KJVR
Korean Journal of Veterinary Research

Original Article

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2023;63(2):e18
https://doi.org/10.14405/kjvr.20230009

*Corresponding author:
Tae-Wook Hahn
College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon 24341, Korea
Tel: +82-33-250-8671
E-mail: twhahn@kangwon.ac.kr
https://orcid.org/0000-0002-3919-1153

†These authors contributed equally to this work.

Conflict of interest:
The authors declare no conflict of interest.

Received: Apr 11, 2023
Revised: Jun 1, 2023
Accepted: Jun 8, 2023

Porcine epidemic diarrhea viruses from Vietnam: isolation, characterization, and neutralizing activity

Van Tan Do[1,†], Quang Lam Truong[2,†], Hoai Thu Dao[1], Thi Lan Nguyen[2], Minna Shin[3], Kyeong Ryeol Shin[3], Tae-Wook Hahn[1,3,*]

[1]College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea
[2]Key Laboratory of Veterinary Biotechnology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Ha Noi 12406, Vietnam
[3]INNOVAC, Chuncheon 24341, Korea

Abstract

Porcine epidemic diarrhea (PED) is characterized by acute enteritis, watery diarrhea, weight loss, dehydration, and death, with high mortality in neonatal piglets. In this study, 3 virus isolates collected in Vietnam between 2016 and 2017 were propagated successfully in Vero cells at high virus titers. Sequence analysis of the full-length spike (S) gene showed that all 3 isolates belong to genogroup 2b, which is closely related to other prevalent Asian strains. A comparison of the amino acid sequence revealed a 98.19% to 99.13% homology with the Vietnam isolates circulating during 2013–2015, suggesting that field PED viruses (PEDVs) are evolving continuously. Experiments in animals showed that the antisera from guinea pigs immunized with the vaccine strain resulted in higher levels (5 $\log_2$) of neutralizing antibodies against the homologous strain and a relatively moderate level of neutralizing antibodies against the field isolates. This finding would be helpful in selecting a PEDV strain for vaccine development.

Keywords: porcine epidemic diarrhea virus; neutralizing activity; spike gene; Vietnam isolates

Introduction

The porcine epidemic diarrhea virus (PEDV) causing porcine epidemic diarrhea (PED) is a large-enveloped RNA virus belonging to the genus *Alphacoronavirus*, family *Coronaviridae*, order *Nidovirales*. The PEDV genome is approximately 28 kb long containing at least 7 open reading frames (ORF1a, ORF1b, and ORF2–6) and a 3' untranslated region [1,2]. A PEDV infection in pigs causes watery diarrhea, anorexia, and depression leading to high mortality rates in suckling piglets [1,3].

Over the last 30 years, the disease has been recorded in European and Asian swine farms, with the virus initially isolating in England [4] and Belgium [5] in the late 1970s. PEDV has been reported since 2008 in Vietnam, and the outbreak was a significant event that affected the country's swine industry. The first reported case of PED occurred in the southern provinces of Vietnam and quickly spread throughout the nation, with significant economic losses [6]. According to a recent descriptive survey, the northern region of Vietnam, where many farms tested positive for PED (30.89%), is an endemic PEDV area [7]. Although some pig farms

have used vaccination or the feedback approach, PEDV remains and continually recurs [7].

PEDV isolates need to propagate efficiently in cell culture to examine pathogenesis and vaccine development because of the difficulty growing PEDV in cell culture. The PEDV can be isolated from clinical samples, but the virus may gradually lose infectivity upon further passages in cell culture [8].

The spike (S) protein of PEDV interacts with the cellular receptor in the entry process of the virus and produces neutralizing antibodies in pigs [1]. The S protein is considered a primary target antigen for the induction of neutralizing antibodies and the development of an effective vaccine against PEDV [9,10]. Recent reports have demonstrated the diversity of the S gene in many isolates. The pathogenicity and antigenicity of the PEDV can be changed according to mutations, deletions, and insertions of amino acids in the S protein. As a result, viral antigenicity and viral neutralizing activity may be changed [11,12]. Zhang et al. [12] reported that a field CHN/FL2013 strain reduced virulence in cell culture with a seven-amino acid deletion at N- termination of the S protein.

In this study, PEDV isolates from Vietnam were propagated in Vero cells, and full-length S genes were sequenced to examine the diversity of PEDVs. In addition, the cross-neutralizing activity of the serum of guinea pigs immunized with a PEDV vaccine strain against the isolates was evaluated.

Materials and Methods

Isolation of PEDVs in Vero cells

Stool specimens and the small intestine were taken from suckling piglets and post-weaning pigs exhibiting acute watery diarrhea. The PEDV HID9047 and HID9048 isolates used in this study were collected from infected samples in Ha Nam and Thai Binh provinces, Vietnam in 2016, and the PEDV HID9049 isolate was collected from infected samples in Hung Yen province, Vietnam in 2017.

