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Prevalence and structural protein encoding gene sequence (VP) of porcine parvovirus 2 (PPV2) in slaughtered pigs in Central provinces of Vietnam

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Abstract. Porcine parvovirus (PPV) is a DNA virus and causative agent of several reproductive problems in sows. This study was conducted to determine the prevalence and analyze the DNA sequence of structural protein encoding gene (VP) of PPV2 genotype in pigs. A total of 146 samples (lung and blood samples) were collected from slaughtered pigs of seven provinces in Central Vietnam during 2018-2019. The overall prevalence of PPV2 was 56.2% (82/146). PPV2 positive rate in each province ranged from 37.5% for Quang Nam to 100% for Quang Binh, with the exception of Da Nang, where no PPV2 positive samples were detected. Nearly complete PPV2-VP gene sequences of three strains were identified with the length of 2,493 nucleotides and deposited in GenBank with accession numbers of OL913365-OL913367. Four nucleotide substitutions were detected in Vietnamese PPV2 isolates and were not observed in PPV2 reference strains. Multiple alignment and comparison of nucleotide and deduced amino acid sequences showed the high similarity within Vietnamese PPV2 strains (95.6-96.5% and 94.7-96.9%, respectively). The PPV2 strains from this study clustered together with the "primitive" PPV2 strains from Myanmar, and strains from China in a main clade in the phylogenetic tree (Cluster A). This is the first report on the prevalence of PPV2 genotype and its VP gene sequence in pigs in Vietnam. This also provides the valuable information on the molecular evolution of locally circulating PPV2 and contributes to the control of PPV-induced SMEDI syndrome in sows, especially in the central provinces of Vietnam.

Keywords: Porcine parvovirus 2, prevalence, pigs, VP gene nucleotide sequence, phylogenetic tree

1 Introduction

To date, the clinical significance of porcine parvovirus (PPV) has been well-described with the predominant effect being impaired fertility in sows [1]. PPV are small, non-enveloped, isometric, 18-26 nm in diameter viruses, with a genome structure that resembles a linear, non-segmented molecule of ssDNA which is approximately 4-6.3 kb in size [2] that causes porcine reproductive failure in swine [3]. PPV genome is characterized by a hairpin structure at two 5′-3′ ends [4, 5] and two open reading frames (ORF) coding for non-structural protein (NSP) and viral coat and capsid protein (VP). PPV is stable to environmental factors, living in a pH range of 3.0-8.0 and is heat resistant for hours at 80°C [6].

PPV is a member of the family Parvoviridae [7]. *Ungulate protoparvovirus 1*, or Porcine parvovirus 1 (PPV1- known as "classical" PPV), was first isolated in 1965 in Germany [8] and is

the causative agent of reproductive dysfunction in sows, characterized by stillbirth, mummified fetuses, embryonic death, and infertility (SMEDI) [3]. In the following years, other genotypes of PPV were also confirmed, including: Porcine parvovirus 2 (PPV2) (formally name is *Ungulate tetraparvovirus 3)*, belongs to the *Tetraparvovirus* genus, was discovered in 2001 during a survey for Hepatitis E virus (HEV) in swine sera collected in Myanmar [9]; PPV3 (*Ungulate tetraparvovirus 2*) was discovered in Hong Kong in 2008; PPV4 (*Ungulate copiparvovirus 2*) and PPV5 (unclassifed) were identified in the USA in 2010 and 2013, respectively; PPV6 (*Ungulate copiparvovirus 4*) was discovered in China in 2014 and most recently, PPV7 (proposed genus *Chappaparvovirus*) was identified in the USA in 2016 [10, 11, 12, 13, 14, 15, 16].

