RESEARCH ABSTRACT
SWINE HEALTH

Title: PEDV Diagnostic Approaches to Assess Sow Immunity and Piglet Protection. **NPB #14-038**

Investigator: Eric A. Nelson

Institution: South Dakota State University

Co-Investigators: A. Singrey, S. Lawson, T. Clement, J. Christopher-Hennings

Date submitted: June 24, 2015

Scientific Abstract:
Robust serological assays are needed for the improved control of PEDV. Therefore, the overall objective of this study was to develop and validate multiple serological assays for PEDV. These assays included a fluorescent focus neutralization assay (FFN) to measure functional virus neutralizing antibodies; an indirect ELISA (iELISA); a highly specific monoclonal antibody-based blocking ELISA (bELISA); and fluorescent microsphere immunoassays (FMIA) that can be multiplexed to monitor exposure to multiple antigens and pathogens simultaneously.

The FFN assay was optimized and evaluated using multiple panels of serum samples from PEDV naïve animals and herds sampled at various times post-exposure. Essentially all samples from naïve animals demonstrated serum FFN endpoint titers of <1:20 while most samples from PEDV positive herds had endpoint titers ranging from 1:40 to 1:1280. Additional sequential sample sets including serum, colostrum and milk were then evaluated. Mean colostrum titers were approximately 4-fold higher than serum titers at the time of farrowing while serum and milk titers were similar in magnitude, although substantial animal to animal variation was apparent.

A recombinant North American nucleoprotein (NP) based iELISA was developed and validated along with a bELISA using newly developed PEDV-NP specific biotinylated monoclonal antibodies and an FMIA using magnetic beads coupled with expressed PEDV-NP. Receiver operating characteristic (ROC) analysis was performed using swine serum samples (iELISA n=1486, bELISA n=1186, FMIA n=1420). The ROC analysis for the FMIA showed estimated sensitivity and specificity of 98.2% and 99.2%, respectively. The iELISA and bELISA showed a sensitivity and specificity of 97.9% and 97.6%; and 98.2% and 98.9%, respectively. Inter-rater (kappa) agreement was calculated to be 0.941 between iELISA and IFA, 0.945 between bELISA and IFA

and 0.932 between FMIA and IFA. Similar comparative kappa values were observed between the iELISA, bELISA and FMIA, demonstrating significant agreement among assays. No cross-reactivity with the related coronaviruses, transmissible gastroenteritis virus (TGEV) or porcine respiratory coronavirus (PRCV) was noted. All three assays detected seroconversion of naïve animals within 6-10 days post exposure. FMIA tests using PEDV spike (S1) antigen preparations were also developed to compare IgA and IgG responses in serum, milk and colostrum with neutralizing antibody levels detected by the FFN. While moderate to strong correlations were noted among assays, the FFN appeared to provide the most consistent results in a cost-effective test format.

In summary, well-validated iELISA, bELISA and FMIA assays for the detection of PEDV antibodies were developed and showed good correlation with IFA and each other. Each assay format has advantages that dictate how they will be used in the field. Measurement of neutralizing antibody responses using the FFN assay should provide a valuable tool for assessment of vaccine candidates or protective immunity. Ongoing field experience with the FFN assay should eventually allow better correlation of measured neutralizing antibody levels and expectations of protective immunity.