Epidemiological Analysis of Porcine Circovirus Type 2 (PCV2) Spatiotemporally of Circulation in Viet Nam from 2004 to 2019

Mai Phuong Ngoc Nguyen [1], **Thien Ngoc Lam** [2], **The Hien Nguyen**[2], **Tat Toan Nguyen**[2], **Duy Tien**[2,*], **Minh Nam Nguyen**[3,4,5,*]

[1]*School of Biotechnology, Tan Tao University, Tan Duc E.City, Duc Hoa, Long An, 850000, Vietnam.*

[2]*Faculty of Animal Science and Veterinary Medicine, Nonglam University HCMC, Thu Duc district, Ho Chi Minh City, Vietnam*

[3]*Research Center for Genetics and Reproductive Health, School of Medicine, Vietnam National University Ho Chi Minh City, Linh Trung ward, Thu Duc district, Ho Chi Minh City, Vietnam.*

[4]*Department of Biomedical Engineering, School of Medicine, Vietnam National University Ho Chi Minh City (VNU-HCM), Ho Chi Minh City 700000, Vietnam*

[5]*Vietnam National University Ho Chi Minh City (VNU-HCM), Ho Chi Minh City 700000, Vietnam*

Correspondence

Duy Tien, Faculty of Animal Science and Veterinary Medicine, Nonglam University HCMC, Thu Duc district, Ho Chi Minh City, Vietnam

Email: duy.dotien@hcmuaf.edu.vn

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ABSTRACT

Introduction: Porcine circovirus 2 (PCV2) is a causative agent of postweaning multisystem wasting syndrome (PMWS), an epizootic disease that causes significant economic losses to pig producers worldwide, including Viet Nam. However, analyses of which groups of PCV2 strains cause disease in pigs in Viet Nam are still limited, leading to the delay or lack of application of specific vaccine sources to prevent PMWSs in other countries. **Method**: In this study, we aimed to analyze the geographical and temporal diversity of PCV2 in pig farms across 26 provinces in Viet Nam, thus identifying the dominant strain for vaccine production. After samples from North, Central, and South Viet Nam were collected, phylogenetic trees were created to study the molecular genetic/evolutionary relationships, thereby classifying the existing PCV2 strains. The spatiotemporal distribution of strains is shown visually for observation. Finally, an analysis of the important evolutionary differences between the two strains, PCV2d and PCV2b, revealed that these strains were superior in Viet Nam. **Results**: Our study examined the diversity of PCV2 strains in Viet Nam, ranging from the northern to southern regions, and the PCV2d strain was revealed to be the predominant strain. Interestingly, we observed the presence of 2 or 3 different genotypes of PCV2 in the same province and at the same time. We identified the presence of PCV2h, PCV2g, and PCV2e genotypes that had not been previously detected. Furthermore, we identified potential sites of positive selection in the capsid epitopes, which are believed to play a crucial role in evading the host immune system. **Conclusions**: The results showed that Viet Nam has 5 genotypes, namely, PCV2b, PCV2e, PCV2g, PCV2h and PCV2d—the main causative strains of the disease. The coexistence of strains in the same geographical area was also shown, indicating the importance of preparing vaccines and preventing livestock infection in Viet Nam.

Key words: epidemiological, PCV2, PMWS, phylogenetics, porcine circovirus type 2

INTRODUCTION

Postweaning multisystem wasting syndrome (PMWS) in swine was first discovered in 1991 in Canada and is characterized by emaciation and jaundice[1,2]. Over the years, common disease manifestations have been described as emaciation, pale skin, jaundice, and diarrhea in piglets[3]. After years of research and sequence analysis, PMWS has been found to be caused by porcine circovirus (PCV)[4,5]. PCV type 1 (PCV1) is a virus derived from cell culture, whereas PCV type 2 (PCV2) is a virus strain associated with swine diseases[6]. PCV2 causes a disease collectively known as porcine circovirus-associated disease (PCVAD or PMWS), which, although emerging globally, has a major impact on the global swine industry, causing significant economic losses to many specialized pork export countries, including Viet Nam. Subsequent studies have shown the relationship of PCV2 with reproductive disorders[7,8], enteritis[9,10], respiratory disease[11,12], dermatitis, and other syndromes, such as porcine dermatitis and nephropathy syndrome (PDNS)[13,14] and proliferative and necrotizing pneumonia (PNP)[3,10].

