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US variant porcine epidemic diarrhea virus: histological lesions and genetic characterization

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Abstract Porcine epidemic diarrhea virus (PEDV) was first recognized in pigs in the United States (US) in May 2013. Since then, the virus has spread to over 30 states and caused significant economic losses in the US swine industry due to the high mortality in newborn piglets less than 2 weeks of age. A mild-variant strain OH851 of PEDV in the US was first reported in January 2014. Here, we report histological changes in the small intestines of five piglets infected with the variant strain OH851 of PEDV. The lesions observed were milder, compared to the US classical strain of PEDV. Our study, for the first time, reports the histological lesions caused by the variant PEDV OH851 strain from a field case. In addition, genomic characterization demonstrated that US variant PEDV is more closely related to European-like strains in the first 1170 nt of the 5′ spike gene but to US classical PEDV strains in the remaining genome, suggesting that the variant PEDV strain may derive from a recombinant event between the US classical and European-like PEDV strains.

Keywords Porcine epidemic diarrhea virus · PEDV · Variant strain · Histological lesion · Genetic characterization

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Porcine epidemic diarrhea (PED) virus (PEDV) belongs to the genus *Alphacoronavirus* of the family *Coronaviridae*. PED was first observed in England in 1971 [1, 2]. The disease quickly spread to other European countries in the 1980s and later to Asian countries including Japan, Korea, China, and Thailand [3]. Clinical signs of PED resemble those of transmissible gastroenteritis (TGE) and are characterized by severe enteritis, vomiting, watery diarrhea, dehydration, and weight loss but are typically less severe than TGE [3]. Since 2010, a virulent form of PEDV has emerged in China [4]. This virulent PEDV causes high mortality in newborn piglets and results in significant loss for pig farmers [4]. This virus was first confirmed in the United States (US) in May, 2013 [5]. Whole genome sequence analysis showed that US PEDV strains (classical strains) are closely related with a China-PEDV strain, AH2012 [5]. Since then PED has spread to more than 30 states and caused devastating economic loss in the US swine industry.

In January 2014, we detected a variant strain (OH851) of PEDV in samples from a swine farm in Ohio [6]. This virus caused mild clinical disease in pigs [6]. Genome sequence comparison showed that there was a high nucleotide similarity in either the complete genome (99 %) or the full-length spike (S) gene (97 %) between variant PEDV and originally identified classical PEDV strains from US, whereas a low nucleotide identity (≤89 %) was observed in the first 1170 nt of the S1 region between them [6]. Importantly, the S1 domain of the OH851 strain is closely related (99 % identical) to another PEDV strain (CH/HBQX/10) reported in China [7], indicating at least two genotypes of PEDV circulate in the US. Recent in vivo studies reported that pigs either naturally or experimentally infected with a variant PEDV strain were partially protected from a challenge by a classical PEDV strain [8, 9].

Here, we report the histological lesions of piglets naturally infected with the variant strain OH851 of PEDV and genetic characterization of variant PEDV strains from different countries.

Five 3–5 day-old crossbred piglets were submitted to Ohio Animal Disease Diagnostic Laboratory (ADDL). Clinical history indicated that sows on the farm were suspected to be infected with PEDV, but piglets showed minimal to no clinical signs and very low mortality (<5 %). Tests requested included PEDV, rotavirus, TGE virus (TGEV), and histopathology.

Histological changes were observed in the small intestines (jejunum, ileum) of all five piglets, including mild segmental to multifocal villous atrophy, villous fusion, and superficial enterocyte attenuation (Fig. 1a, c). Mild lymphoid depletion in colonic lymph nodes was observed in 1/5 pigs. These microscopic changes were suggestive of a mild atrophic enteritis, likely of viral origin. All small intestine samples tested positive for PEDV by a real-time RT-PCR targeting the M gene [10] with average C_t value of 14 (range of C_t value from 11 to 15) and negative for other viral pathogens including TGEV, deltacoronavirus, and rotaviruses. Importantly, these microscopic changes were much milder than anticipated for PEDV-associated infection (Fig. 1a–d). Other cases caused by classical PEDV strains, both observed at the ADDL as well as reported by others [11] have shown marked to severe villous atrophy and enterocyte attenuation (Fig. 1b, d). Images in Fig. 1b and d were taken from small intestines of newborn piglets of the first case of classical PEDV infection presented to the Ohio ADDL in June, 2013 (OH15962, Genbank accession KJ584361). No evidence of colibacillosis, clostridial enteritis, coccidiosis, or cryptosporidiosis was observed microscopically in any of the 44 intestinal sections examined. In support of our clinical findings, a recent in vivo study demonstrated that piglets infected with another variant PEDV strain, Iowa106, had mild histological lesions and limited intestinal infection [9]. It should be noted that the histological lesions presented here maybe an attenuated manifestation of a variant PEDV infection, if the piglets had maternal antibodies, which could not be determined due to

