



Epidemiological and genetic characterization of porcine epidemic diarrhea virus in the Mekong Delta, Vietnam, from 2015 to 2017

Ngoc Hai Nguyen¹ · Tri Minh Huynh² · Hien Duc Nguyen³ · Danh Cong Lai¹ · Minh Nam Nguyen^{4,5,6}

Received: 9 January 2023 / Accepted: 6 April 2023 / Published online: 4 May 2023
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2023

Abstract

Porcine epidemic diarrhea (PED) virus (PEDV) is a highly contagious virus. PED was first identified in 2008 and has greatly affected the Vietnamese pig production economy. The aim of this study was to investigate the epidemiological and genetic characteristics of PEDV in piglet herds in the Mekong Delta, Vietnam. Diarrheal stool and intestinal samples from 2262 piglets from 191 herds in five provinces were collected to test for the presence of PEDV. Ten PEDV strains were randomly selected for sequencing, and four genes encoding PEDV structural proteins were analyzed. The rates of herds and samples positive for PEDV were 27.23% and 27.72%, respectively. In positive herds, the morbidity and mortality of PEDV-positive piglets were 97.97% and 79.06%, respectively, with most of the infected piglets under 7 days of age. Phylogenetic analysis showed that the 10 PEDV strains from this study clustered with genotype G2 strains from Vietnam and neighboring countries. Many amino acid substitutions were identified in important antigenic regions in the spike protein of the 10 strains when compared to four PEDV vaccine strains. This study provides novel insights into the epidemiology and genetic diversity of circulating PEDV strains, which could facilitate the development of an appropriate and proactive strategy for controlling PED.

Introduction

Porcine epidemic diarrhea (PED) virus (PEDV) is a highly contagious virus belonging to the genus *Alphacoronavirus*, family *Coronaviridae* [19]. PEDV is an enveloped virus with a diameter of approximately 95–190 nm [9]. The PEDV genome is a single-stranded positive RNA containing at least seven open reading frames (ORFs), which encode four structural proteins (S, E, M, and N) and three nonstructural proteins (replicase enzymes 1a, 1b, and ORF3) [5].

PED can spread very quickly in PEDV-negative herds and cause high morbidity (100%) and mortality (30% to 90%) in piglets. PED first occurred in Vietnam at the end of 2008 and caused heavy losses in pig herds in the Southeast provinces, with various mortality rates in piglets (50%–100%) [14]. PEDV did not cause death in mature pigs but did greatly affect reproductive performance, with a 12.6% reduction in farrowing rate, 5.7% failure to breed, 1.3% abortion rate, and 2.0% mummified fetuses [27].

The genetic and molecular epidemiological characteristics of PEDV in countries differ [17, 18]. In Vietnam, most PEDV isolates have belonged to the genotypes G1 and G2

Handling Editor: Pablo Pineyro.

✉ Ngoc Hai Nguyen
nguyenngochai@hcmuaf.edu.vn

✉ Minh Nam Nguyen
nmnam@medvnu.edu.vn

¹ Department of Infectious Disease and Veterinary Public Health, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Quarter 6, Linh Trung Ward, Thu Duc City, HCMC 700000, Vietnam

² Vemedim Research and Development Center, Can Tho 94000, Vietnam

³ College of Agriculture, Can Tho University, Can Tho 94000, Vietnam

⁴ Department of Biomedical Engineering, School of Medicine, Vietnam National University Ho Chi Minh City (VNU-HCM), Linh Trung Ward, Thu Duc City, Ho Chi Minh City 700000, Vietnam

⁵ Research Center for Genetics and Reproductive Health (CGRH), School of Medicine, Vietnam National University HCMC, Linh Trung Ward, Thu Duc City, Ho Chi Minh City 700000, Vietnam

⁶ Vietnam National University Ho Chi Minh City (VNU-HCM), Quarter 6, Linh Trung, Thu Duc City, Ho Chi Minh City 700000, Vietnam

and have very high genetic similarity to strains isolated from neighboring countries such as China and Thailand [3, 24].

The Mekong Delta is located in western Vietnam and is an important pig-production area. Although epidemiological and genetic studies of PEDV have been conducted in southeastern and northern Vietnam, a comprehensive survey of these features in the Mekong Delta has not been undertaken. In this study, we investigated the mortality, morbidity, and genetic characteristics of PEDV in the Mekong Delta, thereby providing new insights into PED in Vietnam.

