

Genetic properties of endemic Chinese porcine epidemic diarrhea virus strains isolated since 2010

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Abstract Acute diarrhea outbreaks caused by porcine epidemic diarrhea virus (PEDV) have been observed in various pig-breeding provinces of China since December 2010. Endemic strains of PEDV were isolated from different areas, and the complete genome sequences of 10 isolates were determined. Our objective in this study was to genetically characterize current Chinese field isolates of PEDV to better understand their epidemiology and genetic diversity. Sequence analysis showed that 10 post-2010 isolates shared high homology with each other and were always clustered together with the virulent DR13 strains (South Korea) and/or one earlier Chinese strain, CH-S, in phylogenetic analysis. All post-2010 isolates possessed common sequence changes in each gene. Our results suggest that current Chinese PEDV isolates originated from either South Korean and/or Chinese ancestors that underwent some genetic variation, thereby forming a new PEDV genotype in China.

Introduction

Porcine epidemic diarrhea virus (PEDV) is a positive-sense single-stranded RNA virus belonging to group 1 of the genus *Alphacoronavirus*, within the family *Coronaviridae*. The virus is the etiological agent of porcine epidemic diarrhea (PED), an acute and highly contagious enteric disease characterized by watery diarrhea, vomiting, dehydration, and high mortality in nursery piglets [1]. PEDV was first recognized as the causative

agent of PED in Belgium [2] and the United Kingdom in 1978 [3]. The presence of PED in China was confirmed, by immunofluorescence assays and serum neutralization tests, in 1984. Bivalent killed or attenuated vaccines have been used to prevent transmissible gastroenteritis (TGE) and PED in China since then. Clinical PED outbreaks have reemerged in the major pig-producing areas of China since December 2010, resulting in major losses of suckling piglets. Morbidity rates between 90 and 100 % have been observed, with mortalities of 70–100 % on affected pig farms [4].

Heterogeneity of genomic sequences is believed to be responsible for the diversity in PEDV pathogenicity. A number of molecular investigations have been conducted, revealing different levels of variation in nucleotide sequences among PEDV isolates [5–7]. Because of incomplete genomic information, most molecular epidemiological analyses have targeted a single gene of PEDV. To date, complete genome sequences of 18 PEDV strains, including 10 endemic Chinese isolates, have been submitted to the GenBank database [4, 8–19]. To better understand the diversity, epidemiology and molecular characteristics of PEDV isolates in China, we sequenced the complete genome of a virulent PEDV strain from Henan Province, China. This isolate was from a suckling piglet that exhibited severe diarrhea. We compared its genomic sequence with those of other Chinese PEDV isolates and reference strains.

Materials and methods

Isolation and identification of PEDV strain
CH/ZMDZY/11

PEDV strain CH/ZMDZY/11 was isolated from 2- to 3-day-old suckling piglets suffering from watery diarrhea

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and dehydration. These piglets were from a single farm in Henan Province, China, which contained 1500 breeding sows. Cases were characterized by watery diarrhea, dehydration with milk curd vomitus, thin-walled intestines, 100 % morbidity and 90 % mortality. Pigs of other ages were affected, exhibiting diarrhea and loss of appetite to different degrees, with severity of symptoms dependent on age. Affected sows were characterized by anorexia, depression and transient diarrhea, with most affected sows recovering within one week. Production records showed that breeding herds had been immunized with a killed CV777-based vaccine that had been produced in China.

Virus isolation was conducted using Vero E6 cells (American Type Culture Collection, VA, USA). Samples from the small intestines of pigs that were positive for PEDV but negative for TGE virus (TGEV), porcine rotavirus (PRV), classical swine fever virus (CSFV), and porcine circovirus 2 (PCV2) were homogenized by adding phosphate-buffered saline (PBS) to achieve a 10 % (w/v) homogenate. These were then centrifuged (10,000×g, 15 min, 4 °C) in a Sorvall ST 16R microcentrifuge (Fisher Scientific, MA, USA) and filtered through a 0.22-µm filter (Millipore, MA, USA) for use as inocula. Vero E6 cells were seeded onto 21-cm² tissue culture plates 24 h prior to infection. The growth medium was Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, NY, USA) supplemented with 10 % heat-inactivated fetal calf serum,

