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# Detection and phylogenetic analysis of Porcine epidemic diarrhea virus based on the ORF3 gene in pigs in the north of Vietnam

Tran Duc Hoan<sup>1\*</sup> Doan Thi Thao<sup>1</sup> Nguyen Thi Huong Giang<sup>1</sup> Nguyen Thi Thu Huyen<sup>1</sup>

## *Abstract*

To identify further the infectivity, genetic diversity and molecular characteristics of the porcine epidemic diarrhea virus (PEDV). 286 samples of the intestinal tissue of pigs with severe diarrhea were assessed from 5 provinces in the north of Vietnam. The full-length ORF3 gene of 20 representative field strains from 36 farms in 5 provinces was sequenced and analyzed. The prevalence of PEDV was 90.56% and was detected in 259 of 286 samples, with 94.44% (34 of 36) of pig farms harboring the disease. Based on the phylogenetic analysis of the ORF3 genes, our isolates all fell into group G2 (variants) and showed a close relationship to isolates from Chinese (GH1, QH2, and SX1), Korean (DR13 and DR13 attenuated) sources, and these isolates differed genetically from other Chinese (CH and CV777), Korean (DBI865) and French (CV777) strains. Our isolates also differed from attenuated vaccine strains, CV777 (used in China) and DR13 (used in Korea). According to our derived amino acid sequence analysis, we detected one novel variant PEDV, viz: BF, with 6-nt insertion, 4-nt difference, 68-nt deletion and 17-aa deletion at position 63-79 compared to the CV777 attenuated strain. Our ORF3 gene analyses showed that the prevalent PEDV isolates were variants and the isolated strains differed genetically from the vaccine strains. These results demonstrated the existence of genetic diversity among geographically distinct PEDV strains and our study has contributed to conducting further researches into PEDV and on an efficacious vaccine design in the future.

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**Keywords:** Detection, PEDV, ORF3 gene, Phylogenetic analysis, Variants

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## Introduction

Porcine epidemic diarrhea (PED) is an acute, highly contagious disease of swine caused by the Porcine Epidemic Diarrhea virus (PEDV), which leads to severe vomiting and diarrhea along with dehydration and high mortality in new-born piglets (Pensaert and de Bouck, 1978). PEDV belongs to the family *Coronaviridae*, genus *Alphacoronavirus* and was first reported in England (Takahashi et al., 1983). Since then a lot of research has been reported worldwide, including in Belgium, France, Japan, Korea, Italy, Thailand, the USA, Canada and Mexico (Cima, 2013; Martelli et al., 2008; Pensaert and de Bouck, 1978; Puranaveja et al., 2009; Takahashi et al., 1983), that poses severe economic burdens in pig production. Since 2010 serious PED outbreaks have been detected in China and Vietnam (Dam et al., 2014; Do et al., 2011; Sun et al., 2012). PEDV is an enveloped ssRNA coronavirus with a 28 kb genome, including seven open reading frames (ORFs), a 5' untranslated region (UTR) and a 3' UTR with a polyadenylated tail. The seven ORFs encode four structural proteins, spike (S), envelope (E), membrane (M) and nucleocapsid (N) and three non-structural proteins, replicases 1a, 1b and ORF3 (Bridgen et al., 1998; Kocherhans et al., 2001). The S protein contains a specific receptor binding site that plays an important role in cell membrane fusion and virus entry and is an antigenic target for neutralising antibodies (Duarte et al., 1994). The M protein is the most abundant surface protein and co-expression with E protein to form pseudo-particles results in interfering genic activity (Baudoux et al., 1998). The N protein is highly conserved and binds to virion RNA to provide a structural basis for the helical nucleocapsid and it is used for early diagnosis (Isabel et al., 2005). For non-structural proteins, replicases 1a and 1b are multi-functional and associated with viral genome replication (Brian and Baric, 2005) and the accessory ORF3 protein is thought to influence virulence (Wang et al., 2012).

The accessory ORF3 gene is highly relevant to the virulence of PEDV as previously reported (Wang et al., 2012) and reduction in virulence is produced through cell culture adaptation (Park et al., 2008; Park et al., 2007). The ORF3 gene of attenuated vaccine isolates has a continuous deletion of 17 amino acids (aa 82–99) thus distinguishing the vaccine isolates from variant PEDV (Park et al., 2008). Therefore, the ORF3 gene has been the focus of molecular epidemiology to study the characteristics of PEDV (Chen et al., 2013; Li et al., 2013; Song et al., 2015; Temeeyasen et al., 2014). As aforementioned, the ORF3 genes were chosen as the target genes for molecular characteristic analysis.

Many studies have indicated that the ORF3 gene of PEDV has an unexpected genetic variability (Chen et al., 2013). Especially, wild-type and cell culture adapted PEDVs almost have complete sequence identity except for variations and truncations in the ORF3 gene observed, exclusively, in the cell culture adapted PEDV (Chang et al., 2002; Chen et al., 2013; Sun et al., 2008; Suzuki et al., 2015). Similarly, the highly adapted PEDV attenuated DR13 is differentiated from wild-type PEDVs by both reverse transcriptase-polymerase chain reaction (RT-PCR) and restriction

fragment length polymorphism (RFLP), which use sequence variations in the ORF3 gene of the highly adapted PEDV. Moreover, the ORF3 gene has been suggested as an important determinant for PEDV biological properties.

Based on the ORF3 gene, the phylogenetic analysis of PEDV isolates from China divide into genotypes G1 and G2, the G2 genotype including 2a PEDV and 2b PEDV subgroups (Wang et al., 2016) and other strains isolated from some countries. To investigate further infectivity, genetic diversity and molecular characteristics of PEDV, we performed phylogenetic analyses based on the ORF3 gene of the latest collected Vietnamese isolates. This is the first report in Vietnam about the characterization of the PEDV ORF3 gene.

## Materials and Methods

**Sample collection and cDNA synthesis:** The 286 intestinal tissue samples were used in the clinical diagnosis of acute diarrhea from pigs with different ages suffering from severe diarrhea on 36 farms in 5 provinces (Bacgiang, Hanoi, Haiduong, Quangninh, and Vinhphuc) of northern Vietnam from September 2018 to August 2019. The samples were prepared for RNA extraction and RT-PCR detection.

**Primers design:** The PCR primers for amplification of the Porcine Epidemic Diarrhea Virus (PEDV) were designed by Primer Premier 5.0 software according to the nucleotide sequence published in GenBank (accession number EU054929). The sequences of the sense and anti-sense primers are as follows: Sense: 5'-TTCTGAGTCACGAACAGCCA-3' and Anti-sense: 5'-CATATGCAGCCTGCTCTGAA-3'.

A pair of primers based on the ORF3 gene of PEDV was designed and synthesized by Sangon Biotech Co., Ltd.

**RNA extraction and cDNA synthesis:** Intestinal tissue samples collected from diseased pigs were processed as 20% suspensions in PBS. RNA was extracted from 200 µl of each suspension prepared from the pig samples and the PEDV isolates using an RNA/DNA Isolation Kit for Cells and Tissues (WizPrep, Viral RNA Mini Kit, Korea) according to the manufacturer's instructions.