The presence of the PEDV was confirmed by a reverse transcription polymerase chain reaction (RT-PCR) amplifying approximately 900 bp of the N gene with primers PEDV-N–F (5′-CGG TTC TCA CAG ATA GTG A-3′) and PED-N-R (5′- CTC CTC CAC TCT GGG ATG T -3′).

The isolation and propagation of PEDV in Vero cells were conducted using a slight modification of the methodology reported by Park et al. [13]. Briefly, 80% confluent Vero cells were inoculated with the virus at 37°C for one hour. The infection medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 0.02% yeast extract, 0.3% tryptose phosphate broth, 5 μg/mL trypsin, and 1% antibiotic–antimycotic) was added to the infected cell flask without removing the inoculum. The infected cells were incubated at 37°C for 7 days until extensive cytopathic effects (CPEs) were observed; viruses were harvested by 3 freeze-thaw cycles.

Virus titration

Vero cells (1×10^5) were seeded in each well of a 96-well plate (Corning, USA) and grown at 37°C one day before the assay. The virus was diluted in the infection medium, and 0.2 mL of 10-fold serial virus dilutions were added to each well after removing the medium. Each virus dilution was used to infect 10 wells of monolayer Vero cells. The CPE was examined under a microscope every day for 7 days postinfection, and the virus titer was calculated as the 50% tissue culture infective dose ($TCID_{50}$) using the Reed-Muench method [11].

Immunofluorescent assay

Vero cells were infected with the PEDV 24 hours before the assay. The control and infected cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. The cells were then blocked with 5% bovine serum albumin, followed by incubation with 1/1,000 monoclonal mouse anti-PEDV (Median Diagnostics, Korea) and 1/1,000 fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG) (MP Biomedicals, USA). The signals were observed using fluorescence microscopy [13].

Nucleotide sequence analysis

The full-length S genes of PEDVs were amplified by RT-PCR using the primers listed in Table 1. The individual fragment was

Table 1. List of the primers used for the full-length spike gene sequencing in this study

Primers	Sequence (5′-3′)	Length
S1-F	GCTAGTGCGTAATAATGACAC	707 bp
S1-R	GCACTAGTAACATTAAGCATG	
S2-F	CGTGTTGCGACAAGATGT	731 bp
S2-R	TCATCGTCAGTGCCATGA	
S3-F	GCATCTCGGTTTGTTGGAT	712 bp
S3-R	CTTGGTACACACATCCAGAG	
S4-F	AGTTGATTACTGGCACGC	736 bp
S4-R	CCATCACCATTAAACGAAC	
S5-F	GAAGAGGCTCTACAGTTAGC	722 bp
S5-R	CATTAAGTGCTGATAATCTGC	
S6-F	ACTCCCGACTGGACATTCTT	643 bp
S6-R	TCTGCAATTTCACCAGTGAG	
S7-F	GGTCACCTATGTCAATCTGAC	709 bp
S7-R	CAAAACGCGCTGCCAACA	

https://doi.org/10.14405/kjvr.20230009

cloned into the T&A cloning vector (RBC; Real Biotech Corp., Taiwan) and sequenced in both directions. The sequences of the full-length S genes of the PEDV isolates in this study and reference PEDVs were used in sequence alignments and phylogenetic analyses with the Serial Cloner 2.6 (SerialBasic, USA), the Clustal X 2.0 program (European Bioinformatics Institute, UK), and the Mega 6.0 program (megasoftware, USA) [14].

Serum neutralization

The neutralizing activity of the antisera collected from guinea pigs immunized with a PEDV vaccine strain SM98 against the PEDV isolates HID9047, HID9048, and HID9049 was evaluated. Three to four-month-old guinea pigs (n = 3) were immunized twice intramuscularly with 10^5 $TCID_{50}$ inactivated vaccine strain SM98 at a two-week interval. Two other guinea pigs were given phosphate-buffered saline (PBS) as a control. Serum samples were collected 2 weeks after the last immunization for the neutralizing assay.

The Vero cells were grown at 2×10^5 cells per well in 96-well plates. The viruses were diluted to 200 $TCID_{50}$ in 50 μL DMEM, mixed with 50 μL of a twofold diluted serum, and incubated at 37°C for one hour. The cells were rinsed 3 times with PBS, followed by inoculating 100 μL of the mixture at 37°C for one hour. After removing the mixture, the cells were rinsed 3 times with PBS, and the infection medium was added to the cells and incubated at 37°C in an atmosphere containing 5% CO_2. The neutralization titer was calculated as the highest serum dilution inhibiting virus-specific CPE [14].

Statistical analysis

One-way analysis of the variance in GraphPad Prism 6 (GraphPad Software Inc., USA) was used, and p-values < 0.05 were considered significant.

Ethics statement

Animal experiments were approved by the Institutional Animal Care and Use Committee (approval number: KW-181031-1) and performed at the Center for Animal Experiments, Kangwon National University.