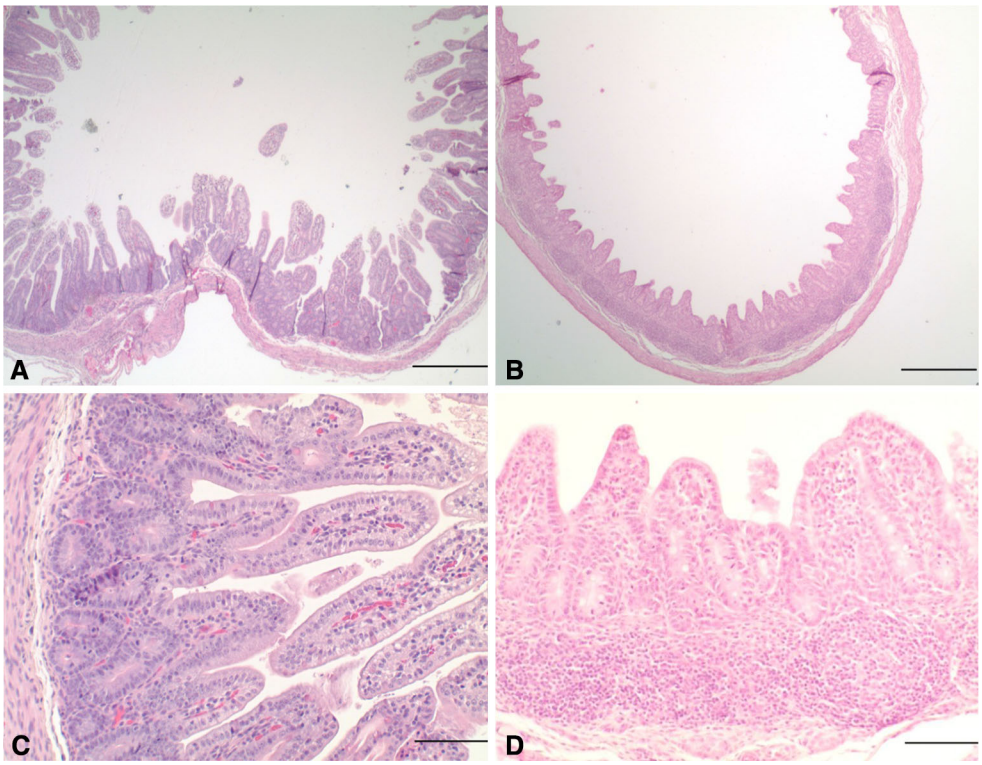

Fig. 1 Photomicrographs comparing sections of jejunum from nursing piglets infected with a variant strain OH851 of porcine epidemic diarrhea virus (PEDV), **a** and **c**, and with a classical PEDV strain OH15962, images **b** and **d**. **a** Jejunum, low magnification of jejunum from nursing piglet showing villi of near normal length and with minimal villus fusion. *Bar* 500 µm. **b** Jejunum, low magnification of jejunum from nursing piglet infected with the classical PEDV. There is marked and diffuse atrophy of villi with fusion of several villi also evident. *Bar* 500 µm. **c** Jejunum, higher magnification of small intestine from piglet infected with the variant strain of PEDV showing villus:crypt ratios of 2:1–3:1 or greater and villi lined by columnar epithelial cells frequently showing vacuolated cytoplasm. *Bar* 100 µm. 1D, jejunum, marked decrease of villus:crypt ratios is demonstrated (1:1 or less) in small intestine of piglet infected with the classical PEDV, with fusion of two villi at right side. Cuboidal attenuation of superficial enterocytes is also seen. *Bar* 100 µm