The synthesis of the cDNA was carried out through reverse transcription as described below. A total of 13 µl of viral RNA was mixed with 1 µl of 10 pmol Oligo (dT) primer (TaKaRa), incubated at 70°C for 10 mins, then placed on ice for 1 min. Next, 4 µl of 5×RT buffer, 1 µl of dNTP (2.5 mM) mixture, 0.5 µl of Rnase inhibitor (40 U/µl) and 0.5 µl of reverse transcriptase M-MLV (200 U/µl) were added and gently mixed. The mixture was kept at 42°C for 1 h and the resulting cDNA stored at -20°C until further use.

**RT-PCR detection:** PCR amplification was performed using Platinum Tag DNA polymerase High Fidelity (Invitrogen, CA, USA). After an initial incubation at 94°C for 5 mins, the reactions were subjected to 30 cycles of PCR as follows: 94°C for 60 s, 50°C for 60 s and 72°C for 60 s. These cycles were followed by a

terminal 10-min extension at 72°C. The PCR products were visualized by agarose gel electrophoresis.

**Sequencing and analysis:** PCR products were purified using the QIAquick PCR purification kit (Qiagen). The purified PCR products were sequenced using the BigDye Terminator Cycle sequencing kit (Applied Biosystems) according to the supplier's instructions. The sequence of the PCR products was determined using the dye terminator sequencing method and analyzed by an ABI PRISM 310 auto-sequencer.

**PEDV genome pairwise comparison:** To compare the PEDV nucleotide sequence pairwise identity, twenty of Open Reading Frame 3 (ORF3) gene sequences were downloaded from GenBank (Table 2) and compared using pairwise sequencing identity using the Mega5.10 software program.

**PEDV genome nucleotide homology and divergence:** The recently identified nucleotide sequence was aligned and analyzed with pairwise analysis and divergence based on the sequence by using the MegAlign 6, DNASTAR software program (Madison, WI, USA), nucleotide homology percentage was compared with available different ORF3 genes from NCBI GenBank database.

**Sequence alignments and phylogenetic analysis:** Sequences of ORF3 were processed by the Clustal W method (Guindon *et al.*, 2009) and phylogenetic trees were constructed by the neighbor-joining method using MEGA 6 software (Tamura *et al.*, 2013). Bootstrap values were indicated for each node from 2000 replicates. In addition, 20 reference strains (Table 2) were chosen from Genbank for inclusion in the phylogenetic analysis.

## Results

**PCR detection:** DNA fragments with expected lengths were amplified from DNA templates extracted from diseased pigs. PEDVs were detected by PCR on 94.44% (34 of 36) pig farms in 5 provinces and 90.56% (259 of 286) of samples were positive for PEDV in clinical diseased samples.

**Analyses of nucleotides and amino acids sequences:** The homology of PEDV BF strain nucleotide sequences compared with known PEDVs on the database was deduced by Blastn (NCBI) and analyzed with DNASTAR 2.0, SeqEdit, MegAlign, Clustal W and Jotun Hein methods. The results confirmed that its sequence was highly conserved and indicated that it had several homologues at the GenBank database. The percent identity results are shown under Table 3.

The sequence result of PEDV-BF and corresponding amino acids compared with the published sequence in GenBank described in Fig. 3 displayed 98.69 – 99.63% similarity in the nucleotide sequence and 98.63 – 99.95% in amino acid sequence with the published one. It indicates that the isolated sequence was highly related to that published one.

The sequence results revealed that the ORF3 sequence of the PEDV BF strain was 636 bp. The open

reading frame started with ATG at the beginning and ended with a TGA stop codon at base 636, encoding an about 25.2 kDa polypeptide of 224 amino acids. The deduced protein contained 17 strongly basic amino acid residues, 18 strongly acidic amino acid residues, 105 hydrophobic amino acid residues and 65 polar amino acid residues. Meanwhile the total net charge was positive. There were 4 nucleotide changes at the positions of 15, 16, 334 and 484; 6 nucleotide insertions at 627-630, 632, 634; 68 nucleotide deletions. There were 3 amino acid changes at the positions of 6, 106 and 159; 17 nucleotide deletion at 63-79. The theoretical pI of the protein was 4.61 and no signal peptide was found in the deduced protein but one glycosylation site and twenty-one phosphorylation sites were detected by sequence analysis.

**Pairwise comparison:** The isolate PEDV of nucleotide sequence pairwise comparison showed extensive similarity and divergence from previously isolated PEDV. The nucleotide sequences analyses of the new isolate were closely related to the strains DR13 attenuated, DR13 from Korea and QH1, QH2, NX1, SX from Shanxi, China.

To compare the difference of genes of PEDV, all genes including accessory genes were aligned and compared pairwise on the nucleotide similarity index (Table 4), the high nucleotide similarity of the PEDV BF strain was found 99 - 100% similar with PEDV ORF3 genes from Shanxi, China and Korea.

**Phylogenetic tree analysis:** The phylogenetic analysis revealed that, based on the ORF3 gene, the PEDV isolates were further divided into 3 clusters: clusters 1, 2 and 3 (Fig. 3). BF PEDV field isolates were in cluster 2, as were the isolates from China. Cluster 2 consisted of CV777 and isolates with a unique truncated portion, a 17-amino acid deletion at position 82-98, which represented attenuated virus vaccines and no BF PEDV isolate was included in this group. According to the analysis of the ORF3 genes, twenty PEDV isolates in this study were cluster 2, which showed similarity to the phylogenetic trees based on the sequences of amino acid. Our strains also showed a close relationship to published isolates and genetically differed from the vaccine strains which were all cluster 3, indicating that the prevailing PEDV strains were mostly variants. According to the sequences of ORF3 genes processes, our isolates exhibited 95.9-96.9% nucleotide identity and 93.8-96.4% amino acid identity compared with the CV777 strain. Meanwhile, our isolates exhibited 91.3-93.1% nucleotide identity and 84.6-89% amino acid identity compared with the CV777 attenuated/Korea strain.

## Discussion

In this research, we describe the open reading frame ORF3 sequence of the PEDV BF strain, isolated from diseased pigs from five provinces, including Bacgiang, Hanoi Haiduong, Quangninh and Vinhphuc, Vietnam.

Previous studies found 79.66% of pig farms in 29 provinces to be positive for the presence of PEDV, with 72.27% of samples confirmed as PEDV-positive (Chen

et al., 2013). The results of our study showed 94.03% (34 of 36) of pig farms in 5 provinces, 92.25% (259 of 286) samples were PEDV-positive, indicating a high prevalence of PEDV in clinical diseased samples.

The ORF3 genes of our field isolates sequenced to compare with the current isolates are closely related to field isolates in Korea and China or to vaccine isolates. The ORF3 genes have been used to differentiate

between field and vaccine-derived isolates. The vaccine-derived isolates have a unique characteristic at nucleotide position 245–293, a continuous deletion of 49 NT. The results of this study demonstrated that the Vietnamese field isolates do not possess that unique 49-nucleotide deletion suggesting that they are not related to vaccine isolates.

