

Prevalence and phylogenetic analyses of porcine diarrhea associated viruses in Southern China from 2012 to 2018

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Abstract

Background Large-scale outbreaks of severe diarrhea caused by viruses have occurred in pigs in China since late 2010. To investigate the prevalence and genetic evolution of diarrhea-associated viruses responsible for the outbreaks, a total of 2,987 field diarrheal samples from 168 pig farms in five provinces in Southern China during 2012-2018 were collected and tested. **Results** Porcine epidemic diarrhea virus (PEDV) was the mostly frequently detected virus with prevalence rates between 50.21 and 62.10% in samples, and 96.43% (162/168) in premises, respectively. Porcine deltacoronavirus (PDCoV) was the second prevalent virus with detection rates ranging from 19.62 to 29.19% in samples, and 70.24% (118/168) in premises, respectively. Both transmissible gastroenteritis virus (TGEV) and porcine rotavirus (PRoV) were detected at low detection rates of < 3% in samples and 10.12% in premises. In the present study, we firstly identified swine acute diarrhea syndrome coronavirus (SADS-CoV) in diarrheal samples of piglets from Fujian province in Southern China and the prevalence rate of SADS-CoV was 10.29% (7/68). Co-infections of these diarrhea-associated viruses were common. The most frequent co-infections observed were PEDV with PDCoV, with an average rate of 12.72% (380/2987, ranging from 8.26% ~ 17.33%). Phylogenetic analyses revealed that the strains of PEDV circulating in Southern China during the last 7 years were G IIa variant PEDVs. The most mutations were present in the collagenase equivalent (COE) and epitope regions of the spike protein of the PEDVs presently circulating in the field. PDCoVs in this area were closely related with Chinese PDCoVs other than the strains from the USA, South Korea, Thailand and Lao's public. **Conclusion** These findings indicated that PEDV, PDCoV, and SADS-CoV were leading etiologic agents; and there were severe mono-infections and co-infections of pathogenic enteric coronaviruses in pigs in Southern China during 2012 -2018 and thus the attention should be paid in order to prevent and control porcine viral diarrhea.

Introduction

Outbreaks of severe diarrhea in pigs in China has been lasting since late 2010, which characterized as watery diarrhea in pigs of all growing stages, and high morbidity and mortality in newborn piglets [1]. Enteropathogenic viruses, including porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and porcine rotavirus (PRoV) are suspected as the causative agents of viral diarrhea in pigs [2]. The data from previous epidemiologic investigations demonstrated that the variant PEDV is the most common etiological pathogen responsible for porcine diarrhea in China in recent years. Porcine epidemic diarrhea (PED) was first recognized as transmissible gastroenteritis (TGE)-like diarrhea in pigs in Shanghai, China, in 1973. The causative agent of PED was defined in 1984. Before 2010, PEDV infections were sporadic or locally endemic in China although high infections were occasionally reported in sows, suckling pigs, fattening pigs and boars. Since late 2010, variant strains of PEDV have emerged in China and rapidly swept throughout the country. Pigs of all ages have suffered from a serious PED pandemic, resulting in severe losses of piglets within 7 days old due to 80–100% morbidity and up to 100% mortality [1]. Between 2010 and 2014, PED that affected Thailand, South Korea, and China has spread to Vietnam. In May 2013, PED was first reported in the USA, and then

rapidly spread to the main pork product states within a year. The majority strains prevailed in China were pandemic variant PEDVs (genotype II), along with classical strains (genotype I: SD-M, AH-M, SQ2014, and SC1402). In 2014, a variant PEDV strain, OH851, with large insertions and deletions in the S1 region of PEDV has emerged in the USA [3]. Afterwards, several similar strains were found in the USA and China [4, 5]. Recombinant analysis demonstrated that these strains were originated from recombination events between classical and variant strains in the S gene, and were named as S-INDEL variant PEDVs. Animal challenge trials showed that the S-INDEL variant PEDV was less pathogenic than that of the prototype strains [6].

