Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virus Research 226 (2016) 20–39

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Evolution, antigenicity and pathogenicity of global porcine epidemic diarrhea virus strains

Chun-Ming Lin[a], Linda J. Saif[a,*], Douglas Marthaler[b], Qiuhong Wang[a,*]

[a] Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA
[b] Department of Veterinary Population Medicine and Veterinary Diagnostic Laboratory, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108, United States

ARTICLE INFO

Article history:
Received 10 April 2016
Received in revised form 21 May 2016
Accepted 21 May 2016
Available online 8 June 2016

Keywords:
Porcine epidemic diarrhea
Porcine epidemic diarrhea virus
Evolution
Pathogenicity
Antigenicity
Cross-reactivity
Cross-protection
Coronavirus

ABSTRACT

Emerging and re-emerging coronaviruses cause morbidity and mortality in human and animal populations, resulting in serious public and animal health threats and economic losses. The ongoing outbreak of a highly contagious and deadly porcine epidemic diarrhea virus (PEDV) in Asia, the Americas and Europe is one example. Genomic sequence analyses of PEDV variants have revealed important insights into the evolution of PEDV. However, the antigenic variations among different PEDV strains are less explored, although they may contribute to the failure of PEDV vaccines in Asian countries. In addition, the evolution of PEDV results in variants with distinct genetic features and virulence differences; thus PEDV can serve as a model to explore the molecular mechanisms of coronavirus evolution and pathogenesis. In this article, we review the evolution, antigenic relationships and pathologic features of PEDV strains. This information and review of researches will aid in the development of strategies for control and prevention of PED.

Contents

Abbreviations: aa, amino acid; CCIF, cell culture immunofluorescence assay; CDCD, cesarean-derived, colostrum-deprived; DPI, days post-inoculation; E, envelope; ELISA, enzyme-linked immunosorbent assay; FIPV, feline infectious peritonitis virus; HPI, hours post-inoculation; IHC, immunohistochemistry; IFN, interferon; INDEL, insertions and deletions; MAb, monoclonal antibody; M, membrane; N, nucleocapsid; nt, nucleotide; NTD, N-terminal domain; ORF, open reading frame; PDCoV, porcine deltacoronavirus; PED, porcine epidemic diarrhea; PEDV, porcine epidemic diarrhea virus; PFU, plaque-forming unit; PRCV, porcine respiratory coronavirus; RBD, receptor binding domain; S, spike; TC, tissue culture-adapted; $TCID_{50}$, 50% tissue culture infectious dose; TGEV, transmissible gastroenteritis virus; US, United States; VH:CD, villus height versus crypt depth ratio; VN, viral neutralization; WT, wild type.

* Corresponding authors.
E-mail addresses: lin.1903@osu.edu (C.-M. Lin), saif.2@osu.edu (L.J. Saif), marth027@umn.edu (D. Marthaler), wang.655@osu.edu (Q. Wang).

http://dx.doi.org/10.1016/j.virusres.2016.05.023
0168-1702/

1. Introduction

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded, positive-sense RNA virus that belongs to the order *Nidovirales*, family *Coronaviridae* and genus *Alphacoronavirus*. The genome of PEDV is approximately 28 kb in length and is composed of seven open reading frames (ORFs) (Kocherhans et al., 2001). The 5′ two thirds of the genome contains two overlapping ORFs, ORF1a and ORF1b, which encode nonstructural proteins that direct genome replication, transcription and viral polyprotein processing. The remaining PEDV genome contains five ORFs encoding four structural and an accessory protein in the following order: spike (S), ORF3, envelope (E), membrane (M) and nucleocapsid (N) proteins (Duarte et al., 1993).

PEDV is highly contagious. Fecal-oral transmission is the major route for the spread of PEDV in swine. Contaminated feed and feed ingredients may serve as vehicles for PEDV transmission to naïve pigs (Dee et al., 2014, 2016; Pasick et al., 2014). In addition, evidence of PEDV aerosol transmission has been reported (Alonso et al., 2014). During disease outbreaks, vomiting, watery diarrhea, dehydration and growth retardation are observed. High mortality occurs in young piglets exposed to the virulent PEDV strains. Microscopic examination of infected nursing pigs revealed atrophic enteritis (Debouck and Pensaert, 1980; Jung et al., 2014; Stevenson et al., 2013). Clinical signs and histopathologic features of PEDV infection resemble that of transmissible gastroenteritis virus (TGEV) infection, which is also an alphacoronavirus (Saif, 1989; Saif et al., 2012). Differential diagnosis of these two viruses relies mainly on molecular (Kim et al., 2001; Oka et al., 2014) and serological assays (Gerber et al., 2014; Song and Park, 2012).

At the end of 2010, pandemic porcine epidemic diarrhea (PED) outbreaks occurred in China and caused large economic losses. Subsequently, starting in the spring of 2013, the pork industry in the United States (US) was also severely affected by this disease. A lack of protective lactogenic immunity in the US swine population (Stevenson et al., 2013) and PEDV antigenic variations between the traditional attenuated vaccine and current highly virulent PEDV field strains in Asia (Lin et al., 2015a,b) were major contributors to severe PED outbreaks. Therefore, the development of updated PEDV vaccines is urgently needed.

In this article, genetic, antigenic and pathogenic features of global PEDV strains are reviewed. Throughout this review, the terms “classical” and “emerging” PEDV strains correspond to the PEDV prototype CV777-like strains that have appeared since the 1970s and those PEDV strains detected globally after 2010, respectively. Also, emerging PEDV strains are divided into two major groups based on features of the S gene and their virulence in young pigs: (1) “non-S INDEL (insertions and deletions)”, which is “highly virulent” and causes pandemic PED outbreaks worldwide; and (2) “S INDEL” strains that are less pathogenic in terms of lower mortality. This nomenclature is proposed based on sequence analysis as explained in detail in Section 2.

2. Evolution of global PEDV strains

2.1. Historic/classical PEDV strains emerged in the 1970s and were discovered in Europe and Asia

The first reported PED outbreak occurred in swine populations in 1971 in the United Kingdom (Chasey and Cartwright, 1978), but no PEDV was isolated from this outbreak. In 1978, PEDV CV777 strain was confirmed as the cause of PED by the experimental inoculation of pigs with the virus from an outbreak that occurred in 1977 in Belgium (Pensaert and de Bouck, 1978). The complete genomic sequence of the prototype PEDV CV777 strain was characterized later by Kocherhans et al. (2001). During the 1970s and 1980s, PEDV was widespread in European countries and caused epidemics with severe losses in suckling pigs (Saif et al., 2012). Thereafter PEDV did not persist in Europe except in isolated outbreaks. As a result, no vaccine was developed and used in Europe. Before 2010, PED had been reported in multiple European countries (the United Kingdom, Belgium, The Czech Republic, Hungary, France, Switzerland, Germany, and Italy) (Song and Park, 2012).

In Asia, PEDV has been reported in Shanghai, China since 1973 (Sun et al., 2015a). However, not until 1984 were the causative agents, PEDV H, CH and J strains, especially the J strain, isolated in

primary fetal pig intestinal cell cultures and confirmed to be PEDV by immunofluorescence assay (IFA), two-way cross virus neutralization (VN), and in vivo pig cross-protection assays comparing tissue culture-adapted (TC) PEDV and TGEV, and their respective antisera (Xuan et al., 1984). However, the sequence information for the early PEDV strains (H, CH, and J) is unavailable (personal communication with Drs. Li Feng and Jianfei Chen, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, and Dr. Dongbo Sun at the College of Animal Science and Veterinary Medicine, at Heilongjiang Bayi Agricultural University, Daqing, China). Since 1994, inactivated or live attenuated PEDV CV777 strain vaccines have been developed (Sun et al., 2015a). The inactivated, bivalent TGEV and PEDV vaccine (1999 to present) and the attenuated, bivalent TGEV and PEDV vaccine (2003–2006) have been used extensively in the Chinese pig population, and they have played an important role in the control of PEDV infections in China. Before October 2010, PEDV infections were endemic in Chinese pig populations, but there were no reports of large-scale outbreaks (Sun et al., 2015a).

PEDV was first reported in South Korea in 1992 (Park et al., 2014). From 2004–2013, PED outbreaks were well controlled by oral vaccination with a cell culture-attenuated DR13 strain vaccine (Park et al., 2013; Song and Park, 2012). In Japan, PEDV outbreaks were reported from the early 1980s (Song et al., 2015b) and temporarily disappeared in 2006, probably due to the use of an attenuated PEDV 83P-5 (or P-5V) strain vaccine (Nisseiken Co. Ltd., Japan) since 1997 (Masuda et al., 2015; Sato et al., 2011; Song et al., 2015b; Song and Park, 2012). Similar classical PEDV strains also spread to and caused PED outbreaks in Vietnam, Thailand, Taiwan, and the Philippines. Since 2011, the attenuated PEDV DR13 vaccine also has been used in the Philippines (Song and Park, 2012).

No PED cases were reported in the American, African and Australian continents prior to 2013. We will refer to these historic PEDV strains as classical strains because such viruses are still detected in the field, such as CHN/SD-M/2012 and THA/EAS1/2014, to differentiate them from the emerging strains detected worldwide after 2010.

2.2. Since 2010, highly virulent PEDV strains emerged in China and subsequently spread to other Asian countries, the Americas, and back into Europe

Starting from October 2010, severe PEDV outbreaks occurred in the Chinese pig population and caused enormous economic losses (Sun et al., 2015a, 2012). Although PEDV vaccines based on classical PEDV strain CV777 were widely used in China, the mortality in neonatal piglets was high, reaching 50–100%, indicating the emergence of highly virulent PEDV variants.

In April, 2013, the first highly virulent PEDV infections emerged in swine farms in the US (Stevenson et al., 2013). By March 12, 2015, PEDV had spread to 36 US states, including the geographically separated state of Hawaii, and one US territory, Puerto Rico (https://www.aasv.org/Resources/PEDv/PEDvWhatsNew.php). In the first year, PEDV killed about 10% of the US swine population, accounting for deaths of 7 million pigs, and resulting in major economic losses to the US swine industry (http://www.dailykos.com/story/2014/4/27/1295143/-PEDv-virus-has-killed-10-of-U-S-pig-population-causing-spike-in-prices). The last large-scale outbreak in the US ended in the spring of 2014 (Jarvis et al., 2015). By 2014, the highly virulent PEDV had spread to other countries in the Americas, including Canada, Mexico, and Columbia (Vlasova et al., 2014).

In October 2013, the highly virulent PEDV emerged in Japan, and about 1000 outbreaks were reported by 2015 (Masuda et al., 2015). In South Korea and Vietnam, the highly virulent PEDV emerged in 2013, based on complete genomic sequences (Kim et al., 2015; Vui et al., 2014). In Thailand, both classical and highly virulent PEDV strains were sequenced recently (Cheun-Arom et al., 2015). PEDV outbreaks emerged in Taiwan in late 2013 (Lin et al., 2014) and in Philippines in 2014 (Kim et al., 2016). However, no complete genomic sequence data are available for these strains. The year 2013 was critical for the spread of the highly virulent PEDV strains to other swine producing countries besides China. However, except for the Ukraine (Dastjerdi et al., 2015), no highly virulent PEDV infections were reported in the European, African and Australian continents.

2.3. The S INDEL PEDV strains emerged in June 2013 in the US and have since been detected in Asia and Europe

A PEDV variant OH851 strain, designated as S INDEL PEDV, was first discovered in Ohio, USA (Wang et al., 2014). Later, it was found that S INDEL strains, such as Indiana12.83/2013 and Minnesota52 strains, existed in samples collected in the US in June 2013, just 1–2 months later than the emergence of the highly virulent PEDV strains in this country (Vlasova et al., 2014). The N-terminal region of the S protein of these strains has an amino acid insertion (aa 161–162) and two deletions (aa 59–62, and aa 140) compared with those of the highly virulent PEDV strains, but similar to that of the classical PEDV strains (Fig. S9) (Oka et al., 2014). Although the amino acid sequences of the S proteins of S INDEL strains are closer to the classical strains than to the highly virulent US strains (Fig. 2), the rest of the genes (ORF1, ORF3, E, M and N) of S INDEL strains group together with the emerging highly virulent PEDV strains from China, North American, etc. (Figs. S1, S4–S7) (Vlasova et al., 2014). Therefore, S INDEL strains fall into the emerging NA clade at the complete genomic sequence level (Fig. 1).

In December 2013, an S INDEL PEDV MYZ-1/JPN/2013 strain was identified in Japan (Suzuki et al., 2015). Since 2014, PEDV strains from Canada, Korea, Belgium, France, Germany, Portugal, Slovenia and Netherlands have been described, and these strains cluster with the US S INDEL strains (Grasland et al., 2015; Hanke et al., 2015; Kim et al., 2016; Lee and Lee, 2014; Mesquita et al., 2015).

2.4. Other variant PEDV strains

2.4.1. PEDV variants that have deletions in the S protein

The S protein of coronaviruses is responsible for binding to cellular receptors to initiate infection and it induces neutralizing antibodies in vivo (Li, 2015). Mutations, especially deletions and/or insertions in the S protein may change the pathogenicity and tissue tropism of coronaviruses. For TGEV, a large deletion (621–681 nt) in the N-terminus of S gene reduced its virulence and changed its tissue tropism from intestinal to respiratory [subsequently designated porcine respiratory coronavirus (PRCV)] (Saif et al., 2012). For PEDV, Korean strain MF3809/2008 was identified with a large deletion (204 aa) at amino acid positions 713–916 of the S protein (Park et al., 2014). Except for this large deletion, its S protein was similar to that of highly virulent PEDV strains (Fig. 3). Later, a minor PEDV variant (USA/Minnesota188/2014) with a 2 aa-deletion in the S protein was reported as very virulent (Marthaler et al., 2014a). In China, a field CHN/FL2013 strain with a 7 aa-earlier termination of S protein, like the classical CHN/LZC strain, was also reported (Zhang et al., 2015). Laboratory pig experiments showed that the virulence of FL2013 was reduced. A field PEDV strain JPN/Tottori2/2014 with a 194 aa-deletion in the position aa 23–216 of the S protein was discovered (Murakami et al., 2015). Interestingly, a single US PEDV strain, PC177, with a 197 aa-deletion in a similar position (aa 34–230) of the S protein, was isolated from Vero cell culture (Oka et al., 2014). Later, it was confirmed that the large deletion originated during cell culture adaptation because the wild-type (WT)

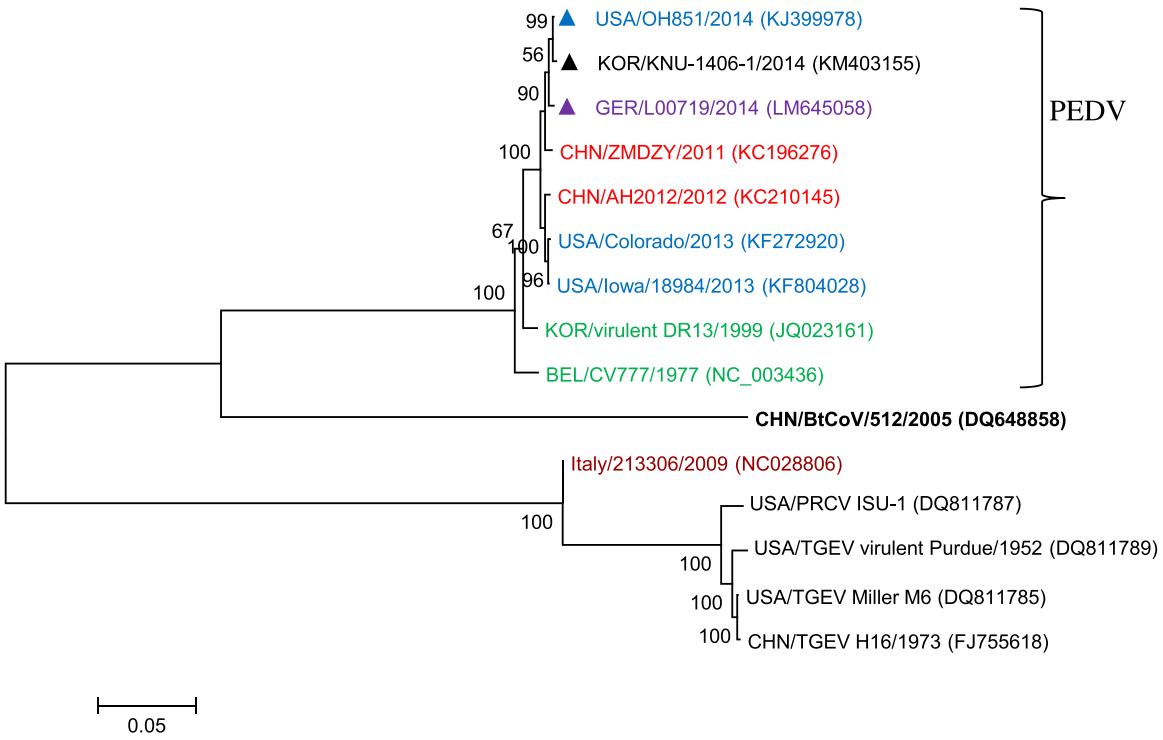

Fig. 1. Phylogenetic tree of the complete genomes of PEDV strains and closely related alphacoronaviruses. Classical and the emerging PEDV strains, a bat alphacoronavirus (in bold), the recombinant porcine enteric coronavirus between TGEV and PEDV (in brown), three TGEV strains and one PRCV strain were included to generate a Maximum Likelihood phylogenetic tree using General Time Reversible nucleotide substitution model and supported with a bootstrap test of 1000 replicates in MEGA 6.06 software (Tamura et al., 2013). The number on each branch indicates the bootstrap value. The scale represents the nucleotide substitutions per site. Classical PEDV strains are in green. Emerging US, Chinese and European PEDV strains are in blue, red, and purple, respectively. Emerging S INDEL PEDV strains are labeled with a solid triangle in front of the strain name.