**Table 1** Numbers of PEDV positive samples and detection rates in respective age groups according to research periods, 2018–2019

| Location (province) | Collection samples | Positive   | Rate (%)           |
|---------------------|--------------------|------------|--------------------|
| Bacgiang            | 56                 | 52         | 92.86 <sup>a</sup> |
| Hanoi               | 58                 | 52         | 89.66 <sup>b</sup> |
| Haiduong            | 55                 | 51         | 92.73 <sup>a</sup> |
| Quangninh           | 61                 | 54         | 88.52 <sup>b</sup> |
| Vinhphuc            | 56                 | 50         | 89.29 <sup>b</sup> |
| <b>Overall</b>      | <b>286</b>         | <b>259</b> | <b>90.56</b>       |

Values within columns not sharing a common superscript letter (a–b) are significantly different at  $p < 0.05$ .

**Table 2** Summary of virus strains used in this study, Porcine Epidemic Diarrhea Virus; Accession number used for phylogenetic tree, pair wise comparison

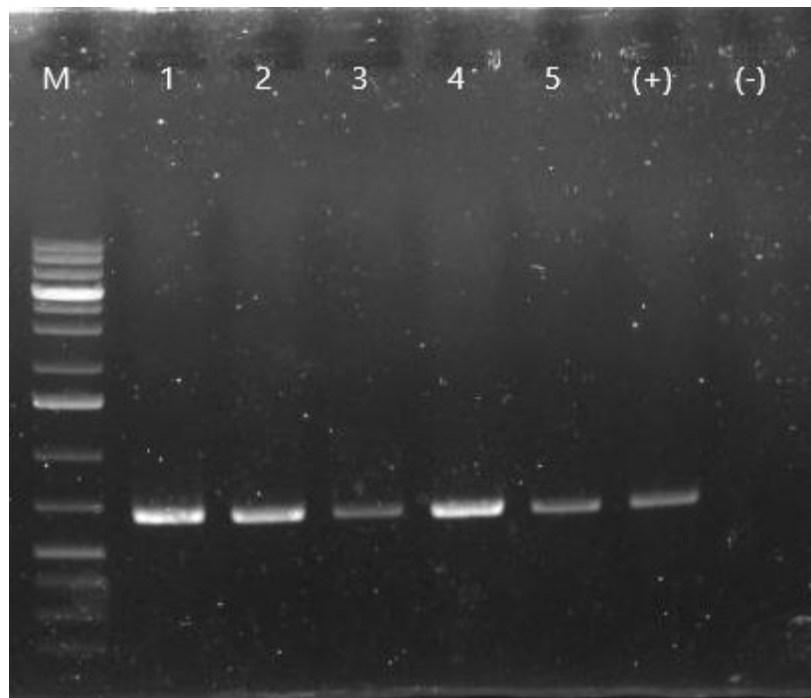
| Reference strain          | Year | Location            | GenBank accession no. | nt size |
|---------------------------|------|---------------------|-----------------------|---------|
| CH/SXYC                   | 2016 | Shanxi, China       | KU977500              | 675     |
| DR13                      | 2008 | Korea               | EU054929              | 675     |
| attenuated DR13           | 2012 | Korea               | JQ023162              | 675     |
| attenuated DR13           | 2008 | Korea               | EU054930              | 624     |
| J-vac                     | 2016 | Korea               | EF628251              | 612     |
| CH3-ORF                   | 2013 | Hubei, China        | KC342813              | 626     |
| DBI865 truncated ORF3     | 2012 | Korea               | HQ537432              | 672     |
| CV777 truncated ORF3      | 2010 | Heilongjiang, China | GU372744              | 725     |
| CH4-ORF3                  | 2013 | Hubei, China        | KC342814              | 626     |
| CH/BJ/2011 truncated ORF3 | 2012 | Heilongjiang, China | JQ027019              | 626     |
| QH2-ORF3                  | 2018 | Shanxi, China       | MF418657              | 675     |
| GS1-ORF3                  | 2018 | Shanxi, China       | MF418656              | 675     |
| QH1-ORF3                  | 2018 | Shanxi, China       | MF418658              | 672     |
| NX1-ORF3                  | 2018 | Shanxi, China       | MF418655              | 672     |
| SX2-ORF3                  | 2018 | Shanxi, China       | MF418657              | 672     |
| SX1-ORF3                  | 2018 | Shanxi, China       | MF418653              | 672     |
| CV777                     | 2016 | France              | Z24733                | 1740    |
| CV777 attenuated          | 2016 | Heilongjiang, China | KT323979              | 276     |
| CH/HNHB-1                 | 2016 | Hebei, Henan, China | KU977480              | 675     |
| CH/HNHB-2                 |      | Hebei, Henan, China | KU977481              | 675     |
| CH/HNHB-3                 |      | Hebei, Henan, China | KU977482              | 675     |
| CH/HNHB-4                 |      | Hebei, Henan, China | KU977483              | 675     |
| CH/HNHB-5                 |      | Hebei, Henan, China | KU977484              | 675     |
| CH/HNHB-6                 |      | Hebei, Henan, China | KU977485              | 675     |
| CH/HNHB-7                 |      | Hebei, Henan, China | KU977486              | 675     |
| CH/HNKF-1                 |      | Henan, China        | KU977487              | 675     |
| CH/HNKF-2                 |      | Henan, China        | KU977488              | 675     |
| CH/HNKF-3                 |      | Henan, China        | KU977489              | 675     |
| CH/HNSMX                  |      | Henan, China        | KU977490              | 675     |
| CH/SXYC                   |      | Shanxi, China       | KU977491              | 675     |
| CH/HNXC                   |      | Henan, China        | KU977492              | 675     |
| CH/HNXX                   |      | Henan, China        | KU977493              | 675     |
| CH/HNAY                   |      | Henan, China        | KU977494              | 675     |
| CH/HNAY-1                 |      | Henan, China        | KU977495              | 675     |
| CH/HNAY-2                 |      | Henan, China        | KU977496              | 675     |
| CH/HNAY-3                 |      | Henan, China        | KU977497              | 675     |
| CH/HNZMD                  |      | Henan, China        | KU977498              | 675     |
| CH/HNLY                   |      | Henan, China        | KU977499              | 675     |
| CH/HNPY                   |      | Henan, China        | KU977500              | 675     |

