REVIEW *Virology*

Epidemiology and vaccine of porcine epidemic diarrhea virus in China: a mini-review

Dongbo SUN[1,2)]*, Xinyu WANG[1)], Shan WEI[1)], Jianfei CHEN[2)] and Li FENG[2)]*

[1)]*College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, No. 2 Xinyang Road, Sartu District, Daqing 163319, P.R. China*

[2)]*Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences, No. 427 Maduan Street, Nangang District, Harbin 150001, P.R. China*

(Received 29 July 2015/Accepted 22 October 2015/Published online in J-STAGE 3 November 2015)

ABSTRACT. Porcine epidemic diarrhea (PED) is an intestinal infectious disease caused by porcine epidemic diarrhea virus (PEDV); manifestations of the disease are diarrhea, vomiting and dehydration. Starting from the end of 2010, a PED outbreak occurred in several pig-producing provinces in southern China. Subsequently, the disease spread throughout the country and caused enormous economic losses to the pork industry. Accumulating studies demonstrated that new PEDV variants that appeared in China were responsible for the PED outbreak. In the current mini-review, we summarize PEDV epidemiology and vaccination in China.

KEY WORDS: epidemiology, porcine epidemic diarrhea virus, vaccine

doi: 10.1292/jvms.15-0446; *J. Vet. Med. Sci.* 78(3): 355–363, 2016

Porcine epidemic diarrhea (PED) is a highly contagious, intestinal infectious disease caused by porcine epidemic diarrhea virus (PEDV). The disease is characterized by severe diarrhea, vomiting and dehydration. PEDV infections can occur in pigs of all ages, and infections are most serious in piglets, with morbidity and mortality often reaching 100% [9, 40]. Pigs that tolerate PEDV infections usually exhibit growth retardation, reduced food conversion rates, increased feeding costs and the co-occurrence of other diseases. Furthermore, PEDV infections result in reduced pig slaughter rates, insufficient pork supplies and pork price instability, because of a large number of piglet deaths.

PEDV is an enveloped, single-stranded, positive-sense RNA virus that belongs to the order *Nidovirales*, family *Coronaviridae* and genus *Alphacoronavirus*. In 1971, the first reported PED outbreak occurred in pig populations in England, and PEDV was confirmed to cause PED in 1978 [30]. Thus far, PED has been reported in swine-farming countries in Asia, Europe, North America [7, 17, 20, 29, 32]. In 1973, an acute diarrheal disease similar to swine transmissible gastroenteritis (TGE) was first reported in Shanghai, China; however, the causative agent of this disease was not confirmed to be PEDV until 1984 [41]. From 1984 to early 2010, PEDV infections occurred in the pig population in China, but there were no large-scale outbreaks [3, 6]. Starting from the end of 2010, PEDV outbreaks occurred in the Chinese pig population and caused enormous economic losses [1, 2, 4, 20, 22, 42]. Because of the severity of PED, extensive, in-depth studies of PEDV have been performed during the past five years in China.

EPIDEMIOLOGY

Since 1966, the occurrence of pig diarrheal disease has been recorded in China. The disease showed a local endemic tendency with significant seasonal variations, usually occurring from the end of October to early February, and PEDV was confirmed to be the cause of such outbreaks in 1984 [43]. After PEDV infections in the pig population were confirmed, a series of investigations of PED occurrence were conducted in China. Ji *et al.* (1987) reported that in farms with PEDV outbreaks, the PEDV incidence was 80% in sows, 100% in suckling pigs and 90% in fattening pigs [18]. Wang *et al.* (1989) reported that among cases of PED in 1989, the PEDV incidence was 95% in fattening pigs, 60.4% in sows, 78.95% in boars and 100% in suckling pigs (with a mortality rate of 87.9%) [39]. These data suggest that PEDV infections were present in the pig population in China during PED occurred in Europe. However, before 2010, PEDV infections were mostly sporadic or locally endemic, with no large-scale outbreaks in China.

After the PEDV outbreaks in 2010, detailed studies of the molecular epidemiology and evolution of PEDV were conducted in China, which led to the accumulation of a large amount of data (Table 1). From February 2011 to March 2014, a long-term investigation showed that PEDV was circulating in 29 provinces in China, excluding Tibet and Hainan (Fig. 1A); the positive rates of samples varied from 61.10% to 78.49%, and the positive rates of pig farms varied from 71.43% to 83.47% (Fig. 1B) [10, 45]. The positive rates of PEDV in samples or farms were significantly

*CORRESPONDENCE TO: SUN, D., College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, No. 2 Xinyang Road, Sartu District, Daqing 163319, P.R. China. FENG, L., Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences, No. 427 Maduan Street, Nangang District, Harbin 150001, P.R. China. e-mail: dongbosun@126.com (D. Sun); fl@hvri.ac.cn (L. Feng)