PC177 strain in the original field piglet intestinal contents was successfully sequenced and did not have the large deletion in the S protein (GenBank accession no. KR078300). The pathogenicity of these PEDV variants is discussed in Section 4 in detail.

2.4.2. PEDV variants that have deletions in the ORF1a region

Most recently, a highly virulent PEDV strain, HUA-14PED96/2014, was detected from a piglet with severe diarrhea in Vietnam. It contains a 72 nt-deletion in the open reading frame 1a (ORF1a), corresponding to a 24 aa-DEL in the nsp3 protein, and is grouped with the Chinese highly virulent PEDV cluster (Fig. 1) (Choe et al., 2016).

2.4.3. Recombinant PEDV strains

Recombination occurs frequently among PEDV strains and was detected in seven main areas of the PEDV genome excluding the 5′- and 3′-UTRs: nsp2, nsp3, nsp14-16, S1 domain, and N gene (Jarvis et al., 2015). For example, S INDEL strains USA/Iowa106/2013 and USA/Iowa107/2013 (Genbank accession no. KJ645696; 1 nt different from Iowa106 strain at the genomic level) were probably the result of several recombination events: the ORF 1a and 1b region was most similar to that of the highly virulent strains CHN/AH2012 and CHN/ZMDZY/2011, respectively; the S1 and S2 region of the S protein was most similar to that of the classical strain CHN/CH/S/1986 and the highly virulent ZMDZY/2011 strain, respectively; and the 3′ end (E, M, and, N genes) was dissimilar to that of either AH2012 or ZMZDY/2011 strains (Vlasova et al., 2014). USA/Minnesota211/2014 strain is a recombinant between a highly virulent strain (genomic fragment from the middle of nsp2 to the middle of nsp14) and an S INDEL strain (Jarvis et al., 2015). Most recently, the CH/HNQX-3/14 strain was reported as a recombinant among the classical strains CV777 and DR13, and the highly virulent CHN/ZMDZY/11 strain. It grouped together with the CH/ZMDZY/11 strain at the genomic level (Li et al., 2016). Not surprisingly, this strain was detected from a swine farm that is located in the same province as the CHN/ZMDZY/11 strain, and both CV777 and DR13 PEDV vaccines were used at the farm.

2.4.4. Recombinant enteric coronavirus

Although both PEDV and TGEV belong to the *Alphacoronavirus* genus and cause indistinguishable enteritis (Saif et al., 2012), no recombinant virus between TGEV and PEDV had been found until recently (Boniotti et al., 2016). This recombinant porcine enteric coronavirus, Italy/213306/2009 strain, has potential parental strains TGEV H16 strain (major, backbone) and classical PEDV CV777 strain (minor, S gene) (Fig. 1). This virus was circulating in 2009–2012 in Italy. Another TGEV/PEDV chimeric virus (SeCoV/GER/L00930/2012), sharing similar recombination pattern and 99.5% nucleotide identity with the Italy/213306/2009 strain, was reported in Germany (Akimkin et al., 2016). It is important to determine the virulence and tissue tropism of these chimeric viruses and to monitor how they evolve in the swine population.

2.5. Evolution of global PEDV strains

2.5.1. Sequence analysis suggests the potential origin of emerging PEDV strains

Phylogenic analysis of the full-length genomes and the genome organization showed that all PEDV strains are most closely related to bat alphacoronaviruses within the *Alphacoronavirus* genus (Fig. 1) (Huang et al., 2013; Tang et al., 2006; Vlasova et al., 2014), suggesting that PEDV strains probably originally evolved from bat alphacoronaviruses and were transmitted cross-species directly or indirectly to pigs. There were about 300 complete genomes of

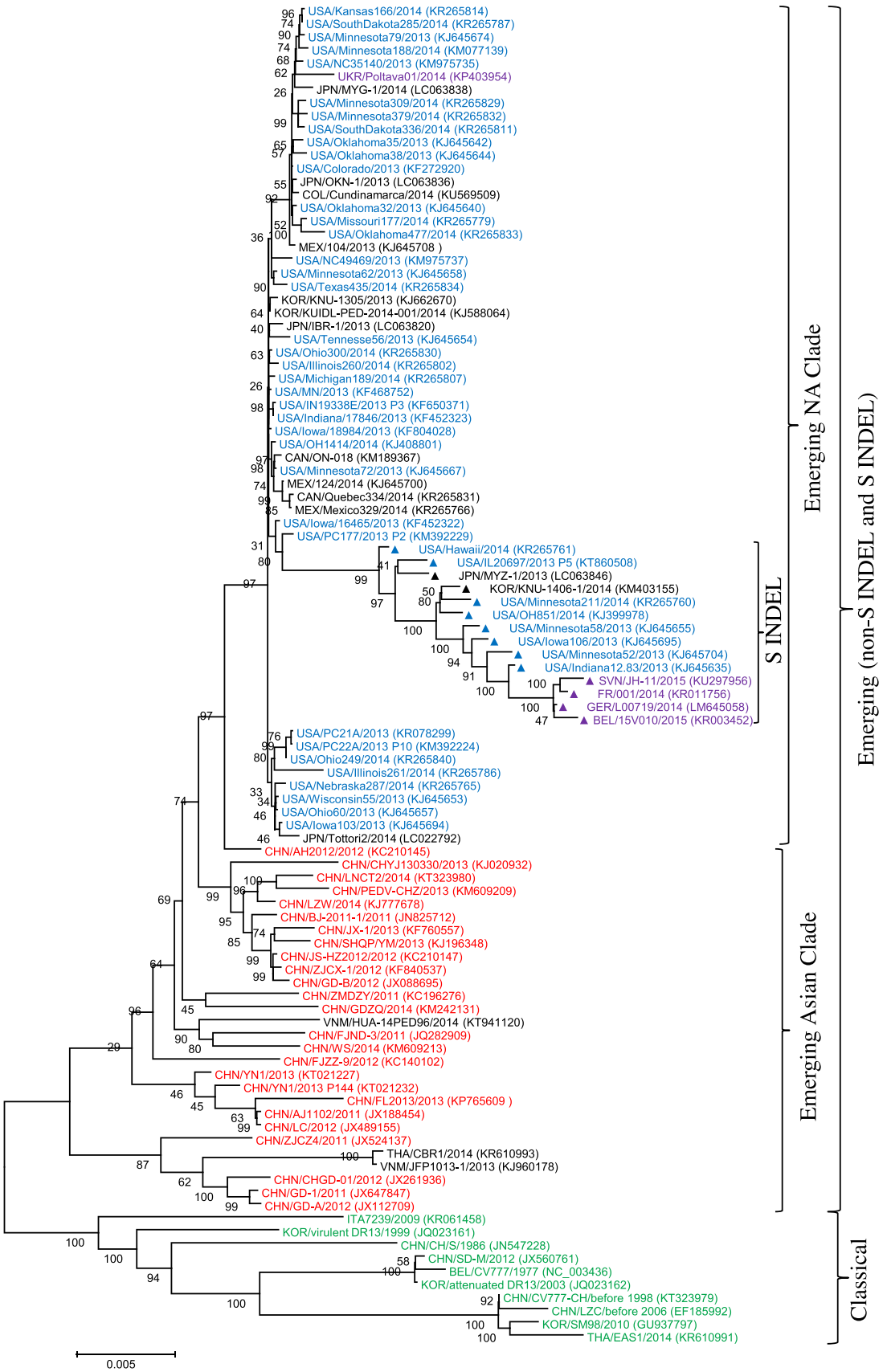

Fig. 2. Phylogenetic tree of the genomes of PEDV strains, excluding the UTRs. A Maximum Likelihood phylogenetic tree was constructed using General Time Reversible nucleotide substitution model and supported with a bootstrap test of 1000 replicates in MEGA 6.06 software (Tamura et al., 2013). The number on each branch indicates the bootstrap value. The scale represents the nucleotide substitutions per site. Each PEDV strain is indicated in the following format: Country origin (three letter code: BEL, Belgium; CAN, Canada; CHN, China; COL, Columbia; FRA, France; GER, Germany; JPN, Japan; KOR, Korea; MEX, Mexico; SVN, Slovenia; THA, Thailand; UKR, Ukraine; USA,

PEDV listed in the GenBank database (http://www.ncbi.nlm.nih.gov/) at the beginning of 2016. For phylogenetic analysis of PEDV strains, we selected around 100 representative strains from published papers based on the following criteria: (1) strains collected in different geographical areas; (2) strains whose pathogenesis have been studied; (3) vaccine strains and strains whose antigenicity will be discussed in this review; and 4) both TC and wild type (WT) strains. The WT genomes of two PEDV strains (PC21A-WT and PC177-WT), whose TC sequences have been reported previously (Oka et al., 2014), were successfully sequenced and included in this analysis. Sequence alignment was performed using ClustalW method. Maximum Likelihood phylogenetic trees were constructed using General Time Reversible nucleotide substitution model (for nucleotide sequences) or Jones-Taylor-Thornton (JTT) model (for amino acid sequences) and supported with a bootstrap test of 1000 replicates in MEGA 6.06 software (Tamura et al., 2013).

Based on the nearly complete genomes, excluding the UTRs due to the lack of these regions for many PEDV strains, all PEDV strains separated into two distinct groups: "classical" and "emerging" PEDV strains (Fig. 2). Phylogenetic trees based on ORF1 and M also confirmed the two distinct groups (Figs. S1 and S6). The nucleotide percent identity of the PEDV genomes between "classical" and "emerging" clusters is 96–98%. Since PEDV has a relatively low mutation rate (6.2×10^{-4} substitutions/site/year) similar to TGEV and other animal coronaviruses (Jarvis et al., 2015), it seems likely that the PEDV strains have diverged due to host adaptation. On the other hand, evidence supporting a different origin of the two distinct clusters of PEDV strains exists. Huang et al. (2013) found that in the 5′-proximal region of the 5′-UTR, all the emerging highly virulent PEDV strains share a unique insertion [a T at nt 48, the loop of stem-loop (SL) 2 structure] and two deletions (an A deletion between nt 72 and 73, immediately after the core sequence of the leader transcription-regulating sequence; a 4 nt (TTCC)-deletion in SL4) with the bat coronavirus (BtCoV)/512/2005 strain compared with the PEDV prototype CV777 strain. Sequence alignments of the 5′-UTR of classical and emerging PEDV strains showed that all emerging PEDV strains and some classical strains, such as Korean DR13/1999 and Chinese CH/S/1986, contain the same insertion and deletions (Fig. S8). One classical strain SM98/2010 does not have the insertion T at nt 48, but has the two deletions compared with CV777 strain. How such a unique feature was obtained by these PEDV strains is still unknown. If the changes result in a replication advantage for PEDV strains, the genetic changes may evolve and be selected. However, the process for acquiring the individual insertion and deletions in PEDV, or intermediate PEDV strains, has not been determined. It is also possible that PEDV strains obtained this region from a BtCoV/512/2005-like coronavirus directly by recombination.

Although complete genomic sequences are lacking for many PEDV strains, information from the S protein may highlight when the emerging non-S INDEL strains evolved since the S genes are available for some samples collected before 2010, and the emerging non-S INDEL and classical groups can be clearly differentiated in an S protein tree (Sun et al., 2015b) (Fig. 3). It appears that the non-S INDEL strains, such as KOR/Chinju99/1999 strain, had emerged before 2010. An earlier report also indicated the emergence of new PEDV strains since early 2006 in PEDV immunized swine herds in China (Chen et al., 2010). Unfortunately, only the ORF3 gene was analyzed in this study. To date, when, where and how the original emerging non-S INDEL PEDV strains evolved is still unknown. Recently, Khatri (2015) reported that the highly virulent PEDV grew in duck intestinal epithelial cells, raising the question of whether there are other reservoirs besides bats for PEDV. All those questions need to be investigated further.

2.5.2. Complex evolution of emerging PEDV strains

We divide the emerging PEDV strains into "S-INDEL" and "non-S INDEL" (or "highly virulent") because the former and later cause mild and severe PED, respectively, in the field and in laboratory pig experiments (see Section 4 for detail). In the complete genomic tree of PEDV strains (Fig. 2), non-S INDEL PEDV strains from China are more closely related to each other than to the strains from the US and European countries, except for CHN/AH2012 that is closer to the US strains (with nt identity of 99.33–99.61%) than to the Chinese strains (with nt identity of 98.10–99.50%). Because there are no cut-off values for genogrouping or genotyping for PEDV strains, we tentatively refer to them as emerging Asian Clade and emerging North American (NA) Clade. Phylogenetic analysis of ORF1 supports such a grouping system (Fig. S1). For the phylogenetic trees based on ORF3, E, M or N genes (Figs. S4–S7), NA non-S INDEL, S INDEL, and some Chinese emerging strains are clustered together. Therefore, those regions are probably not immediate targets in the recent evolution of emerging PEDV strains and there is a lack of enough information to generate evolution inference.

Other researchers also found such inconsistencies when phylogenic analyses were conducted using complete genomic sequences or different regions of the genome (Chen et al., 2014; Kim et al., 2016; Song et al., 2015a; Sun et al., 2015b; Tian et al., 2014; Vlasova et al., 2014). It is probably due to recombination among PEDV strains, which is reported as a major evolution pattern for coronaviruses (Makino et al., 1986). Recombination analysis and analysis of single nucleotide polymorphisms (SNPs) provided additional information to trace the evolution of PEDV strains (Huang et al., 2013; Jarvis et al., 2015; Song et al., 2015a; Tian et al., 2014; Vlasova et al., 2014). Recently, a novel method using sequence motifs, which was defined as "a highly conserved region at the same position in the genome that was exclusively shared between several of the PEDV strains", was developed to study the relationship among emerging PEDV strains detected in 2013–2014 (Yamamoto et al., 2016). So far, the results of these two analyses are in agreement. The NA non-S INDEL strains probably evolved from different Chinese emerging strains by recombination (Tian et al., 2014; Vlasova et al., 2014). The non-S INDEL strains showed an overall high similarity to the highly virulent CHN/AH2012 strain across the genome except for the S1 region where they showed the highest similarity to another highly virulent CHN/ZMDZY/2011 strain. The S INDEL Iowa106 and Iowa107 strains probably resulted from multiple recombination events among the classical (CHN/CH/S-like strain for S1 region) and at least two different highly virulent strains (CHN/ZMDZY/2011-like strain for ORF1b and S2 regions, and CHN/AH2012-like strain for the remaining genome) (Vlasova et al., 2014). It is notable that the Ukraine, Japanese and Korean emerging non-S INDEL strains are within the NA non-S INDEL, but not the emerging Asian clade, suggesting global spread of the NA non-S INDEL strains (Fig. 2). On the other hand, several PEDV strains from Vietnam and Thailand clustered together with the emerging Chinese strains.

Except for the Ukraine Poltava01/2014 strain, the emerging European strains clustered within the S INDEL clade, which contains strains from NA, Japan and Korea. Surprisingly, no S INDEL strains were reported from China where both the classical and the emerging PEDV strains are co-circulating. The S INDEL strains could

the United States; VNM, Vietnam)/strain name/year of sample collection (Genbank accession number) followed by passage (P) number for tissue culture-adapted strains. Classical PEDV strains are in green. Emerging US, Chinese and European PEDV strains are in blue, red, and purple, respectively. Emerging S INDEL PEDV strains are labeled with a solid triangle in front of the strain name.