Analysis of the genetic relationship between PPV1 and PPV2 revealed that PPV2 is "distantly related" to PPV1 with 32.2-34.5% genomic identity and amino acid identity of 20.2-21.5% for ORF1 and 16.5-16.9% for ORF2 [17]. The efforts to propagate PPV2 *in vitro* have so far been unsuccessful, so studies on the pathogenicity of PPV2 have not been possible [18]. However, recent evidence shows that PPV2 may be a co-factor or triggering agent associated with PCVAD [17] or 'high fever disease' [19].

Further epidemiological studies of PPV2 have been reported differently worldwide [20]. About a decade after it was first detected, PPV2 was identified in 8.8% of serum samples obtained from commercial pig farms in Southeastern China [19]. Subsequently, PPV2 has been discovered in other samples, including hearts, blood, faeces and lungs in many other countries including Hungary, the USA, Germany, Japan, and Thailand with prevalence of 6.4%, 20.7%, 55%, 58% and 83%, respectively [18, 21, 22, 23, 24]. Meanwhile, using tonsil samples in PPV2 screening has reported a higher prevalence, ranging from 78% (Germany) to 100% (Japan), leading to the hypothesis that viral prevalence may depend on the organ type examined, although the routes of infection and viral loci are yet to be elucidated experimentally [22, 23, 24].

In Vietnam, SMEDI syndrome in sows caused by PPV is nationwide and has been of concern since the early 1990s. Previous studies have investigated the prevalence of PPVs using serological testing and PCR methods. The objective of this study is to analyze the genotypic prevalence of PPVs and molecular characterization of VP gene of PPV2 from slaughtered pigs in Central Vietnam.

2 Materials and methods

2.1 Sample collection

Lung tissue and blood samples of grow-finish healthy pigs (18-25 weeks) were sampled at abattoirs located in seven provinces in Central Vietnam. In each participating abattoir, a maximum of five samples were collected, and the number of abattoirs visited was 3-6 for each province. Only one blood or lung tissue sample was collected from each individual pig to avoid duplication. In total, 136 lung and 29 blood samples were collected. Detailed information on the collected sample, including number, sample type, collection year, and collection location, is presented in Table 1. After collection, the samples were stored on ice (4°C) and immediately shipped to the laboratory and all samples were frozen at -20°C until testing.

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Table 1. Sample information collected in this study

Province	Number of abattoirs visited[1]	Number of samples collected[2]	Sample type		Collection year
			Lung	Blood	
Quang Binh	4	17	0	17	2019
Quang Tri	5	22	22	0	2019
Thua Thien Hue	6	28	28	0	2019
Da Nang	3	14	8	6	2018
Quang Nam	4	16	10	6	2018-2019
Quang Ngai	5	24	24	0	2018-2019
Binh Dinh	5	25	25	0	2018
Total	32	146	117	29	

[1]The number of samples collected in each abattoir was less than five.

[2]From each pig, only a single sample was collected (either lung or blood).

2.2 DNA extraction and PCR analysis

Total DNA was extracted and purified according to the methods of Sambrook and Russell [25] with small modifications: blood samples were washed several times with PBS buffer until they became white (try to remove all the hemoglobin) before using for DNA extraction. DNA was diluted in TE buffer, and stored at 4°C for analysis.

To detect PPV2, specific PCR assays were carried out in a single reaction with 50-100 ng of DNA template, 2× PCR master mix (Thermo Scientifc, Lithuania), and 5 pmol of each primer. The PPV2 primers for detection were followed Streck et al. [22]. Primers for the PPV2 sequencing (Table 2) were designed using the program Primer3 based on reference sequences listed below (Table 3). Information on primer sequence, annealing temperature (Ta) and product size are shown in Table 2. The PCR consisted of an initial activation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 56/60/50/54°C for 30 sec, extension at 72°C for 30 sec, final synthesis at 72°C for 5 minutes and holding at 14°C.

The resulting PCR products were analyzed by electrophoresis on a 2% agarose gel using a 100 bp DNA ladder (Thermo Fisher Scientific). Appropriate positive and negative extraction and PCR controls (obtained from the University of Edinburgh and confirmed by sequencing) were used for each extraction and PCR run.