100 U/ml penicillin G, and 100 µg/ml streptomycin. When confluent, growth medium was removed, and cell monolayers were washed once with PBS and then with DMEM containing 0.3 % tryptose phosphate broth and 0.02 % yeast extract. The washed cells were infected with the filtered intestinal preparation that had been resuspended in 'infection medium' (DMEM, 0.3 % tryptose phosphate broth, 0.02 % yeast extract, and 10 µg/ml trypsin). After adsorption at 37 °C for 1 h, an equal volume of infection medium was added. Every 24 h, the culture supernatants were supplied with trypsin at 80 % of the initial concentration. PEDV strain CH/ZMDZY/11 was identified by amplifying structural and non-structural genes of the virus using PEDV-specific primers in a reverse transcription polymerase chain reaction (RT-PCR).

Other Chinese PEDV isolates and reference strains

Another 11 Chinese PEDV isolates from 3- to 10-day-old suckling piglets suffering from watery diarrhea, dehydration and vomiting were examined. For these isolates, from different farms across seven provinces within China, morbidity of affected piglets was 70–100 %, and mortality, 60–90 %. Some of the breeding herds from these farms had been immunized with CV777-based vaccines. Information pertaining to the reference strains and 11 PEDV isolates we analyzed is summarized in Table 1.

Table 1 PEDV strains used in the sequence analysis

Isolate	Accession number	Origin	Piglets		Breeding herds	
			Morbidity	Mortality	Size	Vaccination for PED
AJ1102	JX188454.1	Hubei, China, 2012	60-90 %	50-100 %	3000	No vaccination
BJ-2011-1	JN825712.1	Beijing, China, 2011	NA	NA	NA	NA
CH-FJND-3-2011	JQ282909.1	Fujian, China, 2011	40-90 %	50-100 %	5000	No vaccination
CH GD-01	JX261936.1	Guangdong, China, 2012	100 %	≥90 %	5000	Attenuated CV777
CH-ZMDZY-11	KC196276.1	Henan, China, 2011	100 %	≥90 %	1500	Killed CV777
GD-1	JX647847.1	Guangdong, China, 2012	90 %	60 %	5000	No vaccination
GD-A	JX112709.1	Guangdong, China, 2012	NA	NA	NA	NA
GD-B	JX088695.1	Guangdong, China, 2012	NA	NA	NA	NA
LC	JX489155.1	Guangdong, China, 2012	100 %	≥90 %	3500	Attenuated CV777
ZJCZ-4	JX524137.1	Zhejiang, China, 2012	NA	NA	NA	Killed CV777
CH-S	JN547228.1	Shanghai, China, 1986	NA	NA	NA	No vaccination
LZC	EF185992.1	Gansu, China, 2006	NA	NA	NA	No vaccination
CV777	AF353511.1	Belgium, 1993	NA	NA	NA	NA
Attenuated DR13	JQ023162.1	South Korea, 2001	NA	NA	NA	NA
Virulent DR13	JQ023161.1	South Korea, 1999	NA	NA	NA	NA
SM98	GU937797.1	South Korea, 2011	NA	NA	NA	NA

Nucleotide sequencing

Viral RNAs were extracted from CH/ZMDZY/11-infected Vero cell culture supernatants using TRIzol LS Reagent (Invitrogen, NY, USA) according to the manufacturer's instructions. Based on the nucleotide sequence of PEDV strain CV777, 19 pairs of oligonucleotide primers were designed to amplify different regions of the CH/ZMDZY/11 genome. PCR products were purified, cloned into the pMD18-T vector (TaKaRa Biotechnology, Dalian), and sequenced in both directions using an ABI 3730xl DNA Analyzer (Applied Biosystems, CA, USA). Sequence assembly and alignment were conducted using Lasergene sequence analysis software (DNASTAR Inc., Madison, WI, USA).

Nucleotide sequence analysis

Nucleotide sequences were edited, aligned and analyzed with the MegAlign program, a part of the Lasergene sequence analysis software package. Phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) software version 5.02, applying the neighbor-joining method, which was based on the nucleotide sequence of each gene.

Results

Virus isolation and identification

Cytopathic effects, such as cell fusion and syncytia formation, typical of coronavirus infection, were observed after 11 passages in Vero-E6 cells. Genes encoding the spike protein (S), envelope protein (E), membrane protein (M), and nucleoprotein (N), along with open reading frame 3 (ORF3), were successfully amplified by RT-PCR (Fig. 1). Our sequencing results showed that the amplified fragments shared high homology to other reported PEDV strains.