Materials and methods

Study design and sample collection

This study was conducted on 63 commercial pig farms in five provinces in the Mekong Delta of Vietnam from 2015 to 2017. On these farms, a large number of piglets showed anorexia, depression, and yellow or watery diarrhea. In addition, some gastrointestinal lesions were observed in dead piglets, which had thin intestinal walls and distended stomachs filled with undigested milk (Fig. 1). We randomly selected about three herds per farm to collect diarrheal stools and intestinal samples. A total of 2262 samples from 191 herds were obtained and tested for the presence of PEDV using RT-PCR. The morbidity and mortality were also examined based on age and locality in PEDV-infected herds. Ten PEDV strains were selected from five provinces for sequencing of the four structural genes to investigate the genetic diversity of PEDV in the Mekong Delta region.

RNA extraction and RT-PCR

Total RNA from diarrheal stools and intestinal samples was extracted using a GenJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Fisher, USA) according to the manufacturer's protocol. The extracted RNA was used to synthesize cDNA using a RevertAid First Strand cDNA Synthesis Kit

(Thermo Fisher, USA). A 412-bp fragment of the M gene was amplified using a pair of specific primers [12]. The thermocycling conditions included a 5-min initial denaturation step at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and then a 10-min final elongation step at 72°C.

Genetic sequencing and analysis

Ten PEDV-positive samples were selected randomly for sequencing of the four genes encoding structural proteins (S, E, M, and N). The primer pairs used to amplify the complete S, E, M, and N genes are shown in Supplementary Table S1. The structural genes were sequenced on both strands at Macrogen (Korea), using the primers T7 and SP6. Sequence data were processed and assembled using Sequencher 5.4.6 software (Genecodes). The nucleotide sequences determined in this study were deposited in the GenBank database under the accession numbers shown in Supplementary Table S2.

Nucleotide and amino acid sequences were analyzed and compared with reference sequences (Supplementary Table S3) using Bioedit 7.2.6 software [8]. Phylogenetic trees based on the S, E, M, and N genes were built using the maximum-likelihood method in MEGA 6.06 software [23] with 1000 replicates.

Results and discussion

The prevalence of PEDV in piglets in the Mekong Delta provinces

Out of 191 herds of piglets with diarrhea, 52 (27.23%) tested positive for PEDV (Table 1), which was a higher rate than that reported previously for the southern region of Vietnam (16.96%) [15]. Tien Giang and Ben Tre had the highest rates of PEDV-positive herds, with 33.33% and 32.65%, respectively, followed by Vinh Long (24.13%). The percentage of PEDV-infected herds in Can Tho and Dong Thap was the



Fig. 1 Representative images of PEDV-infected piglets. (A) Weak piglets lying on top of each other. (B) Severe stomach hemorrhage. (C) Thin small intestine and severe large intestinal hemorrhage

Table 1 Prevalence of PEDV in piglets from the Mekong Delta provinces

Province/city	No. of herds	No. of positive herds (%)	No. of samples collected	No. of positive samples (%)
Ben Tre	49	16 (32.65)	618	199 (32.20)
Can Tho	32	6 (18.75)	361	71 (19.67)
Dong Thap	30	6 (20)	322	65 (20.19)
Tien Giang	51	17 (33.33)	645	218 (33.79)
Vinh Long	29	7 (24.13)	316	74 (23.42)
Total	191	52 (27.23)	2.262	627 (27.72)

lowest (18.75% and 20%, respectively). The proportion of PEDV-positive samples in the total samples collected was 27.72%. Tien Giang and Ben Tre had the highest proportion of infected samples, with 33.79% and 32.20%, followed by Vinh Long, Dong Thap, and Can Tho (23.42%, 20.19%, and 19.67%, respectively).

