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*Research Article*

# Whole-Genome, Recombinant, and Phylogenetic Analysis of Porcine Epidemic Diarrhea Virus Strain CH/JSXZ/2015

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*Background.* Porcine epidemic diarrhea virus (PEDV) is an important pathogen causing highly contact infectious intestinal infections in pigs belonging to the family *Coronaviridae*, genus *Coronavirus*, which can cause porcine epidemic diarrhea (PED). Since 2010, outbreaks of PEDV variants have caused great economic losses to the swine industry worldwide. Our study will provide the basis for discovering the key points of PEDV variation and help in understanding the trend of popularity and evolution of PEDV in China. *Methods.* We amplify the complete PEDV CH/JSXZ/2015 genome sequence from naturally infected piglets in Xuzhou, Jiangsu Province, China, by RT-PCR. The comparative genome circle graph, characterization and phylogenetic analysis, and recombination in the PEDV CH/JSXZ/2015 and other PEDVs are analyzed by bioinformatics analysis software. *Results.* At the whole-genome level, CH/JSXZ/2015 showed the highest nucleotide identity (99.5%) with CH/GDZHDM/1401 strain (KR153326.1 and KX016034.1) and the lowest similarity (93.5%) to 85-7-mutant1 strain. CH/JSXZ/2015 S amino acid identity was 90.8%–99.8% compared with other strains. CH/JSXZ/2015 ORF3 amino acid identity was 91.2%–100.0% compared with other PEDV strains. Phylogenetic analysis based on the whole genome, S, and ORF3 revealed that CH/JSXZ /2015 is most genetically close to the CH/GDZHDM/1401 strain (KR153326.1 and KX016034.1) isolated in China in 2014. In addition, we revealed that the CH/GDZHDM/1401 (KX016034.1) strain, PEDV 1842/2016 ITA, and CH/JSXZ /2015 had recombination, and the position of recombination was 1–5530 and 28185–28544 bp. 5531–28184 bp of JSXZ are highly homologous to CH/GDZHDM/ 1401 (KX016034.1), while 1–5530 and 28185–28544 bp of JSXZ are highly homologous to 1842/2016 ITA. These results helped discover PEDV variation rules and provided a basis for preventing and controlling PEDV. *Conclusions.* Our study revealed that PEDV CH/JSXZ/2015 was a variant strain, the current epidemic strain in China. The CH/GDZHDM/1401 (KX016034.1) strain, PEDV 1842/2016 ITA (KY111278) and CH/JSXZ /2015 had recombination.

## 1. Introduction

Porcine epidemic diarrhea virus (PEDV) is an important pathogen causing highly contact infectious intestinal infections in pigs belonging to the family *Coronaviridae*, genus *Coronavirus*, which can cause porcine epidemic diarrhea (PED) [1]. PEDV mainly causes diarrhea in pigs and occasional vomiting after eating or milking [1]. The younger the age, the more severe the symptoms. Pigs born within a week often show severe dehydration symptoms and die 3–4 days after the onset of diarrhea, with a mortality rate of up to 50% and a maximum mortality rate of 100% [2]. The sick pigs are depressed, with reduced or no appetite. Weaned pigs and sows with mental atrophy and

TABLE 1: The reagents were used in the paper.

| Reagents name | Company | Country |
| --- | --- | --- |
| Trizol | Vazyme Biotech | Nanjing, China |
| TransScript First-Strand cDNA Synthesis SuperMix | Vazyme Biotech | Nanjing, China |
| Taq enzyme | Vazyme Biotech | Nanjing, China |
| T-Vector pMD19 | Takara Biotech | Dalian, China |
| AgaroseG-10 | Biowest Biotech | Loire Valley, France |

persistent diarrhea gradually recovered to normal after about 1 week [2]. PEDV infects a protease-rich environment, the epithelium of the small intestine, causing dehydration [3]. As a result, PEDV often causes diarrhea and systemic symptoms such as vomiting, fever, anorexia, and lethargy [4]. The disease is more severe in lactating piglets because of their increased susceptibility to dehydration, but outbreaks have also occurred in growing pigs and occasionally in adult pigs [5].

PEDV genome is a single-stranded positive-strand infectious RNA [5]. The pathogen was first identified as *Coronavirus* in Belgium in 1978, and the virus was also named PEDV (PEDV strain CV777) [6]. The complete genomic sequence of PEDV has 28,038 nucleotides (nt), a 5′ cap and 3′ polyadenylate tail, and at least seven open reading frames (ORFs). PEDV genes are Pol gene, S gene, ORF3 gene, E gene, M gene, and N gene in order from 5′end to 3′end [7]. Pol gene is mainly used to encode the RNA polymerase of replicase polyprotein lab, which is important in the early stage of virus infection [8]. The S protein mainly affects the immune response [8]. After the virus infects the host, it can recognize the target cells and promote the cell membrane fusion with the virus [9]. Therefore, the S protein is the main target that can effectively resist the *Coronavirus* [10]. If the S of the virus mutates, it is very likely to cause changes in the range of the host and the culture and virulence of tissue cells [11]. The ORF3 gene is used to encode the ORF3 protein, a nonstructural protein that directly determines the pathogenicity of the virus [12]. The E gene is used to encode the E protein, which is the smallest structural protein of PEDV. This protein is scattered on the envelope of PEDV, which can promote self-assembly and budding of the virus. M protein is a penetrating protein that plays an important role in virus assembly and budding [13]. At the same time, M protein is an important structural protein used to stimulate the body to produce immune protection [14]. As a major structural protein of PEDV, the main function of N protein is to form nucleocapsid. At the same time, the N protein is also involved in the replication and transcription of the virus [15].

In this study, we determined the complete genome sequences of viruses from tissues of diseased pigs in Xuzhou, Jiangsu, China, to study the diversity of PEDV. We compared these sequences with existing sequences while we analyzed PEDV CH/JSXZ/2015 for genetic variation with the full sequences of existing sequences, S and ORF3. In addition, we explored whether PEDV CH/JSXZ/2015 had the recombination. This experiment helps grasp the prevalence of PEDV and the genetic variation characteristics of PEDV and provides a theoretical basis for scientific prevention and control of PED.

## 2. Materials and Methods

*2.1. Source of Material.* One swine farm from Xuzhou, Jiangsu Province in China, had PED outbreaks, and we got clinical samples, such as fecal swabs, fecal samples, and intestines, to amplify the whole genome of PEDV. The complete genome sequence of the PEDV strain was CH/JSXZ/2015. GenBank number was MT625963. All the reagents are listed in Table 1.

*2.2. PEDV Primer Design and Synthesis.* To amplify the complete PEDV genome sequence, we designed primers targeting different genes based on the genome of strain CV777 (AF353511.1). These primers are shown in Table 2.

*2.3. RNA Extraction and RT-PCR.* The tissue grinding fluid was frozen and thawed three times and pelleted by centrifugation for 15 min at 12,000 rpm. The supernatant was collected and used to extract viral RNA. Following the instructions, we used Trizol reagent (Vazyme Biotech) to extract virus RNA. $1 \mu g$ of total RNA to synthesize cDNA according to the instructions of the reverse transcription kit (TransScript First-Strand cDNA Synthesis SuperMix). Using cDNA as a template, PCR amplification using Taq enzyme. Add the following reagents in sequence to the PCR tube to establish a $25 \mu l$ amplification reaction system: upstream primer $(10 \mu M) 1 \mu l$; downstream primer $(10 \mu M) 1 \mu l$; $2 \times$ Taq Master Mix $12.5 \mu l$; DNA template $1 \mu l$; and sterilized ultrapure water $9.5 \mu l$.

Reaction parameters for PEDV whole genome amplification: 95°C predenaturation for 3 min; denaturation at 95°C for 15 s, annealing at 50°C for 15 s, extension at 72°C for 1 min, 30 cycles; at last, extension at 72°C for 5 min and store at 12°C. The PCR amplification products were identified by agarose gel electrophoresis.

*2.4. Viral Genome Cloning and Sequencing.* After recovering the target gene by gel, the following reaction solution was prepared in a microcentrifuge tube (total volume of $5 \mu l$): T-Vector pMD19 (Simple) $1 \mu l$; gum recycling products $1 \mu l$ (0.1–0.3 pM); and sterilized ultrapure water $3 \mu l$.