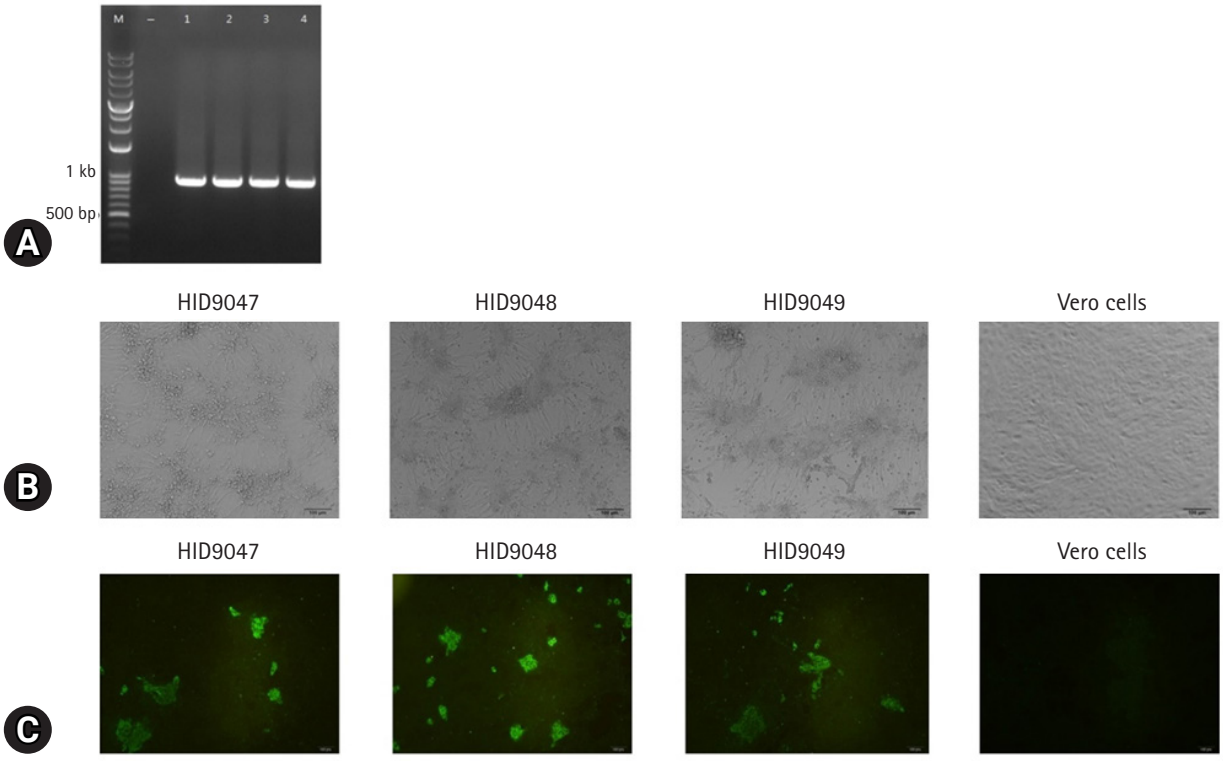

Fig. 1. (A) Reverse transcription polymerase chain reaction amplifying a 900 bp fragment of the N gene. M, DNA standard 3 kb plus; –, Vero cell control; 1, SM98; 2, HID9047; 3, HID9048; 4, HID9049. (B) Isolation of Vietnam porcine epidemic diarrhea virus (PEDV) isolates in Vero cells at 5 days postinfection with obvious cytopathic effects, such as cell fusion and large syncytia. (C) Immunofluorescence assay in Vero cells using monoclonal mouse anti-PEDV and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G antibodies.

Results

Virus isolation and characterization

PEDV-PCR positive samples (Fig. 1A) were processed and incubated in Vero cells. Three PEDV isolates, designated HID9047, HID9048, and HID9049, were propagated successfully in Vero cells with clear CPEs, such as cell fusion, large syncytia, and cell detachment (Fig. 1B). The viral titers of HID9047, HID9048, and HID9049 were $10^{7.5}$, $10^{8.2}$, and $10^{8.5}$ $TCID_{50}$/mL at passage 10, respectively. Immunofluorescence assay using the monoclonal mouse anti-PEDV and FITC-conjugated goat anti-mouse IgG antibodies confirmed virus growth in Vero cells. A specific fluorescence signal was detected in the cytoplasm of Vero cells infected with viruses but not in the non-infected cells (Fig. 1C).

Phylogenetic analysis

Fifty-four PEDVs were used to construct a phylogenetic tree (Table 2). In this study, phylogenetic analysis based on the nucleotide sequences of the S gene showed that PEDV strains could be divided into 2 genogroups, classical genogroup 1 represented by prototype CV777 and variant genogroup 2 of pandemic strains. Each group can be divided into subgroups 1a, 1b, 1 S-INDEL, 2a, and 2b. Three isolates (HID9047, HID9048, and HID9049) belonged to the subgroup 2b, which is most closely related to the Asian strains (Fig. 2).