Pigs of all ages are susceptible to PCV2, and symptoms of PMWS are commonly observed between 6 and 10 weeks of age[15]. Mortality rates in pigs can range from 4 to 20%[16]. PCV2 can be found in various secretions (eyes, nose, bronchi), saliva, urine, milk, and semen, indicating diverse transmission routes both horizontally and vertically[17–22]. Horizontal transmission occurs primarily through the nose and mouth[23]. Opriessing et al. confirmed transmission through the pig's mouth by feeding uncooked food from infected pigs[24]. The most common vertical transmission route occurs through the placenta from mother to offspring, leading to fatal PCV2 infection[22].

PCV1 and PCV2 belong to the Circoviridae family[10,25,26] and the genus Circovirus[27]. This family has a specific avian host and a relatively narrow host

Correspondence

Minh Nam Nguyen, Research
Center for Genetics and
Reproductive Health, School of
Medicine, Vietnam National
University Ho Chi Minh City, Linh
Trung ward, Thu Duc district, Ho Chi
Minh City, Vietnam.

Department of Biomedical
Engineering, School of Medicine,
Vietnam National University Ho Chi
Minh City (VNU-HCM), Ho Chi Minh
City 700000, Vietnam
Vietnam National University Ho Chi
Minh City (VNU-HCM), Ho Chi Minh
City 700000, Vietnam

Email: nmnam@medvnu.edu.vn

range. These viruses are small, nonenveloped, icosahedral viruses with a single-stranded DNA genome (1767/1768 bp) that includes 7 open reading frames (ORFs). Among these, ORF1 encodes a replicase (Rep) protein, and ORF2 encodes a capsid (Cap) protein. Rep and Cap are important components of infectious virions[4,28]. The PCV2 genome has a conserved stem loop structure in its ssDNA, which allows it to infect eukaryotes[29]. The rate of nucleotide substitution for PCV2 was estimated to be on the order of 1.2×10^{-3} substitutions/site/year, the highest ever observed for a ssDNA virus[30–32]. This high evolutionary rate could facilitate the rapid emergence of PCV2 worldwide.

Because ORF2 has a higher evolution rate than the whole genome of PCV2 and is under great selective pressure from the immune system[33], PCV2 can enter host cells. On the other hand, the ORF2 cap is considered a unique protein structure that plays a role in determining the antigenicity and virulence of PCV2[4]. Therefore, changing the amino acid sequence of ORF2 can change its pathogenicity and virulence. For these reasons, ORF2 has become the focus of many studies. The convention to genotype PCV2 based on the prevalent diversity of the ORF2 nucleotide was accepted in 2008[34]. Studies of the PCV2 genome have identified 8 genotypes, namely, PCV2a, PCV2b, PCV2c, PCV2d, PCV2e, PCV2f, PCV2g, and PCV2h[31,32,35–38]. Genotype shifts may be associated with differences in pathogenicity and vaccine immunity[39]. These remarkable "genotype shifts" revealed that PCV2b displaces PCV2a as the dominant virus and has increased virulence[31,40–42]. On the other hand, the mutant PCV2b-calling PCV2d strains isolated from PCV2b strains have begun to gain increasing attention for pathogenicity in many countries, such as China, Korea, the United States, and South America[43,44]. Therefore, there is a need to identify novel PCV2 genotypes to develop more effective vaccines for rapidly evolving PCV2.

In Viet Nam, studies on new strains of PCV2 are limited. This study aimed to investigate the annual geographical distribution of PCV2 across Viet Nam. The results of this study will provide an objective, accurate assessment of the current raging strains of PCV2, as well as a direction for successful vaccine development.

MATERIALS AND METHODS

Sample collection, DNA isolation, PCR, and sequencing

From 76 pig farms, samples were collected from 127 pigs with clinical PRDC manifestations (**Figure 1**).

