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Porcine epidemic diarrhea in China

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ABSTRACT

Porcine epidemic diarrhea (PED) is a contagious intestinal disease caused by *Porcine epidemic diarrhea virus* (PEDV) that characterized by vomiting, diarrhea, and dehydration. PEDV was first identified in the 1980s in China, and since then, it has become one of the most common viral causes of diarrhea. In October 2010, a large-scale outbreak of PED caused by a PEDV variant occurred in China, resulting in tremendous economic losses. This review presents a comprehensive description of PEDV history, prevalence, molecular features, and prevention and control strategies in China.

Contents

1. Introduction

Porcine epidemic diarrhea (PED) is caused by *Porcine epidemic diarrhea virus* (PEDV) and manifests in a similar way to transmissible gastroenteritis (TGE) in swine, with symptoms including diarrhea, vomiting, anorexia, dehydration, and weight loss in piglets (Have et al., 1992; Sueyoshi et al., 1995). Although pigs of all ages can be infected and show symptoms to differing degrees, the condition is especially severe in piglets, among which the mortality rate is up to 100% (Shibata et al., 2000; Sun et al., 2012). This disease is currently causing serious damage to the pig farming industry. The virus strain was isolated for the first time in Belgium and designated coronavirus CV777 (Pensaert and de Bouck, 1978). The spread of PED in Europe has been under control since 2000, but the disease is still pandemic in Asian countries, including China, South Korea, and Japan (Kusanagi et al., 1992; Song and Park, 2012; Takahashi et al., 1983).

In 1973, this TGE-like virus disease was identified for the first time in China, but not until 1984 was the pathogen, PEDV, isolated (Xuan et al., 1984). Despite the progress made in both basic and applied research into PEDV, PED is still common among newborn

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piglets, jeopardizing the pig farming industry. In October 2010, a highly pathogenic variant PEDV strain was identified in China, and later in 2013, the variant caused a pandemic in the USA, spreading to Canada and Mexico (Chen et al., 2012a; Chen et al., 2012b; Cima, 2013; Li et al., 2012; Jung and Saif, 2015; Ojkic et al., 2015; Pan et al., 2012; Sun et al., 2012; Tian et al., 2014; Yang et al., 2014; Yang et al., 2013). PEDV has spread widely to many countries in Europe, Asia, American, and Australia. PED is recognized worldwide for the dramatic changes observed in its epidemic character and pathogenic molecules. This paper comprehensively describes PEDV, including its history in China, its prevalence, its molecular features, and the preventive strategies used to control it.

2. PED history in China

Porcine epidemic diarrhea was first reported in the United Kingdom in 1971 and designated "epidemic viral diarrhea" (EVD) (Oldham, 1972). In 1976, a similar viral diarrhea epidemic occurred in several European countries, and was designated EVD II (Chasey and Cartwright, 1978). Since then, the disease has been reported in many other European countries. In 1978, Belgian scientists collected several EVD samples, including one designated CV777, which was later shown experimentally to be a new coronavirus strain causing diarrhea in pigs (Debouck and Pensaert, 1980; Pensaert and De Bouck, 1978). The disease was collectively designated "porcine epidemic diarrhea" (PED) in 1982 (Pensaert et al., 1982).

Reports of PED have been published continuously in China since 1973. These reports describe the clinical manifestations, which are similar to those of TGE, but the pathogen was not identified (Huang et al., 1980) until 1984, when Xuan et al. confirmed the presence of PED in China with a fluorescently labeled antibody and serum neutralization tests (Xuan et al., 1984). The isolated strain was first successfully cultured in a monolayer of primary cells from fetal porcine intestinal tissue, and in 1988 Hofmann and Wyler first continuously passaged PEDV in African green monkey kidney (Vero) cells by adding trypsin to the medium. The cells showed significant cytopathy, and the virus was isolated from cells for the first time (Hofmann and Wyler, 1988). Later, Li et al. (1991) also isolated PEDV from Vero cells. This research provided a basis for further research into the diagnosis of PEDV and the development of a PEDV vaccine (Li et al., 1991). Qian et al. (1999) generated a trypsin-independent strain of PEDV by subculturing virulence-bearing cells to the fifth generation in medium in which the content of trypsin was reduced stepwise (Qian et al., 1999).