**Table 3** The nucleotide homology of PEDV BF strain ORF3 gene with other PEDV strains available on GenBank

| Description  | Max Score | Total Score | Per. Ident | Accession  |
|--|-----------|-------------|------------|------------|
| Porcine epidemic diarrhea virus strain attenuated DR13 ORF3 gene, complete cds                 | 1131      | 1131        | 99.36%     | EU054930.1 |
| Porcine epidemic diarrhea virus strain Ah2016f2, complete genome                               | 1122      | 1122        | 99.04%     | MN486588.1 |
| Porcine epidemic diarrhea virus strain AH-2018-HF1, complete genome                            | 1122      | 1122        | 99.04%     | MN315264.1 |
| Porcine epidemic diarrhea virus strain CN/Liaoning25/2018, complete genome                     | 1122      | 1122        | 99.04%     | MK796238.1 |
| Porcine epidemic diarrhea virus strain PPC 14, complete genome                                 | 1122      | 1122        | 99.04%     | MG781192.1 |
| Porcine epidemic diarrhea virus strain SQ2014, complete genome                                 | 1122      | 1122        | 99.04%     | KP728470.1 |
| Porcine epidemic diarrhea virus strain AH-M, complete genome                                   | 1122      | 1122        | 99.04%     | KJ158152.1 |
| Porcine epidemic diarrhea virus, complete genome   | 1122      | 1122        | 99.04%     | KC189944.1 |
| Porcine epidemic diarrhea virus isolate CH3 nonfunctional ORF3 protein gene, complete sequence | 1122      | 1122        | 99.04%     | KC342813.1 |
| Porcine epidemic diarrhea virus strain SD-M, complete genome                                   | 1122      | 1122        | 99.04%     | JX560761.1 |
| Porcine epidemic diarrhea virus strain attenuated DR13, complete genome                        | 1122      | 1122        | 99.04%     | JQ023162.1 |
| Porcine epidemic diarrhea virus isolate DBI865 truncated ORF3 gene, complete cds               | 1122      | 1122        | 99.04%     | HQ537432.1 |
| Porcine epidemic diarrhea virus strain CV777 truncated ORF3 protein gene, complete cds         | 1122      | 1122        | 99.04%     | GU372744.1 |
| Porcine epidemic diarrhea virus strain PEDV-SX, complete genome                                | 1116      | 1116        | 98.88%     | KY420075.1 |
| Porcine epidemic diarrhea virus isolate JSLS-1/2015, complete genome                           | 1116      | 1116        | 98.88%     | KX534205.1 |
| Porcine epidemic diarrhea virus isolate HLJBY, complete genome                                 | 1116      | 1116        | 98.88%     | KP403802.1 |
| Porcine epidemic diarrhea virus isolate CH4 nonfunctional ORF3 protein gene, complete sequence | 1116      | 1116        | 98.88%     | KC342814.1 |
| Porcine epidemic diarrhea virus strain CV777, complete genome                                  | 1110      | 1110        | 98.72%     | KT323979.1 |
| Porcine epidemic diarrhea virus strain JS2008, complete genome                                 | 1110      | 1110        | 98.72%     | KC210146.1 |
| Porcine epidemic diarrhea virus strain CH/BJ/2011 truncated ORF3 protein gene, complete cds    | 1110      | 1110        | 98.72%     | JQ027019.1 |
| Porcine epidemic diarrhea virus isolate SC1402, complete genome                                | 1101      | 1101        | 98.56%     | KP162057.1 |
| Porcine epidemic diarrhea virus isolate GDS09, complete genome                                 | 697       | 1141        | 99.22%     | MH726408.1 |
| Porcine epidemic diarrhea virus isolate QH2 ORF3 gene, complete cds                            | 693       | 1143        | 99.48%     | MF418657.1 |
| Porcine epidemic diarrhea virus isolate GS1 ORF3 gene, complete cds                            | 693       | 1143        | 99.48%     | MF418656.1 |
| Porcine epidemic diarrhea virus isolate QH1 ORF3 gene, complete cds                            | 673       | 1123        | 98.69%     | MF418658.1 |
| Porcine epidemic diarrhea virus isolate NX1 ORF3 gene, complete cds                            | 673       | 1123        | 98.69%     | MF418655.1 |
| Porcine epidemic diarrhea virus isolate SX2 ORF3 gene, complete cds                            | 673       | 1123        | 98.69%     | MF418654.1 |
| Porcine epidemic diarrhea virus isolate SX1 ORF3 gene, complete cds                            | 673       | 1123        | 98.69%     | MF418653.1 |

**Table 4** PEDV genes nucleotide sequence pairwise comparison in percentage identity and divergence values. The nucleotide sequences of the ORF3 gene of PEDV and other PEDVs.

