RESEARCH ABSTRACT
SWINE HEALTH

Title: PCVAD Induced Immune Dysfunction-**NPB# 07-208**

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Scientific Abstract:
Objective 1: Pathobiology and Immunology of naïve or vaccinated pigs following PCV2b or PCV2b/PRRS challenge.
Forty-nine conventional pigs with low maternal indirect fluorescent antibody (IFA) titers to porcine circovirus 2b (PCV2b) were divided into seven groups, four challenge control groups and three vaccine groups. Respective control/vaccine groups were subsequently challenged with either porcine reproductive and respiratory virus (PRRSV), PRRS/PCV2b or PCV2b via the intranasal route. One of the remaining non-vaccinate groups and the remaining vaccinate group were left as negative controls. The non-vaccinated group that was challenged with the dual challenge (PRRSV/PCV2b) had a mortality rate of 43%. There was no mortality in any of the other groups. All of the animals challenged with PCV2b generated significant antibody titers to PCV2b as determined by IFA and SN. A differential qPCR assay detected PCV2b in the serum of all of the animals in non-vaccinated groups challenged with PCV2b. The geometric mean copy number of PCV2b in the serum of the dual challenged group was significantly greater than that of the group challenged with only PCV2b. Animals that died in the dual challenge group had 20-100 times more virus than the PCV2b only challenge group. While the PRRSV potentiated the PCV2b infection, there was not an increase in the amount of PRRSV detected by PCR in the serum of the dual challenged group when compared to the PRRSV only challenged group. No PCV2b virus was detected in either the vaccinated single or dual challenge groups, indicating that the PCV2a based subunit vaccine was capable of inducing apparent sterilizing immunity in the face of a moderately lethal heterologous dual challenge. This challenge model system appears to replicate overt clinical disease in conventional pigs and demonstrates the synergism of a PRRSV/PCV2b dual challenge on the replication of PCV2b *in vivo*.

Cellular response following vaccination and/or challenge.
PBMC data has been somewhat problematic due to the large number of data points collected, the lymphocyte proliferation data is being still analyzed. It has been determined that the impact of PCV2b, PRRSV infection, or vaccination does not result in a robust response by lymphocytes to either the poke weed mitogen or PCV2 antigen. The results determined to date indicate that there are subtle differences between groups with the majority of differences occurring at day 14 of the trial. On day 14, the presence of PRRSV did appear to have a minor impact on the ability of cells from the PCV2 vaccinated and infected pigs to respond to the PCV2 antigen. By days 28 and 42, there was little response by the various cell populations to the stimulants. Further analysis of the data is needed to assess more closely the impact of infection and vaccination on the specific immune response by the lymphocyte populations.

B-cell epitope mapping.
Open reading frame 2 (ORF2) of porcine circovirus type 2 (PCV2) codes for the 233 amino acid capsid protein (CP). Baculovirus-based vaccines that express ORF2 are protective against experimental challenge and natural infection. The goal of this study was to identify regions in CP preferentially recognized by sera from experimentally infected and vaccinated pigs and pigs diagnosed with porcine circovirus-associated disease (PCVAD), including porcine multi-systemic wasting syndrome (PMWS) or porcine dermatitis and nephropathy syndrome (PDNS). The approach was to react sera with CP polypeptide fragments followed by finer mapping studies using overlapping oligopeptides that covered amino acids 141-200. The results showed that vaccinated pigs and a subset of pigs experimentally infected with PCV2 recognized only the largest CP(43-233) polypeptide fragment. Another subset of experimentally infected pigs and sera from pigs with PDNS showed strong reactivity against a CP oligopeptide, 169-STIDYFQPNNKR-180. Alanine scanning identified Y-173, F-174, Q-175 and K-179 as important for antibody recognition. The results from this study support the hypothesis that PCV2 modulation of immunity is involved in disease progression. The differences in the recognition of CP(169-180) and other polypeptides provide opportunities to devise diagnostic tests for the surveillance of vaccination, infection and disease.

Objective 2: Development of a Quantitative ELISA.
The ability to use purified capsid protein also allowed for the transition of the assay from an ELISA format to a Fluorescent-microsphere immunoassay (MIA) using Luminex technology. This system allows for more rapid analysis and is paving the way for the development of an assay that will allow for testing for the presence of multiple types of antibodies and antigens at one time. The results from the PCV2b MIA assay are very similar to the ones from the IFA and SN assay. The MIA assay offers the potential of "Multiplexing" or testing antibody levels to multiple pathogens in a single serum sample. This type of assay would allow producers to antibody profile their herd to monitor passive antibody levels, vaccine response, and the circulation of various pathogens within the herd. Preliminary development of a multiplex for PCV2 and PRRS has been completed and correlates well with IFA and ELISA results. The MIA assay for PCV2 and PRRS is currently undergoing validation testing and will be offered as a diagnostic test at KSVDL.

Objective 3: Development of a DIVA ELISA.
The DIVA ELISA is based on the bacterial expression of PCV2b ORF1 and ORF2. Animals vaccinated with a PCV2 ORF2 subunit vaccine produce antibodies only to the ORF2 protein while PCV2 infected animals generate antibodies to both ORF1 and ORF2. Using a traditional ELISA approach, ORF1 and ORF2 polypeptides were used as detection antigens. Initial results demonstrated lack of reaction to ORF1 in vaccinated animals and reaction to ORF1 and 2 in infected pigs. In addition to the ORF1/ORF2 DIVA ELISA, a second DIVA ELISA is currently under development. That assay is based on different reactivity patterns of infected or vaccinated pigs with polypeptides of the ORF 2 capsid protein. In theory, a DIVA ELISA based only on ORF2 reactivity would be useable for baculovirus subunit vaccines as well as conventional whole virus vaccines.

Objective 4 Development of PCV2-specific monoclonal antibodies.

Mice were immunized with recombinant N protein and their splenocytes were used to prepare hybridomas using traditional methodology. Hybridoma screening was done by IFA using PCV2 virus infected PK15 cells and 14 MAbs were obtained. Interestingly, epitope mapping of these MAbs showed that all of these MAbs recognized the N-terminal epitope region, amino acid 49-69 of the N protein. MAbs SD65-16, SD16-10 and SD 72-10 were subcloned and maintained for further study. MAbs against the C-terminal region of the N protein were obtained by immunizing mice with synthetic peptides that cover the amino acid region of 71-90, 119-138, or 171-190. Following fusion, seven MAbs were obtained from the initial hybridoma screen, of which three MAbs, SD39-29, SD2-21, and SD18-43 were subcloned and maintained. Each of these clones recognizes one of the C-terminal peptides. MAbs 72-10 and 36-29 had high titer in ELISA and IFA, were expanded and a large quantity of MAbs were produced. IFA and ELISA results showed that these MAbs can recognize both 2a and 2b genotypes, but did not recognize PCV1. To generate the PCV2b specific MAbs, mice were immunized with two PCV2b N protein specific peptides, 86-SNPRSVPF and 69-VDMMRFNINDFLPPGGGSNPRSV. Unfortunately, mice immunized with these two synthetic peptides did not produce any PCV2 specific MAb.