Although PEDV infection still affected Chinese pig farms before 2010, it was sporadic and regional. No large-scale epidemic occurred until an outbreak, clinically characterized by acute diarrhea, originated in the southern provinces in October 2010, and soon swept throughout the country (Li et al., 2012; Sun et al., 2012; Tian et al., 2014; Wang et al., 2013; Sun et al., 2015a). The mortality rate of PED among piglets soared to 80%–100%, and the pig farming industry suffered a destructive blow (Sun et al., 2012).

3. PEDV prevalence in China

Regional outbreaks of PED have been seen in China since the early 1980s. According to an incomplete statistical analysis of a general survey of PED epidemics in 26 provinces, municipalities, and autonomous regions of China between 1987 and 1989, the mortality attributable to PED accounted for 1.74% of the overall mortality from 36 pig diseases, whereas TGE accounted for 9.53% (Li et al., 2010). In 2004, an epidemiological survey of PEDV was undertaken by Du et al. in six cities of Guangxi Province. The incidence rate of PED was 42% (4658/11,090), with a mortality rate of 5.69% (265/11090), whereas the incidence rate of PED in piglets was 46.4% (4302/9212), with a mortality rate of 6.16% (256/9212); the incidence rate in sows was 19.5% (356/1878), but no death was reported (Du et al., 2004). These data suggest that young piglets are at greater risk of infection and death from PED than adult pigs. A study of different porcine diarrheal diseases by Gan et al. between 2005 and 2007 demonstrated that PED accounted for 46% of all cases of porcine diarrheal disease (Gan et al., 2010). Zhang et al. established a multiplex reverse transcription (RT)–PCR method specific for TGEV, PEDV, and porcine rotavirus (PoRV) to investigate 197 diarrheal samples collected from Hubei, Hunan, Guangxi, Jiangxi, Henan, and other provinces in 2009–2010. Their results showed infection rates for TGEV, PEDV, and PoRV of 0.51% (1/197), 29.44% (58/197), and 7.61% (15/197), respectively, indicating that PEDV is the major pathogen causing porcine diarrhea in China (Zhang, 2010).

In the winter of 2010, a PED outbreak began on pig farms in southern China and immediately spread throughout the country. The disease had a death toll of over one million piglets in South China, with devastating damage to the pig industry. Even vaccinated suckling piglets were not spared, and the morbidity and mortality rates were almost 100% (Li et al., 2012; Sun et al., 2012; Wang et al., 2013). The affected piglets predominantly showed yellow watery stools, weight loss, and death from dehydration. The pathogen was a variant PEDV strain, confirmed by sequencing the whole viral genome (Li et al., 2012). Using a real-time PCR method developed by Bi and colleagues in our team, the average infection rate with PEDV was 58.32% and the PEDV-positive rate was over 50% in all provinces, based on more than 600 tissue and stool samples collected from nine provinces in East China, South China, and Central China between 2010 and 2011. Therefore, the prevalence of PEDV was very high in these provinces. Whole-genome sequencing also indicated that PEDV strain AJ1102 isolated from the positive pathological samples was the currently prevalent variant strain (Bi et al., 2012). Further animal experiments showed that the strain was associated with the typical clinical symptoms of PED, including watery diarrhea, vomiting, dehydration, and high mortality in newborn piglets. Among 3–5-day-old suckling piglets, the symptoms started 2–3 days after infection and the mortality rate was 100%; among 28-day-old weaned piglets, the symptoms started 2–3 days after infection and diarrhea persisted for more than 5 days (unpublished data). Liu et al. (2012) investigated the small intestines and stools of affected pigs collected from 12 Chinese provinces and cities, using RT–PCR, colloidal gold, and other methods to detect common intestinal pathogens. The results indicated that the morbidity rate of single PEDV infection was 33.33% (73/219) and the morbidity rate of mixed infections of PEDV, TGEV, and PoRV was 27.85% (61/219). The overall infection rate for PEDV was 61.19% (134/219), which was much higher than the infection rates for the other two enteric pathogens (Liu, 2012). In an epidemiological survey undertaken from February 2011 to March 2014, PEDV epidemics were reported in 29 provinces of China, with the exception of Tibet and Hainan, and the rates of PEDV-positive samples were 61.10%–78.49%, whereas the rates of PEDV-positive pig farms were 71.43%–83.47% (Zhang et al., 2014; Feng, 2014).