Table 1. Epidemiology investigation of PEDV during 2006–2014 in China

Investigation time	Geographic location	Number of samples	Target genes	Result description	Reference
2006.1–2006.6	Harbin, Heilongjiang province; Changsha, Hunan province; Baotou, Inner mongolia autonomous region; Tongliao, Inner mongolia autonomous region; Xuzhou, Jiangsu province; Shanghai city.	6 (positive for PEDV by RT-PCR)	M gene of PEDV	There was a new genotype of PEDV prevailing in China.	Chen *et al.* (2008) [6]
2006.1–2009.8	Gansu, Heilongjiang, Henan, Hunan, Inner Mongolia, Jiangsu, Jilin provinces and Shanghai city.	12 (positive for PEDV by RT-PCR)	ORF3 gene of PEDV	PEDV field strains in immunized herd have a close phylogenetic relationship to Korean strains and are genetically different from vaccine strain (CV777).	Chen *et al.* (2010) [3]
2010.2–2011.11	Yunfu, Zhaoqing, Foshan, Qingyuan, Yangjiang and Sanshui in Guangdong province; Xinfeng, Jiangxi province; Guilin, Guangxi province; Deyang, Sichuan province; Putian, Fujian province; Huaian, Jiangsu province.	127 (feces or intestinal contents)	partial S and M genes of PEDV	43.0% samples were positive for PEDV; 12.0% samples were positive for PEDV and TGEV; all south China PEDV strains have a close relationship with most of the strains in Korea and Thailand, and differ genetically from European strains, early domestic strains and vaccine strain (CV777).	Li *et al.* (2012) [22]
2010.10–2011.12	Four provinces located in central China.	15 (positive for PEDV by RT-PCR)	M and ORF3 genes of PEDV	15 PEDV strains showed a close relationship with Korean strains, Thai strains and partial other Chinese strains, but differed genetically from European strains and vaccine strain (CV777). PEDV exhibits rapid variation and genetic evolution, and the currently prevailing PEDV strains in central China are a new genotype.	Yang *et al.* (2013) [42]
2010.10–2012.3	Nanning, Guangxi province; Heyuan, Jiangmen, Zhanjiang, Zhongluotan, Zhaoqing, Foshan, Yangjiang, Heshan, and Guangzhou in Guangdong province; Nanyang, Henan province; Zhengzhou, Henan province; Changsha and Cenzhou in Hunan province; Haikou, Hainan province.	70 (rectal swab samples, 16 positive for PEDV by RT-PCR)	partial S, M and ORF3 genes of PEDV	PEDV isolates showed high degree of variation in the genes, particularly S genes, might provide an explanation for the poor immunity and rapid spread of the disease.	Sun *et al.* (2014) [34]
2010–2012	Fujian province.	27 (positive for PEDV by PED Ag Test Kit)	ORF3 gene of PEDV	The Fujian strains were very distant from vaccine strain (CV777), which might be the reason why the vaccine was inefficient to control the disease.	Chen *et al.* (2013) [8]
2010.2–2012.3	Foshan, Yangjiang, Sanshui, Qingyuan and Yunfu in Guangdong Province; Xinfeng, Jiangxi province; Deyang, Sichuan province; Huaian, Jiangsu province; Guilin, Guangxi province.	15 (positive for PEDV by RT-PCR)	N gene of PEDV	These PEDV strains are composed of a separate cluster including three early domestic strains, but differ genetically from the vaccine strain (CV777) and the early Korean strains.	Li *et al.* (2013) [21]
2011.1–2011.10	12 provinces in China.	455 (fecal, intestine, and milk)	S gene of PEDV	78.95% farms were positive for PEDV; 61.11% samples were positive for PEDV. Three new PEDV variants were identified based on full-length S gene sequences.	Li *et al.* (2012) [20]

Table 1. continued

Investigation time	Geographic location	Number of samples	Target genes	Result description	Reference
2011–2012	29 provinces in China (excluding Tibet and Hainan).	577 (intestine and milk)	S gene of PEDV	79.66% pig farms and 72.27% samples were positive for PEDV. There are classical strains and variants prevailing in pig herd.	Chen *et al.* (2013) [2]
2011–2012	Beijing city, Hebei province and Zhejiang province.	288 (fecal and intestinal samples)	S gene of PEDV	92.7% samples were positive for PEDV; Data suggest that a novel PEDV with a characteristic variant S gene is responsible for recent outbreaks of clinical diarrhea in piglets in China.	Gao *et al.* (2013) [11]
2011.9–2012.1	Shanghai city.	135 (intestinal and fecal samples)	M gene of PEDV	10 PEDV strains exhibit three distinct coexisting in the same pig farm. In addition to PEDV, porcine kobuvirus, porcine teschovirus and *Lawsonia intracellularis*, were identified.	Ge *et al.* (2013) [12]
2011–2013	Guangdong province.	10 (PEDV isolate)	S gene of PEDV	10 PEDV strains were clustered into three distinct groups. The circulating PEDV strains in Guangdong from 2011 to 2013 have a genetic composition that is distant from reference strains, especially the vaccine strains (CV777).	Hao *et al.* (2014) [15]
2012.1–2012.3	Gansu province.	5 (positive for PEDV by RT-PCR)	S gene of PEDV	S gene of five PEDV variants showed a series of insertions, deletions and mutations compared with classical and vaccine strains (CV777). These new PEDV variant strains in Gansu Province might be from South Korean or South China.	Tian *et al.* (2013) [35]
2012.9–2013.6	In central China.	14 (positive for PEDV by RT-PCR)	ORF3 gene of PEDV	14 PEDV isolates showed a close relationship to some Chinese strains isolated previously in central China and differed genetically from recent isolates from southern China, Korean strains and vaccine strain (CV777).	Li *et al.* (2014) [19]
2011.12–2014.3	29 provinces in China.	2058 (1383 intestinal and fecal samples; 675 tissue samples)	S1 region of S gene of PEDV	57.83%(4060/7021) pig farms were positive for pig diarrhea, and 49.58% samples were positive for PEDV. These PEDV strains from 2011 to 2013 have a close phylogenetic relationship, but they differed genetically from vaccine strains (CV777) based on phylogenetic analysis of the partial S.	Zhang *et al.* (2014) [45]

higher compared with earlier PEDV outbreaks in China and other countries. In addition to the PEDV, swine transmissible gastroenteritis virus, porcine rotavirus, porcine kobuvirus, porcine bocavirus, porcine enterovirus, porcine teschovirus, *Lawsonia intracellularis* and porcine deltacoronavirus have also been found in diarrhea samples from pigs [10, 12, 44]. These data demonstrated that PED occurred extensively among the pig population in China and that PEDV was the major cause of viral diarrheal diseases in swine during the recent outbreaks. However, co-infections of other intestinal pathogens with PEDV should also be monitored dynamically in future studies in China.