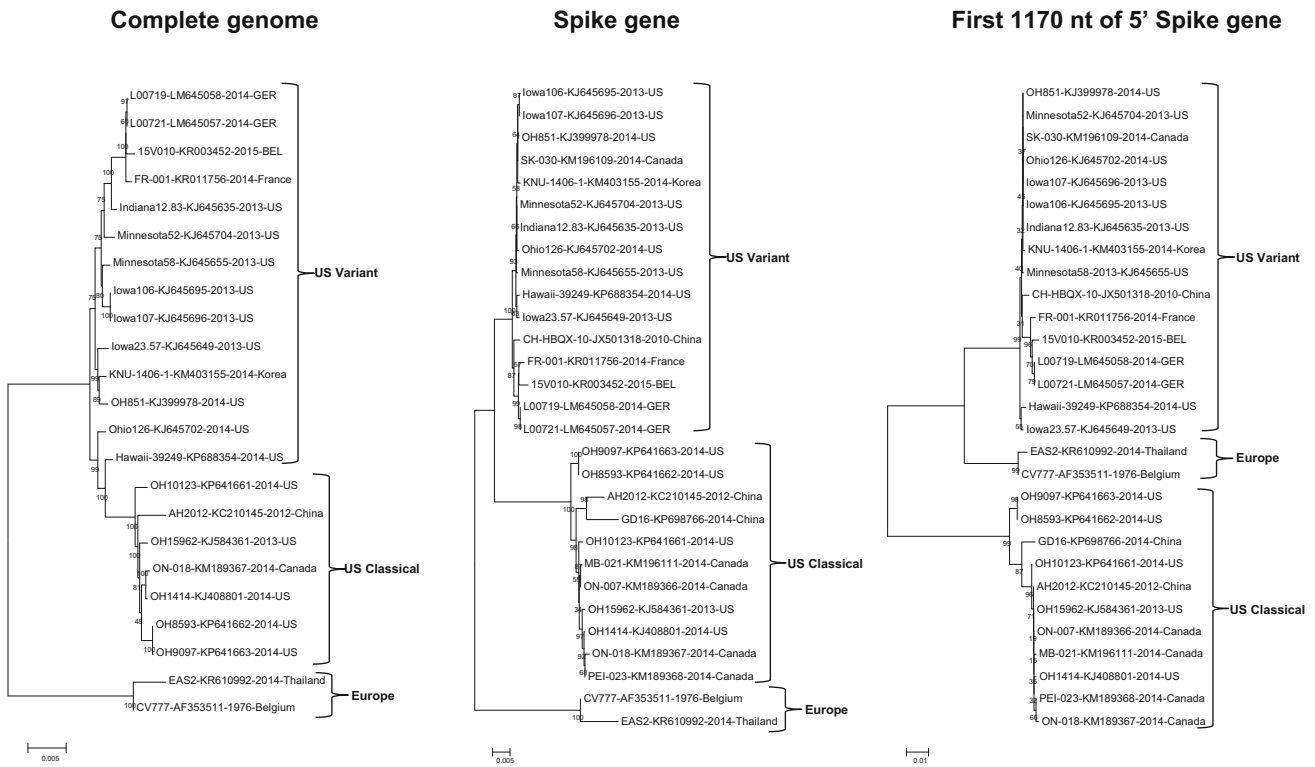

Fig. 2 Phylogenetic tree constructed on the basis of the whole genome nucleotide sequences of PEDV strains from different countries. The dendrogram was constructed using the neighbor-joining method in the MEGA software package, version 6.05 (www.megasoftware.net). Bootstrap resampling (1000 replications) was performed, and bootstrap values are indicated for each node. Reference sequences obtained from GenBank are indicated by names of the strains, GenBank accession numbers, and years and countries of isolation. *Scale bar* represents 0.005, 0.005, and 0.01 nucleotide substitutions per site in the tree of complete genome, spike gene and the first 1170 nt of 5′ spike gene, respectively

a lack of information regarding infection status of sows and PEDV antibody levels in sows and piglets. Future pathogenesis studies are needed to determine if similar histological lesions are observed in piglets experimentally infected with OH851.

Currently, US variant PEDV OH851-like strains have been reported in several European and Asian countries including Germany, Belgium, France, Portugal, Netherlands, Italy, Spain, Japan, and South Korea [12–18]. In the present study, we also analyzed the variant PEDV sequences of multiple countries and compared them with sequences of US classical PEDV and prototype PEDV CV777 strains. Genomic analysis showed that the variant strains from different countries are closely related and share over 99 % nucleotide identity both at the complete genome level and the spike gene level. By contrast, the US variant PEDV strains share 98.4–98.6 and 96.4–96.6 % identity with the US classical PEDV strains, while have 96.7–97.1 and 93.8–95.8 % identity with the prototype PEDV strain CV777, respectively. Further analysis showed that the US PEDV variants only share 86.6–88.2 % identity with US classical PEDV strains while they share 94.6–95 % identity with CV777 in the first 1170 nt of the 5′ spike gene. In support of the genomic analysis findings, the phylogenetic analysis of the complete genome and spike gene also demonstrated that US variant and classical strains relatively correlate under one genogroup separately from European like genogroup (Fig. 2). When the first 1170 nt of 5′ spike gene was used for phylogenetic analysis, the US variant strains showed a close relationship with the European-like strains, but were distinctly far away from US classical strains (Fig. 2). These data highly indicate that the US variant strains may originate from the recombination between European-like and US classical PEDV strains.

In the present study, we report that the variant PEDV caused mild histological lesions in the small intestines of piglets, much milder than those observed in pigs infected by the virulent strains of PEDV currently circulating in the US. Our findings were consistent with the clinical observation that the variant PEDV causes mild clinical disease and low mortality in infected piglets. It is highly likely that the S1 region contributed to the observed differences in clinical and histological features. Since the first variant

PEDV was identified [6], we have developed a duplex real-time RT-PCR assay for detection and differentiation of classical and variant PEDV strains from US [10]. Currently, the variant strain has been detected in different US states including Nebraska, Illinois, Indiana, Ohio, Iowa, Indiana, and Minnesota by our and other laboratories [19], indicating the virus may be widely spread in US. The variant PEDV strain was detected in several European countries except Ukraine where the US classical PEDV was identified [18]. Further research is needed to monitor the evolution of variant PEDV as well as virulent PEDV in swine populations of the US and other countries.

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Compliance with ethical standards

Conflict of Interest All authors in this paper declare they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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