Spike gene sequence comparisons

The entire DNA sequences of the S genes and the deduced amino acid sequences of 3 isolates from Vietnam were compared with the sequences of the reference strains (CV777, SM98, and DR13) and other Vietnam isolates. As shown in Table 3, the nucleotide identities of the S gene between 3 Vietnam isolates and reference strains ranged from 91.80% to 92.71%, and the deduced amino acid sequences were 96.74% to 97.61% homologous. Furthermore, the 3 isolates shared 96.78% to 97.52% nucleotide homology and 98.19% to 99.13% amino acid homology compared with the Vietnam isolates (HUA-PED45, HUA-PED111, VN97, and VN-K28). The nucleotide and amino acid homology of the 3 isolates showed 98.15% to 99.76% and 99.06% to 99.64%, respectively (Table 3).

The sequence alignment of amino acids in the S protein illustrated that mutations in amino acid sequence, including substitutions, insertions, and deletions, were observed in 3 Vietnam isolates. Among them, 4 insertions (^{58}NQGV^{61}) and 2 deletions (^{163}DI^{164}) were found in the N-terminal of the S protein (Fig. 3). Alignment analysis of 4 neutralizing epitope regions core neu-

Table 2. PEDV reference strains used in this study

Virus strains/ isolates	Country	Time	GenBank accession no.
CV777/prototype/1978/Belgium	Belgium	1978	AF353511
SM98/1998/South Korea	South Korea	1998	KJ857455
SM98-1/1998/South Korea	South Korea	1998	GU937797
DR13/virulent/1999/South Korea	South Korea	1999	JQ023161
Spk1/2002/South Korea	South Korea	2002	AF500215
JS2008/2008/China	China	2008	KC109141
KNU-0801/2008/South Korea	South Korea	2008	GU180142
KNU-0901/2009/South Korea	South Korea	2009	GU180144
AJ1102/2011/China	China	2011	JX188454
GD-1/2011/China	China	2011	JX647847
LC/2011/China	China	2011	JX489155
CV777/vaccine/2012/China	China	2012	JN599150
SD-M/2012/China	China	2012	JX560761
GD-A/2012/China	China	2012	JX112709
GD-B/2012/China	China	2012	JX088695
AH2012/2012/China	China	2012	KC210145
CH/FJZZ-9/2012/China	China	2012	KC140102
DR13/attenuated/2012/South Korea	South Korea	2012	JQ023162
CH/ZMDZY/11/2013/China	China	2013	KC196276
KNU-1305/2013/Korea	South Korea	2013	KJ662670
Colorado/2013/USA	USA	2013	KF272920
Indiana 17846/2013/USA	USA	2013	KF452323
MN/2013/USA	USA	2013	KF468752
IA1/2013/USA	USA	2013	KF468754
HUA-PED45/2013/Vietnam	Vietnam	2013	KP455313
VN97/HN/2013/Vietnam	Vietnam	2013	KX982561
VN01/HY/2013/Vietnam	Vietnam	2013	KX982553
PEDV-LYG/2014/China	China	2014	KM609212
KNU-1409-1/2014/South Korea	South Korea	2014	KJ741221
QIAP1401/2014/South Korea	South Korea	2014	KX793713
VN292/HN/2014/Vietnam	Vietnam	2014	KX982568
VN367/VP/2014/Vietnam	Vietnam	2014	KX982571
VN288/SL/2014/Vietnam	Vietnam	2014	KX982567
HUA-PED106/2015/Vietnam	Vietnam	2015	KX708905
HUA-PED94/2015/Vietnam	Vietnam	2015	KX708906
HUA-PED96/2015/Vietnam	Vietnam	2015	KX708907
HUA-PED111/2015/Vietnam	Vietnam	2015	KX708903
HUA-M17/2015/Vietnam	Vietnam	2015	KX708894
HUA-V2/2015/Vietnam	Vietnam	2015	KX708895
VN-K28/TB/2015/Vietnam	Vietnam	2015	KX982575
VN-Jafa/HB/2015/Vietnam	Vietnam	2015	KX982577
XM2-4-S/2016/China	China	2016	KX812524
KNU-1601/2016/South Korea	South Korea	2016	KY963963
KNU-1703/2017/South Korea	South Korea	2017	MH052682
KNU-1705/2017/South Korea	South Korea	2017	MH052684
KNU-1706/2017/South Korea	South Korea	2017	MH052685
KNU-1708/2017/South Korea	South Korea	2017	MH052687
KNU-1709/2017/South Korea	South Korea	2017	MH052688
PC273/O/2017/USA	USA	2017	MG837058
KNU-1802/2018/South Korea	South Korea	2018	MH243314
S10/2018/South Korea	South Korea	2018	MH891590

PEDV, porcine epidemic diarrhea virus.