The newly emerged PDCoV is ranked number 2 agent causing pig diarrhea just after PEDV, with detection rates about 30% in diarrheal samples of pigs [1, 2, 7]. PDCoV belongs to the genus of *deltacoronavirus*, the family of *coronaviridae*, and was first described in swine samples from a surveillance performed in Hong Kong in 2012 [8]. After then, the presence of PDCoV was successively recognized in United States, South Korea, mainland China, and Thailand [9–11]. Several studies revealed that PDCoV was enteropathogenic to swine. Clinical manifestations and histopathologic features referable to PDCoV are indistinguishable from those associated with PEDV and TGEV [12], characterized with acute watery diarrhea, vomiting, anorexia, depression and dehydration. In early 2017, a novel diarrheal coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV) has been recognized in young piglets and resulted in high morbidity and mortality in neonatal piglets [13]. The reported SADS-CoVs showed a high genetic homology with bat-HKU2 (approximately 95%) [14]. Until now, this virus was just found in several pig farms in Guangdong and Fujian provinces in Southern China, and was frequently detected in diarrheal weaning pigs [13, 15].

In this study, we investigated the prevalence of the five major porcine diarrhea-associated viruses, i.e., PEDV, PDCoV, TGEV, PRoV and SADS-CoV from the diarrheal samples of pigs collected during 2012–2018 from five provinces (Jiangxi, Zhejiang, Fujian, Guangdong, and Hunan) in Southern China. To figure out the genetic characterizations of major diarrhea associated viruses, S1 genes of PEDV and PDCoV were amplified, sequenced, and analyzed.

Results And Discussion

Prevalence of PEDV, PDCoV, TGEV, PRoV and SADS-CoV

During the span of seven-year period, we tested 2,987 samples from five provinces in Southern China, and found that PEDV was the dominant virus in our surveillance, with a prevalence varied from 50.21 to 62.1% in 2012–2018 (Table 1). The results were in accordance with that of other studies carried out in other areas in China at the same period [1, 11, 16]. Zhang et al (2014) surveyed samples from 29 provinces in China covering 2011 to 2014, and found the positive rates of PEDV varied from 61.10 to 78.49% in diarrheal pigs. An investigation on 116 diarrheal samples from 6 Chinese provinces in 2016–2017 showed the prevalence of PEDV was 52.60% [17].

In addition to PEDV, PDCoV, TGEV, and PRoV have also been found positive in these diarrhea samples. PDCoV was the second prevalent virus in our surveillance, with detection rates varied from 19.62 to 29.19% from 2012 to 2018, which was slightly lower than that of our previous report [11]. TGEV, and PRoV were both detected at low detection rates in samples (8.3%) and pig farms (10.12%), which was in accordance with the previous studies [18]. SADS-CoV, the newly emerged coronavirus causing acute diarrhea in suckling piglets was firstly reported in China in 2017 [14, 19, 20]. In the present study, we have not found SADS-CoV in the samples from Jiangxi, Zhejiang, Guangdong, and Hunan provinces during 2012 to 2018, but a positive rate of 10.29% (7/68) of this virus was identified in diarrheal piglets from Fujian province. Until now, SADS-CoV was only identified in Guangdong and Fujian provinces.

In the context of the sample sources, the small intestines of suckling piglets showed the highest positive rates of PEDV (63.31%) and followed by the feces from diarrheal pigs (52.72%), and the milk (22.73%), respectively. Likewise, the detection rates of PDCoV from small intestines, feces and milk were 29.66%, 25.75%, and 7.92%, respectively (Table 1). As to growing stage of the pigs, PEDV was frequently detected in all ages of pigs, followed by PDCoV. PEDV infection was more common in sows (52.81%) and suckling piglets (62.37%), and the same situation happened in PDCoV infection. These data suggested that suckling piglets were at greater risk of infections of diarrhea viruses, especially PEDV.