The tissue samples were processed and frozen (-20°C) within 12 hours. Three grams of sample was homogenized in a 15 ml Falcon tube by adding PBS to a 10% suspension. The tissue homogenate was subsequently centrifuged at 3000 rpm for 3-5 min at 4°C. One to three millilitres of the supernatant was used for nucleic acid isolation. Viral nucleic acid was extracted from tissues using the GeneJET Viral DNA/RNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. PCR was used to identify potential cases of infection with PCV2 using the primers PCV2F and PCV2R, which were designed based on 66 ORF2 sequences of the PCV2 strain. PCR was carried out in 25 μl reaction mixtures containing 10 μl of Green Master Mix (GoTaq Green Master Mix, Cat# M7122; Promega, USA), 3 μl of DNA template, 0.5 μl of 10 μM each primer, and 10.5 μl of nuclease–free water. The PCR temperature cycling conditions were as follows: 95°C for 3 min; 33 cycles of denaturation at 94°C for 60 s and 58°C for 60 s; 72°C for 45 s; and 72°C for 5 min. The PCR products were then separated and visualized via 1.2% agarose gel electrophoresis with Gel-red staining. The 702-bp bands of 43 samples (potential PCV2 strain) were collected and purified using the Wizard Preps DNA Purification and PCR Clean-Up System (Promega, USA). The purified products were subsequently sent to Macrogen (Seoul, Korea) for sequencing using the PCV2R and PCV2F primers. These sequences are available in GenBank under accession number MT432425-67. After sequencing, 42/43 ORF2 sequences were selected for phylogenetic study (one sample was disqualified due to ineligibility of the Beast tool). **Table 1** (entries 44-85) displays these sequences.