The S1 regions of the spike (S) protein-encoding gene of 99 selected PEDV strains were divided into two groups (GI and GII) in a phylogenetic tree. The GI group consists of two subgroups (GIa and GIb), while the GII group also consists of two subgroups (GIIa and GIIb) (Fig. 2). Ten Chinese PEDV strains isolated from 2004 to 2015, which exhibited

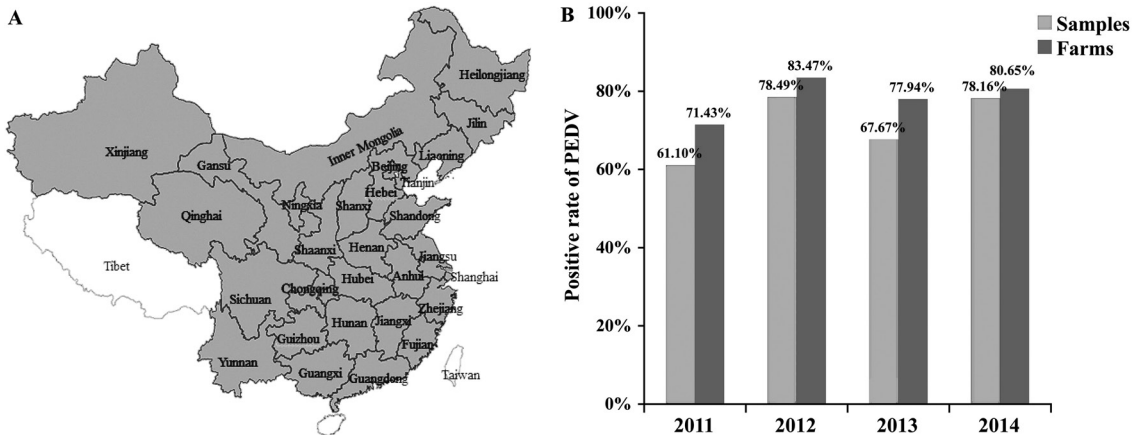

Fig. 1. Circulation of PEDV in China. A, Distribution of PEDV infection in China from February 2011 to March 2014; B, The positive rate of PEDV infection of the samples and pig farms.

a close relationship with PEDV strains from Vietnam and the U.S.A., were placed into the GIIa group; four Chinese PEDV strains, LZC, CH/JL/2011, SC-L and CH/BJSY/2011, which had a close relationship with the classic PEDV strain CV777, were placed into the GIIb group, as well as early PEDV strains from Japan and South Korea. Fifty-one Chinese PEDV strains isolated from 2011 to 2015, as well as 22 selected reference strains that were recently circulating in the U.S.A., Canada, Taiwan, Mexico, South Korea, Vietnam and Thailand, were placed into the GIa group, which exhibited twelve clusters (C1–C12). The clusters from C1–C3 and C7–C10 were composed of the Chinese PEDV strains; the Chinese PEDV strains from C1–C4 clusters differed genetically from these from C7–C11 clusters. In the GIa group, the genetic diversity of the 51 Chinese PEDV strains from 2011 to 2015 may be associated with the introduction of PEDV strains from other countries, self variation of the PEDV as one of the RNA viruses and the prolonged immune pressure from vaccine or infection. Of the GI group, three PEDV strains (Chinju99, Spk1 and KNU-0901) from South Korea and two Japanese strains (KH and NK) formed one specific GIb group. In summary, the phylogenetic analysis of the S1 region of the S gene of PEDV strains from 2004 to 2015 in China revealed that the PEDV field strains circulating in China showed varying degrees of variability and that Chinese PEDV strains from 2011 to 2015 exhibited specific evolutionary lineages compared with PEDV strains from early China, Europe, North America, South America and other Asian countries. In addition, the variations in Chinese PEDV isolates attracted the attention of researchers seeking to determine whether the PEDV vaccine strain (CV777) can effectively prevent PEDV variant strain infections in China. Several reports indicated that for PEDV field strains, the neutralizing epitopes of the major antigenic protein, the S protein, showed high conservation and were highly similar to that of the vaccine strain CV777 [2, 15, 33]. These data suggest that in China, the traditional PEDV vaccine strain (CV777) still could prevent infections by PEDV field strains and provide effective cross-protection. However, the protective effect of the PEDV vaccine needs to be dynamically monitored in future due to the increasing trend of PEDV variants in China.

Recombination of the S gene of coronaviruses has been frequently reported [24, 31]. The PEDV S protein binds to a host cell receptor and triggers fusion of the viral and cellular membranes. It is likely that PEDV variants will continue to emerge, because of naturally occurring recombination events and mutations in the S gene. To identify possible recombination events within the S gene of PEDV, the S1 regions of 65 selected S genes from Chinese PEDV strains isolated from 2004 to 2015 (see Fig. 2) were screened using the Recombination Detection Program (RDP), GENECONV, Bootscan, MaxChi, Chimaera, 3Seq, PhylPro, LARD and SISscan methods embedded in RDP4. The recombination analysis revealed that four potential recombination events occurred between the 65 PEDV strains from China (Table 2), and confirmation of the four potential recombination events was carried out by bootscan analysis and fast neighbor-joining trees, respectively (Fig. 3). Of the four potential recombination events, one potential recombinant event occurred between a GI group strain and a GII group strain, and three potential recombinant events occurred within GI group strains. Most of the GI group Chinese strains consisted of PEDV strains that emerged from 2011 to 2015. The limited data demonstrated that recently emerging PEDV strains in China exhibited high variability and potential recombination of the S gene. In summary, although the evidence of naturally occurring recombination events is limited, possible recombination events between the S genes of Chinese PEDV strains should be monitored by extensive molecular epidemiology investigations in the future.