With the widespread use of inactivated or attenuated bivalent vaccines for PEDV in China, the nationwide spread of PED was controlled until October 2010, when a variant strain emerged and PEDV infection increased dramatically, seriously damaging the pig industry.

4. Molecular characteristics of prevalent PEDV in China

4.1. Genotyping prevalent PEDV strains in China

To analyze the genetic backgrounds of Chinese PEDV strains, we constructed a phylogenetic genetic tree based on complete

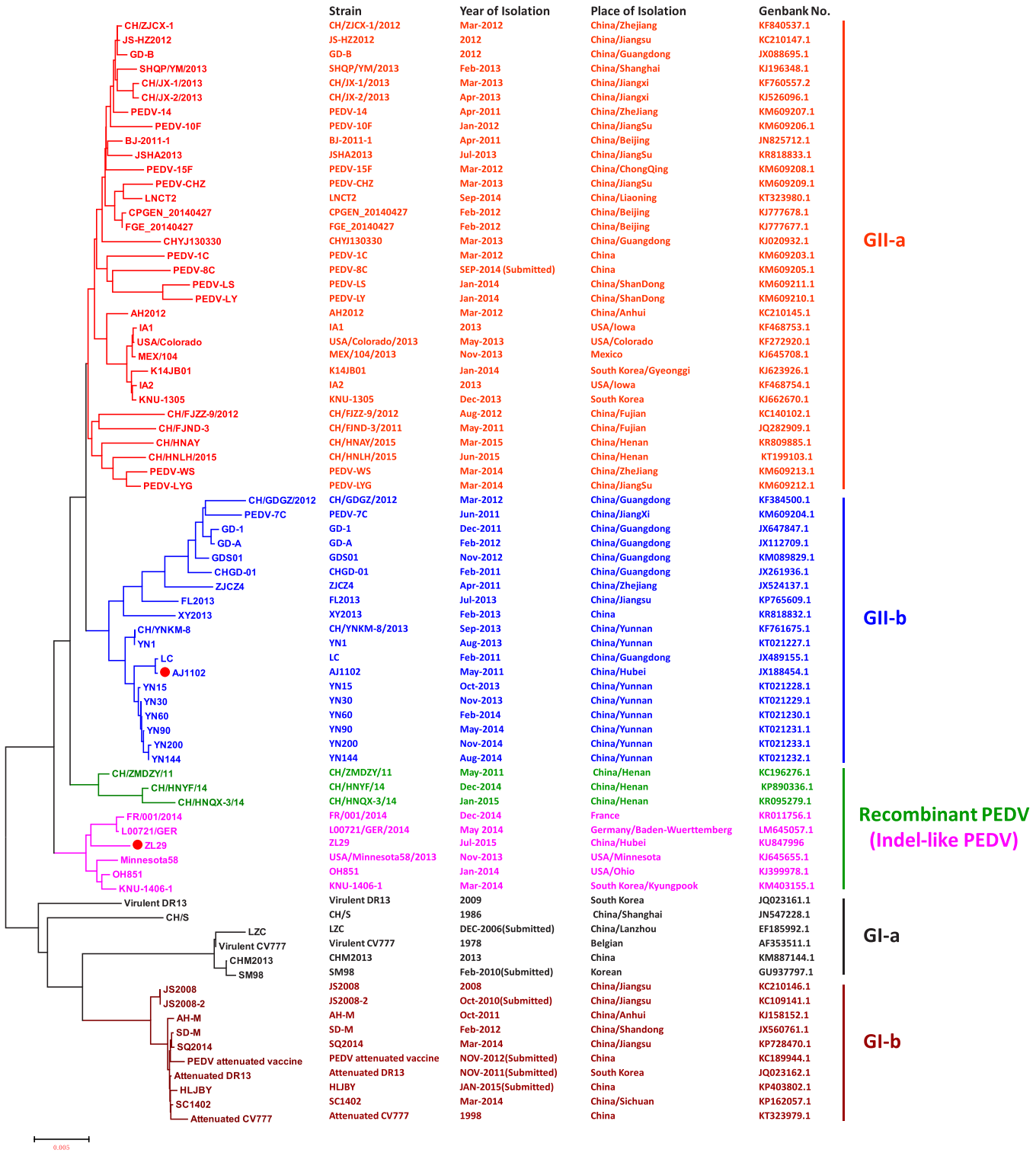

Fig. 1. Genotyping the PEDV strains in China based on a full-length genomic sequence analysis. Phylogeny-based genotyping of 62 PEDV strains in China for which complete genomic sequences were available. The tree was constructed with the neighbor-joining method, and bootstrap values from 1000 resamplings are shown for each node. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7. The names of the strains, years and places of isolation, GenBank accession numbers, and genogroups and subgroups proposed in this study are shown.

genomic sequences of over 60 Chinese PEDV strains and 15 PEDV representative strains of other countries. As showed in Fig. 1, these PEDV strains can be sorted into two genogroups, GI (classical) and GII (variant). Each genogroup has two sub-groups: GI-a, GI-b, GII-a, and GII-b (Fig. 1). The Chinese classical strains (LZC, CH/S, and CHM2013), along with the prototype strains (virulent CV777 and DR13), belong to the GI-a sub-group, whereas GI-b sub-group predominantly consists of the cell-culture-adapted vaccine strains (attenuated CV777 and DR13) and other pandemic classical strains (AH-M, SD-M, SQ2014, and SC1402). The prevalent strains out-

broke previously in Asia in late 2010 and the prevalent strains outbroke recently in the Asian and North American belong to the GII genogroup (Huang et al., 2013; Lee, 2015). Since 2010, the identification and sequencing analyses of PEDV strains have shown that variant PEDV strains (GII genogroup) are highly prevalent in China, and most of the isolated strains are members of GII genogroup, whereas CV777 and pandemic strain DR13 reported in South Korea in 2008 are members of GI genogroup (Fig. 1). Because the GI and GII strains are not genetically closely related, the currently available vaccines (attenuated CV777 and DR13, GI genogroup) failed to provide complete protection against the variant strains (GII genogroup).