Table 2. Primer information used in this study for genotyping and sequencing. The virus target was *U. tetraparvovirus 3* (PPV2)

Purpose	Target	Primer sequence (5′-3′)	Ta (°C)	PCR size (bp)	Reference
Genotyping	PPV2	F: AGATTCTTGCAGGCCGTAGA R: CCAAGGGTCAGCACCTTTTA	60	222	Streck et al., 2013
Sequencing	PPV2-1	F: CAGCTTCATGGCTTACGGGCA R: ATCCCTCTCCGCCCGCCAGA	50	721	This study
	PPV2-2	F: CATGAGCGCTGCCGACGCGT R: TCCATCAGACCCTGGGCCA	54	730	

Purpose	Target	Primer sequence (5′-3′)	Ta (°C)	PCR size (bp)	Reference
	PPV2-3	F: GACCCAGAGATAGATAGCGT R: CCACCAATGAGATCCGCTA	53	773	
	PPV2-4	F: TGGACAAAGTCTTACCGCC R: AGGGTTGTACCACTGAATACT	50	743	
	PPV2-5	F: CATTGGCGCGCAGGCACC R: ATAAACCCCTGAGGAAATA	50	776	

2.3 PPV2-VP2 gene sequencing and sequence analysis

Five primer pairs, designed based on PPV2 gene sequence published on GenBank, were used to amplify structural protein encoding genes (VP genes) of three PPV2 positive strains (Table 2).

The PCR products were sent to Macrogen Inc., South Korea for sequencing using an ABI-3100 Avant Genetic Analyzer automated sequencer according to the standard Sanger method [26].

Multinucleotide and amino acid sequence alignments were performed in BioEdit 7.0.9.0 [27]. The published PPV2 sequence information used as a reference is presented in Table 3. Obtained nucleotide sequences were identified with the Basic Local Alignment Search Tool (BLAST, NCBI) [28].

The phylogenetic tree was constructed with Mega X software using the neighbor-joining (NJ) method [29] and computed with the Kimura 2-parameter method [30]. Bootstrap values were calculated using 1.000 replicates of the alignment.

Table 3. List of PPV2 strains used in this study

Strain	Location	Year	Size (bp)	Reference
AB076669	Myamar	2001	5118	Hijikata et al., 2001
KP245940	China	2014	3099	
KP245943		2014	3099	Sun et al., 2015
KP245944		2014	3099	
MK092387		2018	5205	Ren et al., 2020
MK092408		2018	3172	
MN326142		2019	5119	Sun et al., 2021
MN326185		2019	5119	
MZ577029		2021	5426	Li et al., 2021
MG345016		2017	5427	Qin et al., 2018
OL913365	Vietnam	2019	2493	This study
OL913366		2019	2493	This study
OL913367		2019	2493	This study
KY586144	Brazil	2017	5316	Cerva et al., 2017
KX517759	Hungary	2016	5533	Novosel et al., 2017
KC701296		2013	3099	

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Strain	Location	Year	Size (bp)	Reference
KC687100	Cromania	2013	3099	Cadar et al., 2013
JQ860238	Romania	2012	3096	
JQ860240		2012	3096	
JQ860243		2012	3099	
JQ860248		2012	3099	
JX101461	US	2012	5486	Xiao et al., 2013
KF725662		2013	3259	Opriessnig et al., 2014

3 Results and discussion

3.1 Detection of the PPV2 genotype

The quality and quantity of total extracted DNA were sufficient for analysis with the ratio of OD 260/280 (1.84 to 2.16) and DNA concentration (92 to 446 ng/μL). The PCR products for genotyping of PPV2 are shown in Fig. 1. In Fig. 1, a single clear band was observed in wells 2-6 and 8, corresponding to the size of positive control (+). That means those samples were positive for the PPV2 genotype, but this is necessary to confirm by sequencing. No DNA band appeared in negative control (-) and negative samples (wells 1 and 7).

