RESEARCH ABSTRACT

SWINE HEALTH

Title: PDCoV ELISA development **- NPB 14-176**

Investigator: Dr. Michael Murtaugh

Institution: University of Minnesota

Date Received: February 2, 2015

Scientific Abstract:

Porcine deltacoronavirus (PDCoV) was first identified in the United States in February 2014 in pigs with unexplained diarrheal disease. Further investigation revealed coronavirus-like particles in electron micrographs, which led to the identification of PDCoV by real-time PCR. Diagnosis of PDCoV is performed using a real-time PCR assay to detect viral infection, usually in fecal samples. Serological tests to identify viral antibodies were not available. However, serology is important and useful to identify previous viral infection, even after clearance of the virus, and to suggest that the animal has protection against PDCoV infection. Our main objectives for this proposal were to develop and characterize a rapid, specific, and sensitive ELISA for PDCoV and to share the reagents and protocols with the swine diagnostic and research community. We cloned, expressed and purified 4 PDCoV antigens; nucleocapsid (N), matrix (M), and the spike protein subunits (S1 and S2). These antigens were then used to develop 4 separate ELISA assays to examine the antibody reactivity to each antigen in serum from infected pigs. Serum from confirmed PDCoV-positive animals was difficult to acquire. Serum samples were obtained from 300 animals of unknown PDCoV status, that came from farms in which PDCoV had been observed. These serum samples were then run on our PDCoV ELISA with all 4 antigens. We observed a few animals from each farm that seemed to be antibody positive based on the ELISA results, mainly with reactivity to PDCoV N protein. In order to determine the specificity and cut-off values for the ELISAs, we examined serum samples from known PDCoV-negative (44 samples) and PRCV-positive animals (175 samples). PRCV (porcine respiratory coronavirus), a close relative to transmissible gastroenteritis virus (TGEV), is a common coronavirus infection, and may have cross-reactive antibodies to PDCoV. The negative samples gave low values, but surprisingly the PRCV positive samples showed higher overall values for the N protein than that of the samples with unknown PDCoV status. Comparison to ELISAs with M, S1 and S2 as antigens indicated that N results were false positives, since all three of the other antigens gave consistently low ELISA results. Because of this cross-reactivity and the lack of known antibody positive samples, we are unable to truly test this ELISA. However, an ELISA is available in the event that PDCoV antibody positive samples are identified, which will allow for the optimization of the ELISA assay to decrease the cross-reactivity due to PRCV infection. Serum samples over a time course of infection would allow us to examine the dynamics of PDCoV antibody production and determine values for a true antibody positive sample.