Of the 640 piglets in the positive herds, 627 (97.97%) tested positive for PEDV, and 506 (79.06%) died from PEDV infection (Table 2). This is consistent with the rates of PEDV-infected pigs found previously in the southern region (93.94% and 81.67%, respectively) and some northern provinces (96% and 68.6%, respectively) [14, 16]. A survey in the USA also showed high morbidity and mortality rates in infected piglets, with rates of 100% and 90-95%, respectively [20]. These results show that most of the piglets in the positive herd were infected with PEDV and died. Can Tho had the highest infection rate (100%) but the lowest mortality rate (73.24%). Meanwhile, the remaining provinces had similar infection and mortality rates, ranging from 97.37% to 98.48% and from 78.46% to 83.91%, respectively. The morbidity and mortality of piglets up to 28 days old in PEDV-positive herds were recorded for three age groups: under 7 days old, 7-10 days old, and over 10 days old. Piglets under 7 days of age had the highest PEDV morbidity and mortality (100% and 84.47%, respectively), and the lowest morbidity and mortality were in piglets older than 10 days (88.10% and 64.86%, respectively) ($p < 0.05$). In Europe,

it has been reported that the mortality rate can reach 100% in suckling piglets, while it is less than 10% in piglets older than 10 days of age, and less than 5% in adult and fattening pigs [1]. This seems to be due to differences in resistance and immune status among the animals. In previous studies, piglets were found to be very sensitive to PEDV, especially those under a week of age [18, 20, 22]. In PED outbreaks in 2010, the morbidity and mortality of piglets under 7 days of age were 100% and 80-100%, respectively [22]. In PED outbreaks in Taiwan, these rates were also very high (80-100% and 90-100%, respectively) [13].

Genetic characteristics of PEDV strains

Phylogenetic analysis based on four structural gene sequences showed that the 10 PEDV strains isolated from PEDV-positive piglets in this study belonged to the G2 genotype (Fig. 2). This contrasts with previous studies conducted in southern Vietnam, where all six PEDV strains collected in three provinces in southern Vietnam in 2009-2010 belonged to the G1 genotype [4]. More recently, two strains (HUA-PED176 and HUA-PED254) isolated in the south of Vietnam in 2015-2016 also belonged to the G1 genotype [24]. However, 23 out of 25 PEDV strains collected in northern Vietnam during 2012–2015 belonged to the G2 genotype [3]. Twenty-eight (100%) PEDV strains isolated in the northern and central regions of Vietnam between 2015 and 2016 belonged to the G2 genotype [24]. Furthermore, the majority of PEDV strains collected in northern Vietnam in 2018–2019 also belonged to the G2 genotype [7]. Together, these data show that PEDV strains belonging to genotype G2 have become more prevalent in Vietnam. The PEDV strains from this study had a very close genetic relationship to three strains from Vietnam that were isolated in the northern region in 2013-2014 (VN/VAP1113-1, VN/JFP1013-1, and HUA-14PED96), the CBR1 strain from Thailand, and three Chinese strains (CHGD-01, CH/FJZZ-9/2012, and PEDV-CHZ).

Multiple sequence alignments of the S, E, M, and N genes showed that the 10 PEDV strains from this study

Table 2 Number of PEDV-positive and dead piglets in PEDV-positive herds according to age and province

		No. of piglets	No. of positive piglets (%)	No. of dead piglets (%)
Province/city	Ben Tre	204	199 (97.55)	167 (83.91)
	Can Tho	71	71 (100)	52 (73.24)
	Dong Thap	66	65 (98.48)	51 (78.46)
	Tien Giang	223	218 (97.76)	175 (80.28)
	Vinh Long	76	74 (97.37)	61 (82.43)
Age (days)	< 7	425	425 (100)	359 (87.47)
	7 - 10	131	128 (97.71)	99 (77.34)
	> 10	84	74 (88.10)	48 (64.86)