Add an equal volume $(5 \mu l)$ of DNA Ligation Kit (code no. 6023) to the above DNA solution and mix thoroughly. 16°C reaction for 24 hr. Add the full amount $(10 \mu l)$ to $100 \mu l$ of *Escherichia coli* receptor cells, mix gently, and place on ice for 30 min. After a water bath at 42°C for 90 s, quickly transfer to ice for 5 min. Add $890 \mu l$ of LB medium and incubate at 37°C for 60 min. Apply $100 \mu l$ of the transformation solution on an agar plate medium and incubate overnight at 37°C. Select white colonies for PCR validation.

TABLE 2: Primers for the amplification of the PEDV genomic fragments.

| Primer name | Sequence (5′ to 3′) | Product size (bp) |
| --- | --- | --- |
| 5UTR-F | ACTTAAAAAGATTTTCTATCTACGGATAG | 291 |
| 5UTR-R | GCCGACAGTTACTGGTTTCA | |
| ORF1a-1F | ACTAGACAAACAGCCTTCCTCCG | 2005 |
| ORF1a-1R | CCTCTCACAACGCAGCTGTAAGTTTTAT | |
| ORF1a-2F | ATACCTGATTTTCCTATGCCTGTGGC | 2127 |
| ORF1a-2R | AACAAGGTCACTAAAGTCTCCTTGGT | |
| ORF1a-3F | CTGTATTGGAGCCTGTTGTCAAACC | 2083 |
| ORF1a-3R | CAGAGACCCAAAATGCTAAGGATGTACA | |
| ORF1a-4F | ACGCTACAAAGCTGTTAGACACTATG | 2025 |
| ORF1b-3R | CGCACTATTGTAACAAGTAGCAACCTTAC | |
| ORF1b-4F | CTAATTTTTGTGTTGTGGGCTGGTG | 2036 |
| ORF1b-4R | TCACTGGCCTGAAGTTGCGGATAAA | |
| ORF1b-5F | GCATTCTTAAATCGTTGGATTTGAGCG | 3032 |
| ORF1b-5R | CCGCCTAAAATTAGTGTTAGCTGAGC | |
| S1-F | GAATGGTAAGTTGCTAGTGCG | 1018 |
| S1-R | ATTAAACCTCAGAGCCTCTGG | |
| S2-F | CATGGTAAGGTGGTTTCCAACC | 1045 |
| S2-R | GGGACGTAAACTTAACACCACTACC | |
| S3-F | GGACAGTAATTGCCCTTTCACCT | 1035 |
| S3-R | CCAAGGCCATTAGTAACCACT | |
| S4-F | AATGTGCTGGGTGTTTCTGTG | 1104 |
| S4-R | ATCTGGGATTACTTCTGGTAGTT | |
| S5-F | ACCAGGTGACTTTGTAAATGTT | 800 |
| S5-R | GCGTTTTGCTGTCATCTT | |
| ORF3-F | GGTCCTAGACTTCAACCTTA | 779 |
| ORF3-R | AGCAGGAAAAAGAGTACGAA | |
| EMN-F | GTCTTTTCGCAACATCAAATTGTTGGC | 2100 |
| EMN-R | ACAAGAAGCTCAACATTTGGATCAGAC | |
| 3UTR-F | ACCAAATGTTGCAGCATTGC | 683 |
| 3UTR-R | GTGTATCCATATCAACACCGTCA | |

Then, positive clones were sent to Nanjing Kinco Biotechnology Co., Ltd. for sequencing.

2.5. Comparative Genome Analyzed by BRIG. The comparative genome circle graph is drawn by the software BRIG (Blasting Image Generator) [16].

2.6. Sequence Analysis. Sequence data were assembled and analyzed using the DNAStar package (DNAStar Inc.). The Clustal W method was used to analyze multiple sequence comparisons (the MegAlign program). The neighbor-joining method was used to construct the phylogenetic tree. The nucleotide and amino acid sequences of PEDV were compared with the CH/JSXZ/2015 strain of PEDV by the phylogenetic tree construction method. The nucleotide and the amino acid sequences of the CH/JSXZ/2015 strain were compared with the corresponding sequences of PEDV strains deposited in the GenBank database. The PEDV strains used in this study are shown in Table 3. The sequences of 69 complete genomes and their fully sequenced S and ORF3 of PEDV strains were used for sequence alignments and phylogenetic analyses. Furthermore, we aligned nucleotide sequences of

full-genomes, ORF3 genes, and S genes of PEDV strains by using the ClustalX 2.0 program [17].

2.7. Recombination Analysis. RDP5 software (Recombination Detection Programv 5.05 [18]) was used to identify recombination in the PEDV CH/JSXZ/2015 and other PEDVs.

# 3. Results

3.1. Genome-Wide Gene Comparison (Gene Circle Diagram). We selected 13 representative strains from each region to compare the whole genome with the PEDV CV777 strain as a reference. The genome of CH/JSXZ/2015 (JSXZ) did not show artificial gene deletion of common PEDV vaccine strains, and there was no obvious evidence of other gene sources, and the overall structure was similar to other isolates (Figure 1).

3.2. Complete Genomic Characterization of PEDV CH/JSXZ/2015 Strain. The genome of CH/JSXZ/2015 (JSXZ) is 28,044 nucleotides (nt) in length, excluding the poly(A) tail, with a G + C content of 42% and an A + T content of 58%. The genomic organization of the virus was in the following

TABLE 3: Nucleotide and amino acid sequence identity (%) of different PEDV CH/JSXZ/2015 genome regions compared with the other strains.