The expected bands represented for PPV2 genotypes were purified and sequenced. Obtained nucleotide sequences of 222 bp in length were identified as PPV2 by BioEdit software. As shown in Fig. 2, the BLAST analyses also revealed a high nucleotide identity (98%) between PPV2 sequence and reference PPV2 strain (MG345019).

In the present study, among the 146 lung and blood samples from 32 different abattoirs in seven provinces of Central Vietnam, PPV2 DNA was detected with 56.2% (82/146). PPV2 prevalence rates for each province ranged from 37.5% for Quang Nam to 100% for Quang Binh, with the exception of Da Nang, where no PPV2-positive samples were detected (Table 4).

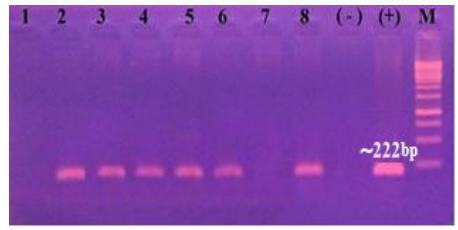

Fig. 1. Agarose gel electrophoresis of PPV2 genotyping PCR product (~222 bp).
M*:* 100 bp DNA ladder (Thermo Fisher Scientific)*; 1, 7: negative samples; 2-6, 8: positive samples; (-): negative control; (+): positive control (~222 bp).*

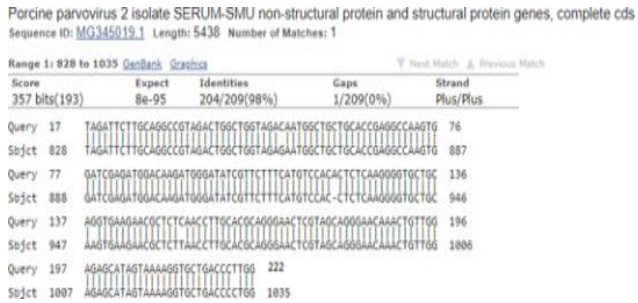

Fig. 2. BLAST result revealed the positive sample with 98% nucleotide identity compared with reference PPV2 strain (MG345019)

Table 4. Number of DNA-positive samples (percentage) in this study

Province	n	PPV2 (%)
Quang Binh	17	17 (100)
Quang Tri	22	10 (45.5)
Thua Thien Hue	28	20 (71.4)
Da Nang	14	0
Quang Nam	16	6 (37.5)
Quang Ngai	24	18 (75)
Binh Dinh	25	11 (44)
Total	146	82 (56.2)

Of the 117 lung and 29 blood samples, the positive PPV2 was detected at 50.4% (59/117) and 79.3% (23/29), respectively (data not shown). However, this result did not reflect the effect of sample types because only a single sample was collected from each pig (either lung tissue or blood).

Recently, studies using diagnostic methods based on target amplification PCR have detected PPV in commercial pigs in many countries around the world. With a PPV2 prevalence of 56.2%, our study results were in a similar range to the PPV2 prevalence in China (45.1%), Hungary (51.0%) and Japan (58.0%) [31, 32, 23]. A lower prevalence of PPV2 infection was reported in North America (36.8%), Romania (25%), South Africa (21.8%) and Poland (19%) [15, 33, 1, 34]. In contrast, a high prevalence of PPV2 in pig herds was observed in Germany (78.0%) and Thailand (82%) [22, 24].

In Vietnam, PPV-induced fertility decline syndrome has been of concern since the early 90s of the last century and has caused considerable damage to the pig industry. Therefore, the detection of PPV genotypes in pigs, including PPV2, is not an exception. In a previous survey of sows in Long An province by serological testing, Nguyen and Tran [35] reported that the positive rate for PPV was quite high at 69%. In addition, analysis of 52 stillbirth samples detected PPV virus and PPV antibodies simultaneously. Recently, surveys in some localities have initially confirmed the prevalence of the PPV2 genotype in pig farms. In a study on pig lung samples collected from abattoirs in four northern provinces of Vietnam, Cuong [36] reported a PPV2 infection rate of 17.6%. Most recently, Thuy et al. [37] found PPV2 prevalence up to 28% in finishing pig from 13 provinces in three regions of Vietnam, including North, Central and South Vietnam.