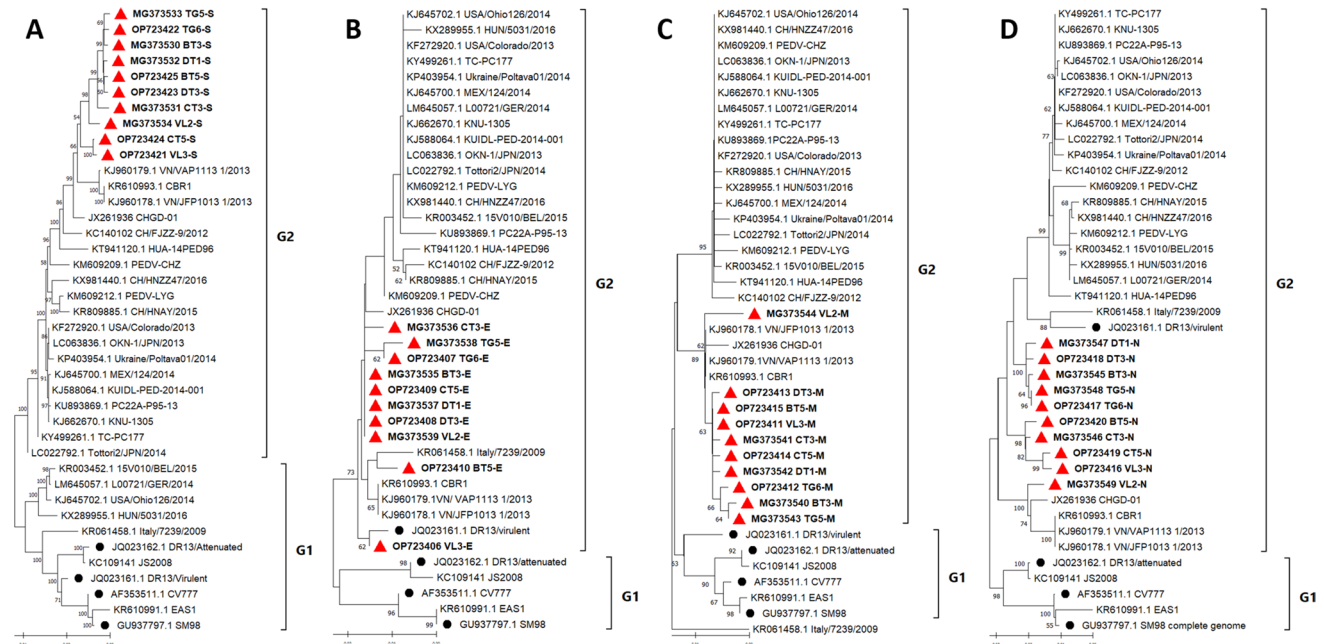


Fig. 2 Phylogenetic trees based on the nucleotide sequences of the genes encoding the S (A), E (B), M (C), and N (D) proteins of 10 PEDV strains. The neighbor-joining method was used for the construction of the phylogenetic trees in MEGA 6.06 software. Numbers

at branches are bootstrap values >50% (1000 replicates). Red triangles and black circles indicate PEDV strains from this study and vaccine strains, respectively. Scale bars represent nucleotide substitutions per site

showed a very high degree of similarity at the nucleotide and amino acid levels (97.2-100% and 93.0-100% identity, respectively) (Supplementary Table S4). They also had very high sequence similarity to three reference strains isolated in northern Vietnam (96.0-99.8% and 89.2-100% identity, respectively). Although the 10 PEDV strains exhibited high similarity at the nucleotide and amino acid levels in the E, M, and N genes to reference strains isolated from Korea, Japan, Thailand, China, and the USA (94.6-99.8% and 97.2-100% identity, respectively), the S gene had low sequence identity (81.5-98.3% and 73.5-95.5%, respectively). A comparison of the S genes of the 10 strains from this study and four PEDV vaccine strains (CV777, SM98, DR13/virulent, and DR13/attenuated) revealed that the sequence identity was only 92.3-94.3% at the nucleotide level and 83.5-88.1% at the amino acid level. In particular, these strains lacked 15 nucleotides (167, 176-186, and 430-432) and had an extra six nucleotides (492-497) when compared to the CV777, DR13/Attenuated, and DR13/Virulent strains, and they lacked 32 nucleotides (167, 176-186, 430-432, 4167-4183) and had an extra 18 nucleotides (344-355, and 492-497) when compared to the SM98 strain. Meanwhile, these 10 strains and the four vaccine strains showed low similarity at the nucleotide and amino acid levels in the E, M, and N genes (Supplementary Table S4).