| | Strain | GenBank accession no. | Location | Date | CH/JSXZ/2015 Genome | S aa | ORF3 aa |
|---|---|---|---|---|---|---|---|
| 1 | JSXZ | MT625963.1 | China | 2015 | 100.0 | 100.0 | 100.0 |
| 2 | CV777 | AF353511.1 | Switzerland | 2001 | 93.6 | 92.4 | 98.5 |
| 3 | 85-7-C40 | KY486714.1 | China | 2017 | 93.6 | 90.8 | 96.1 |
| 4 | 85-7-mutant1 | KX839247.1 | China | 2016 | 93.5 | 90.8 | 95.4 |
| 5 | AH2012/12 | KU646831.1 | China | 2016 | 96.9 | 97.4 | 96.9 |
| 6 | AJ1102 | JX188454.1 | China | 2012 | 98.0 | 98.2 | 97.3 |
| 7 | B5-HB2017 | MF807952.1 | China | 2017 | 98.5 | 98.0 | 100.0 |
| 8 | BJ-2011-1 | JN825712.1 | China | 2011 | 98.9 | 99.0 | 99.6 |
| 9 | CH/FJND-3/2011 | JQ282909.1 | China | 2011 | 98.2 | 97.2 | 99.6 |
| 10 | CH/FJZZ-9/2012 | KC140102.1 | China | 2012 | 98.2 | 98.1 | 99.6 |
| 11 | CH/GDZHDM/1401 | KR153326.1 | China | 2014 | 99.5 | 99.8 | 100.0 |
| 12 | CH/GDZHDM/1401 | KX016034.1 | China | 2014 | 99.5 | 99.8 | 100.0 |
| 13 | CH/HNLH/2015 | KT199103.1 | China | 2015 | 99.2 | 99.4 | 99.6 |
| 14 | CH/HNZZA7/2016 | KX981440.1 | China | 2016 | 98.9 | 98.2 | 100.0 |
| 15 | CH/JLDH/2016 | MF346935.1 | China | 2017 | 98.8 | 98.8 | 96.4 |
| 16 | CH/JXJA/2017 | MF375374.1 | China | 2017 | 99.0 | 99.1 | 100.0 |
| 17 | CH/S | JN547228.1 | China | 2011 | 94.2 | 91.1 | 100.0 |
| 18 | CH/YNKM-8/2013 | KF761675.1 | China | 2013 | 98.2 | 98.3 | 98.2 |
| 19 | CH/ZJCX-1/2012 | KF840537.1 | China | 2013 | 98.9 | 99.3 | 100.0 |
| 20 | CHN/SH-2016-4/2016 | MG837012.1 | China | 2018 | 99.1 | 98.7 | 100.0 |
| 21 | CO/P14/IC | KU558702.1 | America | 2016 | 98.8 | 98.1 | 98.9 |
| 22 | FR/001/2014 | KR011756.1 | France | 2015 | 98.1 | 95.1 | 99.6 |
| 23 | GDS23 | MH107322.1 | China | 2018 | 98.8 | 98.6 | 98.2 |
| 24 | JS-HZ2012 | KC210147.1 | China | 2012 | 98.9 | 99.1 | 100.0 |
| 25 | LNCT2 | KT323980.1 | China | 2015 | 98.9 | 98.6 | 100.0 |
| 26 | MEX/104/2013 | KJ645708.1 | America | 2014 | 98.8 | 99.1 | 100.0 |
| 27 | NPL-PEDv/2013 | KJ778615.1 | America | 2014 | 98.8 | 99.1 | 100.0 |
| 28 | OH8593-14 | KP641662.1 | America | 2015 | 98.8 | 98.7 | 100.0 |
| 29 | OH9097-14 | KP641663.1 | America | 2015 | 98.8 | 98.6 | 100.0 |
| 30 | OH15962 | KJ584361.1 | America | 2014 | 98.7 | 99.1 | 100.0 |
| 31 | PC21A | KR078299.1 | America | 2015 | 98.7 | 99.2 | 100.0 |
| 32 | PC22A-P100-C6 | KU893871.1 | America | 2016 | 98.6 | 98.1 | 98.7 |
| 33 | PC273/O | MG837058.1 | America | 2018 | 98.6 | 99.2 | 99.6 |
| 34 | PEDV 1842/2016 ITA | KY111278.1 | Italy | 2016 | 97.9 | 94.7 | 99.6 |
| 35 | PEDV/MEX/PUE/01/2015 | MH004421.1 | Mexico | 2018 | 98.6 | 98.5 | 100.0 |
| 36 | PEDV/MEX/QRO/02/2017 | MH013466.1 | Mexico | 2018 | 98.6 | 98.7 | 100.0 |
| 37 | PEDV/USA/Minnesota125/2015 | KU982980.1 | America | 2016 | 98.7 | 98.5 | 99.2 |
| 38 | PEDV/USA/NorthDakota93/2015 | KU982970.1 | America | 2016 | 98.7 | 99.1 | 100.0 |
| 39 | PEDV/USA/Oklahoma133/2015 | KU982968.1 | America | 2016 | 98.8 | 98.5 | 100.0 |
| 40 | PEDV-LNsy | KY007140.1 | China | 2016 | 99.0 | 98.5 | 100.0 |

TABLE 3: Continued.

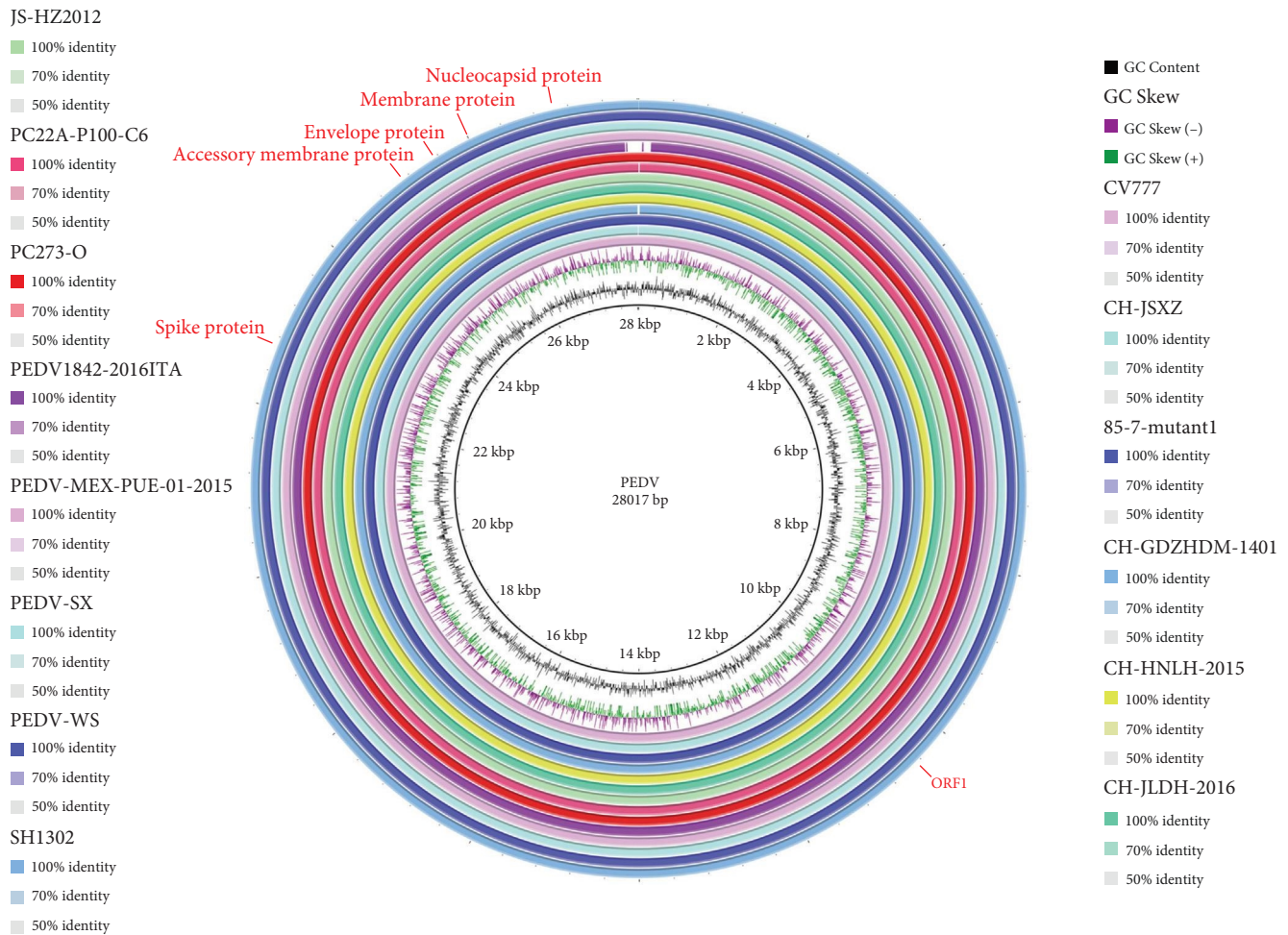| | | | | CH/JSXZ/2015 | | | |
|---|---|---|---|---|---|---|---|
| | Strain | GenBank accession no. | Location | Date | Genome | S aa | ORF3 aa |
| 41 | PEDV-SX | KY420075.1 | China | 2017 | 94.0 | 92.3 | 98.6 |
| 42 | PEDV-WS | KM609213.1 | China | 2014 | 99.3 | 99.4 | 100.0 |
| 43 | TC PC168-P2 | KM392226.1 | America | 2014 | 98.7 | 99.1 | 100.0 |
| 44 | USA/2014/IL/20697 P7 | KT591944.1 | America | 2015 | 98.5 | 95.0 | 95.1 |
| 45 | USA/Colorado/2013 | KF272920.1 | America | 2013 | 98.9 | 99.2 | 99.6 |
| 46 | USA/IL20697/2014 Passage 5 | KT860508.1 | America | 2015 | 98.5 | 95.0 | 100.0 |
| 47 | USA/Illinois98/2013 | KJ645690.1 | America | 2014 | 98.7 | 99.0 | 100.0 |
| 48 | USA/Illinois259/2014 from USA | KR265785.1 | America | 2015 | 98.8 | 99.1 | 100.0 |
| 49 | USA/Indiana34/2013&America | KJ645641.1 | America | 2014 | 98.8 | 98.9 | 100.0 |
| 50 | USA/Iowa/18984/2013 | KF804028.1 | America | 2013 | 98.7 | 99.1 | 100.0 |
| 51 | USA/Kansas46/2013 | KJ645650.1 | America | 2014 | 98.9 | 99.0 | 100.0 |
| 52 | USA/Kansas431/2014 from USA | KR265819.1 | America | 2015 | 98.9 | 98.9 | 100.0 |
| 53 | USA/Minnesota76/2013 | KJ645671.1 | America | 2014 | 98.7 | 99.0 | 100.0 |
| 54 | USA/Minnesota90/2013 | KJ645682.1 | America | 2014 | 98.8 | 99.1 | 99.6 |
| 55 | USA/Missouri373/2014 from USA | KR265844.1 | America | 2015 | 98.7 | 98.7 | 100.0 |
| 56 | USA/MO/2014/03293 | KM975741.1 | America | 2014 | 98.2 | 94.9 | 100.0 |
| 57 | USA/NC/2013/35140 | KM975735.1 | America | 2014 | 98.9 | 99.0 | 100.0 |
| 58 | USA/Nebraska287/2014 from USA | KR265765.1 | America | 2015 | 98.7 | 99.0 | 100.0 |
| 59 | USA/Nebraska288/2014 from USA | KR265803.1 | America | 2015 | 98.7 | 99.0 | 100.0 |
| 60 | USA/NorthCarolina66/2013 | KJ645662.1 | America | 2014 | 98.9 | 99.1 | 99.6 |
| 61 | USA/Ohio123/2014 | KJ645699.1 | America | 2014 | 98.8 | 99.1 | 100.0 |
| 62 | USA/OK10240−8/2017 | MG334555.1 | America | 2017 | 98.8 | 99.0 | 100.0 |
| 63 | USA/Tennesse56/2013 | KJ645654.1 | America | 2014 | 98.7 | 98.7 | 100.0 |
| 64 | YN90 | KT021231.1 | China | 2015 | 98.1 | 97.8 | 91.2 |
| 65 | PEDV/Pig-wt/ESP/Calaf-1/2014 | MT602520.1 | Spain | 2020 | 98.0 | 95.3 | 99.1 |
| 66 | HNAY2016 | MT338518.1 | China | 2021 | 99.0 | 99.5 | 99.2 |
| 67 | CH/HNXX/2016 | MT338517.1 | China | 2021 | 98.5 | 97.9 | 98.2 |
| 68 | CH/SXWS/2018 | MT090146.1 | China | 2020 | 99.1 | 99.2 | 100.0 |
| 69 | SH1302 | MT843277.1 | China | 2020 | 94.0 | 98.6 | 92.4 |
| 70 | MSCH | MT683617.1 | China | 2021 | 98.7 | 97.9 | 98.2 |