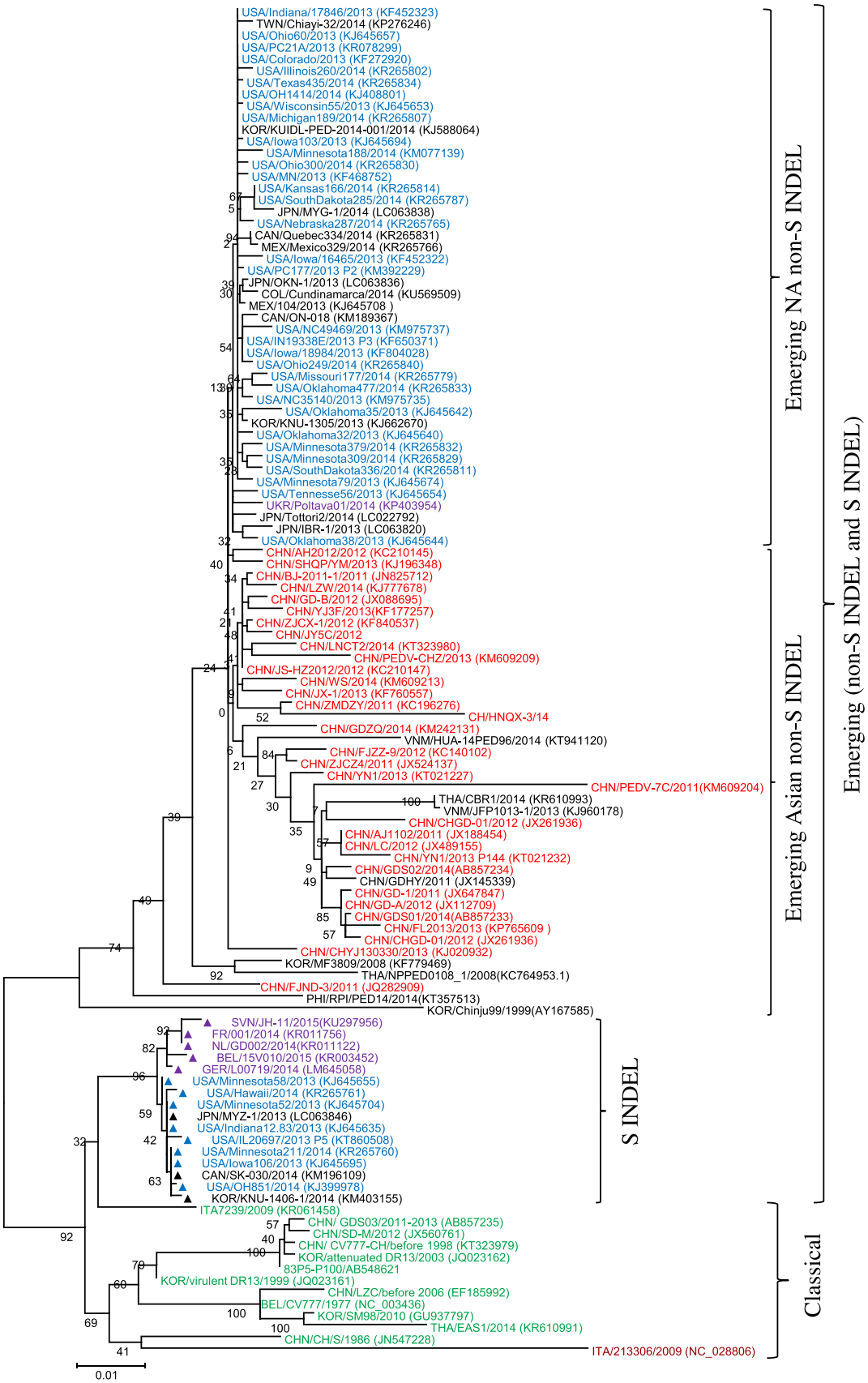

Fig. 3. Phylogenetic tree of the S proteins of PEDV strains. A Maximum Likelihood phylogenetic tree was constructed using Jones-Taylor-Thornton (JTT) model and supported with a bootstrap test of 1000 replicates in MEGA 6.06 software (Tamura et al., 2013). The number on each branch indicates the bootstrap value. The scale represents the nucleotide substitutions per site. Each PEDV strain is indicated in the following format: Country or area origin (three letter code: BEL, Belgium; CAN, Canada; CHN, China; COL, Columbia; FRA, France; GER, Germany; JPN, Japan; KOR, Korea; MEX, Mexico; PHI, Philippine; SVN, Slovenia; THA, Thailand; TWN, Taiwan; UKR, Ukraine; USA, the United

be overlooked due to the following possibilities: (1) in general the S INDEL PEDV strains cause milder diarrhea in pigs compared to the highly virulent emerging strains, so clinical samples may not be collected for diagnosis; and (2) many diagnostic tests or surveillance studies did not differentiate S INDEL strains from classical (if the S1 region of the S gene is the target) or emerging non-S INDEL PEDV strains (if S2, ORF3, E, M or N region is the target), such as using real-time RT-PCR targeting the N gene or sequence analysis of the S1 region only (Oka et al., 2014). Interestingly, based on the genetic motif analysis (Yamamoto et al., 2016), S INDEL strains GER/L00719/2014 and USA/Indiana12.83/2013 contain 6 of the 8 genetic motifs (M1-M6) along the genome. Japanese S INDEL strains (MYZ-1/2013, KCH-1/2014, and KCH-2/2014) contain 2 motifs (M3 and M6) that are different from any of the US S INDEL strains, whereas the JPN/OKY-1/2014 strain contains the same 2 motifs (M5 and M6) as the USA/Iowa23.57/2013 strain. South Korean S INDEL strain KNU-1406-1/2014 contains 3 genetic motifs, similar to the USA/OH851/2014 strain. These results suggest a close relatedness of these global S INDEL strains. However, the origin of emergence of these strains and the direction of transmission of S INDEL strains are unknown.

In summary, based on genetic analysis, the global PEDV strains are divided into two major groups: the classical PEDV strains that first emerged in 1970s in Europe and the highly virulent PEDV strains that emerged in 2010 in China (Fig. S10). Many questions remain unanswered, such as whether the later strains evolved directly from the former strains, and when the non-S INDEL and S INDEL emerging strains were introduced into the US and Europe. Jarvis et al. (2015) reported that the separate evolution of Chinese and US strains (both non-S INDEL and S INDEL) is estimated to have begun around 2006–2009, several years earlier than 2013 when the current US PEDV strains emerged. As of March 2016, no PEDV strains have been reported from the African and Australian continents. Both classical (including attenuated vaccines) and emerging PEDV strains, and TGEV strains are circulating in Asian countries, including China, that accounts for half of global swine production. Because coronaviruses are RNA viruses and recombination occurs frequently, more PEDV variants are expected to emerge in the future. Further availability of more complete genome sequences, especially those from European and Asian strains, and from historical samples before 2010, and a more comprehensive analysis will improve our understanding of the origin, evolution, and diversity of global PEDV strains.

3. Serological cross-reactivity between PEDV and other coronaviruses and antigenic variations among different PEDV strains

3.1. Antigenic relationships between PEDV and other alphacoronaviruses

Traditionally, coronaviruses were classified on the basis of antigenic cross-reactivity, and three distinct antigenic groups (1–3) were delineated (Saif, 1993). This antigenicity-based classification matched well with the results of phylogenetic analysis until the alphacoronavirus PEDV and human coronavirus 229E were identified (Sanchez et al., 1990; Wood, 1977). Based on viral genome sequences, TGEV and PEDV were classified as two independent species belonging to the *Alphacoronavirus* genus. TGEV and other species members within the *Alphacoronavirus* genus, including porcine respiratory coronavirus (PRCV), feline infectious peritonitis virus (FIPV), and canine coronavirus, showed antigenic cross-reactivity in various immunoassays (Horzinek et al., 1982; Sanchez et al., 1990). In contrast, most previous studies reported that PEDV and TGEV had distinct antigenic reactivity. Neither the prototype PEDV CV777 strain nor the other PEDV variants cross-reacted serologically with selected TGEV strains or other coronaviruses (Hofmann and Wyler, 1989; Pensaert et al., 1981; Stevenson et al., 2013). However, exceptions were reported as described subsequently (Have et al., 1992; Lin et al., 2015b; Ma et al., 2016; Zhou et al., 1988).

Some reports showed a low degree of antigenic cross-reactivity between PEDV and other alphacoronaviruses. The antigens of PEDV CV777 strain in pig intestinal epithelial cells were detected by immunofluorescent staining with ascites collected from a cat that had succumbed to FIPV (Zhou et al., 1988). Two-way antigenic cross-reactivities between PEDV and FIPV were also revealed by ELISA and Western blot at the N protein level (Zhou et al., 1988). Have et al. (1992) reported that sera collected from a putative coronavirus-infected mink cross-reacted with both PEDV and TGEV N proteins, supporting the idea that these viruses were distinct species, but partially related antigenically. Recently, we demonstrated that four distinct PEDV strains (prototype CV777, highly virulent PC22A, S INDEL Iowa106 and S 197aa-del PC177) were all recognized by hyperimmune pig antisera to TGEV Miller in a cell culture immunofluorescence assay (CCIF) (Table 1) (Lin et al., 2015b). The pig antisera to TGEV Miller demonstrated high homologous titer (10,640) against TGEV Miller, but lower heterologous titers (160–640) to PEDV CV777 and other US PEDV strains. In agreement with a previous study (Zhou et al., 1988), assessment of a panel of TGEV monoclonal antibodies (MAbs) (Simkins et al., 1989; Simkins et al., 1992) supported at least one conserved epitope on the TGEV N protein (located between aa 1–205, recognized by TGEV N MAb 14G9.3C) that contributed to this cross-reactivity. Sequence alignments showed two highly conserved motifs (66-QIGYWN-71 and 92-FYYLGTGPH-100) that were found in both TGEV and PEDV N proteins. The later motif is also conserved in other coronaviruses (He et al., 2004) and mediates interspecies antigenic cross-reactivity among coronaviruses, even from different genera (Vlasova et al., 2007). Strikingly, only TGEV Miller, but not TGEV Purdue antisera showed one-way antigenic cross-reactivity with PEDV strains. The reasons for these unexpected findings are unknown. However, the antigenic cross-reactivity observed between PEDV and TGEV strains raised concerns about the specificity of PEDV whole virus or N protein-based serologic assays. One recombinant PEDV N protein-based ELISA showed weak cross-reactivity with one TGEV- and one PRCV-infected pig serum sample. Therefore, a more conservative cut-off value had to be used in this assay (Dvorak et al. presented at North American PRRS Symposium. Chicago, IL, USA. 2013. Abstract #22). However, another PEDV N protein-based ELISA assay showed no cross-reactivity with TGEV (Purdue and Miller) and PRCV antisera (Okda et al., 2015). We speculate that convalescent TGEV Miller antisera collected from the field and displaying low cross-reactivity with PEDV N proteins might be interpreted as background or non-specific reactions. This could be the reason why the antigenic cross-reaction between TGEV and PEDV strains was reported infrequently. Truncation of the antigenic site in the N-terminal region could help exclude the possible serological cross-reactivity and enhance the sensitivity/specificity of the immunoassays. Alternatively, PEDV membrane (M) and S proteins could serve as specific antigens for immunoassays (Fan et al., 2015; Gerber et al., 2014; Knuchel et al., 1992; Paudel et al., 2014).

States; VNM, Vietnam)/strain name/year of sample collection (Genbank accession number) followed by passage (P) number for tissue culture-adapted strains. Classical PEDV strains are in green. Emerging US, Chinese and European PEDV strains are in blue, red, and purple, respectively. Emerging S INDEL PEDV strains are labeled with a solid triangle in front of the strain name. The recombinant porcine enteric coronavirus between TGEV and PEDV is in brown.

Table 1
Antigenic cross-reactions as determined by CCIF among various strains of PEDV and TGEV using hyperimmune or convalescent pig PEDV and TGEV antisera[a].

Antiserum to PEDV strain and ID number[a]	Virus strain[a]					
	PEDV PC22A, highly virulent	PEDV S INDEL Iowa106	PEDV S 197DEL PC177	PEDV CV777, classic[b]	TGEV Miller	TGEV Purdue
Anti-PEDV PC22A-like						
PE125 [d, e]	**2560**[c]	2560	2560	640	<10	<10
PE276 [d, e]	**2560**	2560	2560	640	<10	<10
Anti-PEDV S INDEL Iowa106						
PV109 [g, f]	2560	**2560**	2560	2560	<10	<10
PV151 [g,f]	640	**640**	640	640	<10	<10
Anti-PEDV S 197DEL PC177						
PE312 [d, f]	640	640	**640**	640	<10	<10
PE342 [d, f]	640	160	**640**	640	<10	<10
Anti-PEDV CV777						
PC13 [g, f, b]	160	160	160	**640**	<10	<10
PC14 [g, f, b]	640	640 [5]	640	**640** [e]	<10	<10
Anti-TGEV Miller						
S409 [d, e]	640	640	640	640	**10,240**	10,240
M20 [d, e]	160	160	160	160	**10,240**	10,240
Anti-TGEV Purdue						
S411 [d, e]	<10	<10	<10	<10	2560	**10,240**
M2 [d, e]	<10	<10	<10	<10	10,240	**10,240**
RR441 [d, f]	<10	<10	<10	<10	640	**2560**

[a] Negative gnotobiotic (Gn) pig sera (PE71 and HC10078) and mock-inoculated Vero and swine testis (ST) cells were used as negative controls. Data were expressed as the median of sample titrated in triplicates using 4-fold serial dilutions. Detailed materials, methods and data analysis were published in Lin et al. (2015), Journal of Virology 89:3332-3342.
[b] Virus strain and antisera were provided by Dr. Hans Nauwynck (Faculty of Veterinary Medicine, Ghent University, Belgium).
[c] Boldface values represent homologous titers for each PEDV or TGEV strain.
[d] Pig antiserum obtained from gnotobiotic pigs.
[e] Hyperimmune antiserum.
[f] Convalescent antiserum.
[g] Pig antiserum obtained from conventional pigs.

Because viral neutralizing epitopes are located on the S protein, the results of PEDV S protein-based immunoassays correlated well with viral neutralization (VN) titers (Paudel et al., 2014), which is important for clinical protection. In addition, antibodies against the S protein remained detectable for longer periods of time than antibodies to the N protein in the sera of PEDV-infected pigs (Knuchel et al., 1992). However, different antigenicity among S epitopes of the relevant PEDV strains (Lin et al., 2015b; Wang et al., 2015) also raises concerns for the comprehensive detection of diverse PEDV strains.

On the other hand, none of the VN assays revealed reactivity between TGEV and PEDV strains (Table 2) (Lin et al., 2015b; Pensaert et al., 1981). Therefore, the inactivated or attenuated vaccines used to control and prevent swine viral diarrhea diseases were designed as bivalent or trivalent (e.g., TGEV, PEDV and porcine rotavirus G5 type) (Sun et al., 2015a) vaccines in countries where both PEDV and TGEV are endemic. In China, two experimental DNA vaccines bearing the full length TGEV and PEDV S genes simultaneously (Meng et al., 2013; Zhang et al., 2016) were designed as well.

3.2. Antigenic relationships among PEDV, TGEV, and porcine deltacoronaviruses

Porcine deltacoronavirus (PDCoV) is a newly emerged porcine enteropathogenic coronavirus, identified in 2014 (Marthaler et al., 2014b; Wang et al., 2014). Recombinant full-length PDCoV N (Su et al., 2015) or the S1 region of S protein- (Thachil et al., 2015) based ELISA assays have been developed and showed no cross-reactivity with PEDV antisera. However, a whole virus-based ELISA and Western blot showed two-way antigenic cross-reactivities between PEDV VBS2 strain and PDCoV Michigan/8977/2014 strain (Ma et al., 2016). Sequence analysis of the N proteins showed 4 motifs (47-GYW-49, 68-FYYTGTGPRGNLKY-81, 194-PKG-196, and 329-EWD-331) are conserved between PEDV and PDCoV, probably leading to the cross-reactivities in the immunoassays. Interestingly, no antigenic cross-reactivity between PEDV and PDCoV was detected in either virus-infected cells or intestinal tissues using CCIF or immunohistochemistry (IHC) staining (Ma et al., 2016). The discrepancy of different assays was suggested to result from different sensitivity among immune assays and/or the exposure of hidden epitopes on viral N proteins by protein denaturing steps in the ELISA and Western blot (Ma et al., 2016). In our laboratory, two pig hyperimmune TGEV Miller antisera (S409 and M20) and one mouse ascites TGEV N protein MAb (14G9.3C) cross-reacted with PEDV in CCIF assays. However, the same panel of antibodies did not cross-react with PDCoV in our CCIF assays (Lin and Saif, unpublished data). On the other hand, no virus cross-neutralization activity was observed between PEDV and TGEV, or between PEDV and PDCoV (Lin et al., 2015b; Ma et al., 2016). If PDCoV is found to be widespread in swine and a factor in enteric disease, it is possible that PDCoV may be included in future multivalent vaccines against swine enteric viral diseases.

3.3. Antigenic variations among PEDV strains

3.3.1. Antigenic variations between classical and emerging PEDV strains may contribute to the failure of traditional attenuated vaccines in Asia

Under the pressure of herd immunity, the S genes of coronaviruses mutate frequently and some of the amino acid changes lead to changes in viral antigenicity to aid in virus escape from pre-existing immunity. Thus, periodic updates of vaccines may be required to ensure sufficient efficacy against emerging virus variants. For example, antigenic variations between bovine coronavirus field and vaccine strains were demonstrated by VN assay (Kanno

Table 2
PEDV neutralizing antibody titers determined by fluorescent focus virus neutralization assay[a].