VACCINE

Conventional vaccine: Wang *et al.* reported that a tissue-inactivated PEDV vaccine was prepared using PEDV strain

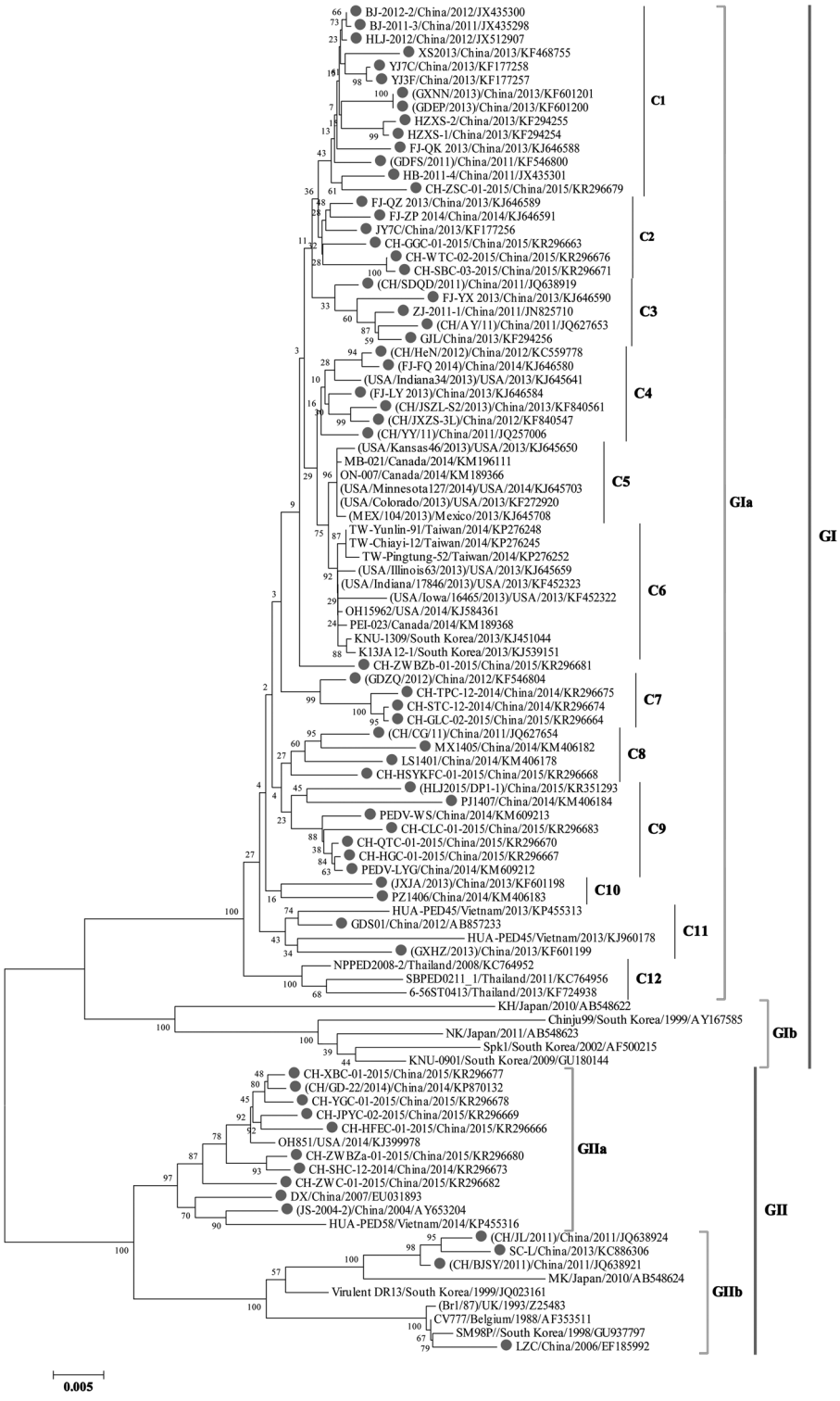

Fig. 2. Phylogenetic analysis of selected PEDV strains among Europe, North America, South America and Asia from 1988 to 2015 based on the S1 gene. The phylogenetic trees were generated from the ClustalX-generated alignments by MEGA6.06 software using the neighbor-joining method, respectively. The reliability of the generated phylogenetic tree was assessed by bootstrapping, using 1,000 bootstrap replications. ● China.