Taken together, the above data have shown that our results on PPV2 prevalence are in the average range compared with other previous studies. It could be explained by the type of sample collected (mainly lung sample) and the status of investigated animal (slaughtered pig) in our study. This result supports the hypothesis of Saekhow and Ikeda [24]: viral prevalence may depend on the type of sample collected and screened, although the routes of infection and the viral tissue tropism are yet to be elucidated.

3.2 Analysis of PPV2-VP gene

The VP gene sequences encoding structural proteins of three PPV2 strains collected from Quang Binh, Hue and Quang Ngai were identified and analyzed. The length of the partial VP gene of three PPV2 isolates in this study is 2,493 nucleotides, including VP1 and VP2 sequences, without insertions and deletions in the coding regions. The sequences were deposited in the GenBank database with the accession numbers OL913365-OL913367.

Comparing many reference strains found that there are four base substitutions (444: T→A, 734: C→T, 820: A→C, 1974: T→A) in PPV2 QN03-VN strain, completely different compared with the reference strains; two of them (734: C→T, 820: A→C) resulted in amino acid changes in the VP gene sequence (245: S→F, 274: K→Q). No variation was detected in the deduced amino acid sequence of the two strains, PPV2 HU10-VN and PPV2 QB05-VN. For the PPV1 genotype, nucleotide changes leading to amino acid changes at several potential sites on the VP2 gene and responsible for antigenicity were observed in previous studies [4, 38, 39, 40, 41]. The studies on molecular genetic variability of PPV2 are still limited; therefore, the analysis of molecular changes related to antigenicity, virulence, immune response, neutralization activity and pathogenesis is necessary.

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Comparison results of the VP nucleotide and amino acid sequence (2,493 nucleotides and 832 amino acids) between the PPV2 strains isolated in Vietnam in this study and strains isolated in Myamar, China and Europe are presented in Table 5.

Comparison between three strains of PPV2 isolated from pigs raised in Central Vietnam showed that the nucleotide similarity between them was quite high, at 95.6-96.5%. Compared with the "original" PPV2 strain first identified from Myanmar in 2001, the PPV2 strains isolated in Vietnam showed nucleotide and amino acid similarity of 96.1-97.9% and 95.4-98.1%, respectively. A high level of sequence identity at the nucleotide and amino acid levels was observed between the Vietnamese PPV2 strains and the European strains (94.9-100% and 93.3-100%, respectively). The data presented in Table 5 also show that the nucleotide and amino acid sequences of the PPV2 strains in Vietnam have a high degree of similarity with the Chinese strains isolated from 2018-2021, and less with the Chinese strains isolated in 2014.

To analyze the genetic relationship between the PPV2 sequences obtained in this study and the reference strains from other geographical locations, a phylogenetic tree was constructed based on the sequences of the partial PPV2-VP gene. As seen in Fig. 3, the PPV2 phylogenetic tree splits into two main branches (Cluster A and B). PPV2 strains isolated in Vietnam (black dots), "primitive" PPV2 strains discovered for the first time in Myanmar, strains isolated in China during 2018-2021 together with isolates in Europe gathered to form Cluster A. Cluster B includes PPV2 strains isolated in China in 2014 and PPV2 strains originating from the USA. The bootstrap value for all branches reached 99%, indicating the reliability of the phylogenetic tree. These results are consistent with a previous phylogenetic analysis based on PPV2 genome sequencing [22, 31] and show the genetic relationship between PPV2 strains circulating in Vietnam and reference strains.