4.2. Variations in the prevalent PEDV in China

Compared with classic strains such as CV777 (GI genogroup), the major PEDV strains prevalent in China are new variant strains (GII genogroup), the products of various evolutionary trajectories (Xia et al., 2013). An analysis of whole-genome differences showed that four hypervariable regions are present in PEDV, V1, V2, V3, and V4. V1 mainly includes the C terminus of the nsp2 gene and the N terminus of the nsp3 gene (Sun et al., 2015b). V2 is located in the S1 region of the S gene, which is involved in receptor binding and in which excessive changes can lead to the cross-species or cross-organ transmission of PEDV (Belouzard et al., 2012). V3 spans from the C terminus of the S gene to open reading frame 3 (ORF3), and changes in this segment can distinguish wild-type prevalent strains (GI-a, GII-a, and GII-b sub-groups) from the cell-culture-adapted vaccine strains (attenuated DR13, GI-b sub-group) (Park et al., 2008). It has been reported that changes in ORF3 are associated with virus adaptation during serial passage in cells (Ye et al., 2015). V4 is located in the N gene, which contradicts the idea that the N protein is highly conserved (Li et al., 2013). Further comparison of the genes of the variant (GII genogroup) and classical (GI genogroup) PEDV strains revealed that the greatest changes occur in the S gene in the variant strains, especially in the S1 region. Compared with the classical strain CV777, the S1 region (nt l–2217) in the variant strains is characterized by three insertions (at nt 162, nt 170–180, and nt 413–415) and one deletion (nt 470–475). A comparison of the S1 neutralization epitopes of the variant and classical PEDV strains also detected mutations in all three important epitopes (248–280 aa, 442–499aa, and 697–742 aa) (Sun et al., 2007), which are possibly the major factors contributing to the increased virulence of these strains (GII genogroup) and the failure of vaccines (GI genogroup) to protect pigs against them (Sun et al., 2015b).