| Seq->              | Percent identity |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|--------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                    | 1                | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    |
| BFVN               | ID               | 0.88  | 0.885 | 0.974 | 0.974 | 0.367 | 0.971 | 0.906 | 0.857 | 0.968 | 0.902 | 0.902 | 0.898 | 0.898 | 0.898 | 0.347 | 0.883 | 0.882 | 0.882 | 0.88  |
| CH/SXYC/ShxCN      | 0.885            | ID    | 0.979 | 0.902 | 0.902 | 0.379 | 0.903 | 0.846 | 0.788 | 0.9   | 0.976 | 0.976 | 0.971 | 0.971 | 0.971 | 0.371 | 0.995 | 0.994 | 0.994 | 1     |
| DR13/Kr            | 0.974            | 0.979 | ID    | 0.906 | 0.906 | 0.38  | 0.908 | 0.85  | 0.791 | 0.908 | 0.98  | 0.98  | 0.976 | 0.976 | 0.976 | 0.374 | 0.983 | 0.982 | 0.982 | 0.979 |
| DR13/attenuated/Kr | 0.974            | 0.902 | 0.906 | ID    | 1     | 0.37  | 0.996 | 0.928 | 0.86  | 0.993 | 0.924 | 0.924 | 0.92  | 0.92  | 0.92  | 0.348 | 0.905 | 0.903 | 0.903 | 0.902 |
| DR13/Kr            | 0.974            | 0.902 | 0.906 | 1     | ID    | 0.37  | 0.996 | 0.928 | 0.86  | 0.993 | 0.924 | 0.924 | 0.92  | 0.92  | 0.92  | 0.348 | 0.905 | 0.903 | 0.903 | 0.902 |
| Jvac/Kr            | 0.367            | 0.379 | 0.38  | 0.37  | 0.37  | ID    | 0.371 | 0.347 | 0.323 | 0.368 | 0.382 | 0.382 | 0.382 | 0.382 | 0.382 | 0.145 | 0.382 | 0.38  | 0.383 | 0.379 |
| CH/HubCN           | 0.971            | 0.903 | 0.908 | 0.996 | 0.996 | 0.371 | ID    | 0.931 | 0.863 | 0.996 | 0.927 | 0.927 | 0.922 | 0.922 | 0.922 | 0.349 | 0.906 | 0.905 | 0.905 | 0.903 |
| DBI865/Kr          | 0.906            | 0.846 | 0.85  | 0.928 | 0.928 | 0.347 | 0.931 | ID    | 0.926 | 0.928 | 0.868 | 0.868 | 0.864 | 0.864 | 0.864 | 0.375 | 0.848 | 0.847 | 0.847 | 0.846 |
| CV777/HIJCN        | 0.857            | 0.788 | 0.791 | 0.86  | 0.86  | 0.323 | 0.863 | 0.926 | ID    | 0.86  | 0.808 | 0.808 | 0.804 | 0.804 | 0.804 | 0.405 | 0.79  | 0.789 | 0.789 | 0.788 |
| CH/BJ/HijCN        | 0.968            | 0.9   | 0.908 | 0.993 | 0.993 | 0.368 | 0.996 | 0.928 | 0.86  | ID    | 0.924 | 0.924 | 0.92  | 0.92  | 0.92  | 0.348 | 0.903 | 0.902 | 0.902 | 0.9   |
| QH2/ShxCN          | 0.902            | 0.976 | 0.98  | 0.924 | 0.924 | 0.382 | 0.927 | 0.868 | 0.808 | 0.924 | ID    | 1     | 0.995 | 0.995 | 0.995 | 0.376 | 0.979 | 0.977 | 0.977 | 0.976 |
| GS1/ShxCN          | 0.902            | 0.976 | 0.98  | 0.924 | 0.924 | 0.382 | 0.927 | 0.868 | 0.808 | 0.924 | 1     | ID    | 0.995 | 0.995 | 0.995 | 0.376 | 0.979 | 0.977 | 0.977 | 0.976 |
| QH1/ShxCN          | 0.898            | 0.971 | 0.976 | 0.92  | 0.92  | 0.382 | 0.922 | 0.864 | 0.804 | 0.92  | 0.995 | 0.995 | ID    | 1     | 1     | 0.374 | 0.974 | 0.973 | 0.973 | 0.971 |
| NX1/ShxCN          | 0.898            | 0.971 | 0.976 | 0.92  | 0.92  | 0.382 | 0.922 | 0.864 | 0.804 | 0.92  | 0.995 | 0.995 | 1     | ID    | 1     | 0.374 | 0.974 | 0.973 | 0.973 | 0.971 |
| SX/ShxCN           | 0.898            | 0.971 | 0.976 | 0.92  | 0.92  | 0.382 | 0.922 | 0.864 | 0.804 | 0.92  | 0.995 | 0.995 | 1     | 1     | ID    | 0.374 | 0.974 | 0.973 | 0.973 | 0.971 |
| CV777/France       | 0.347            | 0.371 | 0.374 | 0.348 | 0.348 | 0.145 | 0.349 | 0.375 | 0.405 | 0.348 | 0.376 | 0.376 | 0.374 | 0.374 | 0.374 | ID    | 0.372 | 0.372 | 0.372 | 0.371 |
| CH/HNAY/CN         | 0.883            | 0.995 | 0.983 | 0.905 | 0.905 | 0.382 | 0.906 | 0.848 | 0.79  | 0.903 | 0.979 | 0.979 | 0.974 | 0.974 | 0.974 | 0.372 | ID    | 0.998 | 0.998 | 0.995 |
| CH/HNHB/CN         | 0.882            | 0.994 | 0.982 | 0.903 | 0.903 | 0.38  | 0.905 | 0.847 | 0.789 | 0.902 | 0.977 | 0.977 | 0.973 | 0.973 | 0.973 | 0.372 | 0.998 | ID    | 0.997 | 0.994 |
| CH/HNKF/CN         | 0.882            | 0.994 | 0.982 | 0.903 | 0.903 | 0.383 | 0.905 | 0.847 | 0.789 | 0.902 | 0.977 | 0.977 | 0.973 | 0.973 | 0.973 | 0.372 | 0.998 | 0.997 | ID    | 0.994 |
| CH/SXYC/CN         | 0.88             | 1     | 0.979 | 0.902 | 0.902 | 0.379 | 0.903 | 0.846 | 0.788 | 0.9   | 0.976 | 0.976 | 0.971 | 0.971 | 0.971 | 0.371 | 0.995 | 0.994 | 0.994 | ID    |



**Figure 1** Identification of PEDV from the diseased pigs samples by PCR amplification  
*M: Marker DNA (1 kb Plus DNA Ladder, Invitrogen); Lane 1: PEDV-BG; Lane 2: PEDV-HN; Lane 3: PEDV-HD; Lane 4: PEDV-QN; Lane 5: PEDV-VP; Lane 6: Positive control; Lane 7: Negative control.*

```

DBI865/Kr : .....*.....20.....*.....40.....*.....60.....*.....8..... : 76
CV777/HljC : ..... : 77
CH3/HubCN : ..... : 30
BF/VN : .....AC..... : 30
DR13a/Kr : ..... : 30
CH4/HubCN : .....T..... : 30
CH/BJ/2011 : ..... : 30
SX2/ShxCN : ..... : 30
SX1/ShxCN : ..... : 30
QH1/ShxCN : ..... : 30
GS1/ShxCN : ..... : 30
QH2/ShxCN : ..... : 30
DR13/Kr : ..... : 30
Jvac/Kr : .....CCG.GG.G.A.TGACA...CGCG.T...C... : 32
AtgtTtcTtGgActtttTCAatacAcGATT

```

```

DBI865/Kr : .....0.....*.....100.....*.....120.....*.....140.....*.....1..... : 155
CV777/HljC : ..... : 156
CH3/HubCN : ..... : 109
BF/VN : ..... : 109
DR13a/Kr : ..... : 109
CH4/HubCN : ..... : 109
CH/BJ/2011 : ..... : 109
SX2/ShxCN : ..... : 109
SX1/ShxCN : ..... : 109
QH1/ShxCN : ..... : 109
GS1/ShxCN : ..... : 109
QH2/ShxCN : ..... : 109
DR13/Kr : .....A..... : 109
Jvac/Kr : .....TGG.T.C...T...C.CTT..A...TTGGGTA.TGGAGAAA..C...A..G.CA.AA.CAACAGCA.A..CCT : 110
GacaCaGtTGTCAAAGATGtCtCgAAgTCTgccaactTgtctttggATgCTGtCcAaGagTtgGagctcaatGtAGttc

```

```

DBI865/Kr : .....60.....*.....180.....*.....200.....*.....220.....*..... : 226
CV777/HljC : ..... : 227
CH3/HubCN : ..... : 180
BF/VN : ..... : 180
DR13a/Kr : ..... : 180
CH4/HubCN : ..... : 180
CH/BJ/2011 : ..... : 180
SX2/ShxCN : ..... : 180
SX1/ShxCN : ..... : 180
QH1/ShxCN : ..... : 180
GS1/ShxCN : ..... : 180
QH2/ShxCN : ..... : 180
DR13/Kr : .....T.....C..... : 180
Jvac/Kr : .....A.GCAG..A...TC.G...GC.GA.AAAA.ACA.C.AAG.AG.ACAAA.CC.G...CC...GAA.G.AC...CC : 189
caAttaGAcAAGctTca AatGtgAcgggTtttCtTttcAccAgtgTttAt ttACTTCTcttGcActGT tt

```

```

DBI865/Kr : .....240.....*.....260.....*.....280.....*.....300.....*..... : 290
CV777/HljC : ..... : 291
CH3/HubCN : ..... : 244
BF/VN : ..... : 244
DR13a/Kr : ..... : 244
CH4/HubCN : ..... : 244
CH/BJ/2011 : ..... : 244
SX2/ShxCN : ..... : 259
SX1/ShxCN : ..... : 259
QH1/ShxCN : ..... : 259
GS1/ShxCN : ..... : 259
QH2/ShxCN : ..... : 259
DR13/Kr : .....C.....CG..... : 259
Jvac/Kr : .....TC.AA.A.A...CCA...-T.G.GGAGA...CCCAA..GC.AAAA.AGC...AG..GC..G...C... : 251
aaAgcGtCtTcttGagGcGcAattatATTatgttGGcaGcgGtTttGcTgtCAtttTctTtT