Abbreviations: aa, amino acid.

FIGURE 1: Genome-wide comparison of CH/JSXZ/2015 (CH-JSXZ) with different isolates available in GenBank. We selected representative strains from each region to compare the whole genome with the PEDV CV777 strain as a reference.

order: 5′ untranslated region (UTR), nucleotide 1–292; replicase polyprotein, nucleotide 293–12,646 for ORF1a and nucleotide 12,601–20,637 for ORF1b; spike (S) protein, nucleotide 20,634–24,794; ORF3, nucleotide 24,794–25,468; envelope (E) protein, nucleotide 25,449–25,679; membrane (M) protein, nucleotide 25,687–26,367; nucleocapsid (N), nucleotide 26,379–27,704; and 3′UTR, nucleotide 27,705–28,044.

Interestingly, compared with CH/JLDH/2016, JSXZ had a single amino acid insertion in the S gene ($^{1197}$T). The genome information of JSXZ will be conducive to understanding the evolutionary characteristics and molecular epidemiology of PEDV in China.

*3.3. Genome-Wide Sequence Comparison.* The complete genome sequences of 70 PEDV strains (Table 3) from different locations and years were compared, and the results revealed that JSXZ had a nucleotide identity of 99.5%–90.1% with 69 other entire PEDV genomes available in GenBank (Table 3). JSXZ showed the highest nucleotide identity (99.5%) with CH/GDZHDM/1401 strain (KR153326.1 and KX016034.1) and the lowest similarity (93.5%) to 85-7-mutant1 strain (KX839247.1). JSXZ shared 93.6% nucleotide similarity with CV777 and 98.9% nucleotide identity with JS-HZ2012

(KC210147.1) strain, which was isolated from the same province with JSXZ, and 98.6% nucleotide similarity to PEDV/MEX/PUE/01/2015 (MH004421.1), PC22A-P100-C6 (KU893871.1), and PC273/O (MG837058.1), respectively.

*3.4. Phylogenetic Analysis of CH/JSXZ/2015.* To analyze the evolution of PEDV, we performed a phylogenetic tree based on the determined nucleotide sequences of the full-length JSXZ, comparing 69 PEDV strains (Table 3). Interestingly, phylogenetic analysis revealed that these PEDV strains cluster into two groups (Figure 2). Viruses in group I includes CV777, PEDV-SX, SH1302, 85-7 mutant1, and 85-7-C40. JSXZ and others belong to Group II. JSXZ is closely related to CH/GDZHDM/1401(KR153326.1 and KX016034.1), PEDV-WS, and CH/HNLH/2015.

*3.5. Characterization and Phylogenetic Analysis of S.* S protein is a fibrotic glycoprotein located on the surface of PEDV and the largest structural protein of PEDV [11]. S protein is a key determinant of PEDV invading host cells and a key host antibody response target site [19]. Moreover, it also includes viral neutralizing epitopes that can serve as an important target for vaccine development [20]. Therefore, we conducted
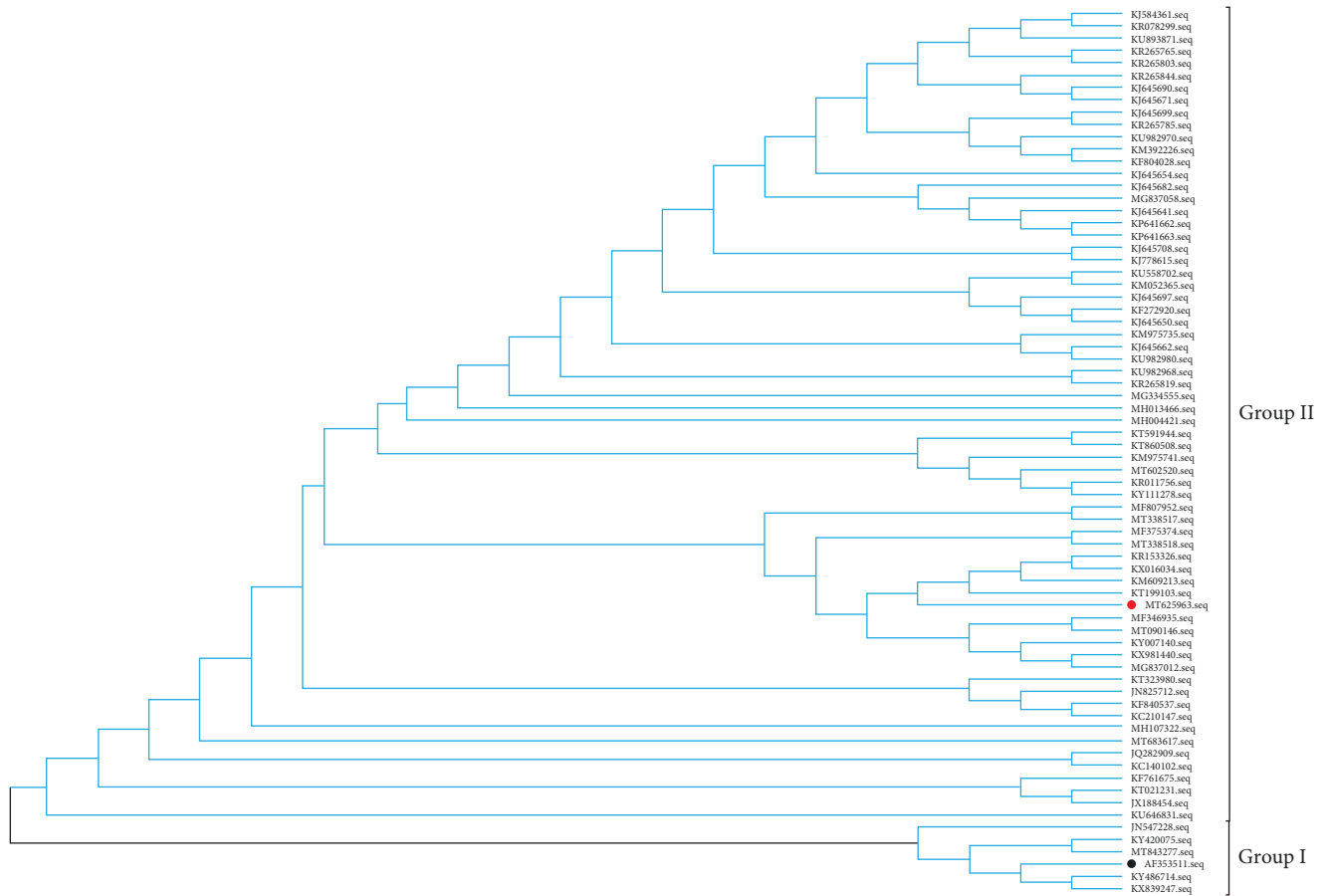
FIGURE 2: Phylogenetic analysis of the whole genome of PEDV strains (red solid circles indicate PEDV CH/JSXZ/2015, black solid circle indicated PEDV CV777).

