

SWINE HEALTH

Title: Development and validation of isolation and diagnostic testing detection for PEDV – NPB #13-238

Investigator: James Collins

Institution: University of Minnesota

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Industry Summary: Since the introduction of porcine epidemic diarrhea virus (PEDV) in the United States, millions of piglets have died. Key items are needed to be addressed to further initial PEDV research, such as development of PEDV propagation techniques, creating PEDV reference samples to share to the research community, and having a reliable PCR test to aid in control and prevention of PEDV. A PEDV strain obtained from NVSL has been successfully grown in Vero cells. Additionally, a PEDV field strain obtained from a swine diagnostic case submitted to the U of MN VDL has been adapted to grow in cells. Now that PEDV has been successfully isolated *in vitro*, efforts are underway to develop serological assays (IPMA and IFA) for the detection of PEDV antibodies. PEDV positive intestine and fecal samples have been deposited in the University of Minnesota Infectious Agent Depository, with additional PEDV samples to be deposited in the future. The samples will be available to PEDV researchers for future assay development and research. Complete genome sequences have been obtained from 13 PEDV strains found in the United States swine population. These American PEDV strains were genetically similar, with 99.8 to 100% nucleotide identity. However, phylogenetic analyses indicate the emergence of two distinct American PEDV clades. As PEDV continues to spread throughout the US, PEDV strain variation is expected to increase. A comparison between the U of MN VDL PEDV qRT-PCR and a commercial PEDV detection kit (Life Technology) indicated

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For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

superior performance by the U of MN VDL qRT-PCR. The commercial kit missed several positive PEDV samples, all of which were confirmed PEDV positive by a secondary U of MN VDL RT-PCR assay targeting the N gene.

Keywords: PEDV, porcine epidemic diarrhea virus, virus isolation, reference samples

Scientific Abstract: Porcine epidemic diarrhea virus (PEDV) is major cause of severe diarrhea and dehydration in pigs. Belonging to the *Coronaviridae* family, PEDV is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 28kb. The first detection of PEDV was reported in 1971 from England while Japan, China, South Korea, and Thailand also have reported PEDV infections. The United States first detected PEDV in May 2013 (1-5). The veterinary diagnostic laboratories quickly development sensitive and specific real time RT-PCR (RRT-PCR) assays to detect PEDV in a variety of porcine and environmental samples. In this study, we compared the PEDV-TGEV multiplex RRT-PCR assay developed at the University of Minnesota (UMN) to a commercial TGEV-PEDV multiplex RRT-PCR assay. In addition, 13 United States PEDV complete genomes were generated to understand the genetic diversity of PEDV, and the phylogenetic relationship to the worldwide PEDV strains.

Introduction: Classified as a member of the *Coronaviridae* family, porcine epidemic diarrhea virus (PEDV) was first reported in the United Kingdom in 1971. PEDV caused clinical signs resembling transmissible gastroenteritis virus. PEDV was subsequently reported in Hungary, Italy, Germany, France, Switzerland, and the Czech Republic (1-7). In addition, countries in Asia have reported PEDV outbreaks including China, South Korea, Thailand, and Vietnam. The initial reports of PEDV were mild compared to the 2010 and present Chinese reports. Recently, PEDV was detected in the United States in April 2013.

Objectives:

Development and standardization of viral propagation techniques to produce virus for use in diagnostic

testing

Development of standardized reference samples that can be utilized by VDLs for diagnostic test validation.

Validation of the current PCR diagnostic tests

Materials & Methods:

Various different cells lines (n=11) were used to isolate PEDV. The NSVL's PEDV isolate was received.

Porcine intestinal samples, fecal samples, fecal swabs, oral fluid samples, and environmental samples are routinely submitted to University of Minnesota (UMN) Veterinary Diagnostic Laboratory for enteric pathogen testing. Sample homogenates were extracted with the MagMax 96 Viral RNA Isolation Kit (Thermo Scientific), according to manufacturer's instructions. The commercial TGEV-PEDV multiplex RRT-PCR assay was performed, according to manufacturer's instructions whiles the UM RRT-PCR assay utilized the Path-ID Multiplex One-Step RT-PCR kit (Thermo Scientific, according to manufacture's instructions.

Positive PEDV samples were selected from different geographical locations. The samples were passed through a 0.22µM filtered and extracted with MagMax 96 Viral RNA Isolation Kit (Thermo Scientific). The samples were submitted for Next Generation Sequencing using the Illumina Miseq as previously described (ref). The 2X250 reads were mapped against USA/Colorada/2013 using the SeqMan NGen software (DNASStar version 11; Madison, WI) using a template assemble. The complete PEDV genomes were aligned using Mauve, and phylogenetic tree was constructing using Maximum Likelihood with the GTR nucleotide substitution model.

Results:

A single field and the NVSL PEDV strain have been adapted to cell culture. The successful adaptation of the single field strain has lead to the development of the adaption technique, which will be applied to additional field strains.