```

```

DBI865/Kr : .....320.....*.....340.....*.....360.....*.....380.....*..... : 330
CV777/HljC : ..... : 331
CH3/HubCN : ..... : 284
BF/VN : ..... : 282
DR13a/Kr : ..... : 282
CH4/HubCN : ..... : 284
CH/BJ/2011 : ..... : 284
SX2/ShxCN : ..... : 333
SX1/ShxCN : ..... : 333
QH1/ShxCN : ..... : 333
GS1/ShxCN : ..... : 333
QH2/ShxCN : ..... : 333
DR13/Kr : .....T.....C..... : 333
Jvac/Kr : .....G.C.C.GGGGGG.C.T..A.AA.T...G.AA...CG.AAAA.G.G. : 307
tGtTgCacActTATTGG CaGgcTTTGtttAgctGcTtT

```

```

DBI865/Kr : .....400.....*.....420.....*.....440.....*.....460.....*..... : 408
CV777/HljC : ..... : 409
CH3/HubCN : ..... : 362
BF/VN : .....A..... : 360
DR13a/Kr : ..... : 360
CH4/HubCN : ..... : 362
CH/BJ/2011 : ..... : 362
SX2/ShxCN : ..... : 411
SX1/ShxCN : ..... : 411
QH1/ShxCN : ..... : 411
GS1/ShxCN : ..... : 411
QH2/ShxCN : ..... : 411
DR13/Kr : .....C.....C..... : 411
Jvac/Kr : .....G.A...G.CAG...GCTC.GATCGC.AG.T.GGCACCA...GT.G.AG..T.GCTC...---GG...T.TG... : 383
Tact CaTggcGCTATAaaaaAtgCGctCtTtTaTtatctttAAATacTaCgaCacTttctTTtctcaaaTGGTAAAGcAGCT

```



|            |     |   |   |   |     |   |     |   |       |
|------------|-----|---|---|---|-----|---|-----|---|-------|
|            | 480 | *   | 500   | * | 520 | * | 540 | * |       |
| DBI865/Kr  | :   | ----  |   |   |     |   |     |   | : 485 |
| CV777/HljC | :   | ----  |   |   |     |   |     |   | : 486 |
| CH3/HubCN  | :   | ----  |   |   |     |   |     |   | : 439 |
| BF/VN      | :   | ----  |   |   |     |   |     |   | : 437 |
| DR13a/Kr   | :   | ----  |   |   |     |   |     |   | : 437 |
| CH4/HubCN  | :   | ----  |   |   |     |   |     |   | : 439 |
| CH/BJ/2011 | :   | A----   |   |   |     |   |     |   | : 439 |
| SX2/ShxCN  | :   | ----  |   |   |     |   |     |   | : 488 |
| SX1/ShxCN  | :   | ----  |   |   |     |   |     |   | : 488 |
| QH1/ShxCN  | :   | ----  |   |   |     |   |     |   | : 488 |
| GS1/ShxCN  | :   | ----  |   |   |     |   |     |   | : 488 |
| QH2/ShxCN  | :   | ----  |   |   |     |   |     |   | : 488 |
| DR13/Kr    | :   | ----  |   |   |     |   |     |   | : 488 |
| Jvac/Kr    | :   | GT.CG....TA..GG..T.-.AC..GC...C.T---A.A..T..A.A..G...G.GCCA..G...--.A.C.AAA |   |   |     |   |     |   | : 454 |
|            |     | taTtaTgA  | cgGCaaATcCaTTgtGAttCTAGaaggtggTgACcATTAcATcACTtTtggcAAcTCTtttGtTgCttt |   |     |   |     |   |       |

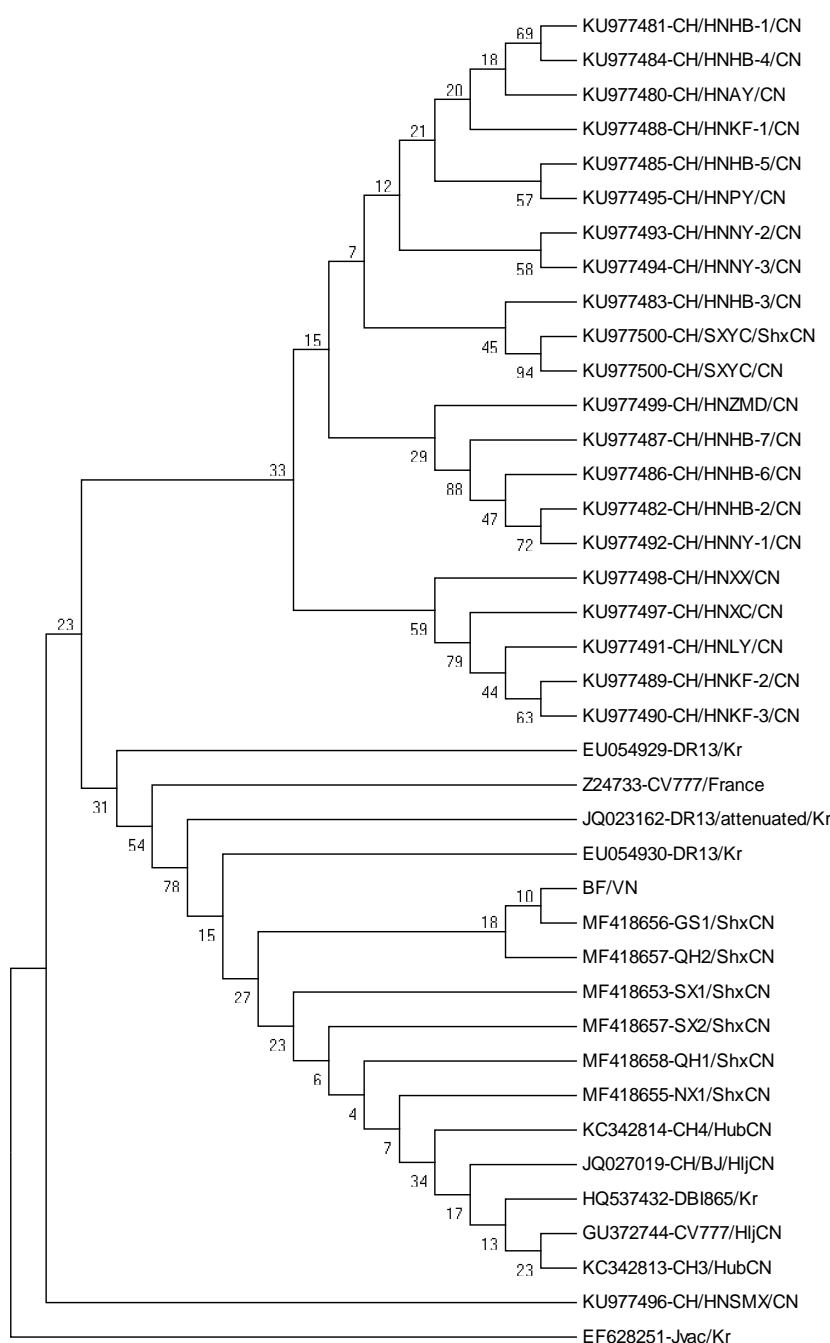
|            |     |  |     |   |     |   |     |   |       |
|------------|-----|--|-----|---|-----|---|-----|---|-------|
|            | 560 | *  | 580 | * | 600 | * | 620 | * |       |
| DBI865/Kr  | :   | ----   |     |   |     |   |     |   | : 564 |
| CV777/HljC | :   | ----   |     |   |     |   |     |   | : 565 |
| CH3/HubCN  | :   | ----   |     |   |     |   |     |   | : 518 |
| BF/VN      | :   | ----   |     |   | T   |   |     |   | : 516 |
| DR13a/Kr   | :   | ----   |     |   |     |   |     |   | : 516 |
| CH4/HubCN  | :   | ----   |     |   |     |   |     |   | : 518 |
| CH/BJ/2011 | :   | ----   |     |   |     |   |     |   | : 518 |
| SX2/ShxCN  | :   | ----   |     |   |     |   |     |   | : 567 |
| SX1/ShxCN  | :   | ----   |     |   |     |   |     |   | : 567 |
| QH1/ShxCN  | :   | ----   |     |   |     |   |     |   | : 567 |
| GS1/ShxCN  | :   | ----   |     |   |     |   |     |   | : 567 |
| QH2/ShxCN  | :   | ----   |     |   |     |   |     |   | : 567 |
| DR13/Kr    | :   | ----   |     |   |     |   |     |   | : 567 |
| Jvac/Kr    | :   | T...GAGCTTC...TT.CAC.GG.G.--.G.A.TTAAAA.TG.G.AT.---...T.C.C-CA.A...AGAAG..A---   |     |   |     |   |     |   | : 524 |
|            |     | cGTTtagtagcaTTGacTtgtAtcTaGctATaCgTggggcgCaaGaagcTgacCTAcAtCtgttGcGAActgttGAGctt |     |   |     |   |     |   |       |