Co-infections of diarrhea viruses in diarrhea pigs in Southern China

In this investigation, co-infection frequency was analyzed. Among the 2,987 field samples, the positive rates of mono-infection of each individual PEDV, PDCoV, TGEV and PRoV from 2012 to 2018 were 45.53% (1360/2987), 14.23% (425/2987), 0.33% (10/2987), and 0.60% (18/2987), respectively (Fig. 1). The most common co-infection was PEDV with PDCoV, with an average positive rate of 12.72% (380/2987), ranging from 8.26% to 17.33%. Dual-infections of PEDV and TGEV, PEDV and PRoV, PDCoV and TGEV, were at average rates of 0.30%, 0.13%, and 0.10%, respectively. Four cases of triple-infections were also observed, with 3 cases in PEDV, PDCoV, and PRoV co-infection, and 1 case in PEDV, PDCoV, and TGEV co-infection. Four samples from Fujian province in 2017 were determined as co-infection of SADS-CoV and PEDV. We didn't find other patterns of co-infection in these tested samples. Mixed infections are common in diarrheal pigs as reported, and might alter pathogenesis and pathogenicity the agents involved. We also observed severe morbidity and mortality in piglets infected with multiple pathogens, such as PEDV, PDCoV, TGEV, and pathogenic *Escherichia coli*.

Molecular characterization and phylogenetic analysis of PEDVs circulating in South China

The S protein of PEDV is a significant structural protein of coronavirus, and is thought to encode the antigenic determinants, especially the neutralizing epitopes of the virus [21]. To elucidate the genetic characteristics of PEDVs circulating in Southern China during 2012–2018, the sequences of the complete S1 genes of 11 PEDV strains were sequenced, and analyzed. Phylogenetically, the S1 regions (aa 1~794) of the 11 strains of PEDV identified in this study and other 73 selected reference PEDV strains were divided into two groups (GI and GII). All of the 11 strains determined in this study, along with 3 formerly reported strains were clustered into the group G II, and subgroup G IIa (Fig. 2A). Variations were observed

among the 14 strains, which were located into different clades in G IIa, two strains (CH/JX/JJ08/2015 and CH/JX/JGS11/2016) were closely with CH/JX/01, a strains isolated in Jiangxi province in 2015; six strains were clusered into a independent clade; a strain, CH/JX/ZS03/2014 was in a clade with CH/ZMDZY/11; two strains CH/JX-1/2013 and CH/JX-2/2013, determined in 2013 were closely related to the recombinant Chinese strain, AH2012, determined in 2012.

Homology analysis was carried on the 11 PEDVs determined in this study, with 3 PEDVs previously reported in our lab and reference stains deposited in GenBank out. The results showed that all field strains shared 97.8–100% nucleotide (nt) and 97.2–100% amino acid (aa) identities with each other, as well as 90.7–92.9% nt and 89.8–91.6% aa identities with GI strains, and 93.2–99.7% nt and 91.6–100% aa identities with genotype 2 strains. Compared with the CV777 vaccine strain, the strains determined in this study had same mutation sites of the ^A518^S, ^G521^D, ^L522^{H/Y}, ^S524^G, ^V528^I, ^T550^S, ^S563^F, ^G594^S, ^A605^E, ^L612^F, ^F617^L, ^K630^T, ^E633^V, and ^I635^V (Fig. S1). In addition, when considered the neutralizing epitope SS2 and SS6, mutations were found among differernt genotypes, including the Jiangxi strains determined in this study clustered in G IIa: ^S764^L and ^T774^M were only found in G 1b, ^Y764^S were observed in genotypes G 1b and GII. Furthermore, three mutations were only observed in the neutralizing epitope SS2 and SS6 between Jiangxi strains and CV777, ^V747^L IN CH/JXJGS11/2016, ^I751^M in CH/JXJA89/2015 and CH/JXWN13/2016, and ^I77^N in CH/JXGZ09/2018 in Jiangxi strains (Fig. 3).

Molecular characterization and phylogenetic analysis of PDCoVs in South China

Eight complete sequences of the S1 gene of PDCoV strains were obtained from representative positive samples. To analyze the homology and phylogenetic relationships among PDCoV isolates from different countries, the eight PDCoVs determined in this study and 39 reference strains of PDCoV from GenBank were used. The eight PDCoV isolates showed 97.7–99.9% nt and 97.9–99.8% aa identities with each other; and with 95.2–99.4% nt identities and 95.5–99.1% aa identities to the reference strains from China and other countries. Interestingly, sequence alignment analysis indicated that all eight PDCoV strains determined in this study along with all Chinese strains (except AN-2004) and a strain from Thailand (TT_1115) had an amino acid deletion at ^N52 when compared with other strains. Phylogenetic analysis based on the aa sequence of the S1 protein demonstrated that PDCoV isolates were divided into three groups, the Chinese- and US-like-groups, as reported previously [17]. The S1 genes of the PDCoVs currently circulating in Southern China were more closely related to other Chinese PDCoVs rather than to those isolated previously from USA, South Korea, and Thailand (Fig. 2B).