The IAR has received positive and negative tissue homogenates for validate a PCR assay. The PEDV

reference panel consists of the following

- PEDV, TGEV, Rotavirus A, B, and C negative samples
- PEDV positives, negative for TGEV, Rotavirus A, B, and C
- PEDV and Rotavirus A positive, negative for TGEV, Rotavirus B and C
- PEDV and Rotavirus B positive, negative for TGEV, Rotavirus A and C
- PEDV and Rotavirus C positive, negative for TGEV, Rotavirus A and B

Porcine oral fluids (n=39), intestinal homogenates (n=107), fecal (n=136), fecal swabs (n=47), feedback (n=12) and environmental samples (n=55), totaling 396 samples, were compared with the UMN TGEV-PEDV multiplex RRT-PCR and the commercial TGEV-PEDV multiplex RT-PCR assays. The UMN TGEV-PEDV multiplex RRT-PCR assay had lower Ct values compared to the commercial TGEV-PEDV multiplex RRT-PCR assay. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 53 more positive PEDV samples (oral fluids (n=6), intestinal homogenates (n=9), fecal (n=6), fecal swabs (n=13), and environmental samples (n=19)) compared to the commercial TGEV-PEDV multiplex assay. The additional positive PEDV samples as indicated by the UMN TGEV-PEDV multiplex assay, but negative by the commercial TGEV-PEDV multiplex assay, were confirmed positive by a secondary UMN PEDV RRT-PCR assay, which targeted the N gene. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 11 more positive TGEV samples (intestinal (n=4) and fecal (n=7)) compared to the commercial TGEV-PEDV multiplex RRT-PCR.

Phylogenetic analysis of the 13 novel PEDV strains generated from this study and the 35 PEDV genomes available from GenBank (13 previously reported US strains and 22 worldwide PEDV strains) indicated two distant American clades (Fig 1). The US PEDV strains cluster with Chinese strain AH2012.

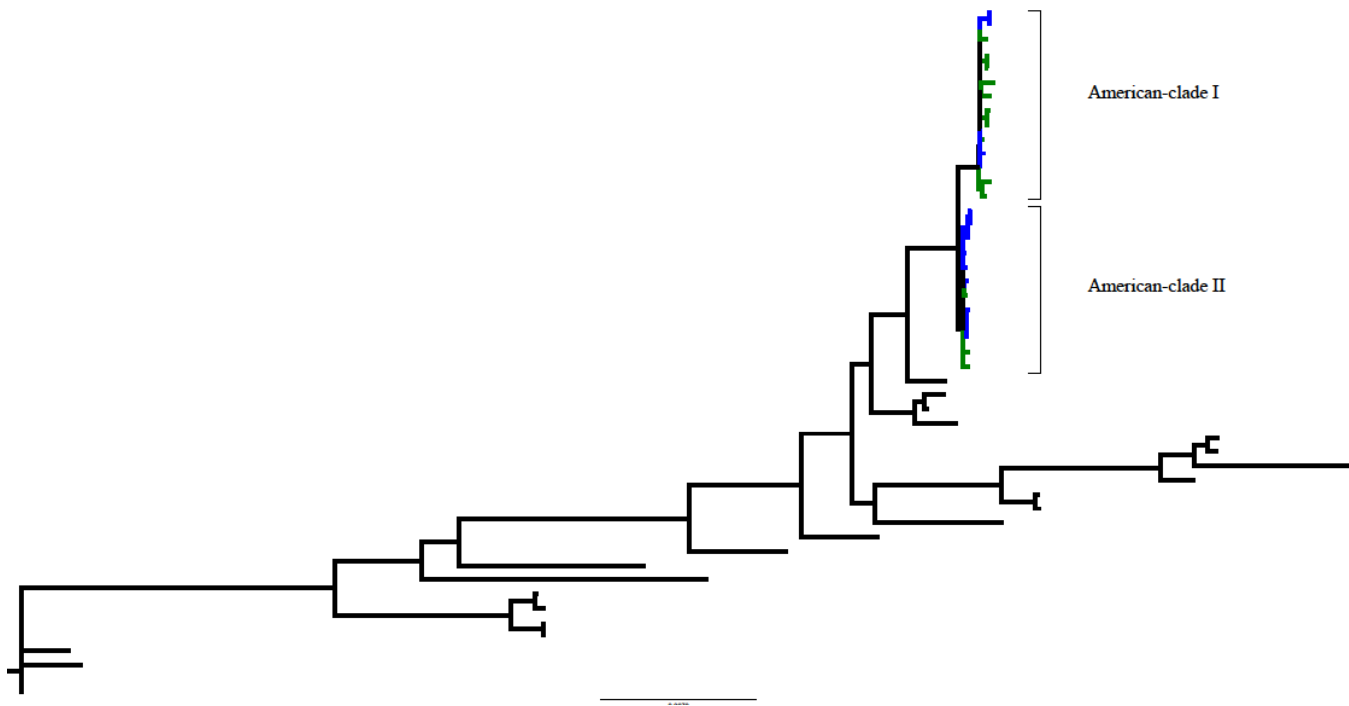


Fig 1. Phylogenetic tree of 13 American strains from this study (n=13, green) and complete PEDV genomes available in GenBank from previously reported non-American PEDV strains (n=22, black) and American PEDV strains (n=13, blue). The brackets indicate the American-clade I and clade II. The American strains cluster and share the highest nucleotide percent identity with Chinese strain AH2012 (GenBank accession number KC210145).

Discussion: The UMN TGEV-PEDV RRT-PCR assay had superior performance over the commercial TGEV-PEDV multiplex RRT-PCR assay. Accurate detection of PEDV and TGEV in clinical samples is important to minimize the spread of these two viruses. The role of the clinical diagnostic laboratories is to provide high sensitivity and specificity assay to help prevent and control pathogens and many assays must be evaluated before choosing the best assay to support the swine industry. Different PEDV clades are emerging with the US swine population. However, there may be no pathogenesis differences between the two clades.

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