4.3. Origin of PEDV variants in China

Only sporadic outbreaks of PEDV occurred before 2005, and when Sun et al. (2015b) genotyped the Asian PEDV strains and subjected them to a regional phylogenetic analysis, the pathogens involved in these outbreaks were four classical strains in China (Genbank IDs: KC109141, JN547228, EF185992, and JX560761), one classical strain in Japan (Genbank ID: KT968509), and three classical strains in South Korea (Genbank IDs: JQ023161, GU937797, and KF898124). However, two pandemic strains were reported in South Korea (Genbank IDs: KC189828 and KC189829) that could have been the origins of the subsequent outbreaks of PEDV in Asia. By 2010, as more regions were affected by PEDV, pandemic strains were reported in South Korea, two pandemic strains and one classical strain were reported in Japan, and four classical strains were detected in China. Two new pandemic strains were also reported for the first time in Thailand. Between 2010 and 2011, frequent sporadic PEDV epidemics occurred in many regions of Thailand and South Korea, but the infection rates were low. However, in China, pigs of all ages suffered a serious PED epidemic (Li et al., 2012; Sun et al., 2012; Wang et al., 2013). The strains that prevailed in China were not only those pandemic variant strains (GII genogroup), but also the pandemic classical strains (AH-M, SD-M, SQ2014, and SC1402, GI-b sub-group). Between 2012 and 2014, the influence of the pandemic that affected Thailand, South Korea, and China had even spread to Vietnam (Sun et al., 2015b). The PEDV-free record of the USA was compromised in May 2013 by the first report of PEDV infection. A phylogenetic analysis indicated that the PEDV strain identified in the USA was highly homologous to the strain prevalent in China since 2010. This strain was then designated “AH2012-like Chinese PEDV” by researchers (Huang et al., 2013; Jung and Saif, 2015). However, a phylogenetic analysis of the Korean PEDV field isolates and other reference strains, based on the complete E and M genes, showed that the Korean PEDV strains isolated between 2007 and 2010 and the currently prevalent USA PEDV strain (USA/Colorado/2013 strain) belonged to the same evolutionary group (Kim et al., 2015). We generated a phylogenetic tree, based on the E gene, of the USA/Colorado/2013 strain from the USA, the PEDV strains collected between May 2010 and May 2011 in South Korea (Park et al., 2013), and strain AH2012 from China, which also indicated that the USA strain belonged to the genogroup containing the South Korean strains (unpublished data). Together, these results suggest that the USA strain most likely originated in South Korea, and that the Chinese variant strain (GII genogroup) also originated in South Korea (Kim et al., 2015; Sun et al., 2015b).

4.4. Emergence of recombinant PEDV in China

Currently, several PEDV strains with different S1 gene deletions have been isolated in the USA, so the virulence-determining S1 gene is undergoing rapid mutation. In 2014, the first S-INDEL-variant PEDV, named OH851, emerged in the USA. It shows a new insertion and deletion in S gene relative to the prototype PEDV discovered in 2013 (Vlasova et al., 2014; Wang et al., 2014). The S-INDEL-variant PEDV is less pathogenic than its prototype, so it has caused less damage to the industry (Goede et al., 2015; Lin et al., 2015). Our team recently examined the S1 genes of 320 PEDV-positive samples, and found eight samples possibly infected with an S-INDEL-like virus. Whole-genome sequencing of one of the samples showed that PEDV ZL29, isolated by our team, and the S-INDEL variant strain clustered on the same evolutionary branch, indicating that the S-INDEL-variant PEDV emerged in China (Fig. 1). A further investigation demonstrated that the recombination of this PEDV strain mainly occurred between classical and variant strains in the S gene, suggesting that recombination occurred in the S gene of Chinese PEDV strains (unpublished data). Li et al. (2016) also isolated a recombinant PEDV, designated CH/HNQX-3/14, and a recombinant analysis showed that CH/HNQX-3/14 arose from the recombination of an attenuated vaccine strain (CV777 or DR13) and a pandemic variant (CH/ZMDZY/11). This recombination occurred not only in the structural-protein-coding region (S and N genes), but also in the nonstructural-protein-coding region (replicase 1a and ORF3) (Li et al., 2016).