Antiserum to PEDV strain and ID number[a]	Virus strain[a]			
	PEDV PC22A, highly virulent	PEDV S INDEL Iowa106	PEDV S 197DEL PC177	PEDV CV777, classic[b]
Anti-PEDV PC22A like-				
PE125 [d, e]	**1024**[c]	1024	1024	64
PE276 [d, e]	**256**	256	256	64
Anti-PEDV S INDEL Iowa106				
PV109 [g, f]	256	**256**	256	256
PV151 [g, f]	16	**64**	64	16
Anti-PEDV S 197DEL PC177				
PE312 [d, f]	16	16	**16**	16
PE342 [d, f]	256	256	**256**	256
Anti-PEDV CV777				
PC13 [g, f, b]	64	64	64	**1024**
PC14 [g, f, b]	512	512	512	**512**
Anti-TGEV Miller				
S409 [d, e]	<4	<4	<4	<4
M20 [d, e]	<4	<4	<4	<4
Anti-TGEV Purdue				
S411 [d, e]	<4	<4	<4	<4
M2 [d, e]	<4	<4	<4	<4
RR441 [d, f]	<4	<4	<4	<4

[a] Negative gnotobiotic (Gn) pig sera (PE71 and HC10078) and mock-inoculated Vero and swine testis (ST) cells were used as negative controls. Data were expressed as the median of sample titrated in triplicates using 4-fold serial dilutions. Detailed materials, methods and data analysis were published in Lin et al. (2015), Journal of Virology 89:3332-3342.
[b] Virus strain and antisera were provided by Dr. Hans Nauwynck (Faculty of Veterinary Medicine, Ghent University, Belgium).
[c] Boldface values represent homologous titers for each PEDV or TGEV strain.
[d] Pig antiserum obtained from gnotobiotic pig.
[e] Hyperimmune antiserum.
[f] Convalescent antiserum.
[g] Pig antiserum obtained from conventional pig.

et al., 2013). PEDV has one single serotype as measured by VN assay using convalescent and hyperimmune antisera. However, more evidence based on field observations, viral gene sequence analysis and serologic assays suggested that the emerging highly virulent (non-S INDEL) PEDV strains differed antigenically in various degrees from classical PEDV strains. Detailed VN results are described below.

In Asian countries, many classical PEDV strains, including prototype CV777, Japan 83P-5 and Korea DR13 strains, were attenuated via continuous cell culture passage and licensed as inactivated or attenuated vaccines (Park et al., 2012; Sato et al., 2011; Song et al., 2007). These traditional attenuated PEDV vaccines showed good clinical efficacy against classical PEDV strains, until emerging PEDV outbreaks started at the end of 2010 in China (Song et al., 2015b; Sun et al., 2015a, 2012). To date, the use of classical PEDV strain-based inactivated and attenuated vaccines may still help to reduce the severity of disease. However, many pig herds followed the routine vaccination program, but still experienced high mortality rates among newborn piglets caused by emerging highly virulent PEDV strains (Jung and Saif, 2015; Song et al., 2015b). Epidemiological studies have shown that the highly virulent PEDV strains have become pandemic in the swine population (Chiou et al., 2015; Kim et al., 2015; Lee and Lee, 2014; Song and Park, 2012).

Initially, sequence comparisons of neutralizing epitopes on the S protein (Li et al., 2014) suggested that the antigenic variations between classical and emerging non-S INDEL (highly virulent) PEDV strains are the major reason for vaccine failure (Table 3). Several regions on PEDV S protein were recognized as containing the neutralizing epitopes, including a CO-26 K equivalent epitope COE (aa 499–638), SS2 (aa 748–755), SS6 (aa 764–771) and 2C10 (aa 1368–1374) (Chang et al., 2002; Ge et al., 2012; Kang et al., 2005; Oszvald et al., 2007). Compared with classical PEDV vaccine strains, several SNPs leading to 8–11 individual amino acid changes located on COE epitope and 1–2 amino acid changes located on SS6 epitope were observed in highly virulent PEDV strains (Li et al., 2014). Among them, three (A522S, A554 S and G599S) and one (Y766S) were serine substitutions (Table 3) (Chiou et al., 2015; Hao et al., 2014; Huang et al., 2013; Tian et al., 2013). In addition, bioinformatics predicted that the S protein of highly virulent PEDV strains changed in primary/secondary structures, high-specificity *N*-glycosylation sites, potential phosphorylation sites, and palmitoylation sites (Chiou et al., 2015; Hao et al., 2014). These changes may affect viral antigenicity and change viral neutralizing activity.

In addition to molecular evidence, antigenic variations between classical and emerging highly virulent (non-S INDEL) PEDV strains were demonstrated in several serological cross-reactivity assays (Kim et al., 2015; Lin et al., 2015b; Wang et al., 2015). A retrospective study analyzing 27 Korean PEDV strains from 1998 to 2013 showed that the S protein sequences of the field strains exhibited a maximum of 10% difference from the classical attenuated vaccine strains (Kim et al., 2015). One serum sample collected from a pig immunized with attenuated PEDV vaccine strain SM98 neutralized the homologous strain (SM98) with a titer of 512. The same serum reacted with another PEDV vaccine 83-P5 (P5-V) strain and four field PEDV strains (KDGG10YO, KDGG12HWN, KDGN12DJ and KDGN13_295BG) at lower titers (ranging from 8 to 32) (Kim et al., 2015). In our laboratory, two-way antigenic cross-reactivities among prototype PEDV CV777 and three genetically distinct US PEDV strains (highly virulent non-S INDEL PEDV PC22A, S INDEL Iowa106, 197-DEL PC177) were tested with a panel of pig antisera (Tables 1 and 2) and mouse MAbs to PEDV (Lin et al., 2015b). In agreement with the phylogenetic distance (Oka et al., 2014; Vlasova et al., 2014), classical PEDV CV777 strain showed more differences in antigenic cross-reactivity compared with the other US PEDV strains. However, inconsistences in cross-reactivity were evident using antisera from different pigs. One of two hyperimmune PEDV PC22A pig antisera and one of two convalescent PEDV

Table 3
Analysis of amino acid variations in CO-26K equivalent epitopes (COEs) of the S glycoprotein of PEDV strains. Amino acids that differ from CV777 but are identical to the majority of emerging strains are labeled with black background; amino acids that differ from CV777 and the majority of emerging strains are labeled with gray background.

				Amino acids in COE of CV777 as reference strain compared with other PEDV strains																
Country/state (province)	Strain	Year collected	S1 Phylogenetic clade	511 N	517 A	521 L	523 S	527 V	549 T	567 S	577 N	594 G	605 A	606 F	611 K	612 L	621 K	633 E	635 I	GenBank number
China/Shanghai	CH/S	1986	Classical	-	-	S	G	-	-	-	-	-	E	-	-	F	-	-	V	JN547228
South Korea	DR13 virulent	1999	Classical	-	-	H	G	I	-	-	-	-	E	-	-	F	-	-	V	JQ023161
South Korea	DR13 attenuated	2003	Classical	-	-	H	G	I	-	-	-	-	E	-	-	F	-	Q	V	JQ023162
South Korea	SM98	2010	Classical	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GU937797
Japan	83P5	2011	Classical	-	-	H	G	I	-	-	-	-	E	-	-	F	-	-	V	AB548618
Japan	83P5-100th	2013	Classical	-	-	H	G	I	-	-	-	-	E	-	-	F	-	Q	V	AB548621
China/Guangdong	GDS03	2013	Classical	-	-	H	G	I	-	-	-	-	E	-	-	F	-	Q	V	AB857235
China/Guangdong[a]	CH-GD	2011	Emerging	I	S	H	G	I	S	-	-	S	D	Y	-	F	-	-	V	JQ638915
China/Guangdong[a]	GD-B	2012	Emerging	-	S	R	G	I	S	-	-	S	D	-	-	F	T	-	V	JX088695
China/Guangdong[a]	CH-GDHY	2011	Emerging	-	-	H	G	I	S	-	S	S	E	-	E	F	-	-	V	JX145339
China/Guangdong[a]	CHGD-01	2012	Emerging	-	-	H	G	I	S	-	-	S	E	-	-	F	-	-	V	JX261936
China/Guangdong[a]	GDZQ	2014	Emerging	-	S	-	G	I	S	-	-	S	E	-	-	F	-	-	V	KM242131
China/Gansu[b]	YJ3F	2012	Emerging	-	S	-	G	I	S	-	-	S	E	-	-	F	-	-	V	KF177254
China/Gansu[b]	JY5C	2012	Emerging	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KF177257
China	CHYJ130330	2013	Emerging	-	-	Q	G	I	S	-	-	S	E	-	-	F	-	-	V	KJ020932
China	FL2013	2013	Emerging	-	-	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KP765609
China	YN1	2013	Emerging	-	-	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KT021227
China/Fujian	FJND-3	2011	Emerging	-	S	P	G	I	S	-	-	S	E	-	-	F	-	-	V	JQ282909
China/ZheJiang	WS	2014	Emerging	-	S	H	G	I	S	-	-	S	D	-	-	F	-	-	V	KM609213
USA/Colorado	Colonado47	2013	Emerging	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KF272920
USA/Minnesota	Minnesota79	2013	Emerging	-	S	H	G	I	S	-	-	S	D	-	-	F	-	-	V	KJ645674
USA/Missouri	Missouri177	2014	Emerging	-	S	H	G	I	S	-	-	S	D	-	-	F	-	D	A	KR265779
USA/Ohio	PC22A-P10	2013	Emerging	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KM392224
USA/Oklahoma	Oklahoma32	2013	Emerging	-	S	H	D	I	S	-	-	S	E	-	-	F	-	-	V	KJ645640
USA/Oklahoma	Oklahoma35	2013	Emerging	-	S	R	G	I	S	-	-	S	E	-	-	F	-	-	V	KJ645642
Belgium	-	2015	S-INDEL	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	AKC34872
Germany	-	2014	S-INDEL	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	CDW77213
USA/Iowa	Iowa106-WT	2014	S-INDEL	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KJ645695
USA/Ohio	OH851	2014	S-INDEL	-	S	H	G	I	S	-	-	S	E	-	-	F	-	-	V	KJ399978

[a] PEDV strains in Gansu province, China (Tian et al., 2013).
[b] PEDV strains isolated during 2011–2013 in Guangdong province, China (Hao et al., 2014).

CV777 pig antisera showed 16-fold differences between the homologous and heterologous VN titers (Table 2). Subsequently, similar observations were reported for the S protein by Wang et al. (2015). Mice immunized with the recombinant S proteins of either CV777 strain or emerging highly virulent China LNCT2 strain both generated polyclonal neutralizing antibodies against homologous and heterologous PEDV strains in serum samples. However, the homologous titers were two-fold higher than the heterologous titers in CCIF, ELISA and VN assays. Overall, the above studies showed that pigs or mice immunized with classical attenuated PEDV vaccine strains usually displayed similar or lower serum antibody titers against the emerging highly virulent PEDV strains (2–16 fold differences), suggesting the classical PEDV vaccine strains may only provide partial cross-protection against the highly virulent PEDV strains (Song et al., 2015b). However, *in vivo* cross-protection studies are needed to confirm the *in vitro* cross-reactivity data.

3.3.2. Emerging PEDV strains are predicted to be antigenically variable based on sequence analysis

Although differences in antigenicity between classical and highly virulent PEDV clusters have been studied, the antigenic variations among PEDV strains within the same phylogenetic cluster are less studied in serological assays. Comparative analysis of TGEV, PRCV, and murine hepatitis virus revealed that the NTD and the C-terminal receptor binding domain (RBD) of the S1 region of the S protein contained two main antigenic sites. Jarvis et al. (2015) reported that the highly virulent PEDV strains from China had an increased number of substitutions within the S1 domain compared with the highly virulent American strains, probably due to the longer circulation time of PEDV in pigs in China. Compared with the attenuated classical vaccine strain DR13, 29 of 185 (16%) of non-S INDEL NA PEDV strains and 19 of 34 (56%) of emerging Asian strains had at least one amino acid substitution in the RBD. In this review, we compared the neutralizing epitopes from different PEDV strains (Table 3). Among the four neutralizing epitopes, two epitopes (SS2 and 2C10) were conserved (Li et al., 2014). The neutralizing epitopes of prototype PEDV CV777 strain were the same as those of classical PEDV SM98 vaccine strain, except for one amino acid difference (S764P) in the SS6 epitope. Compared with PEDV CV777 strain, other classical attenuated PEDV strains (83P-5-P100 and attenuated DR13) and classical Asian field strains had 7–8 and 1–2 amino acid differences located in COE and SS6 epitope, respectively. On the other hand, emerging highly virulent PEDV strains had variations mainly in COE (most frequently, in amino acid positions 517, 521, 523 and 605). Compared with the vaccine strain CV777, five highly virulent PEDV strains (JY5C, JY6C, JY7C, YJ3F, and YJ7C) detected in Gansu Province, China had eight mutations (A517S, S523G, V527I, T549S, G594S, A605E, L612F, and I635V) in the neutralizing epitope COE. However, three (JY5C, JY6C and JY7C) of these highly virulent PEDV strains had one additional mutation (L521H) in the neutralizing epitope COE (Table 3) (Tian et al., 2013). Higher sequence diversity was observed in Guangdong Province, China, where ten PEDV strains, including one classical strain GDS03, isolated during 2011–2013, had five different patterns of amino acid sequences in neutralizing epitope COE (Table 3) (Hao et al., 2014). The majority of emerging highly virulent NA PEDV strains had the same amino acid sequences in neutralizing epitopes. Compared with other NA highly virulent PEDV strains, USA/Minnesota79/2013 had one (E605D), USA/Missouri177/2014 had three (E605D, E633D and V635A), USA/Oklahoma32/2013 had one (G523D) and USA/Oklahoma35 had one (H521R) substitutions in COE epitope (Table 3). The significance of these amino acid differences in VN epitopes among these highly virulent PEDV strains has not yet been examined by using MAbs in serological assays or by *in vivo* cross-protection studies in pigs. However, it is likely that higher antigenic variations will appear in the future.

In addition, the amino acids in neutralizing epitopes of S INDEL strains were the same as that of the majority of NA highly virulent strains (Table 3), suggesting their cross-neutralization ability and confirmed by our previously reported *in vitro* and *in vivo* studies (Lin et al., 2015a,b). Recently, serological cross-reactivity and cross-neutralization between S INDEL (USA/IL20697/2014) and non-S INDEL (USA/IN19338/2013) strains were studied by Chen et al. (2016a,b). In their IFA study, serum samples collected from pigs inoculated with non-S INDEL PEDV reacted with homologous and S-INDEL (heterologous) PEDV-infected Vero (ATCC CCL-81) cells. However, the homologous titers were higher than heterologous titers. Alternatively, serum samples collected from pigs inoculated with S-INDEL PEDV showed similar homologous and heterologous titers by IFA and VN assays (Chen et al., 2016b). However, *in vivo* cross-protection also can be affected by additional factors, such as cellular immune responses and in suckling pigs by the lactating sow's milk production (Goede et al., 2015; Lin et al., 2015a). Also serum viral neutralizing antibody level may not reflect IgA viral neutralizing antibody level in milk (Scherba et al., 2016). Whether the circulating antibody plays a role in the protection of piglets against PEDV is still questionable (Poonsuk et al., 2016). Thus, *in vivo* cross-protection may not always be consistent with *in vitro* virus neutralizing antibody levels (Lin et al., 2015b). Detailed in vivo cross-protections between S INDEL and non-S INDEL PEDV strains were reviewed in Section 5.2.

3.4. Limitations of the PEDV serological cross-reaction assays and potential solutions

Continuous monitoring of genetic and antigenic changes in PEDV strains is critical to control epidemic outbreaks and for vaccine development (Chattha et al., 2015; Mumford, 2007). However, there are limitations in the comprehensive characterization of antigenic variations among PEDV strains, although cross-protection activity is a primary criterion for vaccine strain selection, and immunity is thought to be a major driving force of virus evolution.

First, unlike the viral genomic information accessible from GenBank, it is difficult to collect large numbers of PEDV strains and produce the corresponding highly specific pig antisera for measuring cross-reactive neutralizing antibody titers. Few virus strains are adapted to cell culture, and in the US it is difficult to import foreign isolates for comparison. Antibody specificities can be influenced by the backgrounds of the experimental animals (Lin et al., 2015b). In addition to inferring the antigenicity by sequence data (Li et al., 2014; Sun et al., 2013), expression of recombinant proteins in eukaryotic systems and generating antiserum and/or MAbs in laboratory animals could be alternative methods to explore the antigenic variations among PEDV strains (Wang et al., 2015). However, whether the structure of recombinant proteins reflects the proteins in the intact virus (Makadiya et al., 2016), or whether cross-reactive neutralizing antibody profiles defined using antisera from injected small laboratory animals are similar to those observed using post-infection sera obtained from their natural host in the field needs to be confirmed (Chia et al., 2014).

Secondly, in the field a post-infection/convalescent antiserum contains polyclonal antibodies that may react with the conserved epitopes and may not reveal the antigenic variations among PEDV strains. The antigenic variations among different TGEV strains could be distinguished by MAbs (Simkins et al., 1992), but not by conventional antisera (Laude et al., 1986). Therefore, production of a comprehensive panel of PEDV neutralizing MAbs and epitope mapping studies will help to more precisely identify the regions contributing to antigenic variation. One MAb 5F7 against the S protein of highly virulent PEDV CHN/LNCT strain did not cross-react with CV777 strain. Further epitope mapping of PEDV strains is ongoing (Wang et al., 2015).

