRS from all swine farms within a defined region. Two regions in Minnesota were identified and local pork producers and veterinary practitioners working in the regions were contacted; eastern Rice County and Stevens County, both in Minnesota. Diagnostic testing for PRRS was paid by the project in Rice County and not in Stevens County. Quarterly meetings were held with the producers and veterinarians in each of the two regions to report progress, present recent research findings regarding PRRS control and facilitate communication among the producers. All the pig sites have been located by GPS and mapped. In Rice County, owners of 45 of the 50 farms (90%) in the area have participated in the project. As of June 1, 2006, PRRS had been eliminated from 5 of the 9 sow farms, but then during June - July, 2006, 2 sow herds became re-infected from transmission within the farm and 3 sow farms, linked to each other by pig movement became infected with a PRRS virus from outside the project region. Within the Rice county region, we have identified 6 groups of PRRS virus isolates in the area and according to their geographical distribution, we have no evidence of PRRS virus area spread between independent farms in this region since the project began. In Stevens County, progress has been made towards area-wide eradication without the recent set-backs experienced in Rice. Three challenges have become apparent: 1) "small" producers may be reluctant to invest to eliminate PRRS, 2) pigs entering the region for finishing may be PRRS positive and pose a risk to adjacent producers, and 3) 4H pigs entering the region may be positive for PRRS virus. Although the projects are not complete, we believe that sufficient knowledge exists regarding biosecurity practices and PRRS elimination techniques, to allow producers in a region to eliminate PRRS. The major challenges lie in having all producers actively participate and encouraging them to apply available knowledge to accomplish the goal.

The NC-229 (PRRS) Committee thanks CRWAD for its support of the International PRRS Symposium

The Conference of Research Workers in Animal Diseases (CRWAD) was established in 1920. CRWAD is a non-profit organization and has been so since its origin. The sole purpose of CRWAD is to discuss and disseminate the most current research advances in animal diseases. The annual meeting is held each November or December where research scientists from around the world present their recent research in oral or poster presentation formats. The meeting averages 600 in attendance and 325 presentations.

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The 2007 International PRRS Symposium (Nov 30 - Dec 1, 2007) will be held immediately prior to the Conference of Research Workers in Animal Diseases (December 2-4, 2007) at the Chicago Marriott Downtown

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