Table 5. Nucleotide and amino acid sequence identity (%) within Vietnamese *U. tetraparvovirus 3* (PPV2) strains and compared with reference strains

Strain \ Molecular size[1]		Nucleotide (2,493 nt)	Amino acid (832 aa)
Vietnam		95.6-96.8	94.7-96.9
Myama		96.1-97.9	95.4-98.1
China	Cluster A	95.2-99.5	94.5-99.0
	Cluster B	93.5-94.6	93.2-95.4
Europe		94.9-100	93.3-100

[1]nt, nucleotide; aa, amino acid

4 Conclusions

This is the first study on the circulation of PPV2 in commercial pigs in seven central provinces of Vietnam and the characterization of their VP gene sequence. The partial VP gene sequences of three PPV2 strains (2,493 nucleotides), deposited in the GenBank database, showed four nucleotide substitution mutations that were completely different from the reference strains. The PPV2 strains from this study clustered with the "primitive" PPV2 strains from Myanmar, and strains from China to form a large clade in the phylogenetic tree (Cluster A). The obtained result provides the valuable information on the molecular evolution of locally circulating PPV2 and contributes to the control of PPV-induced SMEDI syndrome in sows, especially in central provinces of Vietnam.

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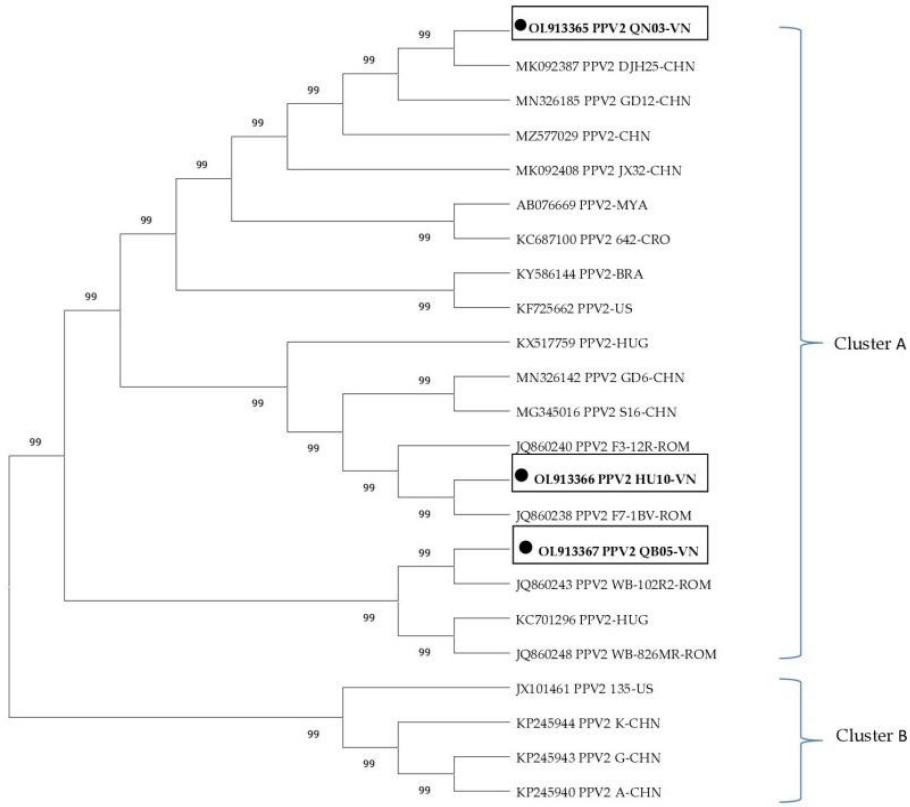

Fig. 3. Phylogenetic tree based on the VP nucleotide sequence of PPV2 (2,493 nucleotides) obtained from three provinces of Vietnam (in this study) and reference sequences from GenBank database. The black square box indicates the Vietnamese PPV2 sequence. The number indicated the bootstrap value of 1.000 replicates