a systematic development analysis of S proteins JSXZ with 69 PEDV strains (Table 3). JSXZ S amino acid identity was 90.8%–99.8% compared with other strains.

To analyze the evolution of JSXZ, phylogenetic analysis based on 70 PEDV strains S gene (Table 3) was performed. We found a phylogenetic tree cluster into two groups (Figure 3). The result had a similar grouping structure as the tree generated from the PEDV whole genomes. The S of JSXZ belonged to Group II. The S of JSXZ is closely related to CH/GDZHDM/1401 (KR153326.1 and KX016034.1).

3.6. Characterization and Phylogenetic Analysis of ORF3. ORF3 is the only adjuvant protein of PEDV, located between the S protein and the E protein, composed of 225 amino acids, and the relative molecular mass is about 25 kdα. PEDV ORF3 proteins show ion channel activity and enhance the copying ability of viruses [21]. Besides, ORF3 is important in PEDV inhibiting host natural immunity [22]. We compared the amino acid of JSXZ ORF3 identity with 69 PEDV strains (Table 3). The results revealed that JSXZ ORF3 amino acid identity was 91.2%–100.0% compared with other PEDV strains. The results showed that JSXZ ORF3 protein was similar to the remaining strains.

We performed a phylogenetic tree based on the determined nucleotide sequences of ORF3 of JSXZ, comparing 69 PEDV strains ORF3 (Table 3). These PEDV strains cluster into two groups (Figure 4). The result had a similar grouping structure as the tree generated from the PEDV whole genomes and S. The ORF3 of JSXZ belonged to the Group II.

3.7. Recombination Analysis. Recombination Detection Program v5.5, RDP5 was used to identify recombination in the CH/GDZHDM/1401 (KX016034.1) strain, PEDV 1842/2016 ITA (KY111278.1) and JSXZ, and the position of recombination is 1–5530 and 28185–28544 bp (Figure 5(a)). According to the separation time of the three strains, we speculated that PEDV 1842/2016 ITA isolated by Itay in January 2016 might be obtained by recombination of CH/GDZHDM/1401(KX016034.1) isolated by China in 2014 and JSXZ isolated by China in 2015. According to the results of evolutionary tree analysis (Figures 5(b) and 5(c)), we revealed that 5531–28184 bp of JSXZ are highly homologous to CH/GDZHDM/1401 (KX016034.1), while 1–5530 and 28185–28544 bp of JSXZ are highly homologous to 1842/2016 ITA (KY111278.1).

## 4. Discussion

As the virus continues to evolve and mutate, PEDV is causing more and more damage to the pig herd, as well as causing huge losses to the global pig economy. The early clinical detection
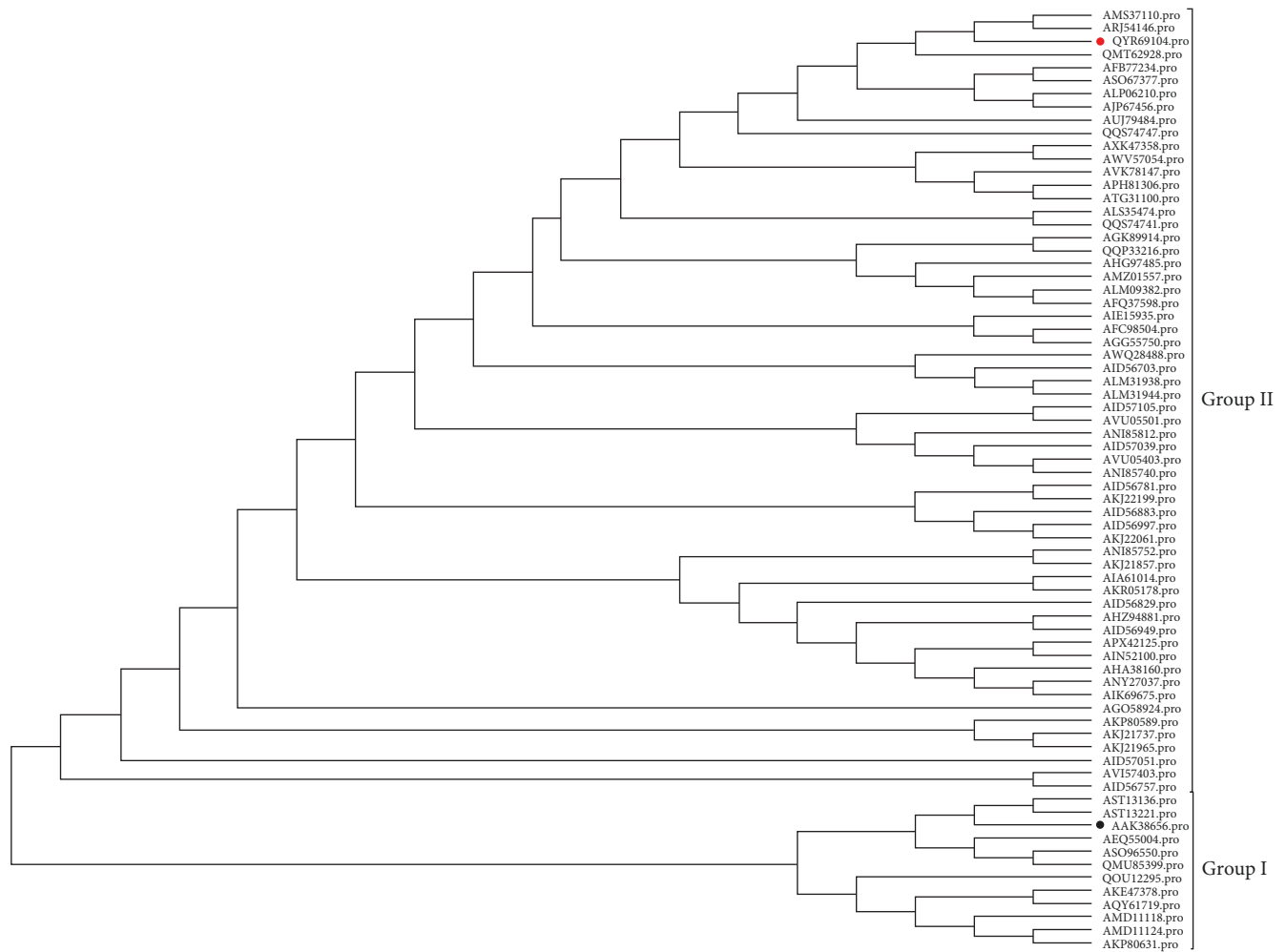
FIGURE 3: Phylogenetic analysis based on the S protein of PEDV JSXZ strain and 69 historic strains available in GenBank (red solid circles indicate PEDV CH/JSXZ/2015, black solid circle indicated PEDV CV777).

diagnosis of PEDV plays a key role in timely and effectively controlling virus dissemination. Vaccination and feedback are the main control measures against PEDV, but these methods do not eradicate and block PEDV effectively [23–25].