PCV2 sequences in Viet Nam were collected from the NCBI

To obtain the most approximate results, it is necessary to have a large amount of information as well as the sequences to be analyzed in all three regions of North-Central-South Viet Nam. Therefore, in this study, two additional sources of sequences from the North were identified to enrich the PCV2 sequence of Viet Nam and help the study obtain more reliable results. First, 43 ORF2 sequences of the PCV2 strain from previous studies were collected from the GenBank. These PCV2 strains were isolated from several provinces of Viet Nam from 2004 to 2017, and their names are listed in **Table 1** (from number 1 to 43). Second, 2 ORF2 sequences of PCV2 from 2018 were also collected from GenBank (2 sequences are shown in numbers 86 and 87 in **Table 1**)[45].
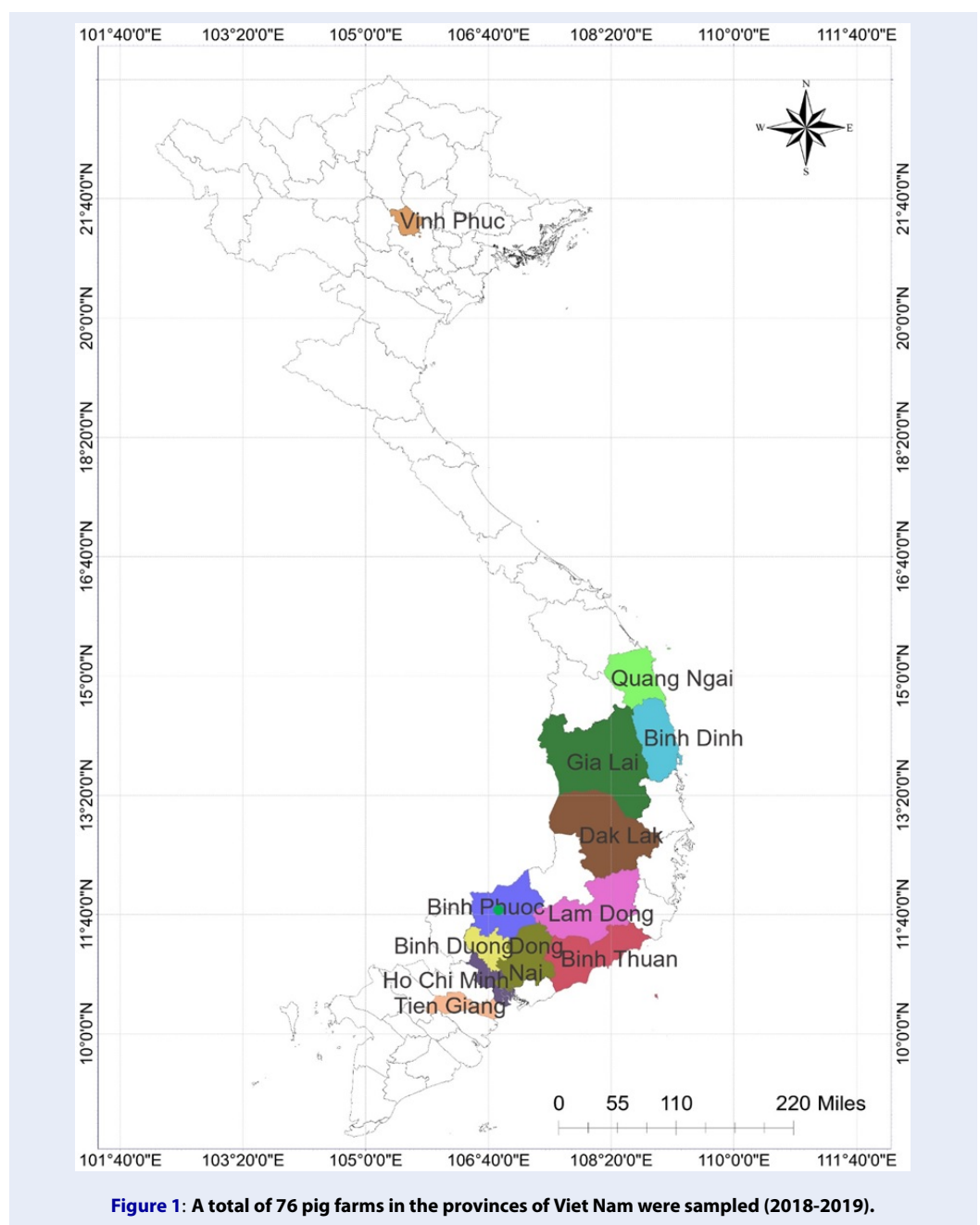
Figure 1: **A total of 76 pig farms in the provinces of Viet Nam were sampled (2018-2019).**

Altogether, a total of 87 ORF2 nucleotide sequences from 26 provinces in the northern, central, and southern regions of Viet Nam from 2004 to 2019 were used for analysis in this study. The genome size of the 87 PCV2 strains collected in this study was 626 nucleotides after alignment and editing of the sequences.

Table 1: The sequence IDs of the PCV2 strains were obtained from 2004–2019 from 26 provinces in Viet Nam

No.	Strain (1)	Location (2)	Year (3)	No.	Strain	Location	Year
1	NAVET_vie	BD	2004	44	DTD-AnPha/BD	BD	2018
2	749-Vp33	VP	2004	45	DTD-CS1/ND		2018
3	P2425NT	TN	2008	46	DTD-Dat/ND		2018
4	550-TH	TH	2008	47	DTD-Guyo/DN	DN	2018
5	641-HB	HB	2008	48	DTD-Hai/BP		2018
6	P473BY	YB	2009	49	DTD-Minh/ND		2018
7	P477LG	GL	2009	50	DTD-N/ND	BTh	2018
8	P485YH	HY	2009	51	DTD-Nhon/DN	DN	2018
9	P624LB	BL	2009	52	DTD-Phuoc2/TG	TG	2018
10	661-BN		2009	53	DTD-RT2/GL	GL	2018
11	529-Hno	Han	2009	54	DTD-RT5.2/DL	DL	2018
12	P789NH	HN	2010	55	DTD-RT5/DL	DL	2018
13	P902NH	HN	2010	56	DTD-T/ND	BTh	2018
14	638-HY	HY	2010	57	DTD-The/ND		2018
15	HD1-9	HD	2011	58	DTD-Tiep/QN	QNg	2018
16	HB1-4	HB	2011	59	DTD-Tuan/ND		2018
17	HB1-6	HB	2011	60	DTD-Tuyen/LD	LD	2018
18	BG0-1	BG	2011	61	DTD-03PG/BD	BD	2019
19	Han13-4	Han	2011	62	DTD-07PG/BD	BD	2019
20	Han6-12	Han	2011	63	DTD-09PG/BD	BD	2019
21	TN1-1	TN	2011	64	DTD-10PG/BD	BD	2019
22	TN4-1	TN	2011	65	DTD-118/HCM	HCM	2019
23	TN4-M1	TN	2011	66	DTD-11PG/BD	BD	2019
24	TN5-3	TN	2011	67	DTD-125/HCM	HCM	2019
25	HD1-1	HD	2011	68	DTD-C2/DN	DN	2019
26	Han0-4	Han	2011	69	DTD-C3/DN	DN	2019