https://doi.org/10.14405/kjvr.20230009

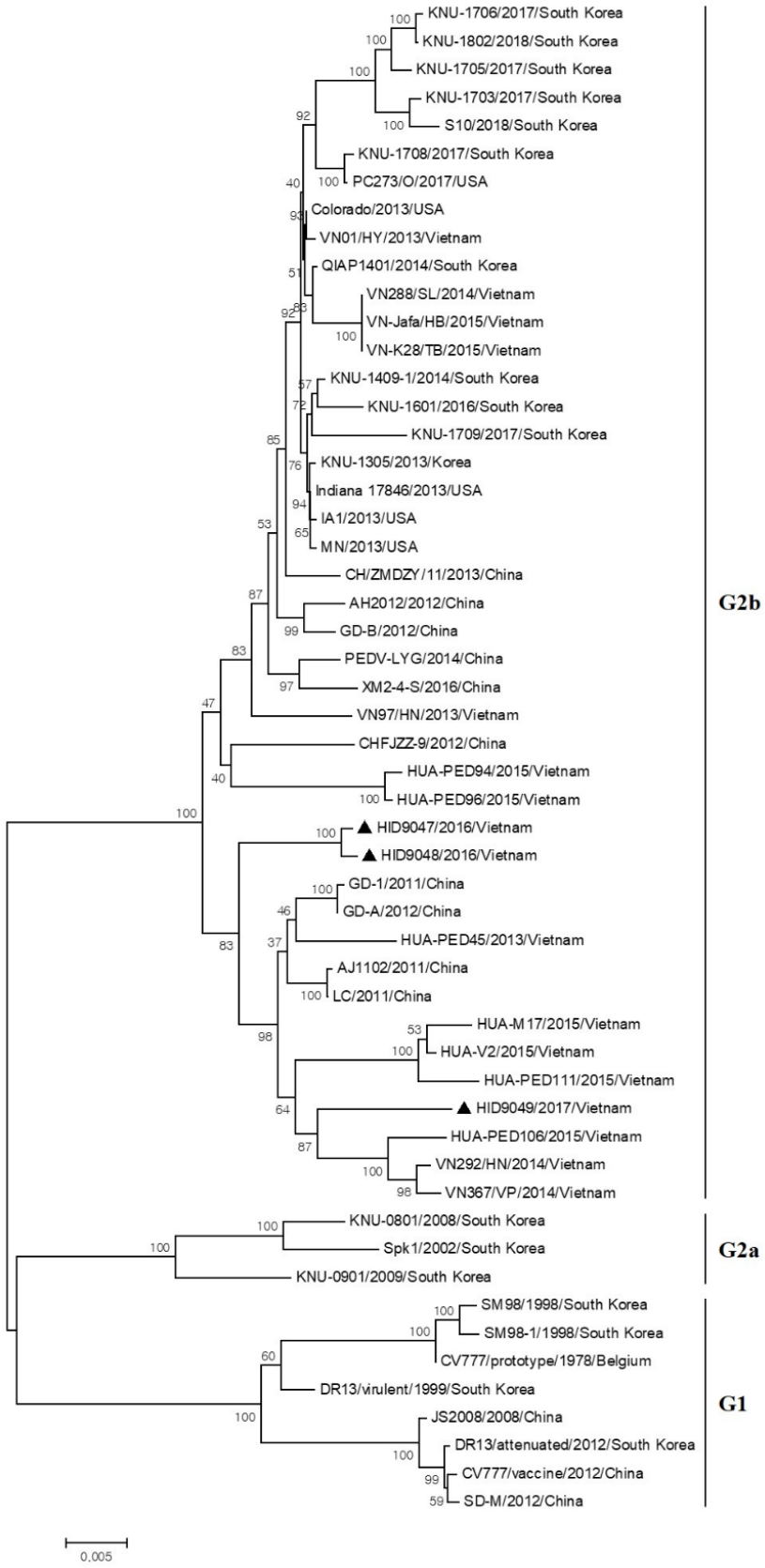

Fig. 2. Phylogenetic analysis of porcine epidemic diarrhea virus isolates and the reference strains. The neighbor-joining method generated the phylogenetic tree using the Mega 6 program with the nucleotide sequences of the full-length S gene and 1,000 replicates bootstrapping.

Table 3. Pairwise comparisons of the nucleotide and deduced amino acid sequences of the full-length S gene of PEDV isolates compared with the reference strains.

	CV777	DR13	SM98	HUA-PED45	HUA-PED111	VN97	VN-K28	HID9047	HID9048	HID9049
CV777		99.88	96.14	92.04	91.75	92.84	92.31	92.64	92.50	91.97
DR13	99.71		96.17	92.11	91.82	92.91	92.33	92.71	92.57	92.04
SM98	97.90	97.97		91.99	91.77	92.69	92.12	92.23	92.09	91.80
HUA-PED45	96.88	97.39	97.59		97.28	97.24	97.14	97.79	97.69	97.79
HUA-PED111	96.81	97.31	97.23	98.70		96.54	96.25	97.16	97.07	97.23
VN97	97.17	97.75	97.67	98.41	98.19		98.29	97.52	97.48	97.26
VN-K28	97.32	97.97	97.52	98.27	98.12	98.63		97.28	97.24	96.78
HID9047	96.81	97.61	97.30	98.63	98.70	98.12	98.70		99.76	98.24
HID9048	96.74	97.31	97.01	98.56	98.70	98.19	98.41	99.64		98.15
HID9049	97.03	97.61	97.45	98.92	99.13	98.70	98.63	99.06	99.06	

Nucleotide and amino acid homology (%) are presented in the upper right and the lower left, respectively.
PEDV, porcine epidemic diarrhea virus.