Conclusions

To survey the prevalence of major diarrhea-associated viruses in pigs, samples (n = 2,987) from five provinces in Southern China during 2012 to 2018 were investigated. The results indicated that PEDV was the most frequently detected virus with a prevalence rate varied from 50.21 to 62.1%. PDCoV was the second prevalent virus detected in the diarrheal samples in pigs in this study. It is worth to note that PDCoV-associated enteric disease might be a long-term threat to pigs. We have firstly reported SADS-CoV

isolated in Fujian province in Southern China. Phylogenetic analyses revealed that the strains of PEDV circulating in Southern China during the 7 years were variant PEDVs belonging to G IIa, and the PDCoVs tested were closely related with Chinese PDCoVs.

Methods

Diarrhea associated virus sampling and testing

During 2012 and 2018, a total of 2,987 samples, which consisted of small intestines (N = 1,581), feces (N = 1,305), and milk (N = 101) were collected from pigs in different growing stages with diarrhea from aforementioned five provinces in Southern China (Table 1). Viral RNA was extracted using RNAlplus Reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. And then the first strand cDNA was synthesized by random primers. The previously established PCR protocols were used to test the four major diarrhea-associated viruses, PEDV, PDCoV, TGEV, and PRoV [22]. The newly emerged SADS-CoV was tested by the method established in our laboratory (primer sequence information is presented in the Supplementary Table S1). Detection data was analyzed based on the discriminations of year, pig growing stage, sampling area.

Genetic characteristics analysis on S1 gene of the field strains of PEDV and PDCoV.

To elucidate the molecular characteristics of the S1 genes of PEDV and PDCoV, representative positive samples of PEDV and PDCoV were amplified, cloned and sequenced (primer sequences is present in the Supplementary Table S1). The sequence fragments of the PCR products were assembled and annotated. Nucleotide (nt) and deduced amino acid (aa) sequences of the S1 gene of PEDV and PDCoV were aligned by using Jotun Hein Method in DNASTar software (Version 7.10). Phylogenetic trees were generated based on the S1 gene of PEDV and PDCoV strains by using neighbour-joining method of Molecular Evolutionary Genetics Analysis (MEGA version 7.0) with a bootstrap value of 1,000 replicate datasets.

Abbreviations

aa: Amino acid; nt: Nucleotide; PED: Porcine epidemic diarrhea; TGE: Transmissible gastroenteritis PEDV: Porcine epidemic diarrhea virus; PDCoV: Porcine deltacoronavirus; SADS-CoV: Swine acute diarrhea syndrome coronavirus; TGEV: Transmissible gastroenteritis virus; PRoV: Porcine rotavirus; S: Spike protein; COE: collagenase equivalent; MEGA: Molecular Evolutionary Genetics Analysis; G: Glycine; A: Alanine; V: Valine; L: Leucine; I: Isoleucine; F: Phenylalanine; Y: Tyrosine; S: Serine; T: Threonine; D: Aspartic acid; E: Glutamic acid; H: Histidine.

Declarations

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Ethical approval and consent to participate

This study was approved by the Animal Ethics Committee of Jiangxi Agricultural University, Nanchang, China. All procedures involving animals in this study were conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Agriculture of China.

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Authors' contributions

Experiments design: DS YT. Experiments performance: FZ SL JG. Data analysis: DS FZ. Sequence analysis: ZL KL WY. Manuscript preparation: FZ YY ZD DS. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Table 1

Table 1 Categorization of detection results on porcine diarrhoea Associated-viruses of samples collected between 2012 and 2018