Third, the data from serologic assays were usually reported as titration values or titers. When large numbers of viral strains and antiserum data were included, the results were difficult to combine and data below the sensitivity threshold of the assay could not be interpreted. Moreover, results from different laboratories may be inconsistent. To resolve these problems, an antigenic cartography (antigenic map) was originally proposed for mapping the antigenic evolution of the influenza virus, in which ordinal multidimensional scaling was applied to position the antigens/antisera in the map (Smith et al., 2004). Multiple datasets of the serological assays from different laboratories worldwide could be combined for constructing a comprehensive PEDV antigenic cartography by a computational framework approach (Cai et al., 2010; Smith et al., 2004). Subsequently, a similar approach was also applied for other RNA viruses with high antigenic variation, such as human enteroviruses (Chia et al., 2014) and noroviruses (Debbink

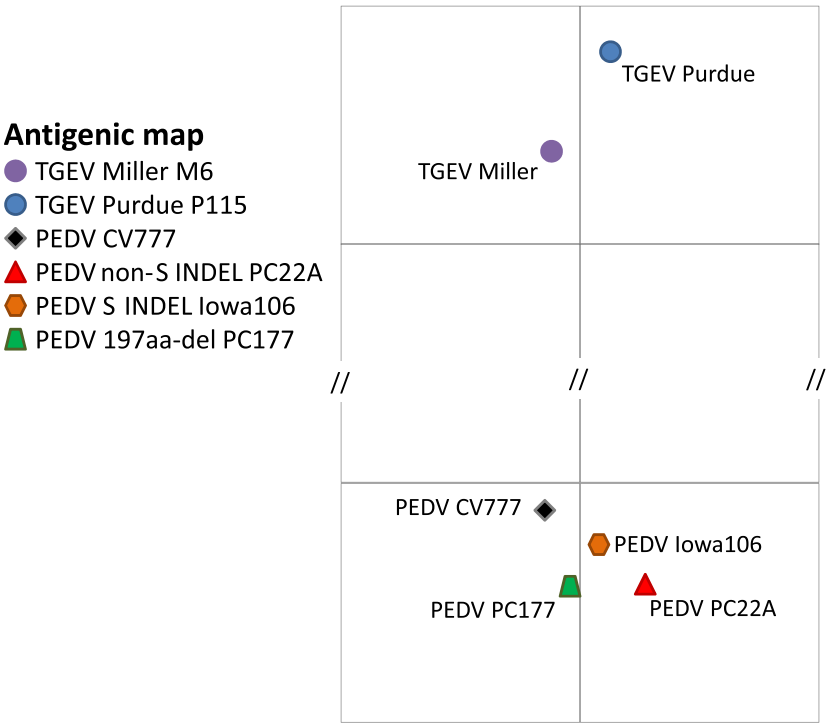

Fig. 4. Antigenic map generated using cross-reactive PEDV and TGEV CCIF serum antibody titers presented in Table 1. The relative position of strains, including the prototype PEDV CV777 (black diamond), highly virulent PEDV PC22A (red triangle), S INDEL PEDV Iowa106 (orange hexagon), S 197del PEDV PC177 (green trapezoid), TGEV Miller (purple circle) and TGEV Purdue (blue circle) was adjusted such that the distances between strains in the map represent the corresponding ratios between homologous and heterologous antibody titers. The original data (Table 1) was published in our previous study (Lin et al., 2014). This antigenic map was constructed using webserver (http://sysbio.cvm.msstate.edu/AntigenMap) established by Cai et al., 2010.

et al., 2014). In our previous study, the results of CCIF and VN assays showed that the antigenic determinants among three US PEDV strains were conserved. The same two-way antigenic cross-reactivity data among three US PEDV and prototype CV777 strains were re-analyzed by antigenic cartography (Fig. 4). In the antigenic map, the S INDEL Iowa106 strain and the S 197aa-del PC177 strain were located between the highly virulent PEDV PC22A and the classical CV777 strain, showing a well correlation between the phylogenetic distance and antigenic differences. This map will be expanded further when more data becomes available in the future.

4. Pathogenicity of PEDV strains

4.1. Classical PEDV infections cause acute gastroenteritis and intestinal lesions, similar to virulent TGEV infections

The infection and pathogenic features of classical PEDV CV777 strain were examined in earlier studies (Table 4) (Coussement et al., 1982; De Bouck et al., 1981; Debouck and Pensaert, 1980; Ducatelle et al., 1981; Pospischil et al., 1981). One to twenty day-old cesarean-derived, colostrum-deprived (CDCD) and conventional piglets inoculated with PEDV CV777 had clinical signs between 23 and 40 h post-inoculation (HPI) (Coussement et al., 1982; Debouck and Pensaert, 1980). About one day after onset of diarrhea, marked villus shortening along with the villus height (VH) versus crypt depth (CD) ratios (VH:CD) reduced from 7 to 8 in the healthy controls to approximately 2–3 for infected pigs were observed (Debouck and Pensaert, 1980). Starting from 60 HPI, villous regeneration was observed in CV777-inoculated piglets. These regenerating cells were re-infected by PEDV CV777, resulting in a second peak of infection (De Bouck et al., 1981). Earlier research compared the pathogenesis of PEDV CV777 with endemic TGEV Miller strain (De Bouck et al., 1981; Pospischil et al., 1981). PEDV CV777 infection showed more extensive vertical and longitudinal distribution in the digestive tract: both small intestinal and colonic epithelial cells and occasionally, epithelial cells at the base of a villus and the crypt cells could be infected by PEDV CV777. In addition, the progression of PEDV CV777 infection was slower than that in TGEV (De Bouck et al., 1981; Saif, 1989).

However, not all of the pathological features of prototype PEDV CV777 infection were consistently observed for other PEDV strains (Table 4). The infection of one-day-old, colostrum-deprived piglets with Korea classical PEDV strain (SNUVR971496) induced severe villous atrophy with the lowest mean VH:CD ratio (1.11 ± 0.34) at 60 HPI. However, in these very young piglets, no PEDV antigens or nucleic acids were detected in epithelial cells lining the crypts, and positive-staining cells were lacking in the colon by IHC staining (Kim et al., 1999) and *in situ* hybridization (Kim and Chae, 2003). Similarly, different levels of virulence among TGEV strains were also reported (Kim and Chae, 2002). Therefore, the pathogenicity of TGEV/PEDV infection in pigs depended on the viral strains, the age of pig, how piglets were derived (CD) or if naturally farrowed, the colostrum/milk status (CD) and environment variations (Table 4). In general, the clinical signs of PEDV and TGEV infections are indistinguishable in the field.

4.2. Emerging non-S INDEL PEDV strains are highly virulent in pigs

Based on epidemiologic and clinical observations in the field, the emerging non-S INDEL PEDV strains since 2010 are highly pathogenic and referred to as "highly virulent" PEDV strains. Experimental infection of highly virulent PEDV strains in gnotobiotic (Jung et al., 2014), CDCD (Liu et al., 2015; Madson et al., 2016) and conventional (Lin et al., 2015a; Madson et al., 2014) pigs resulted in consistent outcomes and conclusions (Table 4).

Table 4
Summary of PEDV pathology studies[a].

PEDV strain	Inoculum type/dose	Pig type	Age at inoculation (day-old)	Villi atrophy (VH:CD ratio)	Onset of clinical signs	Infection Site				Reference
						Vertical (location)		Longitudinal (extent)		
						Villous	Crypt	D, J, I	C	
(Classical strains)										
CV777	Fecal suspension (5–7 log PID)	CDCD/conventional	1–20	Moderate to severe (~2)	24–40	+++ (entire)	NR	D (NR), J, I (cont)	+	Debouck and Pensaert (1980)
CV777	Fecal suspension (4 log PID)	CDCD	2–3	Moderate to severe (NR)	22–36	+++ (entire)	+	D, J, I (cont)	+	Coussement et al. (1982)
CV777	Fecal suspension (4 log PID)	CDCD	2–3	Moderate to severe (1.5–4.2)	22–36	NR	NR	NR	NR	Coussement et al. (1982)
Japan/1993	(naturally infection)	conventional	–	Severe 1.1–2.6	–	+++ (entire)	+	D, J, I (cont)	+	Sueyoshi et al. (1995)
Korea SNUVR971496	Cell culture (P3) (6.5 log $TCID_{50}$)	colostrum-deprived	1	Severe (1.1–3.3)	12–36	+++ (entire)	–	D, J, I (cont.)	–	Kim and Chae (2003)
(Highly virulent strains)										
US PC21A	Fecal suspension (6.3–9.0 log GE)	gnotobiotic	10–35	Severe (<2)	25–46	+++ (entire)	NR	D, J, I (cont.)	+	Jung et al. (2014)
US PC21A	Fecal suspension (11.8 log GE)	conventional	9	Severe (1.1–1.4)	~24	+++ (entire)	NR	D, I, J (cont)	+	Jung et al. (2015)
US PC21A	Fecal suspension (10 log GE)	conventional	4	Severe (0.9–2.0)	<24	+++ (entire)	+	D, J, I (cont)	+	Lin et al. (2015a,b)
US PC21A	Fecal suspension (11.8 log GE)	conventional	26	Moderate to severe (1.2–3.0)	72–182	++ (entire)	NR	D, I, J (cont)	+	Jung et al. (2015)
US PC22A	Cell culture (P3) (1–4 log PFU)	CDCD/conventional	3–4	Severe (0.8–2.3)	18–24	+++ (entire)	+	D, I, J (cont)	+	Liu et al. (2015)
USA/IN19338/2013	Cell culture (P7) (3.7-0.3 log $TCID_{50}$)	conventional	5	Severe (1.2–1.7)	24	+++ (entire)	NR	D, J (NR), I (cont)	NR	Thomas et al. (2015)
US/Iowa/18984/2013	Cell culture (P6) (3 log PFU)	conventional	21	Moderate to severe (1.8–4.2)	48–120	+++ (entire)	NR	D, J, I (cont)	NR	Madson et al. (2014)
US/Iowa/18984/2013	Cell culture (P6) (3 log PFU)	CDCD	5 h	Severe (1.9–3.0)	24	+++ (entire)	NR	D, J, I (cont)	NR	Madson et al. (2016)
Japan ZK-CHR	Cell culture (1.6 log $TCID_{50}$)	SPF	7	NR	24	NR	NR	NR	NR	Yamamoto et al. (2015)
(S-INDEL strains)										
Japan ZK-O	Cell culture (1.1 log $TCID_{50}$)	SPF	7	NR	ND	NR	NR	NR	NR	Yamamoto et al. (2015)
US Iowa106	Fecal suspension (10–12 log GE)	conventional	4	Moderate to severe (1.4–5.4)	24–72	++ (entire)	–	D (patch), J, I (cont)	–	Lin et al. (2015a,b)
US/IL20697/2014	Cell culture (4 log $TCID_{50}$)	Conventional/milk replacer	5	Mild	Minimal	+/++	NR	D, J (NR), I (patch)	+	Chen et al. (2016a,b)

PID: pig infective dose; $TCID_{50}$: 50% tissue culture infectious dose; PFU: plaque formation unit; GE: genomic equivalent; CDCD: caesarian derived, colostrum deprived; SPF: specific pathogen free; VH: CD: villous height: crypt depth; D-duodenum, J-jejunum, I-ileum; cont. = continuous, NR = not reported; –, +, ++, and +++ denotes none, less than 30%, 30–60% and more than 60% of villous enterocytes showing a PEDV antigen positive signal, respectively.

[a] This table is adapted/updated from Saif (1989) "Comparative aspects of enteric viral infections" In: Saif, L.J., Theil, K.W. (Eds.) Viral Diarrhea of Man and Animals. CRC, Boca Raton, Florida, 9–34.

First, the highly virulent PEDV is very infectious. Approximately one plaque-forming unit (PFU) of the highly virulent PEDV PC22A strain (at passage level 3) caused watery diarrhea in 40% of 4-day-old CDCD piglets within 24 HPI (Liu et al., 2015). Similarly, the minimal infectious dose of another highly virulent US PEDV strain USA/IN19338/2013 was titrated as 0.056-0.56 $TCID_{50}$ in 5-day-old conventional piglets and 56 $TCID_{50}$ in 21-day-old conventional weaned pigs, respectively (Thomas et al., 2015).

Secondly, the disease is lethal and the infection progresses rapidly in nursing piglets. Very low inoculation doses induced clinical signs within 24 HPI and deaths within 96 HPI (Liu et al., 2015; Madson et al., 2016; Thomas et al., 2015).

Thirdly, the highly virulent PEDV infections occur throughout the intestine. In addition to jejunum and ileum, the duodenum and cecum/colon were also susceptible to PEDV infection (Jung et al., 2014; Lin et al., 2015a; Madson et al., 2016). PEDV antigens were mainly detected in the cytoplasm of intestinal epithelial cells located both on the tip and lateral walls of villi and in the villus/crypt border (Jung et al., 2014). Occasionally, individual or clustered crypt epithelial cells were infected (Lin et al., 2015a; Liu et al., 2015; Stevenson et al., 2013). Whether the regenerative capacity of the crypts is affected by PEDV infection is still unknown. In our laboratory, two markers for crypt proliferation (Ki67) and stem cells (LGR5) were studied. Compared with mock-inoculated conventional piglets, the number of LGR5-positive cells and proliferation of intestinal crypts of 10-14-day-old conventional piglets increased remarkably at 3–5 DPI of PEDV PC21A, reflecting a compensation for villous atrophy by crypt regeneration (Jung et al., 2015).

Fourth, the highly virulent PEDV strain induced severe villous atrophy. In the majority of CDCD and conventional suckling piglets infected with highly virulent PEDV PC21A or PC22A strain, the VH:CD ratios were <2 and even <1 in some cases (Lin et al., 2015a; Liu et al., 2015). Moreover, the highly virulent PEDV not only severely damaged the infected enterocytes but also rapidly spread to infect the regenerated enterocytes (Lin et al., 2015a). If piglets did not die immediately during the acute stage of infection, they continued to show moderate to severe degrees of villous atrophy during 5–8 DPI. Continuous monitoring of fecal viral RNA shedding showed that the titers were decreasing consistently, after the first peak of fecal viral shedding. This data indicates that limited numbers of enterocytes were still available to sustain PEDV replication, but to lower levels during the prolonged infection (Lin et al., 2015a).

Fifth, pigs of all ages can be infected by the highly virulent PEDV, but pigs develop age-dependent resistant to PEDV disease (Annamalai et al., 2015; Jung et al., 2015; Madson et al., 2014). Experimental inoculation of 2- and 7-day-old CDCD piglets by a Japanese PEDV strain isolated in 1999 resulted in 100% morbidity and mortality. However, only mild diarrhea occurred in 2- and 4-week-old pigs and no clinical signs were observed in 8- and 12-week old pigs (Shibata et al., 2000). In our studies, morbidity was consistently 100% in the PC21A or PC22A-inoculated piglets and their contact-exposed sows (Lin et al., 2015a; Liu et al., 2015). In our laboratories, a slower turnover rate of enterocytes (Jung et al., 2015) and impaired lytic activity and IFN-gamma production by natural killer cells (Annamalai et al., 2015) in 9-day-old nursing piglets being less than in 26-day-old were proposed as explanations for one of the mechanisms of age-dependent resistance to PEDV. However, the highly virulent PEDV strains caused severe growth retardation in weaned pigs and significant impairment in productivity, such as lower farrowing rates, lower total pigs and pigs born alive per litter and decreased nursing performance of sows (Goede and Morrison, 2016; Lin et al., 2016), leading to the overall negative economic impacts on the pork industry.

Overall, the virulence of highly virulent PEDV was characterized by disease/infection induced by a low infectious dose, short incubation times, severe clinical signs and intestinal lesions, and extensive PEDV replication throughout the intestine (Table 4). In addition to antigenic variation, these disease characteristics also explained why current highly virulent PED outbreaks were so devastating in the major pig producing countries in Asia (Choi et al., 2014; Oka et al., 2014; Sun et al., 2012) and North America (Stevenson et al., 2013; Vlasova et al., 2014).

4.3. INDEL PEDV strains showed lower virulence than the emerging non-S INDEL PEDV strains, but still can induce severe disease in some circumstances

The first US PEDV S INDEL strain, OH851, was isolated from conventional pigs without significant clinical signs (Wang et al., 2014). Soon thereafter, comparisons of the pathogenicity between S INDEL and highly virulent PEDV strains were conducted in three independent laboratories (Table 4). In Japan, one-week-old, specific pathogen-free (SPF) piglets inoculated with S INDEL PEDV ZK-O strain had delayed onset of PEDV fecal RNA shedding, lower fecal diarrhea scores and fecal viral RNA titers compared with the highly virulent PEDV ZK-CHR stain, suggesting S INDEL strains may be less pathogenic (Yamamoto et al., 2015). In our laboratory, all the conventional nursing piglets inoculated with the field S INDEL PEDV Iowa106 strain at 4 days of age developed diarrhea. However, the virulence of S INDEL PEDV Iowa106 was lower than that of the highly virulent PEDV strains as evident by: (1) a longer incubation time (delayed onset of clinical signs and the peak of viral RNA shedding); (2) a shorter duration of diarrhea; (3) relatively higher VH:CD ratios; (4) a lower percentage of PEDV positive enterocytes; (5) more limited intestinal regions of virus infection (crypt not involved); and (6) overall lower piglet mortality (18%) compared with the highly virulent PC21A strain (55%) (Lin et al., 2015a). In comparison, Chen et al. (2016a,b) infected conventional farrowed, 5-day-old non-suckling (fed with milk replacer and yogurt) piglets with a TC US S INDEL PEDV strain (IL20697/2014) and three other US highly virulent PEDV strains (IN19338/2013, NC35140/2013, and NC49469/2013). The S INDEL PEDV strain (IL20697/2014) caused no or minimal clinical signs, lower reductions in body weight gain, more limited gross and microscopic lesions and lower fecal viral shedding and tissue PEDV antigen scores (Chen et al., 2016a). However, individual and litter variations, as commonly observed in the field (Stadler et al., 2015), were reported only in our piglet studies: the mortality of S INDEL Iowa106-inoculated piglets was 0% in three litters, but 75% in the fourth litter (Lin et al., 2015a). In addition, the sows contact-exposed to their infected piglets became infected by S INDEL Iowa106 and two of four sows showed anorexia and diarrhea. Outbreaks of European S INDEL PEDV strains in southern Germany (Stadler et al., 2015) and southern Portugal (Mesquita et al., 2015) still caused high mortality (approximately 70%) in suckling piglets. Therefore, although the consensus was that the S INDEL strains were of lower virulence (Lin et al., 2015a,b; Wang et al., 2014; Yamamoto et al., 2015), they still induced severe disease in some circumstances, such as if the sow became infected and ill. If sows had decreased appetite and milk production, the lactogenic immunity could be disrupted or decreased (Chattha et al., 2015) and their suckling piglets were likely to die (Lin et al., 2015a; Mesquita et al., 2015; Stadler et al., 2015). Also piglets with lower birth weight might compete less efficiently for milk and receive less nutrition and lower lactogenic immunity than their littermates. The severity of clinical signs caused by S INDEL PEDV infection showed a negative correlation with the birth body weight of piglets (Lin et al., 2015a).