Because of the large differences at the amino acid level, the predicted amino acid sequences of four major epitopes in the spike proteins of the 10 PEDV strains and four PEDV vaccine strains were compared (Fig. 3). There were many amino acid substitutions in the S gene of the 10 PEDV strains compared to the four PEDV vaccine strains. The S protein consists of 1383 amino acids and is divided into two subunits: S1 (1-789) and S2 (790-1383) [6]. In the S1 subunit, there were amino acid substitutions (S508A, L531I, A533S, Q550R, K568T, V581A, V592L, G599S, Y608H, A/E610D, G614S, and L/F617Y) in the core neutralizing epitope (aa 504-643), which contains the antigen-determining group that induces neutralizing antibodies and is also a variable region [10]. The specific mutation Y/D771S has been identified in a B cell epitope (aa 769-776) of the S1 region [21]. No amino acid substitutions were found in the epitope 1373GPRLQPY1379, which induces anti-PEDV neutralizing antibodies [2]. Although the S protein plays an important role in receptor binding and viral entry, the amino acid mutation rate of the PEDV S gene is very high, up to 10% [11, 25], and multiple epitopes that induce neutralizing antibodies have been identified in this protein. Thus, mutations in the antigenic determinant regions of PEDV can lead to a decrease in the ability of vaccines to protect against field strains [26]. It is therefore important to select vaccines

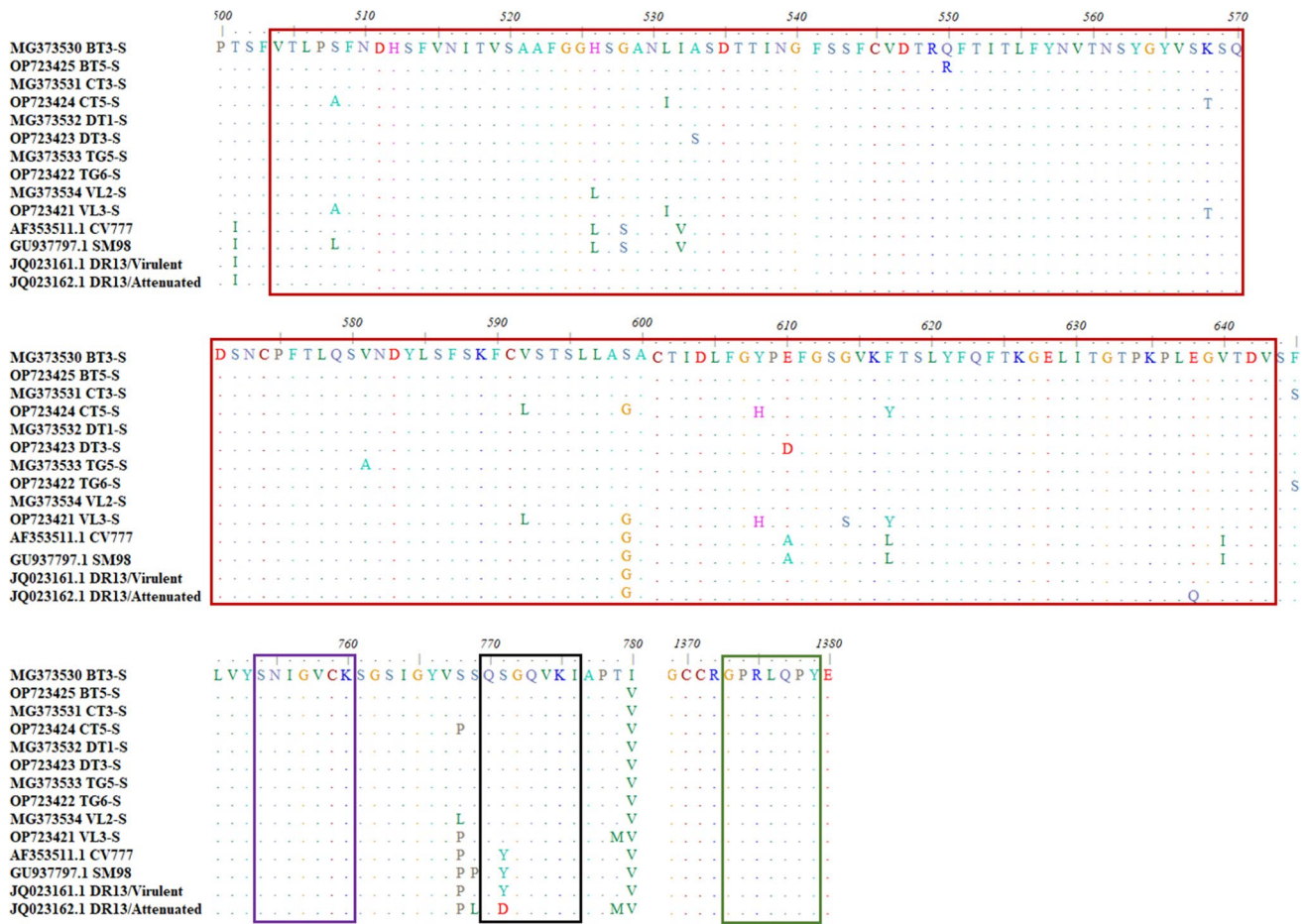


Fig. 3 Amino acid sequences of antigenic regions in the S protein of PEDV. Amino acid sequence alignments of GP5 from the 10 isolates from this study and four reference vaccine isolates are shown. Dots indicate identical amino acids, and letters indicate amino acid