|            |     |   |                          |   |     |   |     |   |       |
|------------|-----|---|--------------------------|---|-----|---|-----|---|-------|
|            | 640 | *   | 660                      | * | 680 | * | 700 | * |       |
| DBI865/Kr  | :   | ----  |                          |   |     |   |     |   | : 643 |
| CV777/HljC | :   | ----  |                          |   |     |   |     |   | : 644 |
| CH3/HubCN  | :   | ----  |                          |   |     |   |     |   | : 597 |
| BF/VN      | :   | ----  |                          |   |     |   |     |   | : 595 |
| DR13a/Kr   | :   | ----  |                          |   |     |   |     |   | : 595 |
| CH4/HubCN  | :   | ----  |                          |   |     |   |     |   | : 597 |
| CH/BJ/2011 | :   | ----  |                          |   |     |   | C   |   | : 597 |
| SX2/ShxCN  | :   | ----  |                          |   |     |   | --- |   | : 643 |
| SX1/ShxCN  | :   | ----  |                          |   |     |   | --- |   | : 643 |
| QH1/ShxCN  | :   | ----  |                          |   |     |   | --- |   | : 643 |
| GS1/ShxCN  | :   | ----  |                          |   |     |   |     |   | : 646 |
| QH2/ShxCN  | :   | ----  |                          |   |     |   |     |   | : 646 |
| DR13/Kr    | :   | ....T   |                          |   |     |   |     | C | : 646 |
| Jvac/Kr    | :   | --A.GAAG..C...G.G.AAC.A--.G..G..G.A..AA.AGGCC.TAT...A...C..GG.--G.G.C.T |                          |   |     |   |     |   | : 594 |
|            |     | cttgAcggcAAGAGCtTtAtgtCttttCaCaAcAtCAaAtTGttGgcattA                     | ATGctGcAttTGacTcaaTtCaAc |   |     |   |     |   |       |

|            |     |                                |     |           |     |     |     |   |       |
|------------|-----|--------------------------------|-----|-----------|-----|-----|-----|---|-------|
|            | 720 | *                              | 740 | *         | 760 | *   | 780 | * |       |
| DBI865/Kr  | :   | -----                          |     |           |     |     |     |   | : 672 |
| CV777/HljC | :   | -----                          |     | T.T       |     | T.C |     |   | : 723 |
| CH3/HubCN  | :   | -----                          |     |           |     |     |     |   | : 626 |
| BF/VN      | :   | -----                          |     | TT.TT.T.T |     |     |     |   | : 636 |
| DR13a/Kr   | :   | -----                          |     |           |     |     |     |   | : 624 |
| CH4/HubCN  | :   | -----                          |     |           |     |     |     |   | : 626 |
| CH/BJ/2011 | :   | -----                          |     |           |     |     |     |   | : 626 |
| SX2/ShxCN  | :   | -----                          |     |           |     |     |     |   | : 672 |
| SX1/ShxCN  | :   | -----                          |     |           |     |     |     |   | : 672 |
| QH1/ShxCN  | :   | -----                          |     |           |     |     |     |   | : 672 |
| GS1/ShxCN  | :   | -----                          |     |           |     |     |     |   | : 675 |
| QH2/ShxCN  | :   | -----                          |     |           |     |     |     |   | : 675 |
| DR13/Kr    | :   | -----                          |     |           |     |     |     |   | : 675 |
| Jvac/Kr    | :   | CT..T...ACCCA.G.C.-----        |     |           |     |     |     |   | : 612 |
|            |     | taGAcGAGtatgcTaCaAtttagtgaatga |     |           |     |     |     |   |       |

