PEDV Research Updates
2013

Porcine Epidemic Diarrhea virus (PEDV) has caused significant challenges to the swine industry. The virus had not been previously identified in the United States prior to April of 2013. To assist producers and their veterinarians in the management, control and potential elimination of the virus, the National Pork Board funded key research projects to better understand PEDV. In order to provide timely information to producers from those projects, the objectives and initial updates will be periodically reported.

NOTE: The updates from the proposal represent interim information only and are not intended to be a final report. The final and formal reports will be provided at the end of the terms of the projects and then posted online at pork.org. The update information is intended to inform stakeholders of progress but are not intended to be the final outcome. For further information, please contact Dr. Lisa Becton at lbecton@pork.org .

#13-239: University of Minnesota
Development and validation for diagnostic testing for antigen and antibody detection for PEDv

Objectives:
1. Develop and validate antibody-based diagnostic tests for serologic monitoring and surveillance.
2. Develop and validate oral fluid testing (antigen and antibody testing capabilities) to provide rapid method for sample collection and to support on-farm surveillance.

Update: 10-3-13
Project 1: Development of serological tests for detecting anti-PEDv antibodies
PI: Michael Murtaugh
We have preliminary evidence that we have developed a nucleocapsid-based ELISA for the detection of PEDV seroconversion. For further characterization and validation of this ELISA, we have requested and obtained serum and oral fluid samples from Dick Hesse. These samples were taken over a time course of PEDV infection. We have also requested known PEDV positive and negative serum samples from the University of Minnesota diagnostic lab.

Update: 9-21-13
Project 1: Development of serological tests for detecting anti-PEDv antibodies
PI: Michael Murtaugh

Title: Development of serological tests for detecting anti-PEDv antibodies
Michael Murtaugh, University of Minnesota

Following further work, we have preliminary data showing expression of PEDV nucleocapsid (N) and spike protein S2 (S2). Large-scale production was performed and purification is in progress. Evidence of expression of membrane protein (M) and S1 domain of spike protein was inconclusive. Large-scale production was performed and the results will be analyzed. Results are expected later today and, if positive, test ELISAs will be performed on Thursday and Friday. In any event we expect to relay the findings to you and NPB when the data are available.

Update: 9-2-13

The goal of this project is to produce PEDV proteins, and use them in immunoassays such as ELISA or Luminex, for serological detection of an antibody response to PEDV infection in pigs. In the next two weeks we plan to isolate the pure proteins and conduct preliminary studies of their ability to detect serological responses in serum samples from PEDV cases. We plan to obtain serum and fecal samples from Dick Hesse at Kansas State University from a PEDV pathogenesis study to compare antibody responses to the four individual proteins and compare the sensitivity of antibody detection in serum and feces. The results will help guide further development of detection assays using single proteins or combinations.

Quick Take

PEDV proteins need to be made to help create useful diagnostic tests.

Update: 8-21-13

<u>Development of serological tests for detecting anti-PEDv antibodies</u>

Gene segments encoding expected antigenic PEDV proteins were amplified by PCR from a US isolate whole genome sequence provided by Doug Marthaler, Veterinary Diagnostic Laboratory, University of Minnesota. Primers were designed to amplify (1) a 711 bp fragment of matrix protein, M, (2) a 1362 bp fragment of nucleocapsid, N, a 2199 bp fragment of the S1 domain of the envelope spike protein, S, and (4) an 1800 bp fragment of the S2 domain of S. Primers were designed to facilitate cloning into two bacterial expression vectors: pMAL-p5X to produce the antigenic protein as a fusion protein with maltose binding protein (MBP), and pET-25b, to produce the antigenic protein with a 6X-histidine tag. PCR products for all four antigens were successfully obtained. Cloning was performed for all four antigens into pMAL-p5X and reaction products were transformed into E. coli Stellar bacteria. Screening and evaluation of transformants is in progress.