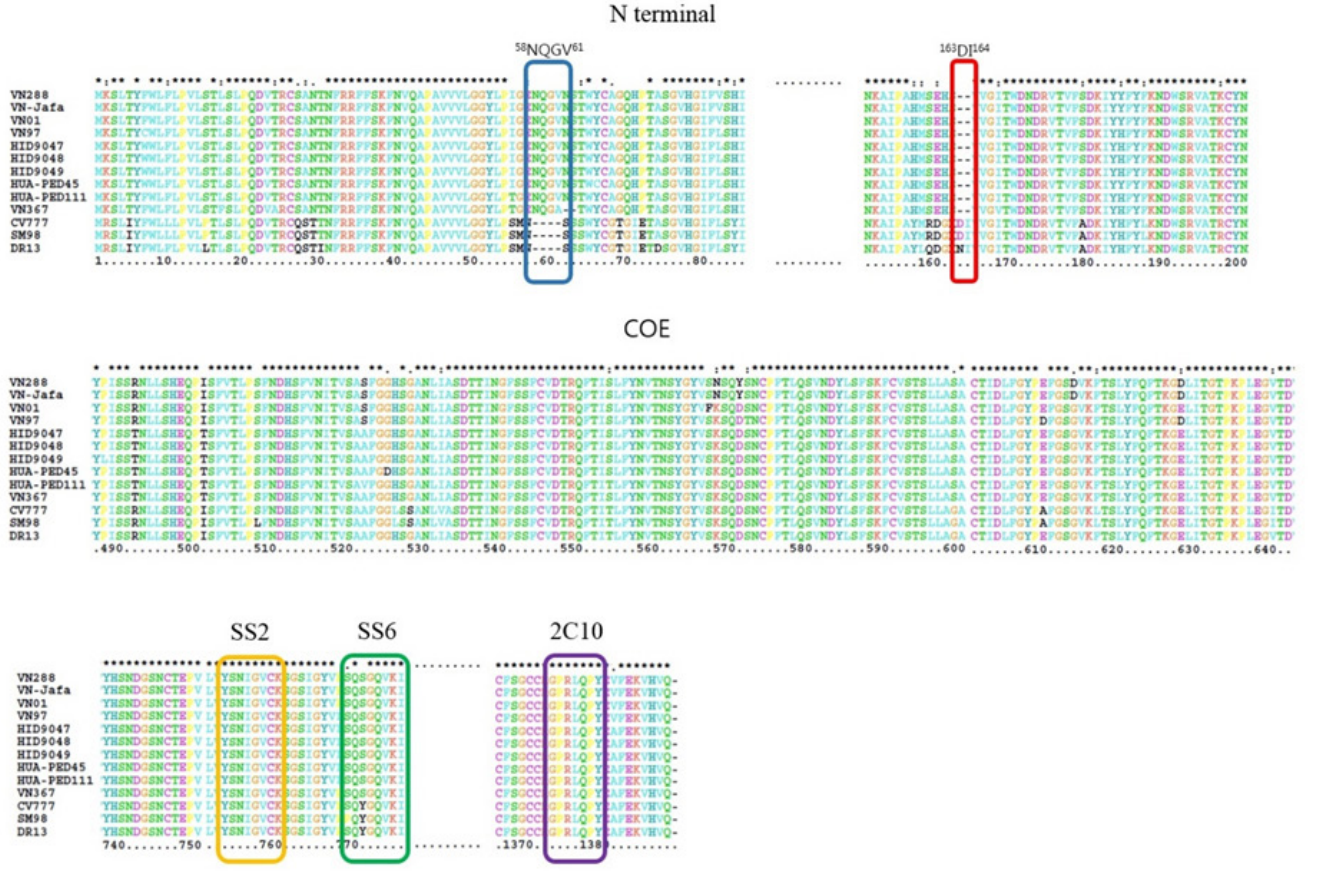

Fig. 3. Analysis of amino acid mutations in the S protein of isolates from Vietnam with reference strains. The insertions and deletions are highlighted in blue and red, respectively. The core neutralizing epitope (COE) and 3 other neutralizing epitopes, SS2 (yellow), SS6 (green), and 2C10 (purple), were aligned.

tralizing epitope (COE), SS2, SS6, and 2C10 showed that all 3 Vietnam isolates in this study have 4 substitutions in COE (^{502}I → ^{502}T, ^{509}L → ^{509}S, ^{529}S → ^{529}G, and ^{607}A → ^{607}E), one substitution in SS6 (^{766}Y → ^{766}S), and no mutations in SS2 and 2C10 compared with CV777, DR13 and SM98 strains (Fig. 3). A comparison with other Vietnam strains (VN288, VN-Jafa, VN01, VN97 isolated during 2012–2015) indicated the substitution in COE (^{502}I → ^{502}T), and the conservation in SS2, SS6, and 2C10 region. PEDV VN288, VN-Jafa, VN01, and VN97 showed addition substitutions in COE, while HUA-PED45 showed more homol-

https://doi.org/10.14405/kjvr.20230009

ogy with 3 isolates, suggesting that 3 isolates (HID9047, HID9048, and HID9049) are closely related to PEDV HUA-PED45.