A whole-genome sequence analysis of 77 PEDV strains from both China and elsewhere indicated that seven strains discovered in China between 2006 and 2015 and the attenuated vaccine strains (attenuated CV777 and DR13, GI-b sub-group) clustered on the same evolutionary branch, and that two of the Chinese strains (LZC and CHM2013) clustered on the same evolutionary branch as the prototype CV777 strain from Belgium (GI-a sub-group). The 50 strains isolated in China between 2011 and 2015 clustered on the same evolutionary branch as the recently reported pandemic PEDV strains from the USA, Mexico, and South Korea (Fig. 1). There is also evidence that the Chinese strains display serious mixed infection with strains from other countries. Cross-regional and transnational

Table 1
Overview of vaccines against PEDV in China.

Vaccine Name	PEDV strain	Genogroup	Vaccine-approval times
Bi-combined inactivated vaccine of PEDV and TGEV	CV777	GI-a	1995
Bi-combined attenuated vaccine of PEDV and TGEV	CV777	GI-a	1998
Three-combined attenuated vaccine of PEDV, TGEV and PRoV	CV777	GI-a	2015
Bi-combined attenuated vaccine of PEDV and TGEV	ZJ08	GI-b	2015
Bi-combined inactivated vaccine of PEDV and TGEV	AJ1102	GII-b	Clinical re-examination
Bi-combined attenuated vaccine of PEDV and TGEV	AJ1102	GII-b	Clinical examination

communication has greatly facilitated the mutation of PEDV and its pandemic outbreaks (Sun et al., 2015b).

5. Prevention and control of prevalent PED in China

As demonstrated in clinical practice, even vaccinated pigs are not protected from PEDV infection, so the vaccines derived from classical strains are insufficiently protective (Li et al., 2012; Sun et al., 2012; Wang et al., 2013). Researchers from Thailand and South Korea have also confirmed that all available commercial vaccines (GI genogroup) do not provide adequate immune protection against the currently prevalent strains (GII genogroup) (Ayudhya et al., 2012; Lee, 2015). This phenomenon may be caused by the dramatic mutation of the virus, posing a major challenge to the prevention and control of PED in China.

When a PEDV infection occurs on a pig farm, the infection usually spreads among pigs of different ages in the following sequence: first infected are the fattening/replacement pigs; then the virus accumulates and infects pregnant sows, bringing the virus to the delivery room; the subclinically infected sows then transmit PEDV to the suckling piglets, giving rise to an eventual epidemic among the piglets. The major transmission route for PEDV is the fecal–oral route, so the sources of infection are vomit, diarrheal stools, and related pollutants. Therefore, when an epidemic emerges, measures must be taken to close the transmission route, including isolating the infected pigs and closing any infected pig houses. Strict biosecurity measures are very effective in protecting unaffected pigs (Li, 2011). Immunization with infected intestinal contents is also effective to some extent, but the inhomogeneous distribution of PEDV in the intestinal contents may compromise the efficacy of this treatment and cause repeated infections of PEDV (Xu, 2014).

5.1. Traditional vaccines

In 1993, an inactivated tissue vaccine against PEDV was prepared by Wang et al. by orally administering virulent PEDV to piglets and collecting their intestinal contents and tissues. The samples were inactivated and prepared as an emulsion for injection into the base of the tail (Wang et al., 1993). Ma et al. (1994) developed an aluminum-hydroxide-adjuvanted inactivated vaccine by attenuating the CV777 strain in Vero cells. Both the active and passive immunization rates among piglets were over 85% after the vaccine was injected into the tail base (Ma et al., 1994). In 1995, the same group successfully developed a commercialized bivalent inactivated TGEV and PEDV vaccine (Ma et al., 1995). Tong et al. (1998) confirmed that the attenuated PEDV CV777 strain was suitable for the preparation of an attenuated vaccine, and prepared an attenuated PEDV strain with successive passages *in vitro* (Tong et al., 1998). An attenuated PEDV vaccine was developed using the strain cloned for 90 generations. In 1999, they developed a commercialized bivalent attenuated TGEV and PEDV vaccine, with attenuated TGEV and PEDV clones in a ratio of 1:1, which produced active and passive immunization rates of 97.7% and 98%, respectively (Tong et al., 1999). The PEDV strain used in both these two commercialized vaccines was the classic CV777 strain. Before 2010, both vaccines were widely used on Chinese pig farms and played a very important role in controlling PEDV and TGEV infections.

