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.

#13-238: University of Minnesota
Development and validation of isolation and diagnostic testing detection for PEDv

Objectives:
1. Development and standardization of viral propagation techniques to produce virus for use in diagnostic testing (outcome to be publicly available to all VDLs for PEDv diagnostics).
2. Development of standardized reference samples that can be utilized by VDLs for diagnostic test validation.
3. Validation of the current PCR diagnostic tests.

Update: 10-3-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDv
PI: Collins

Virus propagation: Attempts to adapt PEDV in various cells lines (n=9) have been unsuccessful. However, the NSVL strain seems to be growing in our Vero-76 cells. We are now studying the effect of virus concentration on cells. We will have a more complete report next time.

Project 5: Virus repository
PI: Maxim Cheeran
Not much to report this week. We continue to optimize and troubleshoot the ISH (FISH) assay.

Update 9-21-13
Project 5: Virus repository
PI: Maxim Cheeran
The IAR is optimizing a FISH protocol and testing various hybridization conditions. We have recently acquired PEDV positive and negative formalin fixed paraffin embedded tissue, as well as a PEDV specific and a scrambled probe. Newly acquired equipment will allow for the development of optimal hybridization conditions.

Project 3: Development and validation of isolation and diagnostic testing detection for PEDv
PI: Collins
Virus propagation: Attempts to adapt PEDV in various cell lines has been unsuccessful. We have received NSVL’s PEDV isolate and are trying to adapt it to grow in our lab.

PCR diagnostics: Lifetech’s TGEV/PEDV/Rotavirus kit thermal cycling conditions have been optimized while and approximately 500 samples are being extracted. Testing with the UMVLD’s TGEV/PEDV multiplex assay has begun while testing with the Lifetech TGEV/PEDV/Rotavirus assay will follow. We are selecting additional PEDV strains for complete genome sequencing to further enhance our knowledge on PEDV’s genetic diversity and spatial spread.

Update: 9-2-13
Diagnostic Test Reference panel: The blind reference panel is available for shipping.
PCR diagnostics: The complete genome sequencing of the three PEDV is in progress. The Lifetech TGEV/PEDV/Rotavirus kit manual is being reviewed. PEDV positive and negative samples are being selected that will be tested with the Life kit within a couple of weeks.

Virus collection and repository: We are waiting on PEDV positive and negative tissue collected by from another ongoing study. Once acquired, the PEDV probe will be used to show binding specifically to positive tissue and not to negative tissue. A scrambled probe will be used to confirm the PEDV probe is binding selectively. Tissue sections are due to arrive later this week.

Quick Take
- A PEDV probe to determine positive from negative pigs/tissues is needed to determine PEDV status.
- Reference samples are now available for other diagnostic laboratories to request for use.
The TGE/PED/Rotavirus test kit manual is being reviewed currently and could be tested with positive and negative samples within several weeks

Update: 8-21-13
Development and validation of isolation and diagnostic testing detection for PEDv

Virus propagation: Efforts are being made to cultivate the virus in 11 different cell lines. So far we have not been successful.
Reference panel: The blind reference panel will be completed by August 30th. The stocks of TGEV, rotaviruses A, B, and C, and Brachyspira sp. are ready to be spiked into feces. We are in the process of testing feces to ensure that they are negative for known pathogens.

PCR diagnostics: The first PEDV complete genome has been deposited into GenBank with accession number KF272920. The complete genome sequencing of the three remaining strains are being done by Illumina Miseq at the University of Minnesota Genomic Center. The Lifetech TGEV/PEDV/Rotavirus A multiplex kits have arrived. Testing of the 500 archived cases will begin in September.

Virus repository
Here is a summary of the PEDV positive samples currently stored in the IAR:

Stock and dilutions of inocula collected from intestine homogenates and fecal supernatant from several pigs from two different cases submitted to the VDL from affected farms:
* Stock inocula: 4 x 2mL tubes
* 10^-1 inocula: 8 x 2mL tubes
* 10^-3 inocula: 7 x 2mL tubes
* 10^-5 inocula: 8 x 2mL tubes

The diluted inocula were used to infect three three-week-old pigs (labeled Pig 7, Pig 8, and Pig 9). The cecal contents of these three infected pigs were collected. Fifteen mL aliquots of the cecal contents of Pig 7 and of Pig 9 were concentrated and 1:20 dilutions of the concentrate were submitted to the VDL for PCR testing. Results showed a PEDV Ct of 15.03, and Rotavirus A Ct of 33.97 for pig 7 and a PEDV Ct
of 17.87 and Rotavirus A Ct of 36.82 for Pig 9. Both were negative for TGEV and Rotavirus B and C. Concentrated aliquots are available. In addition to the cecal contents, intestines from Pigs 7, 8, and 9 were collected. The intestines of Pig 8 were shared with Dr. Goyal. Intestines from Pigs 7 and 9 remain in storage in the IAR. Formalin fixed paraffin embedded tissue sections from Pigs 7, 8, and 9 are available. The IAR is currently developing a FISH assay to identify PEDV infected cells in situ.