changes. The red, purple, black, and green boxes indicate the amino acid positions of the core neutralizing epitope, two B-cell epitopes, and anti-PEDV neutralizing antibodies, respectively

with high similarity to the strains of PEDV that are circulating on farms for PED prevention and mitigation.

Conclusions

In this study, 27.23% of the 191 herds and 27.72% of the 2262 samples from five provinces in the Mekong Delta, Vietnam, that we tested were positive for PEDV, 97.97% of the piglets in the positive herds were PEDV-positive, and 79.06% died from PEDV infection. Ten PEDV strains from this study belonged to the G2 genotype and had a close relationship to PEDV strains isolated in Vietnam and neighboring countries. Fourteen amino acid substitutions were found in key antigenic regions of the spike protein.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00705-023-05779-6>.

Acknowledgements We are grateful for technical support that we received from the HanViet Veterinary Diagnosis Laboratory at Nong Lam University.

Author contributions NHN and MNN designed the study. TMH and HDN performed experiments. NHN, TMH, HDN, DCL, and MNN analysed the data. NHN, DCL, and MNN wrote the manuscript.

Availability of data and material The complete genome sequences of the PEDV strains identified in this study have been deposited in the GenBank database under the accession numbers MG373530-34 and OP723421-25 for the S gene, MG373535-39 and OP723406-10 for the E gene, MG373540-44 and OP723411-15 for the M gene, and MG373545-49 and OP723416-20 for the N gene.

Declarations

Conflict of interest All authors have read the journal's policy on disclosure of potential conflicts of interest, and we declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval The study was conducted in compliance with the institutional rules for the care and use of laboratory animals and using a protocol approved by the Ministry of Agriculture and Rural Development (MARD), Vietnam (TCVN 8402:2010).