Genomic structures in PEDV isolates

The genome of the CH/ZMDZY/11 strain comprises 28,038 nucleotides (nt), excluding the poly (A) tail. The organization of genes is similar to that for other reported PEDVs (5'-ORF1a/1b-S-ORF3-E-M-N-3'), with seven coding regions identified [1, 20]. The 5' and 3' ends of the genome contain untranslated regions (UTRs) of 292 and 334 nt, respectively. ORF1a/1b encodes two large non-structural precursor polyproteins, replicases 1a and 1b. The S gene encodes the S protein (150–220 kDa), E encodes the E protein (7 kDa), M encodes the M protein (20–30 kDa), and the N gene encodes N (58 kDa). ORF3,

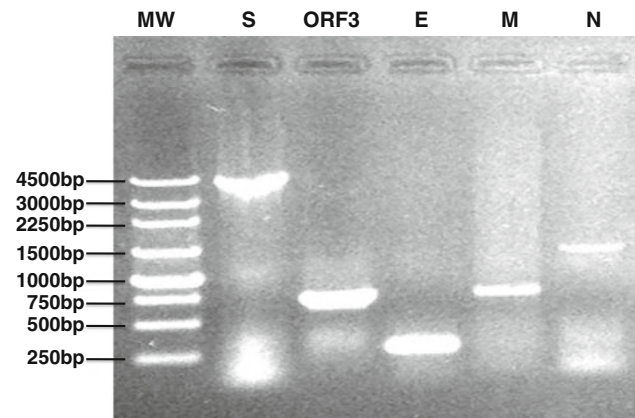


Fig. 1 Amplification of PEDV-specific fragments from CH/ZMDZY/11-infected Vero cells. S, spike gene (4161 bp); ORF3, open reading frame 3 (675 bp); E, envelope gene (231 bp); M, membrane gene (681 bp); N, nucleoprotein gene (1326 bp)

between the S and E genes, encodes a non-structural protein.

Sequence homology and genetic characterization

The nucleotide and deduced amino acid (aa) sequences of certain reference strains and 11 Chinese PEDV isolates were examined and compared with that for the CH/ZMDZY/11 strain (Table 1). Of these isolates, 10 were identified after 2010 from PEDV-affected swine farms of major swine-breeding provinces in China. The remaining two isolates, CH-S and LZC, were isolated in 1986 and 2006, respectively [4].

ORF1a and 1b

The length of ORF1a/1b for the 10 strains isolated after 2010, LZC, and two reference strains (CV777 and virulent DR13) was 20,345 nt. ORF1a/1b for CH-S and the reference strains attenuated DR13 and SM98 was 20,342, 20,321, and 20,339 nt, respectively. The homology of nucleotide and deduced amino acid sequences for ORF1a/b of the post-2010 Chinese PEDV isolates was very high (98.3–100 % nucleotide sequence identity and 97.6–99.9 % amino acid sequence similarity) when compared to each other. When ORF1a/b sequences of the post-2010 isolates were compared with earlier Chinese isolates and reference strains, homology was also high (97.2–98.4 % nucleotide sequence identity and 95.7–97.8 % amino acid sequence similarity). A number of nucleotide substitutions were observed in Chinese PEDV strains isolated post-2010. We saw that attenuated DR13 had a 24-nt deletion from nt 3106 to 3129, which results in an 8-aa deletion. Amino acid changes in post-2010 Chinese PEDV isolates and virulent DR13 were found at residues 5 (H→Q), 449 (L→I), 451

(D→E), 907 (K→R), 1076 (V→A), 1465 (I→T), 1796 (P→S), 2051 (S→G), and 3034 (M→I).

Phylogenetic analysis of the nucleotide and amino acid sequences of ORF1a/1b confirmed that all field strains belonged to three groups (Fig. 3a). Group 1 comprised CV777 (Europe), LZC and SM98 (South Korea). Group 2 only contained attenuated DR13 (South Korea). The 10 Chinese PEDV strains isolated after 2010 were grouped together with virulent DR13 and CH-S (China) in group 3.

S gene

Pairwise alignment and comparison of the S gene from the 10 post-2010 Chinese PEDV isolates showed high levels of nucleotide sequence identity (97.4–100 %). When the S gene sequence for these isolates was compared with the European prototype CV777, we found 92.9–94.6 % nucleotide sequence identity; when they were compared against SM98, attenuated and virulent DR13 strains, nucleotide sequence identities of 92.9–94.6 % were seen.