Classifications	Sample No.	Viruses (Number (positive rate,%))				
		PEDV	PDCoV	TGEV	PoRV	SADS-CoV
Year						
2012	158	91 (57.59)	31 (19.62)	1 (0.63)	0 (0.00)	0 (0)
2013	301	187 (62.13)	81 (26.91)	1 (0.33)	3 (1.00)	0 (0)
2014	714	413 (57.84)	193 (27.03)	4 (0.56)	5 (0.70)	0 (0)
2015	574	343 (59.76)	161 (28.05)	8 (1.39)	9 (1.57)	0 (0)
2016	476	239 (50.21)	124 (26.05)	2 (0.42)	3 (0.63)	0 (0)
2017	389	218 (56.04)	105 (26.99)	1 (0.26)	3 (0.77)	7 (1.80)
2018	375	221 (58.93)	118 (31.47)	4 (1.07)	2 (0.53)	0 (0)
Total	2,987	1,712 (57.32)	813 (27.22)	21 (0.70)	25 (0.84)	7 (0.23)
Province						
Jiangxi	2,690	1,524 (56.65)	737 (27.40)	16 (0.59)	23 (0.86)	0 (0)
Zhejiang	81	50 (61.73)	22 (27.16)	1 (1.23)	1 (1.23)	0 (0)
Fujian	68	45 (66.18)	23 (33.82)	1 (1.47)	0 (0)	7 (10.29)
Guangdong	92	58 (63.04)	18 (19.57)	2 (2.17)	1 (1.09)	0 (0)
Hunan	56	35 (62.50)	13 (23.21)	1 (1.79)	0 (0)	0 (0)
Sample type						
Intestine	1,581	1,001 (63.31)	469 (29.66)	13 (0.82)	13 (0.82)	5 (0.32)
Feces	1,305	688 (52.72)	336 (25.75)	8 (0.61)	12 (0.92)	2 (0.15)

Milk	101	23 (22.73)	8 (7.92)	0 (0)	0 (0)	0 (0)
Pig stage						
Sow	673	355 (52.75)	187 (27.79)	7 (1.04)	2 (0.30)	1 (0.15)
Suckling piglets	1,697	1,064 (62.70)	501 (29.52)	12 (0.70)	18 (1.05)	6 (0.35)
Nursering piglet	357	181 (50.70)	73 (20.45)	1 (0.28)	3 (0.84)	0 (0)
Finishing pig	215	91 (42.33)	36 (16.74)	1 (0.47)	2 (0.93)	0 (0)