4.4. TC PEDV strains at high passage levels showed reduced virulence similar to attenuated TGEV strains

Highly virulent TGEV strains, such as US Miller at low cell culture passage level 3 (passage 3), is associated with short incubation time, extensive spread of infection throughout the small intestine, infection in higher portions of small intestinal enterocytes and marked damage to the villous epithelium. On the other hand, strains of TGEV attenuated by 115 or 350 passages in cell culture (P115 and B1-350) produced scattered foci of infection in villous enterocytes only in the jejunum and ileum (Saif, 1989; Saif et al., 2012). In addition, the P115 virus was found to replicate to higher titers in the upper respiratory tract (nasal shedding) than in the gut (fecal shedding) (VanCott et al., 1993). Similarly, continued passages of TC PEDV also generated attenuated variant strains which caused milder or no diarrhea in pigs (Chen et al., 2015; Sato et al., 2011; Song et al., 2007, 2003). The same properties of infection, including milder/no clinical signs and intestinal lesions and lower tissue antigen scores, were still relevant indicators for attenuation when the high passaged PEDV strains were compared with the parent virulent strains (Chen et al., 2015).

4.5. Several PEDV variants with various deletions in S gene showed reduced virulence

For the TGEV, a large (224 aa)-deletion in the NTD of S1 region of S protein reduced its virulence and changed its tissue tropism from intestinal to respiratory [subsequently designated porcine respiratory coronavirus (PRCV)] (Pensaert et al., 1986; Saif, 1989; Saif et al., 2012). A mutant PEDV strain PC177 has a 197 amino acid (aa)-deletion in the S1 NTD also was attenuated in pigs. Compared with the highly virulent PEDV PC22A, 4-day-old, CDCD piglets inoculated with 1000 $TCID_{50}$ of PEDV PC177 showed milder diarrhea, lower fecal PEDV RNA shedding titers, milder villous atrophy and lower intestinal tissue antigen scores. Mortality rates were 0% and 100% in the PC177- and PC22A-inoculated piglets, respectively (Hou et al., the 35th Annual Meeting of American Society of Virology, Blacksburg, VA, June 18–22, 2016). Unlike TGEV and PRCV, however, the results of our IHC staining did not suggest changes in tissue tropism of the PC177 strain. In a recent report, no mortality occurred in 7-day-old suckling field piglets (n = 120) infected with a Japanese PEDV strain (Tottori2/2014) with a 194 aa-deletion (aa Δ23-216) in the S1 NTD, similar to that of PC177 (aa Δ34-230) (Murakami et al., 2015). Both findings suggest that the large deletion in the S1 NTD may be responsible for the reduced virulence. In addition, a variant Chinese PEDV field strain (FL2013) was isolated from a sow with mild clinical signs and with reduced virulence in her 3-day-old neonatal piglets (Zhang et al., 2015). The backbone of the FL2013 genome was identical to other highly virulent PEDV strains (Fig. 1). However, the extreme C-terminus of the FL2013S gene has a unique 21 nt-deletion, leading to a 7 aa- deletion (FEKVHVQ) in comparison with other highly virulent PEDV sequences (Zhang et al., 2015). The same 7 aa-deletion is also observed in the classical PEDV CHN/LZC/before 2006 strain. Whether these large deletions or early termination of S protein alone alter viral infectivity, pathogenicity and replication efficiency can be further examined by reverse genetics technology (Beall et al., 2016; Jengarn et al., 2015).

5. Considerations for vaccine strain selection: PEDV virulence attenuation and in vivo cross-protection between PEDV strains

The highly virulent PEDV strains spread rapidly and caused very high mortality in suckling piglets. Failure of the live attenuated classical PEDV vaccines in Asia (Song et al., 2015b; Sun et al., 2015a) and the lack of live attenuated PEDV oral vaccines in North America (Jung and Saif, 2015) necessitates the development of future PEDV vaccines for swine based on the emerging strains. Because PEDV causes up to 100% mortality in neonatal pigs (<7-day-old), the aim of vaccination for PEDV is to immunize the sows to induce adequate maternal immunity to passively protect neonatal piglets from PED (Chattha et al., 2015; Saif et al., 2012). This objective may be accomplished by the selection of the optimal viral strain, whose antigenicity is close to that of the epidemic strain, but of minimal pathogenicity in young piglets, as well as the optimal vaccine approach.

5.1. Concerns about the use of autogenous PEDV strains for feedback or for inactivated vaccines to induce protection in pigs

Before effective PEDV vaccines became available in the US and many Asian countries, intentional infection of sows with an autogenous PEDV strain during gestation, feedback exposure was used to induce lactogenic immunity for piglets (Chattha et al., 2015; Jung and Saif, 2015; Song et al., 2015b). When the neonatal piglets were protected by sufficient amounts of lactogenic antibodies, experimental piglet inoculation with doses ~100 and 1000 PFU of PEDV did not induce major clinical signs or microscopic lesions (Liu et al., 2015). Feedback methods utilizing PEDV infected material from the same outbreak farms bypassed the issues regarding antigenic variations between the classical and emerging PEDV strains and had variable success in preventing reinfection. However, the safety of using this strategy as a disease control method was questionable (Chattha et al., 2015). Alternately, inactivated vaccines have advantages of vaccine safety, but usually elicit less robust and lower protective neutralizing/lactogenic immune responses than modified live vaccines. To date, detailed efficacy studies of the PEDV inactivated (Zoetis, Florham Park, NJ) or alphavirus vectored (Harrisvaccines, Ames, IA) vaccines conditionally licensed in the US are lacking.

5.2. INDEL PEDV infections induced insufficient cross-protection in pigs against the highly virulent PEDV strains

Whether the US PEDV S INDEL strains could be used as an effective live vaccine to cross-protect piglets from highly virulent PEDV strains has been investigated in several studies. Compared with prototype PEDV CV777, PEDV S INDEL Iowa106 is antigenically closer to the highly virulent PEDV PC22A in the S protein-based phylogenetic tree (Fig. 3) and antigenic map (Fig. 4). We reported that convalescent antisera obtained from S INDEL-infected pigs cross-reacts with the highly virulent PEDV PC22A strain in two-way CCIF and VN assays (Tables 1 and 2, Fig. 4) (Lin et al., 2015b). However, these *in vitro* immunological assays only examined the IgG antibodies in serum and may not reflect lactogenic antibodies *in vivo* challenge conditions. Because PEDV infects and replicates in the intestinal mucosa, development of mucosal immunity that relies on the quality and quantity of antibodies present in the intestinal mucosa or colostrum/milk, is critical for optimal active protection (Chattha et al., 2015; Saif, 1999). In the study of Goede et al. (2015) with 10 conventional sows, either with no previous PEDV infection or recovered from natural infection by an S INDEL PEDV at 7 months prior to farrowing, were orally boosted with a highly virulent PEDV strain at 109 day of gestation. Subsequently, the piglets were challenged at 3 days of age with a highly virulent PEDV strain. The S INDEL PEDV pre-exposed sows provided higher lactogenic immunity compared with unexposed sows. However, the PEDV-challenged, passively immunized suckling piglets showed extreme litter variations with morbidity rates ranging from 0% to 100%, although their mothers were naturally pre-exposed to an S INDEL PEDV strains and re-boosted with the virulent PEDV

strain (Goede et al., 2015). In our studies, we focused on active immunity to S INDEL PEDV in conventional suckling piglets born to PEDV-naïve sows. The piglets inoculated with S INDEL PEDV Iowa106 strain at 4-days of age and challenged with highly virulent PEDV PC21A at 24-32-days of age. Most piglets (81%, 13/16) recovered from S INDELIowa106 infection still had diarrhea after challenge with PC21A strain, while no piglets (0%, 0/4) recovered from the highly virulent PEDV PC21A infection developed disease after challenge with the homologous strain (Lin et al., 2015a). Therefore, we concluded that the S INDEL PEDV strain provided only partial active cross-protection to the piglets from a subsequent challenge with a highly virulent PEDV strain.

5.3. Attenuated TC PEDV strains at high passage levels can be considered as vaccine candidates

Attenuated PEDV generated via serial cell culture passages, as well as the naturally occurring mild S INDEL PEDV strains, may serve as live attenuated vaccine candidates (Chen et al., 2015; Sato et al., 2011). High cell culture passage level of PEDV strains could facilitate the generation of viruses that replicate well in cell culture, but less efficiently in the natural host, leading to viral attenuation. High titers of virus stocks that are free of other pathogens and can be generated and used to develop cost-effective inactivated and/or live attenuated vaccines. Many live attenuated classical PEDV strain vaccines had been used in Asian countries for decades (Sun et al., 2015a,b). They showed no disease in sows and piglets, but still induced robust immune responses in sows (Song et al., 2007). More recently, attenuated vaccine candidates have been produced via continuous cell culture passage of US PEDV PC22A strain in our laboratory (Lin et al. presented at Conference of Research Workers in Animal Diseases, December 6–8, 2015, Chicago, USA. Abstract no. 177) and Chinese PEDV YN strains in Dr. He's laboratory (Chen et al., 2015). A highly virulent PEDV YN strain was serially propagated in Vero-C1008 cells for 200 passages. At passage 144, the virus caused no clinical signs or histopathologic lesions in 10-day-old piglets. Compared with its parent strain (YN-P1), YN-P144 had 3 amino acid deletions and 13 amino acid substitutions in the S protein, 8 aa-early termination of ORF3 protein and other amino acid changes (9, 2, 2 and 2 in ORF1ab, E, M and N regions). However, none of the changes occurred in neutralizing epitopes. Although detailed data regarding the serological cross-reactivity has not been reported, the S proteins of YN-P1 and YN-P144 had a high (99.13%) sequence identity.In the same study, PEDV YN-P144 was tested safe for pregnant sows and newborn piglets, and induced immunity in sows and their piglets against a highly virulent PEDV challenge. In our laboratory, one US original highly virulent PEDV strain, PC22A, was continuously passaged in Vero cell lineages as described (Oka et al., 2014). After passage 100, it showed reduced virulence in 4-day-old CDCD piglets (Lin and Wang, unpublished data). However, concerns remain that the attenuated PEDV strains may recover their virulence via mutation in the natural hosts or generate new virulent strains by exchanging their genes with field strains by recombination (See Section 2 for examples of PEDV recombination) unless the viral genes responsible for recombination are defective, or suicide strains are engineered (limited replication competency).

5.4. Reverse genetics technology is a useful tool to study the molecular attenuation mechanisms of PEDV and to generate attenuated PEDV vaccines

The use of reverse genetics technology to generate PEDV infectious clones will allow targeted mutations in virulence genes and the engineering of genomic changes that limit PEDV recombination *in vivo* (Beall et al., 2016; Jengarn et al., 2015). Truncation of ORF3 protein observed in attenuated PEDV CV777 (Wang et al., 2012) and ORF3 gene mutations in attenuated DR13 (Song et al., 2003) suggested that ORF3 could be a region other than the S gene associated with viral virulence in vivo. Previous studies indicated that PEDV ORF3 encodes an ion channel protein and regulates virus production (Wang et al., 2012). However, truncation of ORF3 also occurred in low-passaged (P15) YN strain suggesting that it may be related to cell culture adaption, and not necessarily to viral attenuation (Chen et al., 2015). In our recent study, PEDV PC22A ORF3 gene was replaced by a red fluorescent protein gene using revere genetics technology (Beall et al., 2016). This ORF3-deleted PEDV infectious clone showed slightly reduced virulence in gnotobiotic piglets. Deletion of PEDV ORF3 did not change viral antigenicity, but alone it was not sufficient for complete virulence attenuation. With the accumulation of knowledge regarding the molecular mechanisms of PEDV attenuation, reverse genetics will help to rationally design PEDV vaccine candidates in the future.

6. Conclusions

Since the emergence of PEDV in England in 1971, different PEDV strains with various genetic, antigenic and pathologic properties have been detected in pigs. PEDV mutates constantly and recombination occurs frequently among PEDV strains and sometimes between PEDV and other coronaviruses. Currently, emerging non-S INDEL PEDV strains are highly virulent and cause severe threats to swine populations. Antigenic variations among classical, emerging non-S INDEL and S-INDEL PEDV strains were observed. Partial cross-protection between emerging S-INDEL and non-S INDEL PEDV strains and between classical and emerging PEDV strains may exist. The failure of the classical PEDV vaccines in Asia and lack of attenuated vaccines based on the emerging PEDV strains necessitates the development of new PEDV vaccines against the highly virulent strains. Naturally occurring mild PEDV strains, TC PEDV strains, and the infectious clone-derived attenuated strains can be considered as live attenuated PEDV vaccine candidates. Continued monitoring and characterization of global PEDV strains is important for precise classification of these viruses and for the development of strategies to prevent and control PED.

Acknowledgements

Our studies on PEDV were funded by Novartis Animal Health US, Inc. (QW, PI; LJS, co-PI), National Pork Board (Grant #14-265, QW, PI; LJS, co-PI), USDA (Grant # 2015-67015-23067, QW, PI; LJS, co-PI), OARDC/OSU Seeds #2015-008 (QW, PI), and Merck/Intervet (QW, PI; LJS, co-PI). Salaries and research support were provided by state and federal funds appropriated to OARDC, The Ohio State University. The authors would like to acknowledge Dr. Anastasia Vlasova at The Ohio State Univeristy, Drs. Li Feng and Jianfei Chen at Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, and Dr. Dongbo Sun at College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, for their helpful discussions, Dr. Min-Yuan Chia at Department of Veterinary Medicine, National Chung Hsing University, Taiwan, for the consultations on antigenic map preparation. We appreciate Dr. Lanlan Zheng, Mr. Yixuan Hou, and Mr. Yibin Qin for their technical assistance and Ms. Susan Sommer-Wagner for editing assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.virusres.2016.05.023.

References

Akimkin, V., Beer, M., Blome, S., Hanke, D., Hoper, D., Jenckel, M., Pohlmann, A., 2016. New chimeric porcine coronavirus in swine feces, Germany, 2012. Emerg. Infect. Dis. 22, 1314–1315.

Alonso, C., Goede, D.P., Morrison, R.B., Davies, P.R., Rovira, A., Marthaler, D.G., Torremorell, M., 2014. Evidence of infectivity of airborne porcine epidemic diarrhea virus and detection of airborne viral RNA at long distances from infected herds. Vet. Res. 45, 73.

Annamalai, T., Saif, L.J., Lu, Z., Jung, K., 2015. Age-dependent variation in innate immune responses to porcine epidemic diarrhea virus infection in suckling versus weaned pigs. Vet. Immunol. Immunopathol. 168, 193–202.

Beall, A., Yount, B., Lin, C.M., Hou, Y., Wang, Q., Saif, L., Baric, R., 2016. Characterization of a pathogenic full-Length cDNA clone and transmission model for porcine epidemic diarrhea virus strain PC22A. mBio, 7.

Boniotti, M.B., Papetti, A., Lavazza, A., Alborali, G., Sozzi, E., Chiapponi, C., Faccini, S., Bonilauri, P., Cordioli, P., Marthaler, D., 2016. Porcine epidemic diarrhea virus and discovery of a recombinant swine enteric Coronavirus, Italy. Emerg. Infect. Dis. 22, 83–87.

Cai, Z., Zhang, T., Wan, X.F., 2010. A computational framework for influenza antigenic cartography. PLoS Comput. Biol. 6, e1000949.

Chang, S.H., Bae, J.L., Kang, T.J., Kim, J., Chung, G.H., Lim, C.W., Laude, H., Yang, M.S., Jang, Y.S., 2002. Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic diarrhea virus. Mol. Cells 14, 295–299.

Chasey, D., Cartwright, S.F., 1978. Virus-like particles associated with porcine epidemic diarrhoea. Res. Vet. Sci. 25, 255–256.

Chattha, K.S., Roth, J.A., Saif, L.J., 2015. Strategies for design and application of enteric viral vaccines. Annu. Rev. Anim. Biosci. 3, 375–395.

Chen, J., Wang, C., Shi, H., Qiu, H., Liu, S., Chen, X., Zhang, Z., Feng, L., 2010. Molecular epidemiology of porcine epidemic diarrhea virus in China. Arch. Virol. 155, 1471–1476.