|            |   |  |  |   |         |       |          |           |               |       |
|------------|---|--|--|---|---------|-------|----------|-----------|---------------|-------|
|            |   | *  | 20   | *   | 40      | *     | 60       | *         | 80            |       |
| QH1/ShxCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| SX2/ShxCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| SX1/ShxCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| DR13/Kr    | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| GS1/ShxCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| QH2/ShxCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| DR13a/Kr   | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| BF/VN      | : | -----  | .....L   | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| DBI865/Kr  | : | -----  | DFN.TKLLKR.TCS...W.FS.HDHSC.RCLEVCQL.FGCCPRV.AQC..NTS.KCDG.SFH--QCF      | .....   | .....   | ..... | .....    | .....     | .....         | : 66  |
| CH3/HubCN  | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| CH4/HubCN  | : | -----  | .....F   | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| CH/BJ/2011 | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 68  |
| CV777/HljC | : | .....  | H.QC.LDFP..R.TQ...MSRSLPTC.WM.SKSWSS.F.LDK.QMR..F.PVFL.TS.HCLKR.LGA      | .....   | .....   | ..... | .....    | .....     | .....         | : 79  |
| Jvac/Kr    | : | -----  | .....VEHHAM.WWLLS.MPLN.WV..KI.TG.SNSRS..RKSL..AEKIHLRRTNPGP....          | .....   | .....   | ..... | .....    | .....     | .....         | : 60  |
|            |   |  | f glfqyt   | v kd6sk   | an slda | qeeln | vp6rqasn | gf f 3vfi | ffalfkasslrrn |       |
|            |   | 0  | *  | 100   | *       | 120   | *        | 140       | *             | 1     |
| QH1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| SX2/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| SX1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| DR13/Kr    | : | .....  | V  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| GS1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| QH2/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 147 |
| DR13a/Kr   | : | -----  | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 130 |
| BF/VN      | : | -----  | .....L   | .....   | .....   | ..... | .....    | .....     | .....         | : 130 |
| DBI865/Kr  | : | .....  | CT--VS..FEAQ.Y.VGS..CCHFSF   | .....   | .....   | ..... | .....    | .....     | .....         | : 143 |
| CH3/HubCN  | : | .....  | S-----   | AHL.A.F.SAPTHGAI.M.SL.S..LRHFLF.M.KQLIMTA.PLFK..T     | .....   | ..... | .....    | .....     | .....         | : 132 |
| CH4/HubCN  | : | .....  | S-----   | AHL.A.F.SAPTHGAI.M.SL.S..LRHFLF.M.KQLIMTA.PLFK..T     | .....   | ..... | .....    | .....     | .....         | : 132 |
| CH/BJ/2011 | : | .....  | S-----   | AHL.A.F.SAPTHGAI.M.SL.S..LRHFLF.M.KQLIITA.PLFK..T     | .....   | ..... | .....    | .....     | .....         | : 132 |
| CV777/HljC | : | I..CWQ.VL.S.F-----   | .....  | HTYWQALFS..LLMAL.CALYYLYDFFF.QWSSLL.QIHC.S.RWP.H      | .....   | ..... | .....    | .....     | .....         | : 144 |
| Jvac/Kr    | : | VTSKTSQSGGE.PR-----  | .....  | AK.AQLASDPGGASKILEM.NLSKKV..RQAMLR.P.WHQMQLQCHSLV..W. | .....   | ..... | .....    | .....     | .....         | : 126 |
|            |   | y  | laarfa   | fl  | l       | g     | 4        | l         | s             |       |
|            |   | 60   | *  | 180   | *       | 200   | *        | 220       | *             |       |
| QH1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | N-       | .....     | --            | : 223 |
| SX2/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | N-       | .....     | --            | : 223 |
| SX1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | N-       | .....     | --            | : 223 |
| DR13/Kr    | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | --            | : 224 |
| GS1/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | --            | : 224 |
| QH2/ShxCN  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | --            | : 224 |
| DR13a/Kr   | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | --            | : 207 |
| BF/VN      | : | .....  | .....  | .....   | .....   | ..... | D        | .....     | --            | : 209 |
| DBI865/Kr  | : | .....  | .....  | .....   | .....   | ..... | .....    | .....     | --            | : 220 |
| CH3/HubCN  | : | ITS----  | LLATLL.LS.VA.TCI.Y.G..K-LTYICCEL.SF.TA.SFMS.HNIK..A.LMLHLTQFNTSMLQL.N--- | .....   | .....   | ..... | .....    | .....     | .....         | : 203 |
| CH4/HubCN  | : | ITS----  | LLATLL.LS.VA.TCI.Y.G..K-LTYICCEL.SF.TA.SFMS.HNIK..A.LMLHLTQFNTSMLQL.N--- | .....   | .....   | ..... | .....    | .....     | .....         | : 203 |
| CH/BJ/2011 | : | ITS----  | LLATLL.LS.VA.TCI.Y.G..K-LTYICCEL.SF.TA.SFMS.HNIK..A.LMPHLTQFNTSMLQL.N--- | .....   | .....   | ..... | .....    | .....     | .....         | : 203 |
| CV777/HljC | : | HFWQL.CC.RHLVSS.TWAARSPTS.ANCASRQEA.CLFTTSNCWHYCC.LNSTRR.CYNNMIMVQL.SYFG.SYSFS-- | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 221 |
| Jvac/Kr    | : | FVSRILT.LHI.IKLCQ.L.Q.LSF.FH.WMHLKLG.LYPRERRK..TS.KPRSSR.KRPYMMIIVVCH.MRPMP----- | .....  | .....   | .....   | ..... | .....    | .....     | .....         | : 200 |
|            |   |  | l  | 6   | g       | l     | f        | 6         |               |       |

**Figure 2** The ORF Nucleotides sequence comparison for the 636 nucleotide fragment of PEDV-BF and corresponding amino acids sequence with twelve PEDVs from Genbank



**Figure 3** Phylogenetic analysis of the ORF3 nucleotide sequences of 20 PEDV isolates, including the reference strains. The trees were constructed by the neighbor-joining method in MEGA 6 software. Bootstrap values were indicated for each node from 2000 replicates. The names of the strains, years and places of isolation and GenBank accession numbers proposed are shown in Table 1.

The results of sequence analysis revealed that the ORF sequence of the PEDV BF strain was 636 bp. The open reading frame (ORF) started with ATG at the beginning and ended with a TGA stop codon at base 636, encoding about 25.2 kDa polypeptide of 224 amino acids. The nucleotide sequence of the PEDV BF strain compared with the published PEDV ORF3 sequence from GenBank revealed four change positions at 15, 16, 334 and 484, and the amino acids showed three different positions at 6, 106 and 159. It appears that the nucleotide and amino acid sequence of PEDV ORF3 are conserved between the strains of PEDV.

The ORF3 gene is highly relevant to the virulence of PEDV (Chen *et al.*, 2013; Park *et al.*, 2007), since it

regulates virus production (Wang *et al.*, 2012). The ORF3 genes of the PEDV isolates in this study and other isolates did not show the large deletion characteristic of the vaccine CV777 strain. According to the phylogenetic analysis of ORF3, the PEDV isolates in this study were divided into subtype G2 (Fig. 3). The ORF3 gene analysis not only suggested that the isolates in the northern province of Vietnam were not only vaccine-unrelated but the presence of multiple, distinct mutations indicated there is wide diversity in this virulence gene. However, further studies are needed to clarify whether the virulence is a change among these PEDV strains.

Phylogenetic analysis of the ORF3 gene showed that our isolates exhibited high similarity to variant reference strains and differed from CV777 and Jvac. Phylogenetic analysis of ORF3 did not reveal differences between our isolates and partial classical PEDV strains which were similar to other reports (Wang *et al.*, 2016; Su *et al.*, 2016). Further studies are required to conduct the biological functions among PEDV phylogenetic groups.

In conclusion, our study highlighted the present landscape of PEDV in the northern province of Vietnam and the isolated strains in this study were all variable and genetically diverse. These findings make it clear that a new vaccine is required to control this disease. Besides, the discovery of a novel strain, PEDV BF provides an avenue for future investigations into the biological functions of PEDV.

**Conflicts of Interest:** The authors declare no conflict of interest.

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