In this study, we amplify the whole genome of PEDV from intestinal contents from PED-affected pigs in Jiangsu, China. The complete genome of PEDV CH/JSXZ/2015 (JSXZ) was sequenced and then submitted to GenBank. To provide insight into understanding the genetic, phylogenetic, recombination, and current epidemiological status of PEDV, JSXZ was compared with CV777 and other PEDV strains.

The results showed that the homology between the entire genomic nucleotide sequences of PEDV JSXZ and CV777 was 93.6%. Next, we explored the homology of the S protein and ORF3 proteins of PEDV JSXZ and CV777. The homology of the ORF3 protein between PEDV JSXZ and CV777 was 98.5%. However, the S protein's amino acid sequence identity was 92.4% between PEDV JSXZ and CV777. Besides, the complete genome sequences of PEDV from different locations and years were compared, and the results revealed that JSXZ had the highest nucleotide identity (99.5%) with CH/GDZHDM/1401 strain and the lowest similarity (93.5%)

to 85-7-mutant1 strain. The deduced amino acid sequence of JSXZ S protein was compared with 69 historic PEDV strains. The CH/GDZHDM/1401 strain identified the highest nucleotide identity; the lowest was 90.8% compared with 85-7-C40 and 85-7-mutant1 strains. The deduced amino acid sequence of JSXZ ORF3 protein was compared with 69 historic PEDV strains. PEDV JSXZ ORF3 protein is extremely high in most historic strains. The lowest nucleotide identity of ORF3 was 91.2% compared with the YN90 strain. In the phylogenetic tree of the whole genome, the phylogenetic trees based on the whole genome, S and ORF3 were similar. JSXZ is closely related to CH/GDZHDM/1401. In addition, we revealed that the CH/GDZHDM/1401 (KX016034.1) strain, PEDV 1842/2016 ITA (KY111278.1), and JSXZ had recombination, and the position of recombination was 1–5530 and 28185–28544 bp. 5531–28184 bp of JSXZ are highly homologous to CH/GDZHDM/1401 (KX016034), while 1–5530 and 28185–28544 bp of JSXZ are highly homologous to 1842/2016 ITA (KY111278). PEDV JSXZ caused severe diarrhea, vomiting, and dehydration in piglets, leading to a 60% mortality rate at one swine farm from Xuzhou, Jiangsu Province in China. PEDV JSXZ belongs to Group II and is a
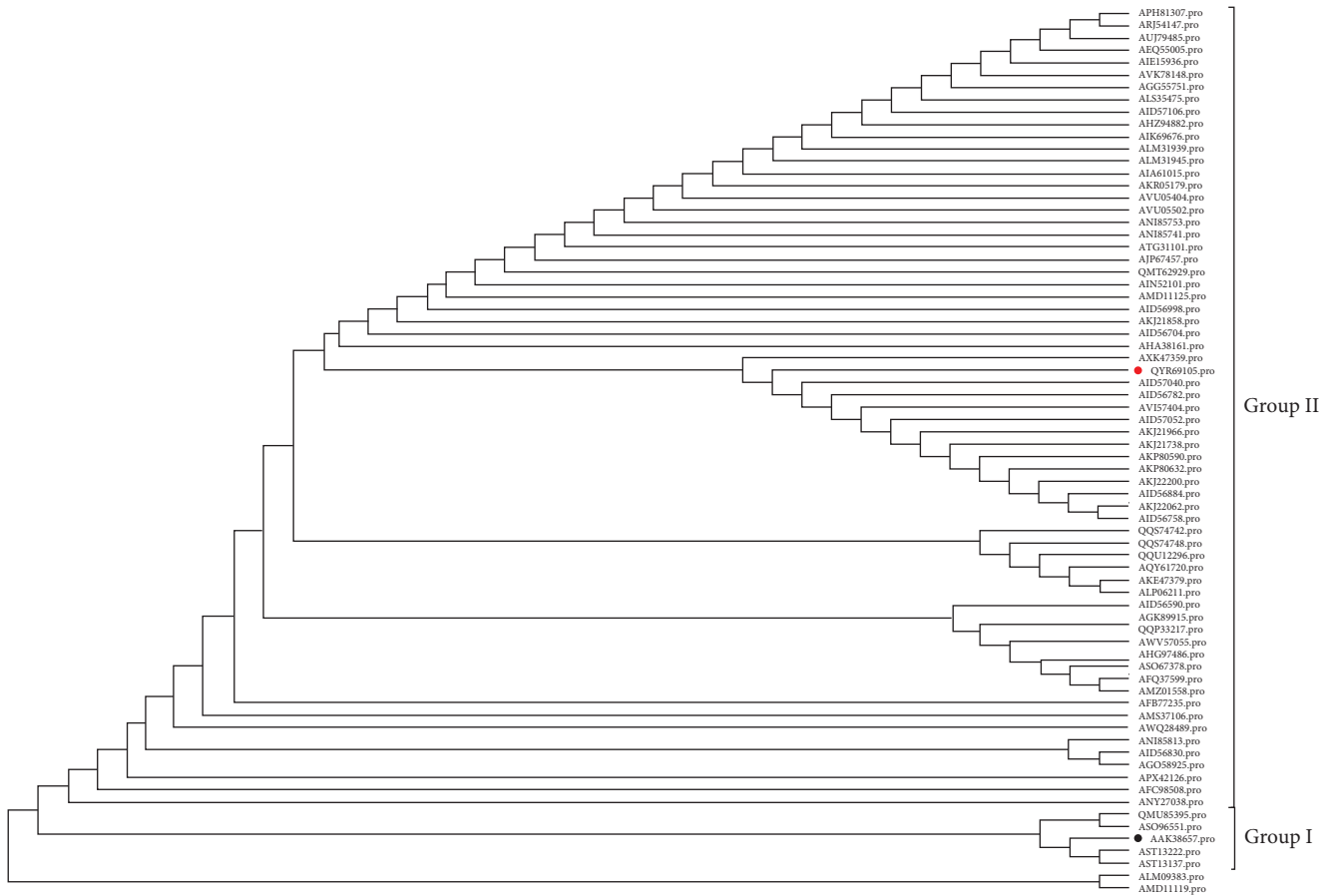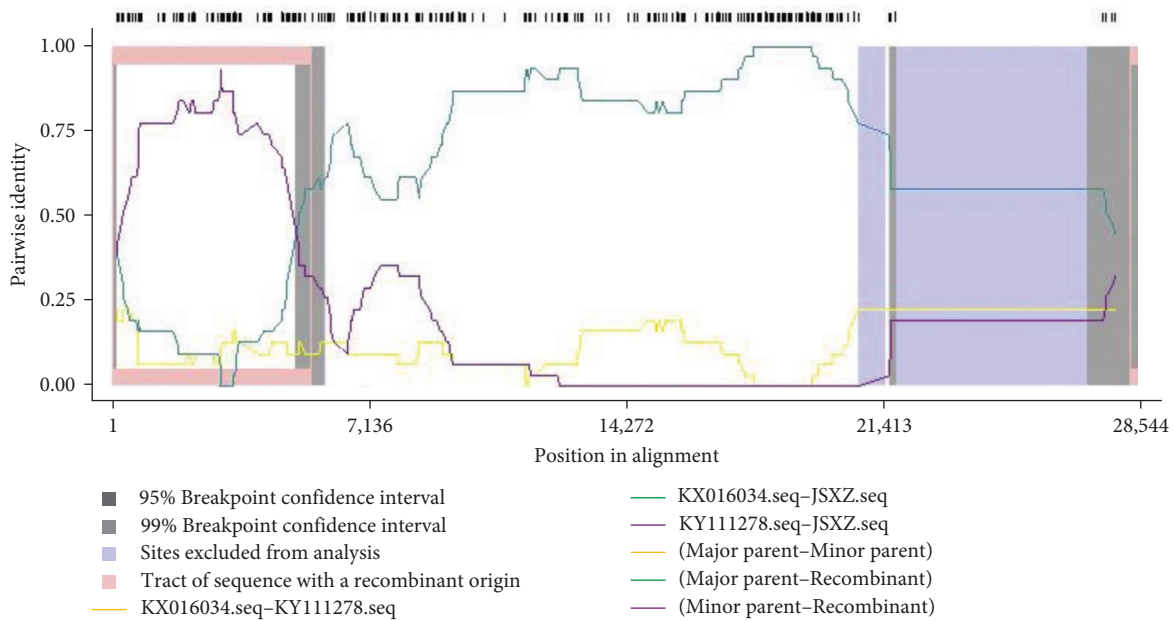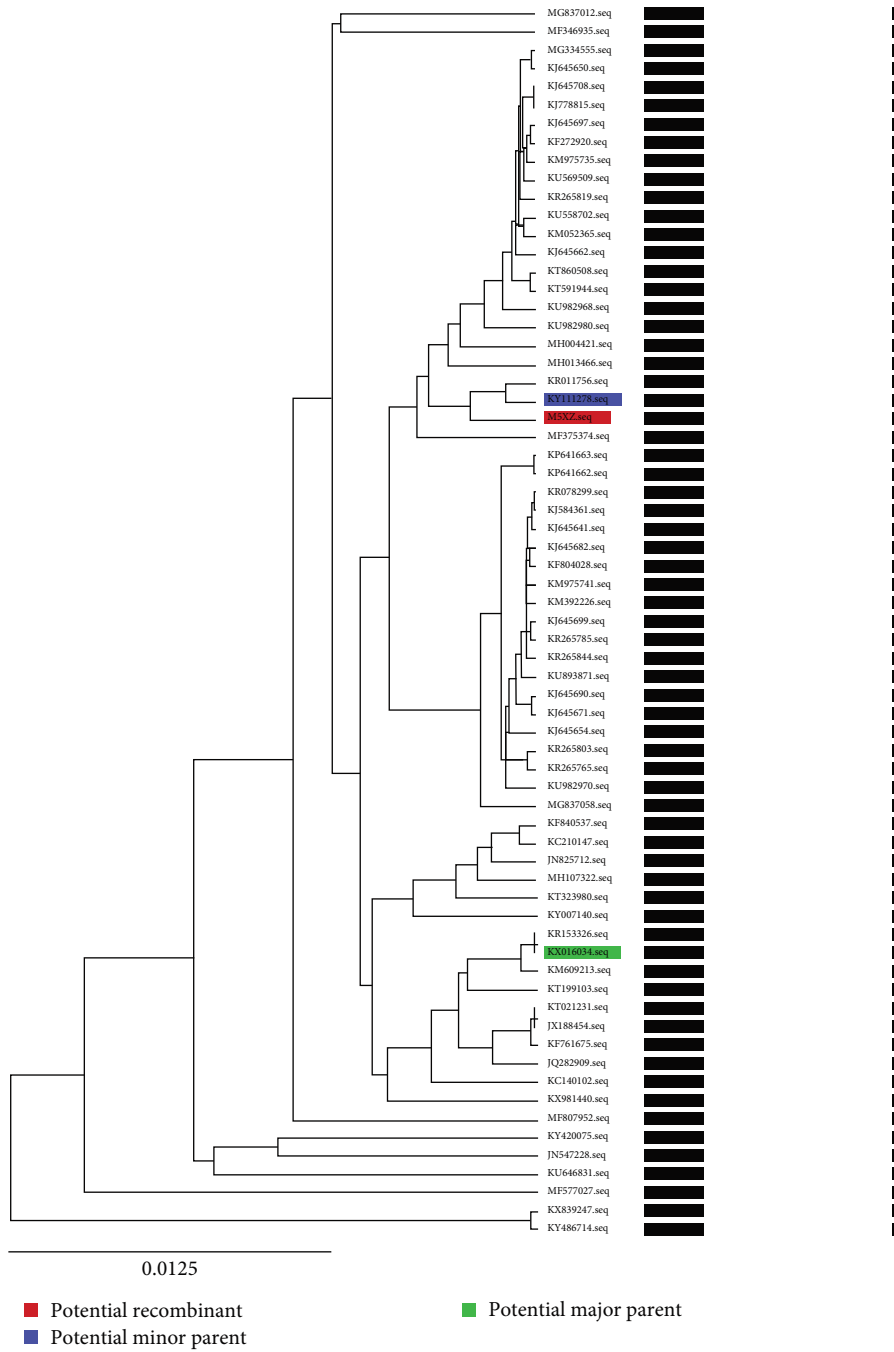
FIGURE 4: Phylogenetic analysis based on the ORF3 protein of PEDV JSXZ strain and 69 historic strains available in GenBank (red solid circle indicated CH/JSXZ/2015, black solid circle indicated PEDV CV777).