Table 2. Potential recombination analysis of the S1genes from Chinese PEDV strains

Potential recombinant events	Recombinant strains (accession no./year/group*)	Major parent strains (accession no./year/group*)	Minor parent strains (accession no./year/group*)	Breakpoint Positions in recombinant sequences	Program or method of recombination detection
1	CH-ZWBZa-01-2015 strain (KR296680/2015/GII)	CH/JL/2011 strain (JQ638924/2011/ GII)	CH-ZWBZb-01-2015 strain (KR296681/2015/GI)	11–762	RDP (*P*=7.15E-22); GENECONV (*P*=6.89E-20); Maxchi (*P*=3.49E-16); Chimaera (*P*=3.04E-16); SiSscan (*P*=3.04E-17); 3Seq (*P*=8.95E-18).
2	JS-2004-2 strain (AY653204/2004/GII)	PJ1407 strain (KM406184/2014/GI)	ZJ-2011-1 strain (JN825710/2011/GI)	50–767	RDP (*P*=5.91E-03); Maxchi (*P*=4.73E-10); Chimaera (*P*=3.12E-06); SiSscan (*P*=2.71E-21); 3Seq (*P*=2.31E-12).
3	GDS01 strain (AB857233/2012/GI)	JY7C strain (KF177256/2013/GI)	MX1405 strain (KM406182/2014/GI)	1455–2093	RDP (*P*=6.38E-03); GENECONV (*P*=2.58E-02); Chimaera (*P*=2.79E-02); SiSscan (*P*=1.47E-05); 3Seq (*P*=7.89E-05).
4	FJ-YX 2013 strain (KJ646590/2013/GI)	JY7C strain (KF177256/2013/GI)	MX1405 strain (KM406182/2014/GI)	1359–1974	GENECONV (*P*=3.71E-02); Maxchi (*P*=1.87E-02); SiSscan (*P*=1.27E-06); 3Seq (*P*=2.23E-03).

*Group was defined based on the phylogenetic tree of S1 gene of the selected PEDV strains in our study (see Fig. 2).

S [38]. Through Houhai acupoint immunization, the active immunization protection rate of the prepared PEDV tissue-inactivated vaccine in three-day-old piglets was 77.28%, and the passive immunization protection rate was 97.06%, while the immunization protection period was six months. Improving the tissue-inactivated PEDV vaccine involves a complicated preparation process, high production cost, high time requirement and quality control problems. Ma *et al.* adapted PEDV strain CV777 to Vero cells and successfully performed continuous passages by adding trypsin to the culture medium [27]. An inactivated PEDV vaccine was prepared using the cultures of 28th passage viruses [27]. Through Houhai acupoint immunization, the active immunization protection rate in piglets was 85.19%, and the passive immunization protection rate was 85.0%. However, inactivated PEDV vaccines still exhibited some limitations, including the need for high immunization doses and multiple booster immunizations, as well as short immunity durations. Tong *et al.* reported that an attenuated PEDV vaccine strain (a 90th generation cloned virus strain) was successfully prepared by serial passage *in vitro* [36]. Through Houhai acupoint immunization, the attenuated PEDV vaccine had a 95.52% active immunization protection rate and a 96.2% passive immunization protection rate in three- to six-day-old piglets.

On the basis of inactivated PEDV cell cultures, Ma *et al.* prepared an inactivated, bivalent TGEV and PEDV vaccine [26]. Tong *et al.* prepared an attenuated, bivalent TGEV and PEDV vaccine based on the attenuated PEDV vaccine [37]. The inactivated, bivalent TGEV and PEDV vaccine (1999 to present) and the attenuated, bivalent TGEV and PEDV vaccine (2003–2006) are extensively used in the Chinese pig population, and they play important roles in the control of TGEV and PEDV infections. After the outbreak of PED at the end of 2010, there was a renewed demand for live vaccines that could effectively prevent viral diarrhea diseases of swine. Based on the attenuated, bivalent TGEV and PEDV vaccine, Feng *et al.* prepared a trivalent TGEV, PEDV and PRoV (G5 type) live vaccine (consisting of an attenuated H strain, an attenuated CV777 strain and the attenuated NX strain), which was licensed on December 31, 2014, by the Chinese Ministry of Agriculture. The results of vaccine clinical trials showed that the immunization protection rate was over 95%, and the incidence of viral diarrheal diseases of swine in experimental farms was reduced from 8–12% to 3–5% [5]. In the future, the TGEV, PEDV and PRoV trivalent live vaccine will serve as an effective tool for the control of viral diarrheal diseases of swine in China, especially PEDV infections.

Genetically engineered vaccines: Mucosal immunity plays an important role in the immune mechanism of viral diarrheal diseases. Immunoglobulin A (IgA) antibodies secreted by the intestinal mucosa can defend against invading pathogens. Oral vaccines can stimulate mucosal immunity and produce protective mucosal and serum IgA antibodies, and they are an effective method for prevention of intestinal infectious diseases. For PEDV oral vaccines, Hou *et al.* reported that lactic acid bacteria expressing the PEDV N protein could activate the production of mucosal IgA and circulating IgG antibodies against the PEDV N protein [16]. Ge *et al.* reported that recombinant lactobacilli expressing

