RESEARCH ABSTRACT

SWINE HEALTH

Title: Characterization of neutralizing antibody responses to PRRSV and association with host - **NPB#12-120**

Investigator: Raymond R.R. Rowland

Institution: Kansas State University, College of Veterinary Medicine

Date Submitted: February 18, 2014

Scientific Abstract:
Virus neutralization (VN) activity during infection with porcine reproductive respiratory syndrome virus (PRRSV) is generally considered to be weak and primarily directed against the virus used for infection; i.e. homologous neutralizing antibody (nAb). Based on results from other viruses, we hypothesize that there are pigs that produce neutralizing antibodies to a broad range of genetically diverse PRRSV isolates, and that the production of nAb is linked to host genetics. Over 1,200 serum samples from experimentally infected pigs were analyzed for VN activity against four different PRRSV isolates. Overall, VN titer was negatively correlated with virus load, and positively correlated with weight gain. VN was only weakly heritibale. Based on the breadth of VN titer, samples were placed into one of the following groups: Group 1, no VN; Group 2, homologous VN against only the isolate used for infection; Group 3, heterologous VN with reactivity against 1 or 2 additional isolates; and Group 4, broad VN with activity against all four isolates. VN escape mutants were prepared by selecting for viruses that were resistant to homologous antibody (Group 2) or bnAb (Group 4). Viruses made resistant to homologous serum *in vitro* remained sensitive to broadly neutralizing serum; and conversely, viruses made resistant to broadly neutralizing serum retained sensitivity to neutralization with homologous sera. Sequencing identified mutations in a short hypervariable domain of GP5 associated with escape from homologous neutralization. For viruses resistant to broadly neutralizing serum, non-conserved amino acid changes were identified in GP3 and GP5, as well as an amino acid deletion in the ectodomain region of M. The results demonstrate that homologous and broadly neutralizing responses are distinct and recognize different epitopes. Furthermore, mutations in GP3, GP5 and M, suggest that these proteins may interact to form a conformational broadly neutralizing epitope. Sera were assayed for total antibody (tAb) using an N-specific fluorescent microsphere immunoassay (FMIA). A genome wide association study identified two regions on SSC 7 linked with tAb. Both markers located to MHC. These regions mapped to genes that are directly involved in the host immune response. The identification of regions within PRRSV linked with nAb groups creates the opportunity to design vaccines tailored to induce the production of bnAb. Another outcome of the study was the development of an improved VN assay for the analysis of experimental samples. The assay involves reacting a single dilution of serum with a standard amount of EGFP-PRRSV, in triplicate. Following one hour incubation, contents are transferred to confluent MARC-145 cells in a 96-well tissue culture plate. Cells are then incubated for 48 hours. The plate is then analyzed for EGFP fluorescence using a FLUOstar Omega plate reader (BMG Labtech). To standardize the assay to traditional methods, a set of samples, with known VN used to make a standard curve. Overall, the EGFP based assay provides a quicker, and more accurate method for measuring PRRSV neutralizing activity compared to traditional methods.