Chen, Q., Li, G., Stasko, J., Thomas, J.T., Stensland, W.R., Pillatzki, A.E., Gauger, P.C., Schwartz, K.J., Madson, D., Yoon, K.J., Stevenson, G.W., Burrough, E.R., Harmon, K.M., Main, R.G., Zhang, J., 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J. Clin. Microbiol. 52, 234–243.

Chen, F., Zhu, Y., Wu, M., Ku, X., Ye, S., Li, Z., Guo, X., He, Q., 2015. Comparative genomic analysis of classical and variant virulent parental/attenuated strains of porcine epidemic diarrhea virus. Viruses 7, 5525–5538.

Chen, Q., Gauger, P.C., Stafne, M.R., Thomas, J.T., Madson, D.M., Huang, H., Zheng, Y., Li, G., Zhang, J., 2016a. Pathogenesis comparison between the United States porcine epidemic diarrhea virus prototype and S-INDEL-variant strains in conventional neonatal piglets. J. Gen. Virol. 97, 1107–1121.

Chen, Q., Thomas, J.T., Gimenez-Lirola, L.G., Hardham, J.M., Gao, Q., Gerber, P.F., Opriessnig, T., Zheng, Y., Li, G., Gauger, P.C., Madson, D.M., Magstadt, D.R., Zhang, J., 2016b. Evaluation of serological cross-reactivity and cross-neutralization between the United States porcine epidemic diarrhea virus prototype and S-INDEL-variant strains. BMC Vet. Res. 12, 70.

Cheun-Arom, T., Temeeyasen, G., Srijangwad, A., Tripipat, T., Sangmalee, S., Vui, D.T., Chuanasa, T., Tantituvanont, A., Nilubol, D., 2015. Complete genome sequences of two genetically distinct variants of porcine epidemic diarrhea virus in the eastern region of Thailand. Genome Announc. 3.

Chia, M.Y., Chung, W.Y., Chiang, P.S., Chien, Y.S., Ho, M.S., Lee, M.S., 2014. Monitoring antigenic variations of enterovirus 71: implications for virus surveillance and vaccine development. PLoS Negl. Trop. Dis. 8, e3044.

Chiou, H.Y., Huang, Y.L., Deng, M.C., Chang, C.Y., Jeng, C.R., Tsai, P.S., Yang, C., Pang, V.F., Chang, H.W., 2015. Phylogenetic analysis of the spike (S) gene of the new variants of porcine epidemic diarrhoea virus in Taiwan. Transbound. Emerg. Dis., http://dx.doi.org/10.1111/tbed.12357 (in press).

Choe, S.E., Park, K.H., Lim, S.I., Le, V.P., Hien, N.B., Thach, P.N., Phuong le, H.T., An, B.H., Han, S.H., Cho, I.S., An, D.J., 2016. Complete genome sequence of a porcine epidemic diarrhea virus strain from vietnam, HUA-14PED96, with a large genomic deletion. Genome Announc. 4.

Choi, J.C., Lee, K.K., Pi, J.H., Park, S.Y., Song, C.S., Choi, I.S., Lee, J.B., Lee, D.H., Lee, S.W., 2014. Comparative genome analysis and molecular epidemiology of the reemerging porcine epidemic diarrhea virus strains isolated in Korea. Infect. Genet. Evol. 26, 348–351.

Coussement, W., Ducatelle, R., Debouck, P., Hoorens, J., 1982. Pathology of experimental CV777 coronavirus enteritis in piglets. I. Histological and histochemical study. Vet. Pathol. 19, 46–56.

Dastjerdi, A., Carr, J., Ellis, R.J., Steinbach, F., Williamson, S., 2015. Porcine epidemic diarrhea virus among farmed pigs, Ukraine. Emerg. Infect. Dis. 21, 2235–2237.

De Bouck, P., Pensaert, M., Coussement, W., 1981. The pathogenesis of an enteric infection in pigs, experimentally induced by the coronavirus-like agent, CV 777. Vet. Microbiol. 6, 157–165.

Debbink, K., Lindesmith, L.C., Ferris, M.T., Swanstrom, J., Beltramello, M., Corti, D., Lanzavecchia, A., Baric, R.S., 2014. Within-host evolution results in antigenically distinct GII.4 noroviruses. J. Virol. 88, 7244–7255.

Debouck, P., Pensaert, M., 1980. Experimental infection of pigs with a new porcine enteric coronavirus, CV 777. Am. J. Vet. Res. 41, 219–223.

Dee, S., Clement, T., Schelkopf, A., Nerem, J., Knudsen, D., Christopher-Hennings, J., Nelson, E., 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naive pigs following consumption via natural feeding behavior: proof of concept. BMC Vet. Res. 10, 176.

Dee, S., Neill, C., Singrey, A., Clement, T., Cochrane, R., Jones, C., Patterson, G., Spronk, G., Christopher-Hennings, J., Nelson, E., 2016. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. BMC Vet. Res. 12, 51.

Duarte, M., Gelfi, J., Lambert, P., Rasschaert, D., Laude, H., 1993. Genome organization of porcine epidemic diarrhoea virus. Adv. Exp. Med. Biol. 342, 55–60.

Ducatelle, R., Coussement, W., Pensaert, M.B., Debouck, P., Hoorens, J., 1981. In vivo morphogenesis of a new porcine enteric coronavirus, CV 777. Arch. Virol. 68, 35–44.

Fan, J.H., Zuo, Y.Z., Shen, X.Q., Gu, W.Y., Di, J.M., 2015. Development of an enzyme-linked immunosorbent assay for the monitoring and surveillance of antibodies to porcine epidemic diarrhea virus based on a recombinant membrane protein. J. Virol. Methods 225, 90–94.

Ge, J.W., Liu, D.Q., Li, Y.J., 2012. Construction of recombinant lactobacilli expressing the core neutralizing epitope (COE) of porcine epidemic diarrhea virus and a fusion protein consisting of COE and Escherichia coli heat-labile enterotoxin B, and comparison of the immune responses by orogastric immunization. Can. J. Microbiol. 58, 1258–1267.

Gerber, P.F., Gong, Q., Huang, Y.W., Wang, C., Holtkamp, D., Opriessnig, T., 2014. Detection of antibodies against porcine epidemic diarrhea virus in serum and colostrum by indirect ELISA. Vet. J. 202, 33–36.

Goede, D., Morrison, R.B., 2016. Production impact & time to stability in sow herds infected with porcine epidemic diarrhea virus (PEDV). Prev. Vet. Med. 123, 202–207.

Goede, D., Murtaugh, M.P., Nerem, J., Yeske, P., Rossow, K., Morrison, R., 2015. Previous infection of sows with a mild strain of porcine epidemic diarrhea virus confers protection against infection with a severe strain. Vet. Microbiol. 176, 161–164.

Grasland, B., Bigault, L., Bernard, C., Quenault, H., Toulouse, O., Fablet, C., Rose, N., Touzain, F., Blanchard, Y., 2015. Complete genome sequence of a porcine epidemic diarrhea s gene indel strain isolated in france in december 2014. Genome Announc. 3.

Hanke, D., Jenckel, M., Petrov, A., Ritzmann, M., Stadler, J., Akimkin, V., Blome, S., Pohlmann, A., Schirrmeier, H., Beer, M., Hoper, D., 2015. Comparison of porcine epidemic diarrhea viruses from Germany and the United States, 2014. Emerg. Infect. Dis. 21, 493–496.

Hao, J., Xue, C., He, L., Wang, Y., Cao, Y., 2014. Bioinformatics insight into the spike glycoprotein gene of field porcine epidemic diarrhea strains during 2011–2013 in Guangdong, China. Virus Genes 49, 58–67.

Have, P., Moving, V., Svansson, V., Uttenthal, A., Bloch, B., 1992. Coronavirus infection in mink (Mustela vison): Serological evidence of infection with a coronavirus related to transmissible gastroenteritis virus and porcine epidemic diarrhea virus. Vet. Microbiol. 31, 1–10.

He, Y., Zhou, Y., Wu, H., Kou, Z., Liu, S., Jiang, S., 2004. Mapping of antigenic sites on the nucleocapsid protein of the severe acute respiratory syndrome coronavirus. J. Clin. Microbiol. 42, 5309–5314.

Hofmann, M., Wyler, R., 1989. Quantitation, biological and physicochemical properties of cell culture-adapted porcine epidemic diarrhea coronavirus (PEDV). Vet. Microbiol. 20, 131–142.

Horzinek, M.C., Lutz, H., Pedersen, N.C., 1982. Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect. Immun. 37, 1148–1155.

Huang, Y.W., Dickerman, A.W., Pineyro, P., Li, L., Fang, L., Kiehne, R., Opriessnig, T., Meng, X.J., 2013. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. mBio 4, e00737–00713.

Jarvis, M.C., Lam, H.C., Zhang, Y., Wang, L., Hesse, R.A., Hause, B.M., Vlasova, A., Wang, Q., Zhang, J., Nelson, M.I., Murtaugh, M.P., Marthaler, D., 2015. Genomic and evolutionary inferences between American and global strains of porcine epidemic diarrhea virus. Prev. Vet. Med., http://dx.doi.org/10.1016/j.prevetmed.2015.10.020 (in press).

Jengarn, J., Wongthida, P., Wanasen, N., Frantz, P.N., Wanitchang, A., Jongkaewwattana, A., 2015. Genetic manipulation of porcine epidemic diarrhoea virus recovered from a full-length infectious cDNA clone. J. Gen. Virol. 96, 2206–2218.

Jung, K., Saif, L.J., 2015. Porcine epidemic diarrhea virus infection: etiology, epidemiology, pathogenesis and immunoprophylaxis. Vet. J. 204, 134–143.

Jung, K., Wang, Q., Scheuer, K.A., Lu, Z., Zhang, Y., Saif, L.J., 2014. Pathology of US porcine epidemic diarrhea virus strain PC21A in gnotobiotic pigs. Emerg. Infect. Dis. 20, 662–665.

Jung, K., Annamalai, T., Lu, Z., Saif, L.J., 2015. Comparative pathogenesis of US porcine epidemic diarrhea virus (PEDV) strain PC21A in conventional 9-day-old nursing piglets vs. 26-day-old weaned pigs. Vet. Microbiol. 178, 31–40.

Kang, T.J., Seo, J.E., Kim, D.H., Kim, T.G., Jang, Y.S., Yang, M.S., 2005. Cloning and sequence analysis of the Korean strain of spike gene of porcine epidemic diarrhea virus and expression of its neutralizing epitope in plants. Protein Exp. Purif. 41, 378–383.

Kanno, T., Ishihara, R., Hatama, S., Uchida, I., 2013. Antigenic variation among recent Japanese isolates of bovine coronaviruses belonging to phylogenetically distinct genetic groups. Arch. Virol. 158, 1047–1053.

Khatri, M., 2015. Porcine epidemic diarrhea virus replication in duck intestinal cell line. Emerg. Infect. Dis. 21, 549–550.

Kim, B., Chae, C., 2002. Experimental infection of piglets with transmissible gastroenteritis virus: a comparison of three strains (Korean, Purdue and Miller). J. Comp. Pathol. 126, 30–37.