The homology of the S gene of CH-S to CV777, SM98, attenuated and virulent DR13 was 95.2–97 %, while that of LZC to CV777, SM98, attenuated and virulent DR13 was 95.2–99.1 %.

Amino acid sequence analysis of the S glycoprotein gene revealed that the 10 post-2010 PEDV isolates displayed sequence homology ranging from 97 to 100 % when compared with each other. These isolates shared relative low levels of identity with earlier Chinese isolates and those from Europe and South Korea, at 92.6–94.6 %. A comparison of the amino acid sequences of the S proteins of isolates CH-S, LZC, CV777, SM98, attenuated and virulent DR13, and the 10 post-2010 PEDV isolates revealed amino acid changes at positions 27–29, 55–58, 64, 68–72, 84, 86, 87, 89, 124, 134, 135, 142, 160–162, 201, 205–207, 215, 232, 234, 251, 252, and 275. All post-2010 isolates had a discontinuous 5-aa insertion (59QGVN62 and 144N) and a 2-aa deletion (163D/NI164) at the N-terminal region of the S gene (Fig. 2).

Phylogenetic analysis of the S gene and protein confirmed that all field strains could be classified into three

Fig. 2 Partial peptide sequence alignment of the S genes of the 10 current Chinese PEDV strains (AJ1102, BJ-2011-1, CH-FJND-3-2011, CH GD-01, CH-ZMDZY-11, GD-1, GD-A, GD-B, LC, ZJCZ-4) and selected reference PEDV strains (European strain CV777, Chinese isolates CH-S and LZC, and Korean isolates SM98, virulent DR13, and attenuated DR13)

	SANTN	IGENQGVNSTWY	CAGQHP	HIRGGHGF	ATARLRICQ	FPSIKTLG	PNTANN
	30	60	70	90	130	140	
AJ1102
BJ-2011-1
CH-FJND-3-2011
CH GD-01
CH-ZMDZY-11	R.....
GD-1
GD-A
GD-B
LC
ZJCZ4
CH-S	QST..	SMNS----	S...GTGIE	Y.DS.Q...	I.....	DN.....	AV.-
LZC	QST..	SMNS----	S...GTGIE	Y.DS.Q...	I.....	DN.....	V.-
CV777	QST..	SMNS----	S...GTGIE	Y.DS.Q...	I.....	DN.....	V.-
Attenuated DR13	QSTI.	SMNS----	S...GTGIE	Y.DS.Q...	I.....	DN.....	V.-
Virulent DR13	QSTI.	SMNS----	S...GTGIE	Y.DS.Q...	I.....	DN.....	V.-
SM98	QST..	SMNS----	S...GTGIE	Y.DSSQ...	I.....	DN.....	V.-

	EHS--VVG	ATKCYNSGGCAMQ	YVYEPTY	YMLNVT	SAGEDGISYQ	ATEPNGHI	PEPGFSP	NNWFL	LLSND	STLV
	170	200	210	220	230	250	260	270		
AJ1102
BJ-2011-1
CH-FJND-3-2011
CH GD-01	I.....	E.....
CH-ZMDZY-11
GD-1	D.....
GD-A	I.....
GD-B
LC
ZJCZ4
CH-S	DGKNI...	..R...KKS...T.....Y.E	..DA.....L
LZC	DGKDI...	..R...RRS.....T.....Y.E	..DS.....L
CV777	DGKDI...	..R...RRS.....T.....Y.E	..DS.....L
Attenuated DR13	DGKNI...	..R...KRS.....T.....Y.E	..DS.....L
Virulent DR13	DGKNI...	..R...KRS.....T.....Y.E	..DS.....L
SM98	DGKDI...	..R...RRS.....T.....Y.E	..DS.....C.....L

groups (Fig. 3b). Group 1 consisted of CV777 (Europe), LZC (China) and SM98 (South Korea). Group 2 comprised CH-S (China), virulent DR13 and its derivative (South Korea), and group 3 comprised all 10 post-2010 Chinese PEDV isolates.