No.	Strain (1)	Location (2)	Year (3)	No.	Strain	Location	Year
27	HB1-1	HB	2011	70	DTD-Cao/ND		2019
28	Han14	Han	2011	71	DTD-CH4/BT	BTh	2019
29	HB2	HB	2011	72	DTD-Cong/ND		2019
30	178-HnDfu	Han	2011	73	DTD-CS7W/VP	VP	2019
31	002-Hna	HN	2011	74	DTD-HMT/DL	DL	2019
32	BG58	BG	2012	75	DTD-N1/ND		2019
33	PT6	PT	2013	76	DTD-N3/ND		2019
34	NN4	Han	2013	77	DTD-NA4/DN	DN	2019
35	M2		2014	78	DTD-NB3/DN	DN	2019
36	Hau7	Han	2014	79	DTD-NC2/DN	DN	2019
37	Hau8	Han	2014	80	DTD-Phuc/HCM	HCM	2019
38	Hau9	Han	2014	81	DTD-Phuoc1/ND		2019
39	Hau2	Han	2014	82	DTD-SP1/Bdi	BDi	2019
40	Hau3	Han	2014	83	DTD-SP2/Bdi	BDi	2019
41	Hau5	Han	2014	84	DTD-Trong/ND		2019
42	M1		2014	85	DTD-Vinh/DN	DN	2019
43	Hau6	Han	2014	86	PCV2/G221	TB	2018
				87	PCV2/G403	PT	2018

Note: (**1**) The strains in bold are the strains collected for this study. (**2**) Yen Bai (YB), Hoa Binh (HB), Thai Nguyen (TNg), Phu Tho (PT), Bac Giang (BG), Bac Ninh (BN), Ha Nam (HN), Ha Noi (Han), Hai Duong (HD), Hung Yen (HY), Ninh Binh (NB), Thai Binh (TB), Vinh Phuc (VP), Thanh Hoa (TH), Quang Ngai (QNg), Binh Dinh (BDi), Binh Thuan (BTh), Gia Lai (GL), Dak Lak (DL), Lam Dong (LD), Binh Phuoc (BP), Binh Duong (BD), Dong Nai (DN), Ho Chi Minh (HCM), Tien Giang (TG), and Bac Lieu (BL). (**3**) For the period from 2005 to 2008, the sample sizes are not shown due to the number of retrospective samples.

Phylogenetic analysis

The sequences for phylogenetic analysis were divided into four datasets: 42 PCV2 strains from this study (2018-2019), 43 PCV2 strains (2004-2017), 2 PCV2 strains (2018) from a previous study in Viet Nam, and 42 PCV2 reference strains from other countries (**Table S1**). Details of the 87 PCV2 strains from Viet Nam are provided in **Table 1**. The reference strains included PCV2a, PCV2b, PVC2c, PVC2d, PCV2e, PCV2f, PVC2g, and PVC2h. Reference sequences were selected based on a megaBLAST search with more than 99% identity to the analyzed strings.