(a)

FIGURE 5: Continued.
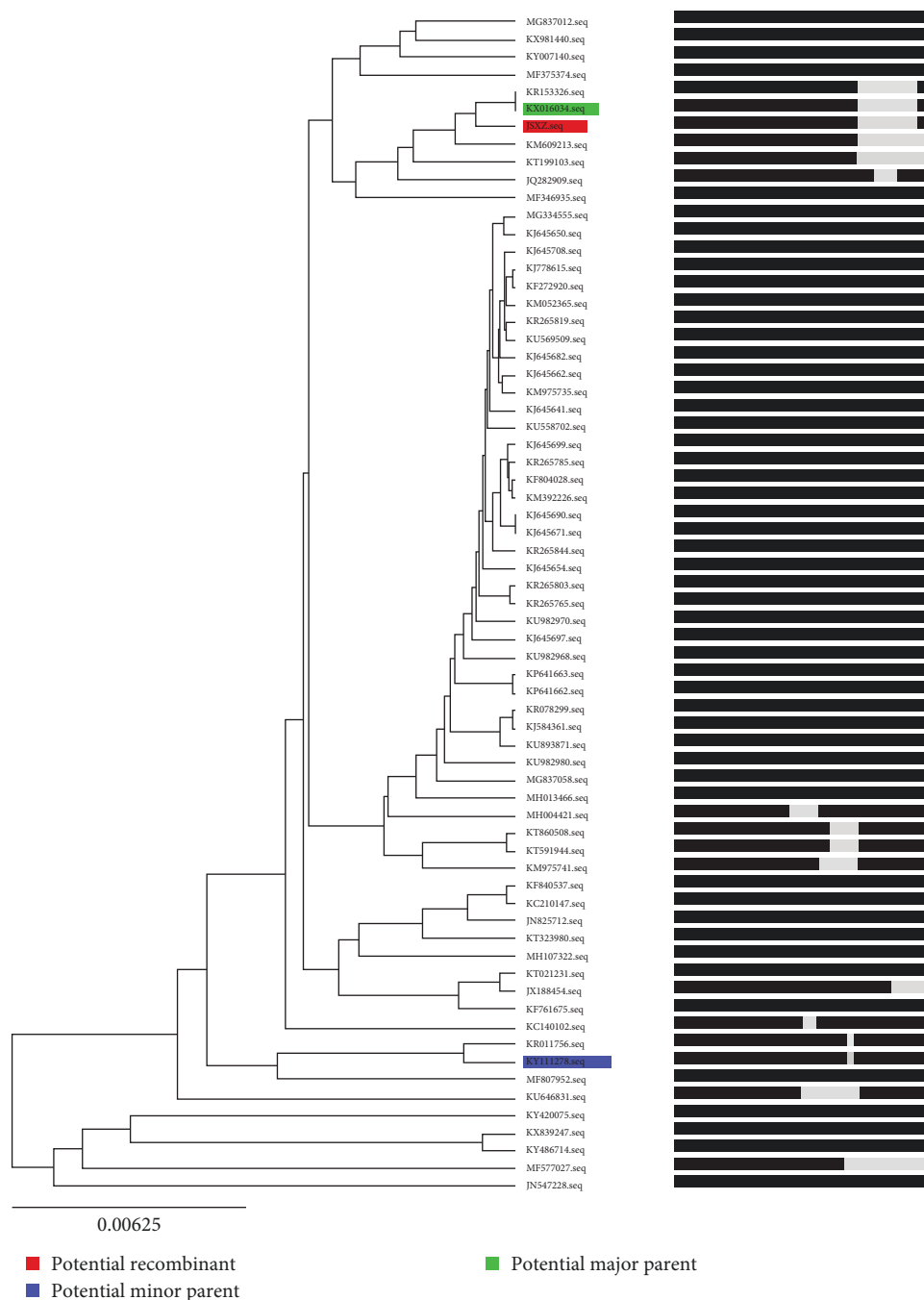
(b)

FIGURE 5: Continued.

