

Status Update on Swine Coronaviruses Recently Identified in US Swine 2-27-2014

Since early January of 2014, there have been significant diagnostic investigative study, advanced genomic analyses, and diagnostic tool development efforts taking place at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) in efforts to better understand the increasing diversity of corona viruses that have been identified to be circulating in US swine populations. The following serves to provide an abbreviated update on findings and developments to date.

Outline:

Page 1: Update on more recently identified PEDV variant

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Question # 1: What have you learned concerning presumed origins of the more recently identified strains of PEDV (i.e., PEDV variant) that are genetically distinct from the PEDV strains identified in US swine since April 2013?

After an in-depth preparation and study of the PEDV genomic information using Next Generation Sequencing (NGS) technologies, ISU VDL's virologists have concluded that it does not seem likely that the more recently identified PEDV variants originated from the strains of PEDV previously identified in the US through a random mutation event. It seems more probable that more than one genotype of PEDV has been introduced into the US. These more recently identified PEDV variants most closely resemble another cluster of PEDVs previously identified in Asia. (See PEDV dendrograms on Pages 2 and 3.) According to the diagnostic data available at ISU VDL to date, the PEDV variants were retrospectively detected in US swine at least from early October 2013. Plans for additional retrospective testing are underway. **ISU VDL is offering PEDV S1 sequencing (first 2.2 kb portion of the spike gene)** to help clientele continue to monitor the genetic relatedness and epidemiology of PEDV. **ISU VDL has also recently developed a PEDV S1-based differential real-time RT-PCR to distinguish the original from the variant US PEDV strains.** It should also be understood that PEDV real-time RT-PCR offered at ISU VDL is targeting the nucleocapsid (N) gene. The N-gene is known to be a conserved portion of the PEDV genome. Thus far, the PEDV N-gene real-time RT-PCR being conducted at the ISU VDL seem to be readily detecting all the PEDVs known to be present in US swine.

Figure 1. PEDV S1 gene dendrogram. The ISU VDL is routinely offering the PEDV S1 (first 2.2 kb portion of the spike gene) sequencing to clientele to help understand the genetic relatedness and monitor the epidemiology of PEDV in US swine.

