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 .

#13-238: 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: 12-12-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDV
PI: Collins

Complete genome sequencing of an additional 13 American PEDV strains has been completed while the remaining PEDV strains still require genome assembly. In depth phylogenetic analysis has yet to be completed. Phylogenetic analysis included non-American PEDV strains (n=22), previously reported American PEDV strains (n=13) from GenBank, and the 13 newly sequenced American strains (n=13). While the American PEDV strains share the highest nucleotide percent identity to AH2012 (99.4-99.5%), the American PEDV strains share a nucleotide percent identify of 99.8 to 100%. Analysis of data from the newly generated 13 complete PEDV genomes indicates two larger American branches are present. Initial results suggest no major difference between these two clades. As PEDV continues to spread, studies on strain variation and sequence lineages will continue to occur.

Project 5: Virus repository
PI: Maxim Cheeran
The IAR has received a request for PEDV positive and negative tissue homogenates to validate a PCR assay. We are corresponding with the requesting researcher to distribute a PEDV positive reference panel consisting of samples:
-PEDV negative, TGEV negative, and rotavirus a, b, and c negative
-PEDV positive, TGEV negative, and rotavirus a, b, and c negative

-PEDV positive, rotavirus a positive, TGEV negative, and rotavirus b, and c negative
-PEDV positive, rotavirus b positive, TGEV negative, and rotavirus a, and c negative
-PEDV positive, rotavirus c positive, TGEV negative, and rotavirus a, and b negative

More PEDV fecal and tissue homogenates with sequencing information will be entering the IAR inventory this week.

Update: 11-27-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDV
PI: Collins

RT-PCR: The PEDV RT-PCR comparison will be completed within a couple weeks. The NGS Miseq run from ~24 complete PEDV genome sequencing will be completed by the end of the week. Complete PEDV genome analysis will be start the first week of December.

Virus growth: The NVSL virus has been grown in cell cultures. Another virus from a field case has also been grown and titrated. Undiluted cell culture-grown virus caused diarrhea when given orally to pigs

Project 5: Virus repository
PI: Maxim Cheeran

The IAR has obtained new PEDV tissue and homogenates. These include:
-Twenty two formalin fixed paraffin embedded intestinal tissue blocks from PEDV positive and negative control piglets.
-Intestinal tissue homogenates from six piglets – one PEDV negative and five PEDV positive. Results for TGEV and Rotavirus PCR are pending.
-PEDV positive and PEDV negative intestinal tissue fixed in 4% paraformaldehyde to be frozen and sectioned. Sections will be probed using FISH protocol in an effort to detect PEDV mRNA in tissue.
An IFA is under development. An IPMA tests is also being developed. We have obtained approximately 600 serum samples for test validation.

Update: 11-13-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDV
PI: Collins
Approximately 100 samples have been compared using Lifetech's GEV/PEDV/Rotavirus and the UMVLD's TGEV/PEDV multiplex assay. Additional complete genome sequencing for ~24 PEDV strains is underway.

Project 5: Virus repository
PI: Maxim Cheeran
The IAR does not have any relevant updates to report.

Update: 10-28-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDv
PI: Collins
No update to report.

Project 5: Virus repository

PI: Maxim Cheeran
The IAR has received three PEDV positive intestines from 12 day old piglets for storage and distribution. PEDV positive fecal samples and tissue homogenates from PEDV cases encountered by the VDL will also be entered into storage pending PCR results. New PEDV positive and negative formalin fixed paraffin tissues are also available.

Update: 10-16-13
Project 3: Development and validation of isolation and diagnostic testing detection for PEDv
PI: Collins
PCR diagnostics: Recently, Tetracore contacted the UMN to validate their TGEV/PEDV assay. We are investigating this possibility. While all the samples have been tested with the UMN TGEV/PEDV, the RNA is now being tested with the Lifetech assay. PEDV sample selection for complete genome sequencing is still in proress. Additionally, we are researching virus purification methods to increase viral sequence recovery during the NGS process.

Immunohistochemistry: The Minnesota Veterinary Diagnostic Laboratory has also developed and validated an immunohistochemistry (IHC) test for the detection of PEDv. The laboratory is happy to offer and share this test as needed.

Project 5: Virus repository
PI: Maxim Cheeran
The IAR is collaborating with the VDL to establish and archive a PEDV+ reference panel, comprised of samples testing positive for PEDV, and negative for PRCV and TGEV; positive for PRCV and negative for PEDV and TGEV; positive for TGEV and negative for PRCV and PEDV. The IAR continues to acquire new known PEDV positive and negative tissue and clinical samples and makes these materials available upon request.

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 cells 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*.