ORF3

ORF3 of all post-2010 Chinese PEDV strains was 675 nt in length and encoded a 225-aa protein. Sequence analysis of ORF3 showed that the 10 post-2010 PEDV strains had 95–100 % nucleotide sequence identity and 96–100 % amino acid sequence identity to each other. These isolates shared 92.9–100 % nucleotide and amino acid sequence identity to earlier Chinese strains and reference strains. Sequence analysis and comparison with earlier Chinese PEDV isolates (CH-S and LZC) and reference strains revealed nucleotide changes in strains AJ-1102, CHGD-1, GD-1, GD-A, LC, and ZJCZ4. These changes were as follows: T→C at 39, 74, 294, and 318; C→T at 99, 264, 336, and 636; G→T at 135, 320, and 381; and G→A at 208 and 502. These nucleotide changes resulted in amino acid changes at four sites: L→S at 25; I→V at 70; C→F at 107;

and D→N at 168. There was a continuous 17-aa deletion in ORF3 of attenuated DR13 (82YCPLLYYCGALLD-ATII98); however, such a deletion did not exist in CH-ZMDZY-11 and other Chinese PEDV strains. Phylogenetic analysis based on ORF3 nucleotide and amino acid sequences showed that PEDV Chinese field strains clustered into three groups (Fig. 3c) Group 1 consisted of CV777 (Europe), LZC (China) and SM98 (South Korea). Group 2 comprised attenuated DR13 (South Korea), and group3 contained the 10 post-2010 Chinese PEDV strains isolated after 2010, CH-S (China) and virulent DR13 (South Korea).

E gene

The E gene of all Chinese PEDV and reference strains, except attenuated DR13, was 231 nt long and encoded a protein with 76 aa. Homology of nucleotide and amino acid sequences of the entire E gene for the post-2010 Chinese PEDV isolates when compared among each other was very high (98.3–100 % nucleotide sequence identity and 97.4–100 % amino acid sequence similarity). Homology was also high (96.1–99.1 % nucleotide sequence