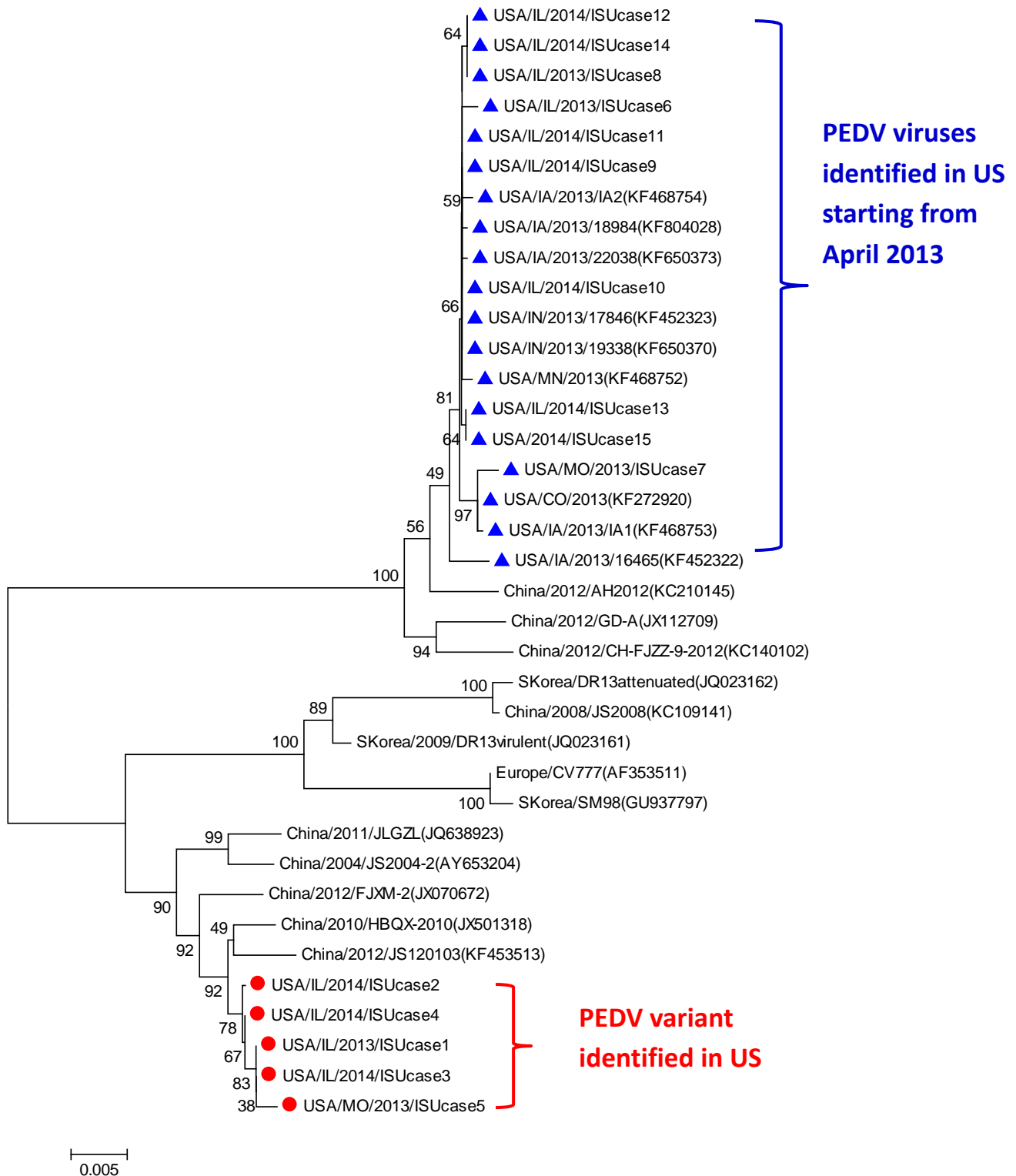
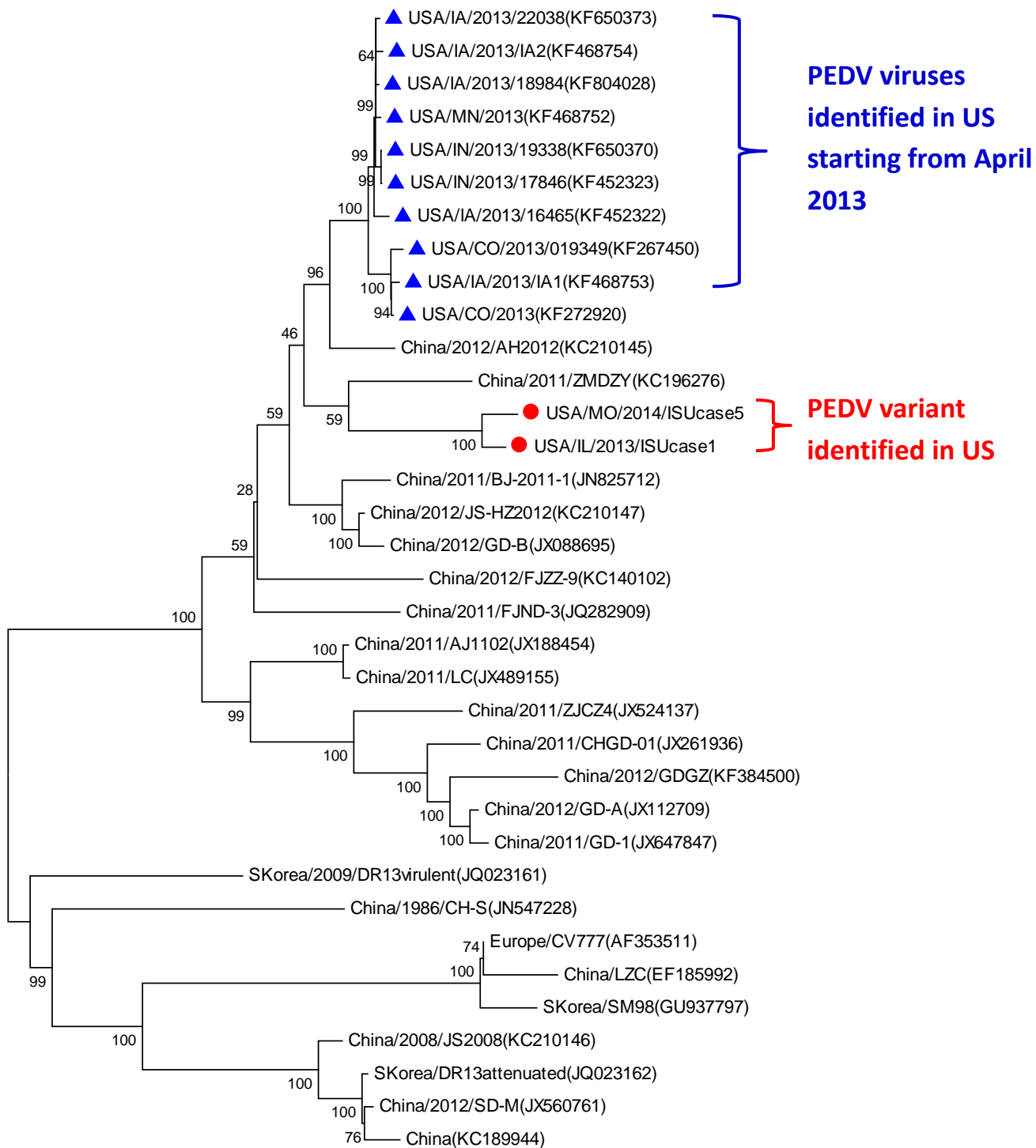