(c)

Figure 5: Recombination analysis of PEDV JSXZ strain and 69 historic strains available in GenBank. (a) We used the recombination detection program v5.5, RDP5, to check the recombination. (b) and (c) The evolutionary tree analysis of 1–5530, 28185–28544 and 5531–28184 bp of JSXZ, CH/GDZHDM/1401(KX016034.1) and 1842/2016 ITA (KY111278.1).

variant strain. Feedback may be the cause of PEDV recombination and increased virulence. Therefore, feedback should be prohibited in swine farms.

JSXZ showed the highest nucleotide identity (99.5%) with the CH/GDZHDM/1401 strain. JSXZ is closely related to the CH/GDZHDM/1401 strain based on the phylogenetic trees. The CH/GDZHDM/1401 (KX016034.1) strain, PEDV 1842/2016 ITA (KY111278.1), and JSXZ had recombination,

and the position of recombination was 1–5530 and 28185–28544 bp. This study might provide a reference for the trend of popularity and evolution of PEDV.

## Data Availability

The data that support the findings of this study are openly available in the National Center for Biotechnology Information

at https://www.ncbi.nlm.nih.gov/nuccore/MT625963.1/, reference number MT625963.1.

## Disclosure

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Changchao Huan, Xinyue Guo, and Jinlong Cheng contributed equally to this work.

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## References

[1] M. B. Pensaert and P. de Bouck, "A new coronavirus-like particle associated with diarrhea in swine," *Archives of Virology*, vol. 58, no. 3, pp. 243–247, 1978.

[2] B. Fan, D. Jiao, R. Zhang et al., "Origin and epidemic status of porcine epidemic diarrhea virus variants in China," *Transboundary and Emerging Diseases*, vol. 67, no. 3, pp. 1364–1370, 2020.

[3] W. Li, H. Li, Y. Liu et al., "New variants of porcine epidemic diarrhea virus, China, 2011," *Emerging infectious diseases*, vol. 18, no. 8, pp. 1350–1353, 2012.

[4] Y. Su, Y. Liu, Y. Chen et al., "Detection and phylogenetic analysis of porcine epidemic diarrhea virus in central China based on the ORF3 gene and the S1 gene," *Virology Journal*, vol. 13, no. 1, Article ID 192, 2016.

[5] D. M. Madson, D. R. Magstadt, P. H. E. Arruda et al., "Pathogenesis of porcine epidemic diarrhea virus isolate (US/Iowa/18984/2013) in 3-week-old weaned pigs," *Veterinary Microbiology*, vol. 174, no. 1-2, pp. 60–68, 2014.

[6] B. Mole, "Deadly pig virus slips through US borders," *Nature*, vol. 499, Article ID 388, 2013.

[7] D. Marthaler, Y. Jiang, T. Otterson, S. Goyal, K. Rossow, and J. Collins, "Complete genome sequence of porcine epidemic diarrhea virus strain USA/Colorado/2013 from the United States," *Genome Announcements*, vol. 1, no. 4, 2013.

[8] D. Li, Y. Li, Y. Liu et al., "Isolation and identification of a recombinant porcine epidemic diarrhea virus with a novel insertion in S1 domain," *Frontiers in Microbiology*, vol. 12, Article ID 667084, 2021.

[9] S. E. A. Salamat, T. M. A. Collantes, W. M. L. Lumbera et al., "Sequence analysis of new variants of porcine epidemic diarrhea virus in Luzon, Philippines, in 2017," *Archives of Virology*, vol. 166, no. 7, pp. 1859–1867, 2021.

[10] L. Yu, Y. Liu, S. Wang et al., "Porcine epidemic diarrhea virusmolecular characteristics and pathogenicity of isolated in some areas of China in 2015–2018," *Frontiers in Veterinary Science*, vol. 7, Article ID 607662, 2020.

[11] H. Zhang, F. Han, X. Yan, L. Liu, X. Shu, and H. Hu, "Prevalence and phylogenetic analysis of spike gene of porcine epidemic diarrhea virus in Henan province, China in 2015–2019," *Infection, Genetics and Evolution*, vol. 88, Article ID 104709, 2021.

[12] F. Si, X. Hu, C. Wang et al., "Porcine epidemic diarrhea virus (PEDV) ORF3 enhances viral proliferation by inhibiting apoptosis of infected cells," *Viruses*, vol. 12, no. 2, Article ID 214, 2020.

[13] S. Dong, R. Wang, R. Yu et al., "Identification of cellular proteins interacting with PEDV M protein through APEX2 labeling," *Journal of Proteomics*, vol. 240, Article ID 104191, 2021.

[14] R. Wang, R. Yu, B. Chen et al., "Identification of host cell proteins that interact with the M protein of porcine epidemic diarrhea virus," *Veterinary Microbiology*, vol. 246, Article ID 108729, 2020.

[15] J. Wu, H. Chi, Y. Fu et al., "The antiviral protein viperin interacts with the viral N protein to inhibit proliferation of porcine epidemic diarrhea virus," *Archives of Virology*, vol. 165, no. 10, pp. 2279–2289, 2020.

[16] N.-F. Alikhan, N. K. Petty, N. L. Ben Zakour, and S. A. Beatson, "BLAST ring image generator (BRIG): simple prokaryote genome comparisons," *BMC Genomics*, vol. 12, no. 1, Article ID 402, 2011.

[17] J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins, "The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucleic Acids Research*, vol. 25, no. 24, pp. 4876–4882, 1997.

[18] D. P. Martin, A. Varsani, P. Roumagnac et al., "RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets," *Virus Evolution*, vol. 7, no. 1, Article ID veaa087, 2021.

[19] R. N. Kirchdoerfer, M. Bhandari, O. Martini et al., "Structure and immune recognition of the porcine epidemic diarrhea virus spike protein," *Structure*, vol. 29, no. 4, pp. 385–392.E5, 2021.

[20] S.-H. Chang, J.-L. Bae, T.-J. Kang et al., "Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic diarrhea virus," *Molecules and Cells*, vol. 14, no. 2, pp. 295–299, 2002.

[21] Y. Lu, X. Su, C. Du et al., "Genetic diversity of porcine epidemic diarrhea virus with a naturally occurring truncated ORF3 gene found in Guangxi, China," *Frontiers in Veterinary Science*, vol. 7, Article ID 435, 2020.

[22] S. Ye, Z. Li, F. Chen et al., "Porcine epidemic diarrhea virus ORF$_3$ gene prolongs S-phase, facilitates formation of vesicles and promotes the proliferation of attenuated PEDV," *Virus Genes*, vol. 51, no. 3, pp. 385–392, 2015.

[23] T. Gillespie, Q. Song, M. Inskeep, S. Stone, and M. P. Murtaugh, "Effect of booster vaccination with inactivated porcine epidemic diarrhea virus on neutralizing antibody response in mammary secretions," *Viral Immunology*, vol. 31, no. 1, pp. 62–68, 2018.

[24] T. Yamagami, T. Miyama, H. Toyomaki et al., "Analysis of the effect of feedback feeding on the farm-level occurrence of porcine epidemic diarrhea in Kagoshima and Miyazaki prefectures, Japan," *Journal of Veterinary Medical Science*, vol. 83, no. 11, pp. 1772–1781, 2021.

[25] T. H. Nguyen Thi, C.-C. Chen, W.-B. Chung et al., "Antibody evaluation and mutations of antigenic epitopes in the spike protein of the porcine epidemic diarrhea virus from pig farms with repeated intentional exposure (feedback)," *Viruses*, vol. 14, no. 3, Article ID 551, 2022.

[26] C. Huan, X. Guo, P. Cheng et al., *Whole-Genome and PHylogenetic Analysis of Porcine Epidemic Diarrhea Virus Strain CH/JSXZ /2015*, Research Square, PREPRINT (Version 1), 2022.