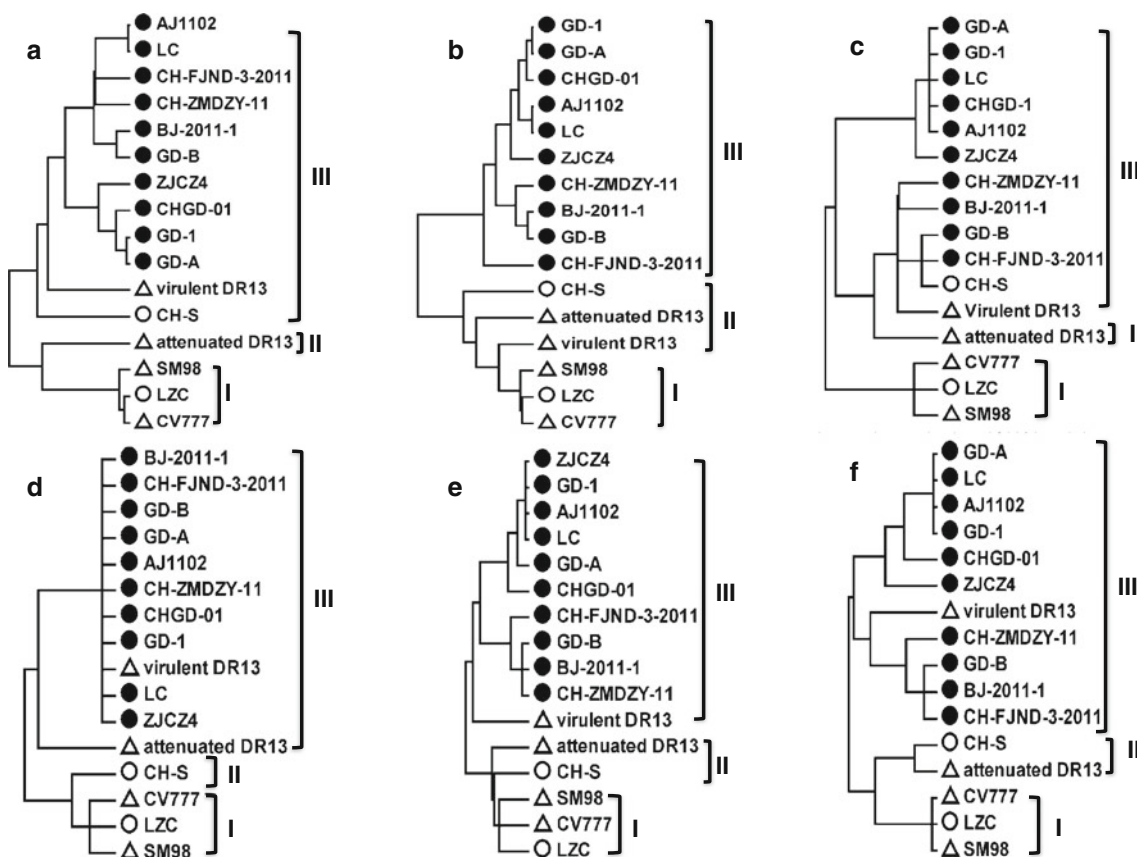


Fig. 3 Phylogenetic analysis of the 10 current Chinese PEDV strains and reference isolates based on the nucleotide sequences of ORF1a/b (a), S (b), ORF3 (c), E (d), M (e), and N (f). Phylogenetic trees were

constructed using MEGA 5.02 software. ●, current Chinese strains; ○, earlier Chinese isolates; △, reference strains. GenBank accession numbers of all strains examined in this study are listed in Table 1

identity and 94.3–100 % amino acid similarity) when the E sequences from post-2010 isolates were compared with earlier Chinese strains and reference strains. The following nucleotide changes in post-2010 PEDV strains and virulent DR13 were observed at the following nucleotide positions: C→T at 90, 165, and 166, and G→A at 194. The change at 194 resulted in an altered amino acid at residue 65 (R→Q). Attenuated DR13 has a 21-nt deletion from 67–87, which corresponds to a 7-aa deletion.

Phylogenetic analysis showed that Chinese field isolates belonged to three groups (Fig. 3d). Group 1 consisted of CV777 (Europe), LZC (China) and SM98 (South Korea). Group 2 comprised attenuated CH-S (China) only at this point, and group 3 comprised the 10 post-2010 Chinese PEDV strains, along with virulent and attenuated DR13 (South Korea).

M gene

The M genes of all Chinese PEDV and reference strains, except SM98, consisted of 681 nt. Sequence comparisons of the M genes for post-2010 Chinese PEDV isolates revealed high levels of homology at the nucleotide and amino acid level (97.1–100 % nucleotide sequence identity and 97.4–99.1 % amino acid similarity) when compared to each other. The homology of M gene sequences for post-2010 Chinese PEDV strains when compared with earlier Chinese and reference strains was also high (96.9–98.4 % nucleotide sequence identity and 96.5–99.1 % amino acid sequence similarity). Except for the SM98 strain, all other virus sequences had a 4-aa deletion (MLVL) from residues 14–17. The 10 post-2010 Chinese strains contained an amino acid change (V→A) at position 46.

Phylogenetic analysis of nucleotide and amino acid sequences showed that all field strains fell into three groups (Fig. 3e). Group 1 consisted of CV777 (Europe), LZC and SM98 (South Korea), while group 2 comprised CH-S (China) and attenuated DR13 (South Korea). Group 3 comprised 10 post-2010 Chinese PEDV strains and virulent DR13.

N gene

The post-2010 PEDV isolates, earlier Chinese isolates, and the reference strains did not contain insertions or deletions in the N gene. The ORF corresponding to the N gene was made up of 1326 nt, coding for a 441-aa protein. All post-2010 PEDV strains shared 96–99.9 % nucleotide sequence identity and 97.1–100 % amino acid sequence similarity with each other, and 95.4–97.3 % nucleotide sequence identity and 95.7–99.7 % amino acid sequence similarity when the post-2010 Chinese isolates were compared with earlier Chinese strains and reference strains.

Phylogenetic analysis of the N gene confirmed that all field strains could be classified into three groups (Fig. 3f). Group 1 contained CV777 (Europe), LZC (China) and SM98 (South Korea). Group 2 comprised CH-S (China) and attenuated DR13 (South Korea), and group3 contained the 10 post-2010 Chinese PEDV strains and virulent DR13 (South Korea).

Discussion

In the present study, a virulent PEDV strain was isolated from a suckling piglet, and its genome was sequenced and compared with those of other PEDV strains. To the best of our knowledge, we are the first to report the genetic characteristics of viruses causing PED outbreaks in China. Comparative analysis of the whole genome sequence indicated that CH/ZMDZY11 shared high nucleotide sequence identity and amino acid sequence similarity with PEDV strains isolated between 2010 and 2012. The PEDV strain prevailing in China has developed several mutations in its genome. The majority of the 10 post-2010 Chinese PEDV strains were isolated from farms where breeding herds were immunized with inactivated or attenuated PEDV vaccines. These were manufactured with the CV777 vaccine strain; however, the morbidity and mortality of suckling piglets suffering from PED remains high, indicating low efficacy of the current vaccine in use.