Neutralizing activity

Guinea pig antisera collected 2 weeks after the final immunization were used for neutralizing tests to determine if vaccine strain SM98 could neutralize the 3 PEDV isolates (HID9047, HID9048, and HID9049). The antisera effectively inhibited infection with the vaccine strain SM98 with mean neutralizing antibody (NA) titers of 5 $\log_2$. The antisera showed lower dilutions with mean of NA titers of 3.67 $\log_2$ ($p < 0.01$), 4 $\log_2$ ($p < 0.05$), and 3.27 $\log_2$ ($p < 0.001$) for inhibiting infection with isolates HID9047, HID9048, and HID9049, respectively (Fig. 4). A significant difference in the mean NA titers against the SM98 strain and 3 isolates was observed, suggesting that the antisera reacted strongly with the homologous virus, but with the heterologous field isolates regarding to antigenic variations between PEDVs.

Discussion

The S protein is an essential factor mediating PEDV entry into host cells and is considered a primary target antigen for the induction of neutralizing antibodies and the development of an effective vaccine against PEDV [9–11]. Several studies suggested that the S gene is prone to mutations, including replacement, insertion, and deletion of nucleotide sequences [11,12,15,16].

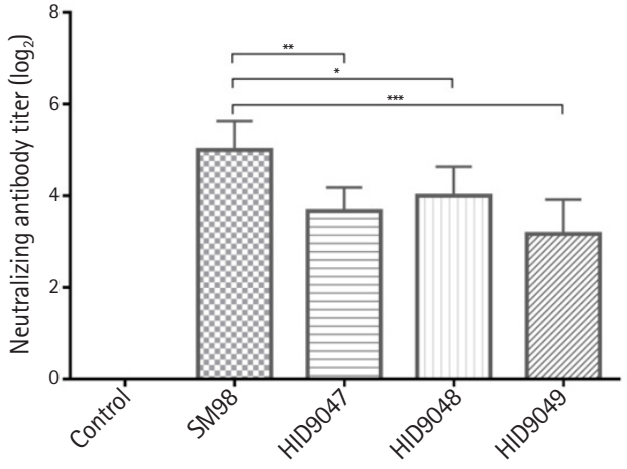

Fig. 4. Neutralizing antibodies in the serum samples of guinea pigs immunized against porcine epidemic diarrhea virus isolates. Serum samples were collected 2 weeks after the second immunization and subjected to the virus neutralization assay using PEDV HID9047, HID9048, and HID9049 isolates. Data represent the mean ± standard deviation. Statistical significance was assessed by one-way ANOVA. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

The PEDV has been reported in Vietnam since 2010 and has spread rapidly [17]. The disease has been circulating and becoming a significant issue in the pork industry. This study was conducted to isolate PEDVs from infected pigs in Vietnam, investigate the genetic variation of PEDV isolates based on the full-length S gene, and assess the neutralizing activity of the traditional vaccine strain with the circulating isolates.

This study isolated 3 PEDV isolates from infected samples and propagated them in Vero cells with high virus titers. Nucleotide sequencing and a phylogenetic tree based on the S gene revealed that the 3 isolates from Vietnam (HID9047, HID9048, and HID9049) belong to subgroup 2b. The 3 PEDVs were isolated in 2016 and 2017, suggesting that they are novel PEDVs that share unique genetic features with the subgroup 2b PEDV strains circulating in Vietnam from 2013–2015. In addition, the 3 isolates showed fewer mutations than PEDV HUA-PED111, HUA-V2, and HUA-M17, which were isolated from the northern provinces of Vietnam in 2015, but showed higher homology in amino acid sequences with strain HUA-PED45. Hence, the 3 isolates and PEDV HUA-PED45 are genetically closely related. Amino acid sequence alignment indicated mutations, such as substitutions, mutations, insertions, and deletions in S protein, particularly in the N-terminal region. The N-terminal domain interacts with 5-N-acetylneuraminic acid, and mutations in the N-terminal may impact the PEDV infection in the host [18].

The neutralizing activity of antisera against the 3 Vietnam isolates was determined. Immunized sera showed higher neutralizing activity against the homogenous strain SM98 but moderate against the isolates HID9047, HID9048, and HID9049. This finding is consistent with a previous study showing that the antisera strongly recognized the homologous strain regarding antigenic variations [14]. Lee et al. [14] reported that the antisera of guinea pigs given 2 immunization with strain KNU-141112 effectively inhibited the homogenous strain with mean NA titers of 1:112, but relatively low NA titers of 1:37 against strain SM98-1. In this study, the 3 isolates have one and 4 amino acid substitutions in the SS6 and COE regions, respectively, which may support the hypothesis of variations in neutralizing the epitope and speculate that the changes may influence the antigenicity of PEDV. Further studies on the pathogenicity and neutralizing activity of these 3 isolates need to be carried out in pigs.