Figures

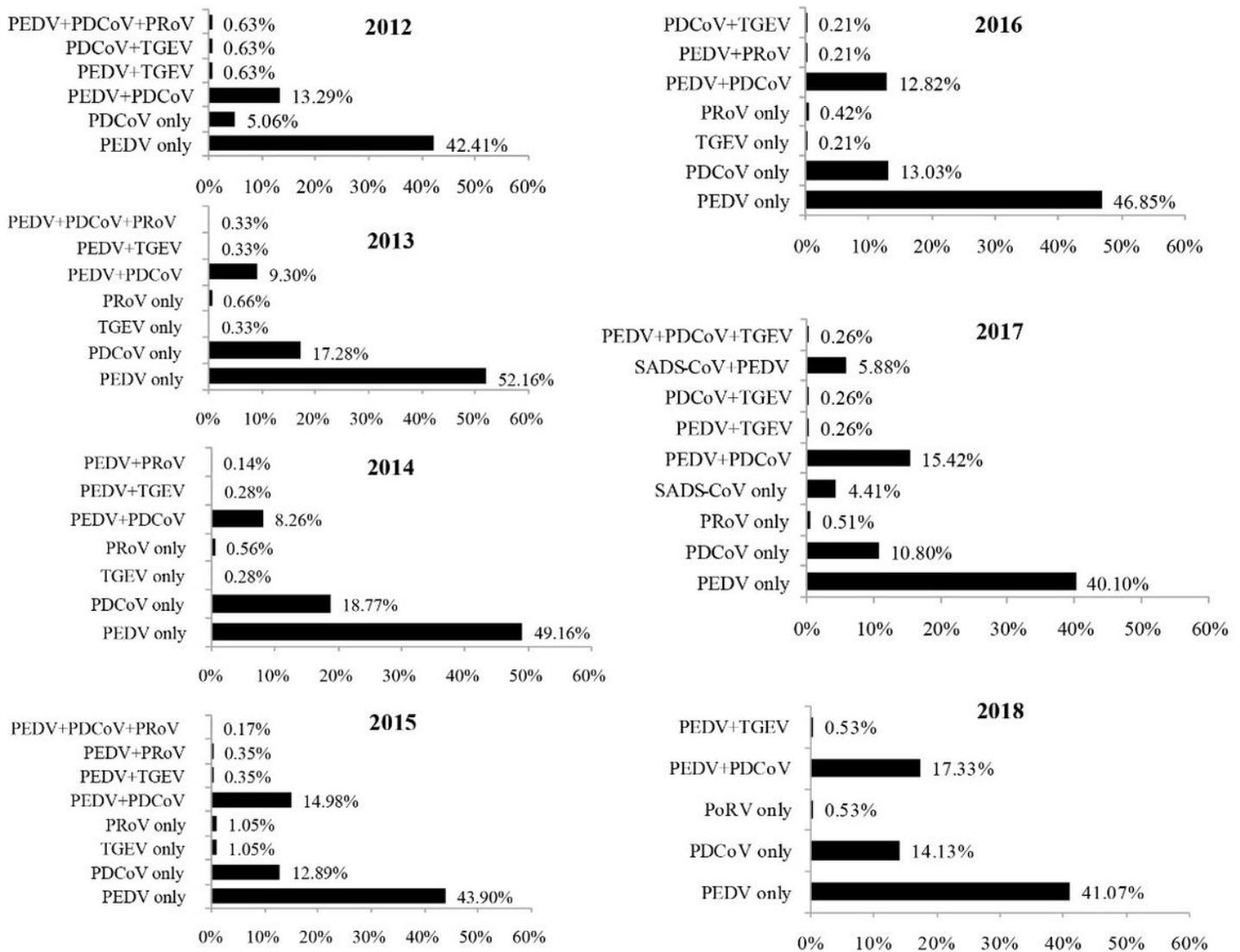


Figure 1

Mono- and co-infections of PEDV, PDCoV, TGEV, PRoV, and SADS-CoV in samples from Southern China during 2012 to 2018