Figure 2. PEDV dendrogram based upon entire PEDV genome. Whole genome sequencing is most useful for advanced research study and molecular epidemiology applications.



Question # 2: What are you learning about the clinical significance of the recently identified Swine Delta Coronavirus (SDCV) and what diagnostic tools are available to identify SDCV?

Less is known concerning the clinical relevance of SDCV. The ISU VDL diagnosticians have been working closely with practitioners in efforts to better understand its potential role in case submissions that include a history of diarrhea in breeding age and suckling pigs that have not been able to be readily confirmed as being due to one or more of the more commonly recognized enteric pathogens. Most recently, the ISU VDL has been working with a practitioner on a case that seems to more strongly suggest that SDCV may be playing a role.

Case Description: Sudden onset of projectile diarrhea of 2 -3 day's duration occurred in breeding-age swine. The epidemic started in one end of the breeding barn of a 2,500 sow breed-to-wean unit and progressed throughout the breeding and gestation barns over a 7 day period. Clinical signs of abrupt onset of diarrhea occurred in the farrowing rooms (sows and piglets) approximately 8 – 9 days after the initial onset of clinical signs in the breeding barn. This particular farm had no previous clinical signs, suspicion, or diagnosis of either PEDV or TGEV. Fecal swabs from sows were submitted to the ISU VDL by farm staff upon the onset of the first clinical signs in the breeding barn and **tested negative for PEDV by PCR**. The attending veterinarian subsequently submitted fresh and fixed tissues from necropsy of acutely affected, euthanized sows, as well as feces from cohorts with diarrhea from the breeding and gestation barns (as the sows and/or suckling pigs in the farrowing barn were not yet affected). Post-mortem observations in the acutely affected gestating sows included stomachs full of feed, small intestines and colons markedly distended with fluid, watery contents, patchy hyperemia of mucosa of small intestine and cecum, and increased quantities of clear fluid in the peritoneal cavity. Histological evaluation revealed villus atrophy and fusion with attenuation of apical enterocytes in multiple sections of small intestine and mucosal edema was noted in the large intestine. Sections of stomach, kidney, liver, lymph node and spleen were unremarkable. Extensive molecular diagnostic investigation and bacterial culture work was conducted. Tissues from these sows and multiple feces from cohorts with diarrhea **tested negative for PEDV and TGEV by PCR**. However, feces from these sows and pooled fecal samples submitted tested **positive for the presence of SDCV by PCR, with cycle-threshold (ct) values ranging from 14 – 19**. The low ct value implies a very high number of SDCV genomic copies present in these samples. Piglets became clinically affected about 9 days after initial clinical signs in the herd. Acutely affected piglets were euthanized and necropsied for submission of samples to ISU VDL. Post-mortem observations included stomachs filled with milk, thin-walled and fluid-filled intestines. Histological evaluation again revealed atrophic enteritis and **equivalent molecular diagnostic test results to those described above for the sows**. Although the findings in this case seem to support a role for SDCV in the epidemic of diarrhea in this herd, prospective research and more diagnostic case-based study are needed to conclusively determine the clinical significance of SDCV. **ISU VDL has developed and recently made a real-time SDCV-PCR assay available for routine diagnostic testing purposes**. This new SDCV-PCR screening assay will enhance the ability of practitioners, diagnosticians, and researchers to better understand the clinical significance and prevalence of SDCV in the US swine herd going forward.

Many questions remain.

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