References

- Antas M, Woźniakowski G (2019) Current status of porcine epidemic diarrhoea (PED) in European pigs. *J Vet Res* 63:465–470
- Cruz DJM, Kim C-J, Shin H-J (2008) The GPRLQPY motif located at the carboxy-terminal of the spike protein induces antibodies that neutralize Porcine epidemic diarrhoea virus. *Virus Res* 132:192–196
- Diep N, Sueyoshi M, Izzati U, Fuke N, Teh A, Lan N, Yamaguchi R (2018) Appearance of US-like porcine epidemic diarrhoea virus (PEDV) strains before US outbreaks and genetic heterogeneity of PEDV s collected in Northern Vietnam during 2012–2015. *Transbound Emerg Dis* 65:e83–e93
- Do DT, Nguyen TT, Suphasawat P, Roongroje T (2011) Genetic characterization of porcine epidemic diarrhoea virus (PEDV) isolates from southern Vietnam during 2009–2010 outbreaks. *Thai J Vet Med* 41:55
- Duarte M, Gelfi J, Lambert P, Rasschaert D, Laude H (1994) Genome organization of porcine epidemic diarrhoea virus. *Coronaviruses*. Springer, Berlin, pp 55–60
- Duarte M, Laude H (1994) Sequence of the spike protein of the porcine epidemic diarrhoea virus. *J Gen Virol* 75:1195–1200
- Duong BTT, Thao PTP, Hoa NT, Thu HT, Phuoc MH, Le TH, Van Quyen D (2022) Molecular analysis reveals a distinct subgroup of porcine epidemic diarrhoea virus in northern Vietnam in 2018–2019. *Adv Virol* 167:2337–2346
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series*, pp 95–98
- Jung K, Saif LJ, Wang Q (2020) Porcine epidemic diarrhoea virus (PEDV): an update on etiology, transmission, pathogenesis, and prevention and control. *Virus Res* 286:198045
- Kang T-J, Han S-C, Yang M-S, Jang Y-S (2006) Expression of synthetic neutralizing epitope of porcine epidemic diarrhoea virus fused with synthetic B subunit of *Escherichia coli* heat-labile enterotoxin in tobacco plants. *Protein Expr Purif* 46:16–22
- Kim S-H, Lee J-M, Jung J, Kim I-J, Hyun B-H, Kim H-I, Park C-K, Oem J-K, Kim Y-H, Lee M-H (2015) Genetic characterization of porcine epidemic diarrhoea virus in Korea from 1998 to 2013. *Adv Virol* 160:1055–1064
- Li R-F, Tian X-Q, Liu Y, Xu J, Liu D-Y (2016) Isolation and genetic analysis of a variant porcine epidemic diarrhoea virus in China. *Pol J Vet Sci* 19:65–73
- Lin C-N, Chung W-B, Chang S-W, Wen C-C, Liu H, Chien C-H, Chiou M-T (2014) US-like strain of porcine epidemic diarrhoea virus outbreaks in Taiwan, 2013–2014. *J Vet Med Sci* 76:14–00498
- Nguyen TT, Do DT (2012) Some related factors and pathological characteristics of Porcine Epidemic Diarrhoea in piglets in some southern provinces. *J Vet Sci Tech* XX:5–11
- Nguyen TT, Nguyen ĐQ, Trinh TTH, Do TD, Tran TD, Nguyen TPN, Nguyen TTN (2012) Detection and identification of porcine epidemic diarrhoea virus (PEDV) isolates from Southern-Eastern provinces of Vietnam. *J Vet Sci Tech* XIX:26–30
- Nguyen VD, Nguyen TL, Nguyen TH, Yamaguchi H (2014) Some epidemic and pathological characteristics of the porcine epidemic diarrhoea in some northern provinces, Vietnam. *Vet Sci Tech* 11:43–55
- Park S-J, Moon H-J, Yang J-S, Lee C-S, Song D-S, Kang B-K, Park B-K (2007) Sequence analysis of the partial spike glycoprotein gene of porcine epidemic diarrhoea viruses isolated in Korea. *Virus Genes* 35:321–332
- Puranaveja S, Poolperm P, Lertwatcharasarakul P, Kesdaengsakonwut S, Boonsoongnern A, Urairong K, Kitikoon P, Choojai P, Kedkovid R, Teankum K (2009) Chinese-like strain of porcine epidemic diarrhoea virus, Thailand. *Emerg Infect Dis* 15:1112
- Schmidt A, Wolff MH, Weber O (2005) Coronaviruses with special emphasis on first insights concerning SARS. Springer, New York
- Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, Cooper VL, Pillatzki A, Gauger P, Schmitt BJ (2013) Emergence of porcine epidemic diarrhoea virus in the United States: clinical signs, lesions, and viral genomic sequences. *J Vet Diagn Investig* 25:649–654
- Sun D, Feng L, Shi H, Chen J, Cui X, Chen H, Liu S, Tong Y, Wang Y, Tong G (2008) Identification of two novel B cell epitopes on porcine epidemic diarrhoea virus spike protein. *Vet Microbiol* 131:73–81
- Sun R-Q, Cai R-J, Chen Y-Q, Liang P-S, Chen D-K, Song C-X (2012) Outbreak of porcine epidemic diarrhoea in suckling piglets, China. *Emerg Infect Dis* 18:161
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Than VT, Choe SE, Vu TT, Do TD, Nguyen TL, Bui TT, Mai TN, Cha RM, Song D, An DJ (2020) Genetic characterization of the spike gene of porcine epidemic diarrhoea viruses (PEDVs) circulating in Vietnam from 2015 to 2016. *Vet Med Sci* 6:535–542
- Tortorici M, Veesler D (2019) Chapter four-structural insights into coronavirus entry. *Adv Virus Res* 105:93–116
- Tran TX, Lien NT, Thu HT, Duy ND, Duong BT, Van Quyen D (2021) Changes in the spike and nucleocapsid protein of porcine epidemic diarrhoea virus strain in Vietnam—a molecular potential for the vaccine development? *PeerJ* 9:e12329
- Weng L, Weersink A, Poljak Z, de Lange K, von Massow M (2016) An economic evaluation of intervention strategies for porcine epidemic diarrhoea (PED). *Prev Vet Med* 134:58–68

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.