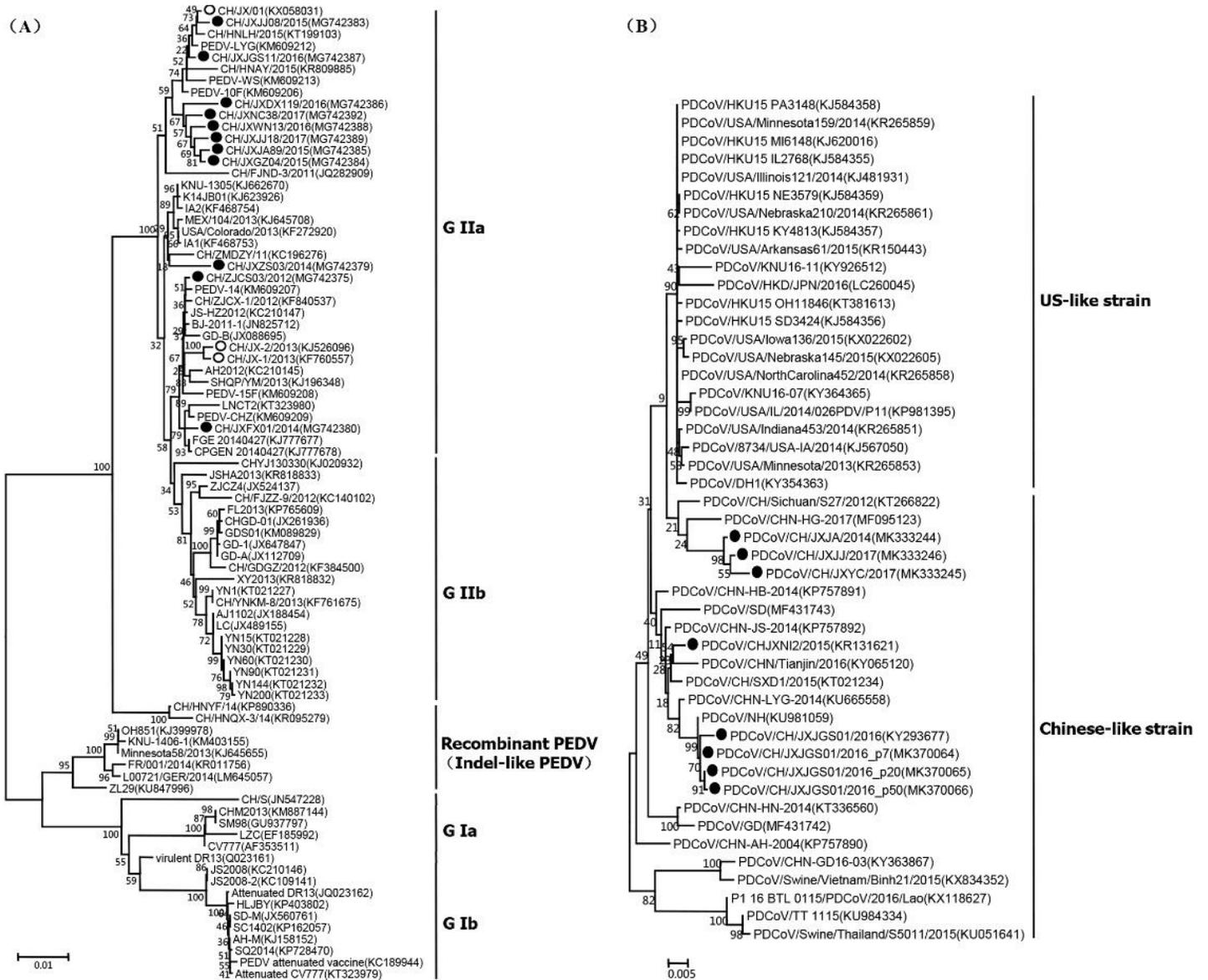


Figure 2

Phylogenetic analysis on the amino acid sequences of the S1 protein of selected PEDV (A) and PDCoV (B) strains from different countries in this study. “●” indicated the strains determined in this study. The tree was constructed using the neighbor-joining method (bootstrap resampling=1000 replications) in the MEGA software package, version 7.0

		SS2										SS6																				
		L	V	Y	S	N	I	G	V	C	K	S	G	S	I	G	Y	V	P	S	Q	Y	G	Q	V	K	I	A	P	T	V	T
G1a	CV777 (AF353511)	746																														
	LZC (EF185992)	746																														
	SM98 (GU937797)	746																		P												
G1b	SD-M (JX560761)	745																		L	D									M		
	Attenuated CV777 (KT323979)	745																		L	D									M		
	Attenuated DR13 (JQ023162)	745																		L	D									M		
Indel	ZL29 (KU847996)	746																				S										
	KNU-1406-1 (KM403155)	746																				S										
	OH851 (KJ399978)	746																				S										
G2a	CH/ZJCS03/2012 (MG742375)	749																				S										
	CH/JXZS03/2014 (MG742379)	749																				S										
	CH/JXFX01/2014 (MG742380)	749																				S										
	CH/JXJJ08/2015 (MG742383)	749																				S										
	CH/JXGZ04/2015 (MG742384)	749																				S										
	CH/JXJA89/2015 (MG742385)	749									M											S										
	CH/JXDX119/2016 (MG742386)	749																				S										
	CH/JXJGS11/2016 (MG742387)	749	L																			S						V				
	CH/JXWN13/2016 (MG742388)	749									M											S										
	CH/JXJJ18/2017 (MG742389)	749																				S										
	CH/JXNC38/2017 (MG742392)	749																				S										
	CH/JX/01 (KX058031)	749																				S										
	CH/JX-1/2013 (KF760557)	749																				S										
	CH/JX-2/2013 (KJ526096)	749																				S										
	AH2012 (KC210145)	749																				S										
	GD-B (JX088695)	749																				S										
	BJ-2011-1 (JN825712)	749																				S										
	CH/FJND-3/2011 (JQ282909)	749																				S										
	G2b	AJ1102 (JX188454)	749																		R	S										
GD-A (JX112709)		749																		R	S											
CH/GDGZ/2012 (KF384500)		749																			S											
CH/ZJCX-1/2012 (KF840537)		749																			S											
CH/FJZZ-9/2012 (KC140102)		749																			S									M		

Figure 3

Amino acid alignment results based on the S1 protein sequences of PEDVs. The neutralizing epitopes SS2 and SS6 are shown in red. Circles indicate the sequences determined in the present study

Supplementary Files

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