Although a variety of methods, including autogenous vaccines, commercialized vaccines, and intestinal content feeding, have been tested to control outbreaks of PED since the end of 2010, their efficacy has always been poor. Repeated diarrhea epidemics are still reported on some pig farms. Inadequate knowledge of the pathogenic mechanisms of the PEDV variants and the immune response to them has limited the effectiveness of the preventive methods used. After PED outbreaks in the USA in 2013, an inactivated vaccine was developed by Collin et al. based on the isolated US variant PEDV strain (GII genogroup) and was used to vaccinate 4-week-old piglets. The inactivated vaccine triggered sufficient humoral immunity against PEDV to prevent infection (Collin et al., 2015). In March 2015, a trivalent vaccine developed from attenuated TEGV (H strain), PEDV (CV777 strain, GI-a sub-group), and PoRV (NX strain) was approved in China. In November 2015, a dual attenuated vaccine combining TGEV (HB08 strain) and PEDV (ZJ08 strain, GI-b sub-group) was also officially launched on the market (Table 1). Considering that the differences between vaccines commercially available (GI genogroup) and field epidemic strains (GII genogroup), it should develop next generation vaccines against field epidemic PEDV strains to control PED. Recently, Chinese researchers have developed bivalent (PEDV and TGEV) inactivated vaccines based on the field epidemic PEDV strain within GII genogroup, and experimental and clinical data showed that this novel vaccine exerts better protective effects against GII epidemic PEDV. Pigs vaccinated with this inactivated vaccine based on PEDV variant strain within GII genogroup induce significantly higher neutralization antibody titers against PEDV variant strain (GII genogroup) than vaccines commercially available (GI genogroup). Nowadays, this vaccine based on the prevalent PEDV (GII genogroup) is under clinical re-examination in China.

5.2. New-generation vaccines

Hou et al. constructed a *Lactobacillus* expressing the PEDV-N protein, which stimulated the porcine intestinal mucosa to produce N-protein-specific immunoglobulin A (IgA) and IgG (Hou et al., 2007). According to the findings of Ge et al., the oral administration of the lactobacillus vaccine expressing the N protein and the core neutralizing epitope stimulated the local immune response in the small intestine as well as a systemic immune response to PEDV (Ge et al., 2009; Ge et al., 2012). Oral immunization with the recombinant lactobacillus expressing the PEDV-N gene or the S1 region of the S gene, constructed by Liu et al., effectively enhanced the mucosal and systemic immune responses (Liu et al., 2012). Meng et al. constructed a recombinant eukaryotic plasmid expressing the PEDV S gene, and the vaccinated animals showed high anti-PEDV antibody levels and cellular immunity (Meng et al., 2013). Research into oral PEDV vaccines and DNA vaccines is ongoing, and no approved products have yet been used. The research into new-generation vaccines should provide new strategies for vaccine

development and allow the development of high-efficacy PEDV vaccines.

In conclusion, PED is now one of the most potent threats to the pig farming industry in China. The hypervariability of PEDV makes field pandemics more complex and heterogeneous, posing major challenges to the development of efficient vaccines. Because variant PEDV strains (GII genogroup) are more epidemic than classical strains (GI genogroup), basic research into PEDV has advanced rapidly. Currently, the whole genomes of over 60 strains of Chinese PEDV have been determined and their genetic features have been characterized. The interactions between PEDV and the immune system of the host have also been largely clarified (Cao et al., 2015; Ding et al., 2014; Wang et al., 2016; Xing et al., 2013). All these studies have provided critical information that has allowed the sources of variants to be traced and the evolutionary mechanisms involved identified. They will also facilitate the development of diagnostic kits, vaccines, and new therapeutic strategies, which are expected to turn the tide in the prevention of pandemic outbreaks of PEDV.

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