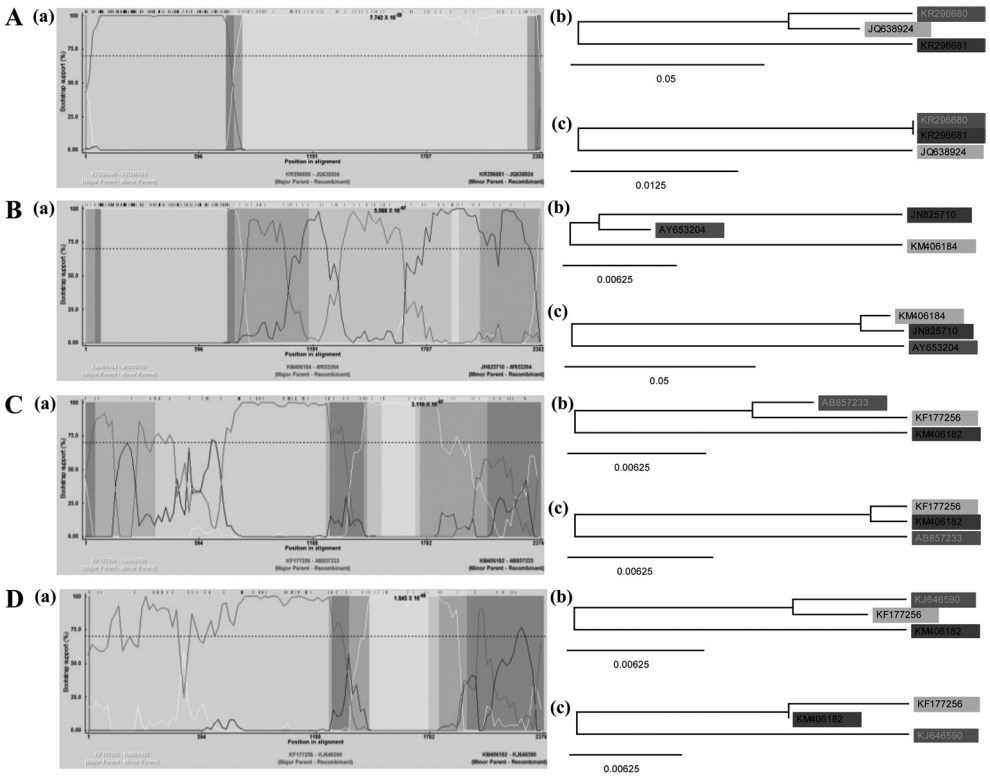

Fig. 3. Confirmation of the potential recombination events occurred between the 65 PEDV strains from China. A, The recombination event between the minor parent strain CH-ZWBZb-01-2015 (KR296681) and the major parent strain CH/JL/2011 (JQ638924), which led to the recombinant CH-ZWBZa-01-2015 strain (KR296680); B, The recombination event between the minor parent strain ZJ-2011-1 (JN825710) and the major parent strain PJ1407 (KM406184), which led to the recombinant JS-2004-2 strain (AY653204); C, The recombination event between the minor parent strain MX1405 (KM406182) and the major parent strain JY7C (KF177256), which led to the recombinant GDS01 strain (AB857233); D, The recombination event between the minor parent strain MX1405 (KM406182) and the major parent strain JY7C (KF177256), which led to the recombinant FJ-YX 2013 strain (KJ646590). (a), Bootscan evidence for each potential recombinant event on the basis of pairwise distance, modeled with a window size 200, step size 20 and 100 bootstrap replicates; (b) Fast neighbor-joining (NJ) tree (1,000 replicates, Kimura two-parameter distance) constructed using the recombinant region of each potential recombinant event; (c) Fast neighbor-joining (NJ) tree (1,000 replicates, Kimura two-parameter distance) constructed using the non-recombinant region of each potential recombinant event.

the N protein and the core neutralizing epitope (COE) could stimulate the intestines to produce local immune responses, as well as systemic immune responses, after oral immunization [13, 14]. Liu *et al.* constructed recombinant lactobacilli expressing the PEDV N gene or the S1 region of the S gene, and an oral inoculation showed that lactobacilli expressing the N protein could enhance the mucosal and systemic immune responses mediated by recombinant lactobacilli expressing the S1 fragment of the S protein [25].

The S protein of PEDV is a peplomer glycoprotein that is located on the surface of viral particles, and it can mediate the production of neutralizing antibodies; therefore, it is a target for vaccine research. However, the S protein is a highly glycosylated protein, and *in vitro* expression of recombinant S protein usually results in the loss of immunogenicity. To produce a highly effective PEDV vaccine, Meng *et al.* used a eukaryotic expression vector to construct a DNA vaccine expressing the PEDV S gene. This vaccine was able to effectively activate cell-mediated immunity and mediate the production of high levels of antibodies in immunized animals [28]. Liang *et al.* used a eukaryotic expression vector to construct a recombinant plasmid expressing the S1 region of the PEDV S gene, and they further constructed an attenuated *Salmonella enterica* serovar Typhimurium strain that carried a recombinant plasmid expressing the S1 region of the S gene [23]. Currently, although PEDV oral vaccines and DNA vaccines are still at the research stage and have not been applied in Chinese pig populations, these studies have broadened our understanding of PEDV vaccines, and they have provided a foundation for the future development of highly effective PEDV vaccines.

CONCLUDING REMARKS

Recently, a large number of studies of PEDV have provided an in-depth understanding of PEDV infections in the pig population in China. In particular, a trivalent live vaccine for viral diarrheal diseases of swine has been authorized for

use, and it is a powerful tool for the control of PED. However, virus variability and co-infections of PEDV with other enteric viruses are unpredictable. These factors make it difficult to understand and control PEDV infections. Therefore, future work should continue to trace the variability of PEDV strains and co-infections of other enteric viruses, improve PEDV vaccines using variant strains as candidates and elucidate the pathogenicity of PEDV variant strains.

ACKNOWLEDGMENTS. This work is supported by the National Natural Science Foundation of China (grant no. 31472209), the State National Key Laboratory of Veterinary Biotechnology (grant no. SKLVBF201302) and the Program for New Century Excellent Talents in Heilongjiang Provincial University (grant no. 1252-NCET-016).