Kim, O., Chae, C., 2003. Experimental infection of piglets with a korean strain of porcine epidemic diarrhoea virus. J. Comp. Pathol. 129, 55–60.
Kim, O., Chae, C., Kweon, C.H., 1999. Monoclonal antibody-based immunohistochemical detection of porcine epidemic diarrhea virus antigen in formalin-fixed paraffin-embedded intestinal tissues. J. Vet. Diagn. Invest. 11, 458–462.
Kim, S.Y., Song, D.S., Park, B.K., 2001. Differential detection of transmissible gastroenteritis virus and porcine epidemic diarrhea virus by duplex RT-PCR. J. Vet. Diagn. Invest. 13, 516–520.
Kim, S.-H., Lee, J.-M., Jung, J., Kim, I.-J., Hyun, B.-H., Kim, H.-I., Park, C.-K., Oem, J.-K., Kim, Y.-H., Lee, M.-H., Lee, K.-K., 2015. Genetic characterization of porcine epidemic diarrhea virus in Korea from 1998 to 2013. Arch. Virol. 160, 1055–1064.
Kim, Y.K., Cho, Y.Y., An, B.H., Lim, S.I., Lim, J.A., Cho, I.S., Le, V.P., An, D.J., 2016. Molecular characterization of the spike and ORF3 genes of porcine epidemic diarrhea virus in the Philippines. Arch. Virol. 161, 1323–1328.
Knuchel, M., Ackermann, M., Muller, H.K., Kihm, U., 1992. An ELISA for detection of antibodies against porcine epidemic diarrhoea virus (PEDV) based on the specific solubility of the viral surface glycoprotein. Vet. Microbiol. 32, 117–134.
Kocherhans, R., Bridgen, A., Ackermann, M., Tobler, K., 2001. Completion of the porcine epidemic diarrhoea coronavirus (PEDV) genome sequence. Virus Genes 23, 137–144.
Laude, H., Chapsal, J.M., Gelfi, J., Labiau, S., Grosclaude, J., 1986. Antigenic structure of transmissible gastroenteritis virus. I. Properties of monoclonal antibodies directed against virion proteins. J. Gen. Virol. 67 (Pt. (1)), 119–130.
Lee, S., Lee, C., 2014. Outbreak-related porcine epidemic diarrhea virus strains similar to US strains, South Korea, 2013. Emerg. Infect. Dis. 20, 1223–1226.
Li, F., 2015. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. J. Virol. 89, 1954–1964.
Li, R., Qiao, S., Yang, Y., Su, Y., Zhao, P., Zhou, E., Zhang, G., 2014. Phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field strains in central China based on the ORF3 gene and the main neutralization epitopes. Arch. Virol. 159, 1057–1065.
Li, R., Qiao, S., Yang, Y., Guo, J., Xie, S., Zhou, E., Zhang, G., 2016. Genome sequencing and analysis of a novel recombinant porcine epidemic diarrhea virus strain from Henan, China. Virus Genes 52, 91–98.
Lin, C.N., Chung, W.B., Chang, S.W., Wen, C.C., Liu, H., Chien, C.H., Chiou, M.T., 2014. US-like strain of porcine epidemic diarrhea virus outbreaks in Taiwan, 2013–2014. J. Vet. Med. Sci. Jpn. Soc. Vet. Sci. 76, 1297–1299.
Lin, C.M., Annamalai, T., Liu, X., Gao, X., Lu, Z., El-Tholoth, M., Hu, H., Saif, L.J., Wang, Q., 2015a. Experimental infection of a US spike-insertion deletion porcine epidemic diarrhea virus in conventional nursing piglets and cross-protection to the original US PEDV infection. Vet. Res. 46, 134.
Lin, C.M., Gao, X., Oka, T., Vlasova, A.N., Esseili, M.A., Wang, Q., Saif, L.J., 2015b. Antigenic relationships among porcine epidemic diarrhea virus and transmissible gastroenteritis virus strains. J. Virol. 89, 3332–3342.
Lin, J.D., Lin, C.F., Chung, W.B., Chiou, M.T., Lin, C.N., 2016. Impact of mated female nonproductive days in breeding herd after porcine epidemic diarrhea virus outbreak. PLoS One 11, e0147316.
Liu, X., Lin, C.M., Annamalai, T., Gao, X., Lu, Z., Esseili, M.A., Jung, K., El-Tholoth, M., Saif, L.J., Wang, Q., 2015. Determination of the infectious titer and virulence of an original US porcine epidemic diarrhea virus PC22A strain. Vet. Res. 46, 109.
Ma, Y., Zhang, Y., Liang, X., Oglesbee, M., Krakowka, S., Niehaus, A., Wang, G., Jia, A., Song, H., Li, J., 2016. Two-way antigenic cross-reactivity between porcine epidemic diarrhea virus and porcine deltacoronavirus. Vet. Microbiol. 186, 90–96.
Madson, D.M., Magstadt, D.R., Arruda, P.H., Hoang, H., Sun, D., Bower, L.P., Bhandari, M., Burrough, E.R., Gauger, P.C., Pillatzki, A.E., Stevenson, G.W., Wilberts, B.L., Brodie, J., Harmon, K.M., Wang, C., Main, R.G., Zhang, J., Yoon, K.J., 2014. Pathogenesis of porcine epidemic diarrhea virus isolate (US/Iowa/18984/2013) in 3-week-old weaned pigs. Vet. Microbiol. 174, 60–68.
Madson, D.M., Arruda, P.H., Magstadt, D.R., Burrough, E.R., Hoang, H., Sun, D., Bower, L.P., Bhandari, M., Gauger, P.C., Stevenson, G.W., Wilberts, B.L., Wang, C., Zhang, J., Yoon, K.J., 2016. Characterization of porcine epidemic diarrhea virus isolate US/Iowa/18984/2013 infection in 1-day-old cesarean-derived colostrum-deprived piglets. Vet. Pathol. 53, 44–52.
Makadiya, N., Brownlie, R., van den Hurk, J., Berube, N., Allan, B., Gerdts, V., Zakhartchouk, A., 2016. S1 domain of the porcine epidemic diarrhea virus spike protein as a vaccine antigen. Virol. J. 13, 57.
Makino, S., Keck, J.G., Stohlman, S.A., Lai, M.M., 1986. High-frequency RNA recombination of murine coronaviruses. J. Virol. 57, 729–737.
Marthaler, D., Bruner, L., Collins, J., Rossow, K., 2014a. Third strain of porcine epidemic diarrhea virus, United States. Emerg. Infect. Dis. 20, 2162–2163.
Marthaler, D., Jiang, Y., Collins, J., Rossow, K., 2014b. Complete genome sequence of strain SDCV/USA/Illinois121/2014, a porcine deltacoronavirus from the United States. Genome Announc. 2.
Masuda, T., Murakami, S., Takahashi, O., Miyazaki, A., Ohashi, S., Yamasato, H., Suzuki, T., 2015. New porcine epidemic diarrhoea virus variant with a large deletion in the spike gene identified in domestic pigs. Arch. Virol. 160, 2565–2568.
Meng, F., Ren, Y., Suo, S., Sun, X., Li, X., Li, P., Yang, W., Li, G., Li, L., Schwegmann-Wessels, C., Herrler, G., Ren, X., 2013. Evaluation on the efficacy and immunogenicity of recombinant DNA plasmids expressing spike genes from porcine transmissible gastroenteritis virus and porcine epidemic diarrhea virus. PLoS One 8, e57468.
Mesquita, J.R., Hakze-van der Honing, R., Almeida, A., Lourenco, M., van der Poel, W.H., Nascimento, M.S., 2015. Outbreak of porcine epidemic diarrhea virus in Portugal, 2015. Transbound. Emerg. Dis. 62, 586–588.
Mumford, J.A., 2007. Vaccines and viral antigenic diversity. Rev. Sci. Technol. 26, 69–90.
Murakami, S., Miyazaki, A., Takahashi, O., Hashizume, W., Hase, Y., Ohashi, S., Suzuki, T., 2015. Complete genome sequence of the porcine epidemic diarrhea virus variant Tottori2/JPN/2014. Genome Announc. 3.
Oka, T., Saif, L.J., Marthaler, D., Esseili, M.A., Meulia, T., Lin, C.M., Vlasova, A.N., Jung, K., Zhang, Y., Wang, Q., 2014. Cell culture isolation and sequence analysis of genetically diverse US porcine epidemic diarrhea virus strains including a novel strain with a large deletion in the spike gene. Vet. Microbiol. 173, 258–269.
Okda, F., Liu, X., Singrey, A., Clement, T., Nelson, J., Christopher-Hennings, J., Nelson, E.A., Lawson, S., 2015. Development of an indirect ELISA, blocking ELISA, fluorescent microsphere immunoassay and fluorescent focus neutralization assay for serologic evaluation of exposure to North American strains of Porcine Epidemic Diarrhea Virus. BMC Vet. Res. 11, 180.
Oszvald, M., Kang, T.J., Tomoskozi, S., Tamas, C., Tamas, L., Kim, T.G., Yang, M.S., 2007. Expression of a synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of Escherichia coli heat labile enterotoxin in rice endosperm. Mol. Biotechnol. 35, 215–223.
Park, S.J., Kim, H.K., Song, D.S., An, D.J., Park, B.K., 2012. Complete genome sequences of a Korean virulent porcine epidemic diarrhea virus and its attenuated counterpart. J. Virol. 86, 5964.
Park, S.J., Song, D.S., Park, B.K., 2013. Molecular epidemiology and phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field isolates in Korea. Arch. Virol. 158, 1533–1541.
Park, S., Kim, S., Song, D., Park, B., 2014. 2014 Novel porcine epidemic diarrhea virus variant with large genomic deletion, South Korea. Emerg. Infect. Dis. 20, 2089–2092.
Pasick, J., Berhane, Y., Ojkic, D., Maxie, G., Embury-Hyatt, C., Swekla, K., Handel, K., Fairles, J., Alexandersen, S., 2014. Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. Transbound Emerg. Dis. 61, 397–410.
Paudel, S., Park, J.E., Jang, H., Shin, H.J., 2014. Comparison of serum neutralization and enzyme-linked immunosorbent assay on sera from porcine epidemic diarrhea virus vaccinated pigs. Vet. Q. 34, 218–223.
Pensaert, M.B., de Bouck, P., 1978. A new coronavirus-like particle associated with diarrhea in swine. Arch. Virol. 58, 243–247.
Pensaert, M.B., Debouck, P., Reynolds, D.J., 1981. An immunoelectron microscopic and immunofluorescent study on the antigenic relationship between the coronavirus-like agent, CV 777, and several coronaviruses. Arch. Virol. 68, 45–52.
Pensaert, M., Callebaut, P., Vergote, J., 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. Vet. Q. 8, 257–261.
Poonsuk, K., Gimenez-Lirola, L.G., Zhang, J., Arruda, P., Chen, Q., Correa da Silva Carrion, L., Magtoto, R., Pineyro, P., Sarmento, L., Wang, C., Sun, Y., Madson, D., Johnson, J., Yoon, K.J., Zimmerman, J., Main, R., 2016. Does circulating antibody play a role in the protection of piglets against porcine epidemic diarrhea virus? PLoS One 11, e0153041.
Pospischil, A., Hess, R.G., Bachmann, P.A., 1981. Light microscopy and ultrahistology of intestinal changes in pigs infected with epizootic diarrhoea virus (EVD): comparison with transmissible gastroenteritis (TGE) virus and porcine rotavirus infections. Zent. Vet. B 28, 564–577.
Saif, L.J., 1989. Comparative aspects of enteric viral infections. In: Saif, L.J., Theil, K.W. (Eds.), Viral Diarrhea of Man and Animals. CRC, Boca Raton, Florida, pp. 9–34.
Saif, L.J., 1993. Coronavirus immunogens. Vet. Microbiol. 37, 285–297.
Saif, L.J., 1999. Enteric viral infections of pigs and strategies for induction of mucosal immunity. Adv. Vet. Med. 41, 429–446.
Saif, L.J., Pensaert, M.B., Sestak, K., Yeo, S.G., Jung, K., 2012. Coronaviruses. In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), Disease of Swine. John Wiley & Sons, Inc., pp. 01–524.
Sanchez, C.M., Jimenez, G., Laviada, M.D., Correa, I., Sune, C., Bullido, M., Gebauer, F., Smerdou, C., Callebaut, P., Escribano, J.M., et al., 1990. Antigenic homology among coronaviruses related to transmissible gastroenteritis virus. Virology 174, 410–417.
Sato, T., Takeyama, N., Katsumata, A., Tuchiya, K., Kodama, T., Kusanagi, K., 2011. Mutations in the spike gene of porcine epidemic diarrhea virus associated with growth adaptation in vitro and attenuation of virulence in vivo. Virus Genes 43, 72–78.
Scherba, G., Bromfield, C.R., Jarrell, V.L., Shipley, C.F., 2016. Evaluation of responses to both oral and parenteral immunization modalities for porcine epidemic diarrhea virus in production units. J. Swine Health Prod. 24, 21–28.
Shibata, I., Tsuda, T., Mori, M., Ono, M., Sueyoshi, M., Uruno, K., 2000. Isolation of porcine epidemic diarrhea virus in porcine cell cultures and experimental infection of pigs of different ages. Vet. Microbiol. 72, 173–182.
Simkins, R.A., Saif, L.J., Weilnau, P.A., 1989. Epitope mapping and the detection of transmissible gastroenteritis viral proteins in cell culture using biotinylated monoclonal antibodies in a fixed-cell ELISA. Arch. Virol. 107, 179–190.
Simkins, R.A., Weilnau, P.A., Bias, J., Saif, L.J., 1992. Antigenic variation among transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus strains detected with monoclonal antibodies to the S protein of TGEV. Am. J. Vet. Res. 53, 1253–1258.

Smith, D.J., Lapedes, A.S., de Jong, J.C., Bestebroer, T.M., Rimmelzwaan, G.F., Osterhaus, A.D., Fouchier, R.A., 2004. Mapping the antigenic and genetic evolution of influenza virus. Science 305, 371–376.
Song, D., Park, B., 2012. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 44, 167–175.
Song, D.S., Yang, J.S., Oh, J.S., Han, J.H., Park, B.K., 2003. Differentiation of a vero cell adapted porcine epidemic diarrhea virus from Korean field strains by restriction fragment length polymorphism analysis of ORF 3. Vaccine 21, 1833–1842.
Song, D.S., Oh, J.S., Kang, B.K., Yang, J.S., Moon, H.J., Yoo, H.S., Jang, Y.S., Park, B.K., 2007. Oral efficacy of Vero cell attenuated porcine epidemic diarrhea virus DR13 strain. Res. Vet. Sci. 82, 134–140.
Song, D., Huang, D., Peng, Q., Huang, T., Chen, Y., Zhang, T., Nie, X., He, H., Wang, P., Liu, Q., Tang, Y., 2015a. Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea viruses associated with outbreaks of severe diarrhea in piglets in Jiangxi, China 2013. PLoS One 10, e0120310.
Song, D., Moon, H., Kang, B., 2015b. Porcine epidemic diarrhea: a review of current epidemiology and available vaccines. Clin. Exp. Vaccine Res. 4, 166–176.
Stadler, J., Zoels, S., Fux, R., Hanke, D., Pohlmann, A., Blome, S., Weissenbock, H., Weissenbacher-Lang, C., Ritzmann, M., Ladinig, A., 2015. Emergence of porcine epidemic diarrhea virus in southern Germany. BMC Vet. Res. 11, 142.
Stevenson, G.W., Hoang, H., Schwartz, K.J., Burrough, E.R., Sun, D., Madson, D., Cooper, V.L., Pillatzki, A., Gauger, P., Schmitt, B.J., Koster, L.G., Killian, M.L., Yoon, K.J., 2013. Emergence of Porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J. Vet. Diagn. Invest. 25, 649–654.
Su, M., Li, C., Guo, D., Wei, S., Wang, X., Geng, Y., Yao, S., Gao, J., Wang, E., Zhao, X., Wang, Z., Wang, J., Wu, R., Feng, L., Sun, D., 2015. A recombinant nucleocapsid protein-based indirect enzyme-linked immunosorbent assay to detect antibodies against porcine deltacoronavirus. J. Vet. Med. Sci.
Sueyoshi, M., Tsuda, T., Yamazaki, K., Yoshida, K., Nakazawa, M., Sato, K., Minami, T., Iwashita, K., Watanabe, M., Suzuki, Y., et al., 1995. An immunohistochemical investigation of porcine epidemic diarrhoea. J. Comp. Pathol. 113, 59–67.
Sun, R.Q., Cai, R.J., Chen, Y.Q., Liang, P.S., Chen, D.K., Song, C.X., 2012. Outbreak of porcine epidemic diarrhea in suckling piglets, China. Emerg. Infect. Dis. 18, 161–163.
Sun, H., Yang, J., Zhang, T., Long, L.P., Jia, K., Yang, G., Webby, R.J., Wan, X.F., 2013. Using sequence data to infer the antigenicity of influenza virus. mBio 4, e00230–13.
Sun, D., Wang, X., Wei, S., Chen, J., Feng, L., 2015a. Epidemiology and vaccine of porcine epidemic diarrhea virus in China: a mini-review. J. Vet. Med. Sci. Jpn. Soc. Vet. Sci. 78, 355–363.
Sun, M., Ma, J., Wang, Y., Wang, M., Song, W., Zhang, W., Lu, C., Yao, H., 2015b. Genomic and epidemiological characteristics provide new insights into the phylogeographical and spatiotemporal spread of porcine epidemic diarrhea virus in Asia. J. Clin. Microbiol. 53, 1484–1492.
Suzuki, T., Murakami, S., Takahashi, O., Kodera, A., Masuda, T., Itoh, S., Miyazaki, A., Ohashi, S., Tsutsui, T., 2015. Molecular characterization of pig epidemic diarrhoea viruses isolated in Japan from 2013 to 2014. J. Mol. Epidemiol. Evolut. Genet. Infect. Dise. Infect. Genet. Evol., http://dx.doi.org/10.1016/j.meegid.2015.10.017 (in press).
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Tang, X.C., Zhang, J.X., Zhang, S.Y., Wang, P., Fan, X.H., Li, L.F., Li, G., Dong, B.Q., Liu, W., Cheung, C.L., Xu, K.M., Song, W.J., Vijaykrishna, D., Poon, L.L., Peiris, J.S., Smith, G.J., Chen, H., Guan, Y., 2006. Prevalence and genetic diversity of coronaviruses in bats from China. J. Virol. 80, 7481–7490.
Thachil, A., Gerber, P.F., Xiao, C.T., Huang, Y.W., Opriessnig, T., 2015. Development and application of an ELISA for the detection of porcine deltacoronavirus IgG antibodies. PLoS One 10, e0124363.
Thomas, J.T., Chen, Q., Gauger, P.C., Gimenez-Lirola, L.G., Sinha, A., Harmon, K.M., Madson, D.M., Burrough, E.R., Magstadt, D.R., Salzbrenner, H.M., Welch, M.W., Yoon, K.J., Zimmerman, J.J., Zhang, J., 2015. Effect of porcine epidemic diarrhea virus infectious doses on infection outcomes in naive conventional neonatal and weaned pigs. PLoS One 10, e0139266.
Tian, Y., Yu, Z., Cheng, K., Liu, Y., Huang, J., Xin, Y., Li, Y., Fan, S., Wang, T., Huang, G., Feng, N., Yang, Z., Yang, S., Gao, Y., Xia, X., 2013. Molecular characterization and phylogenetic analysis of new variants of the porcine epidemic diarrhea virus in Gansu, China in 2012. Viruses 5, 1991–2004.
Tian, P.F., Jin, Y.L., Xing, G., Qv, L.L., Huang, Y.W., Zhou, J.Y., 2014. Evidence of recombinant strains of porcine epidemic diarrhea virus, United States, 2013. Emerg. Infect. Dis. 20, 1735–1738.
VanCott, J.L., Brim, T.A., Simkins, R.A., Saif, L.J., 1993. Isotype-specific antibody-secreting cells to transmissible gastroenteritis virus and porcine respiratory coronavirus in gut- and bronchus-associated lymphoid tissues of suckling pigs. J. Immunol. 150, 3990–4000.
Vlasova, A.N., Zhang, X., Hasoksuz, M., Nagesha, H.S., Haynes, L.M., Fang, Y., Lu, S., Saif, L.J., 2007. Two-way antigenic cross-reactivity between severe acute respiratory syndrome coronavirus (SARS-CoV) and group 1 animal CoVs is mediated through an antigenic site in the N-terminal region of the SARS-CoV nucleoprotein. J. Virol. 81, 13365–13377.
Vlasova, A.N., Marthaler, D., Wang, Q., Culhane, M.R., Rossow, K.D., Rovira, A., Collins, J., Saif, L.J., 2014. Distinct characteristics and complex evolution of PEDV strains, north america, may 2013–february 2014. Emerg. Infect. Dis. 20, 1620–1628.
Vui, D.T., Tung, N., Inui, K., Slater, S., Nilubol, D., 2014. Complete genome sequence of porcine epidemic diarrhea virus in Vietnam. Genome Announc. 2.
Wang, K., Lu, W., Chen, J., Xie, S., Shi, H., Hsu, H., Yu, W., Xu, K., Bian, C., Fischer, W.B., Schwarz, W., Feng, L., Sun, B., 2012. PEDV ORF3 encodes an ion channel protein and regulates virus production. FEBS Lett. 586, 384–391.
Wang, L., Byrum, B., Zhang, Y., 2014. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. Emerg. Infect. Dis. 20, 1227–1230.
Wang, X., Chen, J., Shi, D., Shi, H., Zhang, X., Yuan, J., Jiang, S., Feng, L., 2015. Immunogenicity and antigenic relationships among spike proteins of porcine epidemic diarrhea virus subtypes G1 and G2. Arch. Virol. 161, 537–547.
Wood, E.N., 1977. An apparently new syndrome of porcine epidemic diarrhoea. Vet. Rec. 100, 243–244.
Xuan, H., Xing, D., Wang, D., Zhu, W., Zhao, F., Gong, H., Fei, E., 1984. Culture of porcine epidemic diarrhea virus by using pig intestinal monolayer cell. J. People's Lib. Army Vet. Univ. 4, 202–208.
Yamamoto, R., Soma, J., Nakanishi, M., Yamaguchi, R., Niinuma, S., 2015. Isolation and experimental inoculation of an S INDEL strain of porcine epidemic diarrhea virus in Japan. Res. Vet. Sci. 103, 103–106.
Yamamoto, T., Suzuki, T., Ohashi, S., Miyazaki, A., Tsutsui, T., 2016. Genomic motifs as a novel indicator of the relationship between strains isolated from the epidemic of porcine epidemic diarrhea in 2013–2014. PLoS One 11, e0147994.
Zhang, X., Pan, Y., Wang, D., Tian, X., Song, Y., Cao, Y., 2015. Identification and pathogenicity of a variant porcine epidemic diarrhea virus field strain with reduced virulence. Virol. J. 12, 88.
Zhang, Y., Zhang, X., Liao, X., Huang, X., Cao, S., Wen, X., Wen, Y., Wu, R., Liu, W., 2016. Construction of a bivalent DNA vaccine co-expressing S genes of transmissible gastroenteritis virus and porcine epidemic diarrhea virus delivered by attenuated *Salmonella typhimurium*. Virus Genes 52, 354–364.
Zhou, Y.L., Ederveen, J., Egberink, H., Pensaert, M., Horzinek, M.C., 1988. Porcine epidemic diarrhea virus (CV 777) and feline infectious peritonitis virus (FIPV) are antigenically related. Arch. Virol. 102, 63–71.