The polymerase gene comprises two large ORFs, 1a and 1b, which encode two replicases that determine the replication efficacy of the virus. Sequence analysis of these ORFs showed that the post-2010 Chinese PEDV isolates and virulent DR13 shared multiple mutations at the same location and were clustered in one group. The 10 post-2010 PEDV strains appear to be related with the earlier prevailing Chinese strain, CH-S.

The S protein is an antigen on the surface of the virion that plays a pivotal role in regulating interactions with specific host-cell receptor glycoproteins to mediate viral entry. This stimulates induction of neutralizing antibodies in the natural host [21, 22]. It is also associated with growth adaptation *in vitro* and attenuation of virulence *in vivo* [23, 24]. The S genes of the 10 post-2010 Chinese PEDV isolates were highly homologous. The S genes in all 10 isolates contained some altered amino acids, with a discontinuous 5-aa insertion and a 2-aa deletion at the same sites of the N-terminal region. The S gene has been considered a target gene for analyzing genetic variation of PEDV strains in the field [4, 6]. Our findings indicate that the current PEDV isolate prevailing in China is a novel strain with a variant S gene that is distinct from previous Chinese and reference strains. This could explain the less-effective immunological protection against PEDV strains

induced by the current vaccine in China, and the effectiveness of feeding sows feces or intestinal contents of infected piglets. Further investigation of the antigenic differences among current isolates and the vaccine strain used in China is necessary for the development of an effective vaccine against PED in China.

ORF3 is an accessory gene, located between the S and E genes, and its function has rarely been examined. Sequence analysis revealed a continuous 17-aa deletion in ORF3 of attenuated DR13 and the CV777 vaccine strain. However, this deletion does not exist in current Chinese PEDV strains isolated after 2010. Differences in ORF3 sequences between cell-adapted and field viruses could be a marker of their adaptation to environmental conditions. A recently study suggested that PEDV ORF3 is related to viral infectivity and pathogenicity and encodes an ion channel protein that regulates virus production [25]. The post-2010 isolates were clustered together with virulent DR13, implying that current Chinese PEDV strains retain high infectivity and pathogenicity. The effects of this deletion on virulence and other properties requires further investigation.

The E protein plays an important role during coronavirus budding. It transiently resides in a pre-Golgi compartment before progressing to the Golgi apparatus [26]. Recently, many studies have focused on the genetic and phylogenetic analysis of PEDV S, M and ORF3 genes, whereas a comparison of E genes has rarely been conducted. Our results show a 21-nt deletion in the E gene of attenuated DR13, which does not exist in post-2010 Chinese PEDV isolates and virulent DR13. All post-2010 Chinese PEDV strains exhibited a close relationship to the South Korean virulent DR13 and its derivative.

The M protein plays an important role in the viral assembly process and also induces antibodies that neutralize the virus [27, 28]. Heterogeneity of the M gene sequence is known to confer diverse PEDV pathogenicities [5]. Sequence comparisons indicate that the 10 post-2010 Chinese PEDV strains contain an amino acid change (V46A) compared with earlier Chinese isolates and other reference strains. The post-2010 isolates were clustered together with virulent DR13. The N protein, which binds to virion RNA and provides a structural basis for the helical nucleocapsid, is a basic phosphoprotein associated with the genome [29]. Alignment with reference strains indicated that the N gene is highly conserved, with the exception of some point mutations. Phylogenetic analysis confirmed that all 10 post-2010 Chinese PEDV isolates were grouped with virulent DR13.

Taken together, our results show that a novel PEDV strain is the etiological agent contributing to the re-emergence of PED in China. Prevailing PEDV field isolates share common molecular characteristics in their S, E, and M genes. Phylogenetic analyses of ORF1a/b, ORF3, E, M,

and N genes confirmed that the 10 post-2010 Chinese PEDV isolates have a close relationship to a virulent DR13 strain from South Korea. The differences between the current isolates and the vaccine strain must be defined to develop an effective vaccine that better prevents and controls PED epidemics.

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