Acknowledgments

The authors wish to acknowledge the veterinarians who col-

lected the infected samples and the contribution of all authors for this study.

ORCID

Van Tan Do, https://orcid.org/0000-0003-4512-7622
Quang Lam Truong, https://orcid.org/0000-0002-9403-2614
Hoai Thu Dao, https://orcid.org/0000-0002-2797-9955
Thi Lan Nguyen, https://orcid.org/0000-0002-1409-9967
Minna Shin, https://orcid.org/0000-0002-1638-6177
Kyeong Ryeol Shin, https://orcid.org/0000-0002-1608-5569
Tae-Wook Hahn, https://orcid.org/0000-0002-3919-1153

References

1. Lee C. Porcine epidemic diarrhea virus: an emerging and re-emerging epizootic swine virus. Virol J 2015;12:193.
2. Song D, Park B. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 2012;44:167–175.
3. Diel DG, Lawson S, Okda F, Singrey A, Clement T, Fernandes MH, Christopher-Hennings J, Nelson EA. Porcine epidemic diarrhea virus: an overview of current virological and serological diagnostic methods. Virus Res 2016;226:60–70.
4. Wood EN. An apparently new syndrome of porcine epidemic diarrhoea. Vet Rec 1977;100:243–244.
5. Pensaert MB, de Bouck P. A new coronavirus-like particle associated with diarrhea in swine. Arch Virol 1978;58:243–247.
6. Than VT, Choe SE, Vu TT, Do TD, Nguyen TL, Bui TT, Mai TN, Cha RM, Song D, An DJ, Le VP. Genetic characterization of the spike gene of porcine epidemic diarrhea viruses (PEDVs) circulating in Vietnam from 2015 to 2016. Vet Med Sci 2020;6:535–542.
7. Mai TN, Bui TP, Huynh TM, Sasaki Y, Mitoma S, Daous HE, Fahkrajang W, Norimine J, Sekiguchi S. Evaluating the risk factors for Porcine Epidemic Diarrhea Virus infection in an endemic area of Vietnam. Front Vet Sci 2020;7:433.
8. Chen Q, Li G, Stasko J, Thomas JT, Stensland WR, Pillatzki AE, Gauger PC, Schwartz KJ, Madson D, Yoon KJ, Stevenson GW, Burrough ER, Harmon KM, Main RG, Zhang J. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J Clin Microbiol 2014;52:234–243.
9. Chang SH, Bae JL, Kang TJ, Kim J, Chung GH, Lim CW, Laude H, Yang MS, Jang YS. Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic diarrhea virus. Mol Cells 2002;14:295–299.
10. Sun DB, Feng L, Shi HY, Chen JF, Liu SW, Chen HY, Wang YF. Spike protein region (aa 636789) of porcine epidemic diarrhea virus is essential for induction of neutralizing antibodies. Acta Virol 2007;51:149–156.
11. Sun J, Li Q, Shao C, Ma Y, He H, Jiang S, Zhou Y, Wu Y, Ba S, Shi L, Fang W, Wang X, Song H. Isolation and characterization of Chinese porcine epidemic diarrhea virus with novel mutations and deletions in the S gene. Vet Microbiol 2018;221:81–89.
12. Zhang X, Pan Y, Wang D, Tian X, Song Y, Cao Y. Identification and pathogenicity of a variant porcine epidemic diarrhea virus field strain with reduced virulence. Virol J 2015;12:88.
13. Park JE, Kang KJ, Ryu JH, Park JY, Jang H, Sung DJ, Kang JG, Shin HJ. Porcine epidemic diarrhea vaccine evaluation using a newly isolated strain from Korea. Vet Microbiol 2018;221:19–26.
14. Lee S, Kim Y, Lee C. Isolation and characterization of a Korean porcine epidemic diarrhea virus strain KNU-141112. Virus Res 2015;208:215–224.
15. Sun RQ, Cai RJ, Chen YQ, Liang PS, Chen DK, Song CX. Outbreak of porcine epidemic diarrhea in suckling piglets, China. Emerg Infect Dis 2012;18:161–163.
16. Wang L, Byrum B, Zhang Y. New variant of porcine epidemic diarrhea virus, United States, 2014. Emerg Infect Dis 2014;20:917–919.
17. Diep NV, Sueyoshi M, Izzati U, Fuke N, Teh AP, Lan NT, Yamaguchi R. Appearance of US-like porcine epidemic diarrhoea virus (PEDV) strains before US outbreaks and genetic heterogeneity of PEDVs collected in Northern Vietnam during 2012-2015. Transbound Emerg Dis 2018;65:e83–e93.
18. Kim SH, Cho BH, Lee KY, Jang YS. N-terminal domain of the spike protein of porcine epidemic diarrhea virus as a new candidate molecule for a mucosal vaccine. Immune Netw 2018;18:e21.

https://doi.org/10.14405/kjvr.20230009