References

1. Afolabi KO, Iweriebor BC, Okoh AI, Obi LC. Increasing diversity of swine parvoviruses and their epidemiology in African pigs. Infect Genet Evol. 2019;73:175-183.
2. Tijssen P, Agbandje-McKenna M, Almendral JM, Bergoin M, Flegel TW, Hedman K, et al. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier; 2011.
3. Mengeling WL, Lager KM, Vorwald AC. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. Anim Reprod Sci. 2000;60-61:199-210.
4. Bergeron J, Hérbert B, Tijssen P. Genome organization of the Kresse strain of porcine parvovirus: identification of the allotropic determinant and comparison with those of NADL-2 and field isolates. Virol J.1996;70:2508-2515.
5. Tattersall P, Kerr JR, Cotmore SF, Bloom ME, Linden RM, Parrish CR. The evolution of parvovirus taxonomy. In: Parvoviruses (ed). 2006;10.
6. Choi CS, Molitor TW, Joo HS, Gunther R. Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses. Vet Microbiol. 1987;15:19-29.
7. Wilhelm S, Zimmermann P, Selbitz HJ, Truyen U. Real-time PCR protocol for the detection of porcine parvovirus in field samples. J Virol. Methods. 2006;134:257-260.
8. Mayr A, Mahnel H. Cultivation of hog cholera virus in pig kidney cultures with cytopathogenic effect. Zentralbl Bakteriol Orig. 1964;195:157-166.
9. Hijikata M, Abe K, Win KM, Shimizu YK, Keicho N, Yoshikura H. Identification of new parvovirus

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eISSN 2615-9678

DNA sequence in swine sera from Myanmar. Jpn J Infect Dis. 2001;54:244-245.

10. Lau SK, Woo PC, Tse H, Fu CT, Au WK, Chen XC, et al. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. J Gen Virol. 2008;89:1840-1848.
11. Cheung AK, Wu G, Wang D, Bayles DO, Lager KM, Vincent AL. Identification and molecular cloning of a novel porcine parvovirus. Arch Virol. 2010;155:801-806.
12. Huang L, Zhai SL, Cheung AK, Zhang HB, Long JX, Yuan SS. Detection of a novel porcine parvovirus, PPV4, in Chinese swine herds. Virol J. 2010;7:333-336.
13. Xiao C-T, Halbur PG, Opriessnig T. Complete Genome Sequence of a Novel Porcine Parvovirus (PPV) Provisionally Designated PPV5. 2013;1(1):10.1128/genomea.00021-12.
14. Ni J, Qiao C, Han X, Han T, Kang W, Zi Z, et al. Identification and genomic characterization of a novel porcine parvovirus (PPV6) in China. Virol J. 2014;11:203-210.
15. Cui J, Fan J, Gerber PF, Biernacka K, Stadejek T, Xiao CT, et al. First identifcation of porcine parvovirus 6 in Poland. Virus Genes. 2017;53:100-104.
16. Xing X, Zhou H, Tong L, Chen Y, Sun Y, Wang H, et al. First identifcation of porcine parvovirus 7 in China. Arch Virol. 2018;163:209-213.
17. Opriessnig T, Xiao CT, Gerber PF, Halbur P.G. Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the US concurrently infected with PPV2. Vet Microbiology. 2013;177-183.
18. Csagola A, Lorincz M, Cadar D, Tombacz K, Biksi I, Tuboly T. Detection, prevalence and analysis of emerging porcine parvovirus infections. Arch Virol. 2012;157:1003-1010.
19. Wang F, Wei Y, Zhu C, Huang X, Xu Y, Yu L, et al. Novel parvovirus sublineage in the family of Parvoviridae. Virus Genes. 2010;41:305-308.
20. Nelsen A, Lin C-M, Hause BM. Hause. Porcine Parvovirus 2 Is Predominantly Associated With Macrophages in Porcine Respiratory Disease Complex. Front. Vet Sci. 2021; 8:1-10.
21. Xiao C-T, Giménez-Lirola LG, Jiang Y-H, Halbur PG, Opriessnig T. Characterization of a Novel Porcine Parvovirus Tentatively Designated PPV5. PLOS ONE. 2013;8(6):e65312.
22. Streck AF, Homeier T, Foerster T, Fischer S, Truyen U. Analysis of porcine parvoviruses in tonsils and hearts from healthy pigs reveals high prevalence and genetic diversity in Germany. Arch Virol. 2013;158:1173-1780.
23. Saekhow P, Mawatari T, Ikeda H. Coexistence of multiple strains of porcine parvovirus 2 in pig farms. Microbiol Immunol. 2014;58:382-387.
24. Saekhow P, Ikeda H. Prevalence and genomic characterization of porcine parvoviruses detected in Chiangmai area of Thailand in 2011. Microbiol Immunol. 2015;59:82-88.
25. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual, Vol. 1. New York: Cold Spring Harbor Laboratory Press; 2001.
26. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proceedings of The National Academy of Sciences of The United States of America. 1977;74(12):5463-5467.
27. Hall T. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser. 1999;41:95-98.
28. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403-410.
29. Saitou N, Nei M. The neighbor-joining method: a new method for reconstruction of phylogenetic trees. Mol Biol Evol. 1987;4:406-425.
30. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111-120.
31. Sun J, Huang L, Wei Y, Wang Y, Chen D, Du W, et al. Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. J Gen Virol. 2015;160(5):1339-1344.
32. Novosel D, Cadar D, Tuboly T, Jungic A, Stadejek T, Ait-Ali T, et al. Investigating porcine parvoviruses genogroup 2 infection using in situ polymerase chain reaction. BMC Vet Res. 2018;14:163-170.
33. Cadar D, Csagola A, Kiss T, Tuboly T. Capsid protein evo- lution and comparative phylogeny of novel porcine parvoviruses. Mol Phylogenet Evol. 2013;66:243-253.
34. Opriessnig T, Xiao CT, Gerber PF, Halbur PG. Identifica- tion of recently described porcine parvoviruses in archived North American samples