REFERENCES

1. Chen, J., Liu, X., Shi, D., Shi, H., Zhang, X. and Feng, L. 2012. Complete genome sequence of a porcine epidemic diarrhea virus variant. *J. Virol.* **86**: 3408. [Medline] [CrossRef]
2. Chen, J., Liu, X., Shi, D., Shi, H., Zhang, X., Li, C., Chi, Y. and Feng, L. 2013. Detection and molecular diversity of spike gene of porcine epidemic diarrhea virus in China. *Viruses* **5**: 2601–2613. [Medline] [CrossRef]
3. Chen, J., Wang, C., Shi, H., Qiu, H., Liu, S., Chen, X., Zhang, Z. and Feng, L. 2010. Molecular epidemiology of porcine epidemic diarrhea virus in China. *Arch. Virol.* **155**: 1471–1476. [Medline] [CrossRef]
4. Chen, J., Wang, C., Shi, H., Qiu, H., Liu, S., Shi, D., Zhang, X. and Feng, L. 2011. Complete genome sequence of a Chinese virulent porcine epidemic diarrhea virus strain. *J. Virol.* **85**: 11538–11539. [Medline] [CrossRef]
5. Chen, J. F., Feng, L., Shi, H. Y. and Cui, S. J. 2010. Update on vaccines of porcine epidemic diarrhea virus. *Swine Ind. Sci.* **12**: 51 (in Chinese).
6. Chen, J. F., Sun, D. B., Wang, C. B., Shi, H. Y., Cui, X. C., Liu, S. W., Qiu, H. J. and Feng, L. 2008. Molecular characterization and phylogenetic analysis of membrane protein genes of porcine epidemic diarrhea virus isolates in China. *Virus Genes* **36**: 355–364. [Medline] [CrossRef]
7. 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. and 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. [Medline] [CrossRef]
8. Chen, X., Zeng, L., Yang, J., Yu, F., Ge, J., Guo, Q., Gao, X. and Song, T. 2013. Sequence heterogeneity of the ORF3 gene of porcine epidemic diarrhea viruses field samples in Fujian, China, 2010–2012. *Viruses* **5**: 2375–2383. [Medline] [CrossRef]
9. Debouck, P. and Pensaert, M. 1980. Experimental infection of pigs with a new porcine enteric coronavirus, CV 777. *Am. J. Vet. Res.* **41**: 219–223. [Medline]
10. Feng, L. 2014. The updated epidemic and controls of swine enteric coronavirus in China. *In*: International Meeting of Swine Enteric Coronavirus Diseases (SECD), Chicago.
11. Gao, Y., Kou, Q., Ge, X., Zhou, L., Guo, X. and Yang, H. 2013. Phylogenetic analysis of porcine epidemic diarrhea virus field strains prevailing recently in China. *Arch. Virol.* **158**: 711–715. [Medline] [CrossRef]
12. Ge, F. F., Yang, D. Q., Ju, H. B., Wang, J., Liu, J., Liu, P. H. and Zhou, J. P. 2013. Epidemiological survey of porcine epidemic diarrhea virus in swine farms in Shanghai, China. *Arch. Virol.* **158**: 2227–2231. [Medline] [CrossRef]
13. Ge, J., Jiang, Y., Wang, M., Qiao, X., Liu, M., Tang, L. and Li, Y. 2009. Induction of immune response after oral inoculation of mice with Lactobacillus casei surface-displayed porcine epidemic diarrhea viral N protein. *Chin. J. Biotechnol.* **25**: 813–818 (in Chinese). [Medline]
14. Ge, J. W., Liu, D. Q. and 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. [Medline] [CrossRef]
15. Hao, J., Xue, C., He, L., Wang, Y. and 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. [Medline] [CrossRef]
16. Hou, X. L., Yu, L. Y., Liu, J. and Wang, G. H. 2007. Surface-displayed porcine epidemic diarrhea viral (PEDV) antigens on lactic acid bacteria. *Vaccine* **26**: 24–31. [Medline] [CrossRef]
17. Huang, Y. W., Dickerman, A. W., Piñeyro, P., Li, L., Fang, L., Kiehne, R., Opriessnig, T. and Meng, X. J. 2013. Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. *MBio* **4**: e00737–e13. [Medline]
18. Ji, B. Y. and Zhong, X. G. 1987. Report of investigation and diagnosis of porcine epidemic diarrhea virus infection. *Jiangxi J. Anim. Husb. Vet. Med.* **1**: 19–20 (in Chinese).
19. Li, R., Qiao, S., Yang, Y., Su, Y., Zhao, P., Zhou, E. and 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. [Medline] [CrossRef]
20. Li, W., Li, H., Liu, Y., Pan, Y., Deng, F., Song, Y., Tang, X. and He, Q. 2012. New variants of porcine epidemic diarrhea virus, China, 2011. *Emerg. Infect. Dis.* **18**: 1350–1353. [Medline] [CrossRef]
21. Li, Z., Chen, F., Yuan, Y., Zeng, X., Wei, Z., Zhu, L., Sun, B., Xie, Q., Cao, Y., Xue, C., Ma, J. and Bee, Y. 2013. Sequence and phylogenetic analysis of nucleocapsid genes of porcine epidemic diarrhea virus (PEDV) strains in China. *Arch. Virol.* **158**: 1267–1273. [Medline] [CrossRef]
22. Li, Z. L., Zhu, L., Ma, J. Y., Zhou, Q. F., Song, Y. H., Sun, B. L., Chen, R. A., Xie, Q. M. and Bee, Y. Z. 2012. Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field strains in south China. *Virus Genes* **45**: 181–185. [Medline] [CrossRef]
23. Liang, E. T., Liao, X. D., Huang, X. B., Wen, X. T., Cao, S. J., Yang, Y. P., Duan, S. F., Zhang, D. and Zhang, X. H. 2014. Construction and identification of attenuated Salmonella typhimurium harboring S gene of porcine epidemic diarrhea virus. *Chin. Vet. Sci.* **44**: 264–270 (in Chinese).
24. Licitra, B. N., Duhamel, G. E. and Whittaker, G. R. 2014. Canine enteric coronaviruses: emerging viral pathogens with distinct recombinant spike proteins. *Viruses* **6**: 3363–3376. [Medline] [CrossRef]
25. Liu, D. Q., Ge, J. W., Qiao, X. Y., Jiang, Y. P., Liu, S. M. and Li, Y. J. 2012. High-level mucosal and systemic immune responses induced by oral administration with Lactobacillus-expressed porcine epidemic diarrhea virus (PEDV) S1 region combined with Lactobacillus-expressed N protein. *Appl. Microbiol. Bio-*

technol. **93**: 2437–2446. [Medline] [CrossRef]
26. Ma, S. Q., Wang, M. and Feng, L. 1995. Development of bicombined inactivated vaccine against transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *Chin. Anim. Infect. Dis.* **17**: 23–27 (in Chinese).
27. Ma, S. Q., Wang, M., Zhou, J. F. and Feng, L. 1994. Adaptation of porcine epidemic diarrhea virus to Vero cells and evaluation of the inactivated vaccine against porcine epidemic diarrhea virus. *Chin. Anim. Infect. Dis.* **2**: 15–18 (in Chinese).
28. Meng, F., Ren, Y., Suo, S., Sun, X., Li, X., Li, P., Yang, W., Li, G. and Li, L. 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. [Medline] [CrossRef]
29. Pasick, J., Berhane, Y., Ojkic, D., Maxie, G., Embury-Hyatt, C., Swekla, K., Handel, K., Fairles, J. and 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. [Medline] [CrossRef]
30. Pensaert, M. B. and de Bouck, P. 1978. A new coronavirus-like particle associated with diarrhea in swine. *Arch. Virol.* **58**: 243–247. [Medline] [CrossRef]
31. Regan, A. D., Millet, J. K., Tse, L. P., Chillag, Z., Rinaldi, V. D., Licitra, B. N., Dubovi, E. J., Town, C. D. and Whittaker, G. R. 2012. Characterization of a recombinant canine coronavirus with a distinct receptor-binding (S1) domain. *Virology* **430**: 90–99. [Medline] [CrossRef]
32. Song, D. and Park, B. 2012. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. *Virus Genes* **44**: 167–175. [Medline] [CrossRef]
33. Sun, D., Feng, L., Shi, H., Chen, J., Cui, X., Chen, H., Liu, S., Tong, Y., Wang, Y. and Tong, G. 2008. Identification of two novel B cell epitopes on porcine epidemic diarrhea virus spike protein. *Vet. Microbiol.* **131**: 73–81. [Medline] [CrossRef]
34. Sun, R., Leng, Z., Zhai, S. L., Chen, D. and Song, C. 2014. Genetic variability and phylogeny of current Chinese porcine epidemic diarrhea virus strains based on spike, ORF3, and membrane genes. *Scientific World Journal* **2014**: 208439. [Medline] [CrossRef]
35. 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. and 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. [Medline] [CrossRef]
36. Tong, Y. E., Feng, L. and Li, W. J. 1998. Development of attenuated vaccine strain of porcine epidemic diarrhea virus. *Chin. Anim. Infect. Dis.* **20**: 329–332 (in Chinese).
37. Tong, Y. E., Feng, L. and Li, W. J. 1999. Development of bicombined attenuated vaccine against transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *Chin. J. Prev. Vet. Med.* **21**: 406–410 (in Chinese).
38. Wang, M., Ma, S. Q., Zhou, J. F., Feng, L., Tong, Y. E., Yu, W. T., Wei, F. X., Cui, X. L., Huang, S. T. and Liu, C. M. 1993. Development of the inactivated vaccine of porcine epidemic diarrhea virus. *Chin. Anim. Infect. Dis.* **5**: 17–19 (in Chinese).
39. Wang, X. N., Li, X. M. and Li, C. L. 1989. Report of investigation and diagnosis of porcine epidemic diarrhea virus infection. *Zhejiang J. Anim. Sci. Vet. Med.* **4**: 22–23 (in Chinese).
40. Wood, E. N. 1977. An apparently new syndrome of porcine epidemic diarrhoea. *Vet. Rec.* **100**: 243–244. [Medline] [CrossRef]
41. Xuan, H. 1984. Culture of porcine epidemic diarrhea virus by using pig intestinal monolayer cell. *J. People's Liberation Army Vet. Univ.* **4**: 202–208 (in Chinese).
42. Yang, X., Huo, J. Y., Chen, L., Zheng, F. M., Chang, H. T., Zhao, J., Wang, X. W. and Wang, C. Q. 2013. Genetic variation analysis of reemerging porcine epidemic diarrhea virus prevailing in central China from 2010 to 2011. *Virus Genes* **46**: 337–344. [Medline] [CrossRef]
43. Yu, Z. F. 1986. Porcine epidemic diarrhea diseases. *Contemp. Livest.* **2**: 65–66 (in Chinese).
44. Zhang, B., Tang, C., Yue, H., Ren, Y. and Song, Z. 2014. Viral metagenomics analysis demonstrates the diversity of viral flora in piglet diarrhoeic faeces in China. *J. Gen. Virol.* **95**: 1603–1611. [Medline] [CrossRef]
45. Zhang, Z., Dong, Y. Q., Liu, S., Wu, F. X., Shao, W. X. and Li, X. C. 2014. Survey and surveillance of porcine epidemic diarrhea in some provinces in China. *Chin. Anim. Heal. Insp.* **31**: 47–51 (in Chinese).