from 1996 and association with porcine circo- virus associated disease. Vet Microbiol. 2014;173:9-16.

35. Nguyen H, Tran DT. Survey of some factors related to reproductive disorders in sows in Long An province. Ho Chi Minh: University of Agriculture and Forestry, Ho Chi Minh City; 2000.
36. Cuong NH. Molecular characteristics of the VP2 gene of Parvo virus in pigs [Master thesis]. Hanoi: Institute of Ecology and Biological Resources; 2018.
37. Thuy NTD, Trung NT, Dung TQ, Khoa DVA, Thuy DTN, Opriessnig T. First investigation of the prevalence of parvoviruses in slaughterhouse pigs and genomic characterization of ungulate copiparvovirus 2 in Vietnam. Archives of Virology. 2021;166(3):779-88.
38. Simpson AA, Hébert B, Sullivan GS, Parrish CR, Zadori Z, Tijssen P, et al. The structure of porcine parvovirus: comparison with related viruses. J Mol Biol. 2002;315:1189-1198.
39. Soares RM, Cortez A, Heinemann MB, Sakamoto SM, Martins VG, Bacci M, et al. Genetic variability of porcine parvovirus isolates revealed by analysis of partial sequences of the structural coding gene VP2. J Gen Virol. 2003;84:1505-1515.
40. Streck AF, Bonatto SL, Homeier T, Souza CK, Gonc¸alves KR, Gava D, et al. High rate of viral evolution in the capsid protein of porcine parvovirus. J Gen Virol. 2011;92:2628-2636.
41. Hao X, Lu Z, Sun P, Fu Y, Cao Y, Li P, et al. Phylogenetic analysis of porcine parvoviruses from swine samples in China. Virol J. 2011;8:320-328.