All 129 sequences used in the study were aligned with ClustalW and edited via the Molecular Evolutionary Genetics Analysis (MEGA) X tool (10.2.4). The phylogenetic tree was subsequently constructed with the MEGA X tool based on the ORF2 sequence using neighbor-joining (NJ) trees (classified according to evolutionary distance) and maximum composite likelihood (ML) methods (scores of likelihoods in classification). PCV1 was selected as an outgroup element. The implemented bootstrap value was 1000. With the NJ method, a phylogenetic tree was built based on the Kimura 2-parameter model. Like in the NJ method, in the ML method, the uniformity rate among sites was determined. In addition, the maximum clade credibility (MCC) tree (representing the time scale phylogeny) was also built through BEAST (v1.10.4) software and TreeAnnotator (v1.10.4) and then visualized with FigTree (v1.4.4). The BEAST parameters used were established according to previous methods https://beast.community/first_tutorial. The distribution map of Viet Nam was drawn using ArcMap software 10.8.

Positive selection analysis

After phylogenetic analysis, the sequences of the PCV2b and PCV2d strains were further analyzed via selective pressure analysis. Sequences with a length of 624 nucleotides (Cap protein coding region - ORF2) were uploaded to Data Monkey (www.datamonkey.org), analyzed and selected based on p value calculations[46]. To estimate the sites under selection, four algorithms were used, including single-likelihood ancestor counting (SLAC), mixed effects model of evolution (MEME), fixed effects likelihood (FEL), and fast unconstrained Bayesian approximation (FUBAR)[46–48]. A position was potentially positively selected if it satisfied one of four algorithms with $p < 0.1$ in SLAC, $p < 0.05$ in FEL and MEME, and $p > 0.9$ in FUBAR; otherwise, it was considered negative selection. Finally, the cap sequences of the

strains PCV2b and PCV2d were used to predict the 3D protein structure submitted to TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) and visualized with PyMOL software (South San Francisco, CA, USA).

RESULTS

In this study, 3 reliable tree generation methods, namely, NJ, ML, and MCC, were used to construct a phylogenetic tree based on 129 ORF2 sequences of PCV2. Using the NJ and ML tree methods, the data showed that PCV2 strains collected from pig farms in Viet Nam exhibited various genotypes (PCV2b, PCV2d, PCV2e, PCV2h and PCV2g) (**Figure 2. A and B**). None of the PCV2 strains were clustered into PCV2a, PCV2c, or PCV2f. The most prevalent PCV2 strain was PCV2d (54.02%), the second was PCV2h (25.29%), and the third was PCV2b (16.09%). The phylogenetic tree constructed by the MCC method also produced similar results (**Figure 3**). Interestingly, the results of classifying the sequences on the phylogenetic tree were the same despite the use of different algorithms to generate the phylogenetic tree.

To determine the optimal use of these vaccines based on the distribution of PCV2 strains in Viet Nam in each province at a given time, we analyzed 87 ORF2 sequences of the PCV2 strains collected in Viet Nam from 2004 to 2019 spanning more than 26 provinces (**Figure 4** and **Table 2**). The epidemiological results showed that PCV2h and PCV2b occurred more frequently in the northern provinces during the period from 2004 to 2013. In the central and southern provinces, PCV2h strains were dominant in the early years, while PCV2b and PCV2d were dominant beginning in 2018. The results also showed that two and especially three genotypes appeared simultaneously in the same province at a specific time—for example, PCV2h and PCV2b, PCV2h and PCV2e, and PCV2h and PCV2d—for coexpression and that PCV2h, PCV2e and PCV2b were coexpressed for three-gene expression (**Table 2**).

In general, our phylogenetic analysis showed that PCV2d, PCV2h and PCV2b were the most prevalent strains from 2004 to 2019. However, after 2013, PCV2h was no longer present in the livestock samples (**Table 2**) and was replaced by the PCV2d or/and PCV2b strains from north to south (2014-2019). Through phylogenetic tree analysis, 61/87 PCV2 strains were identified as PCV2b and PCV2d, and they were included in the selection analysis. **Table 3** shows that the MEME algorithm ($p < 0.05$) selected sites 59 ($p = 0.02$) and 68 ($p = 0.04$) as positive selection sites based on the p value. Similarly, the
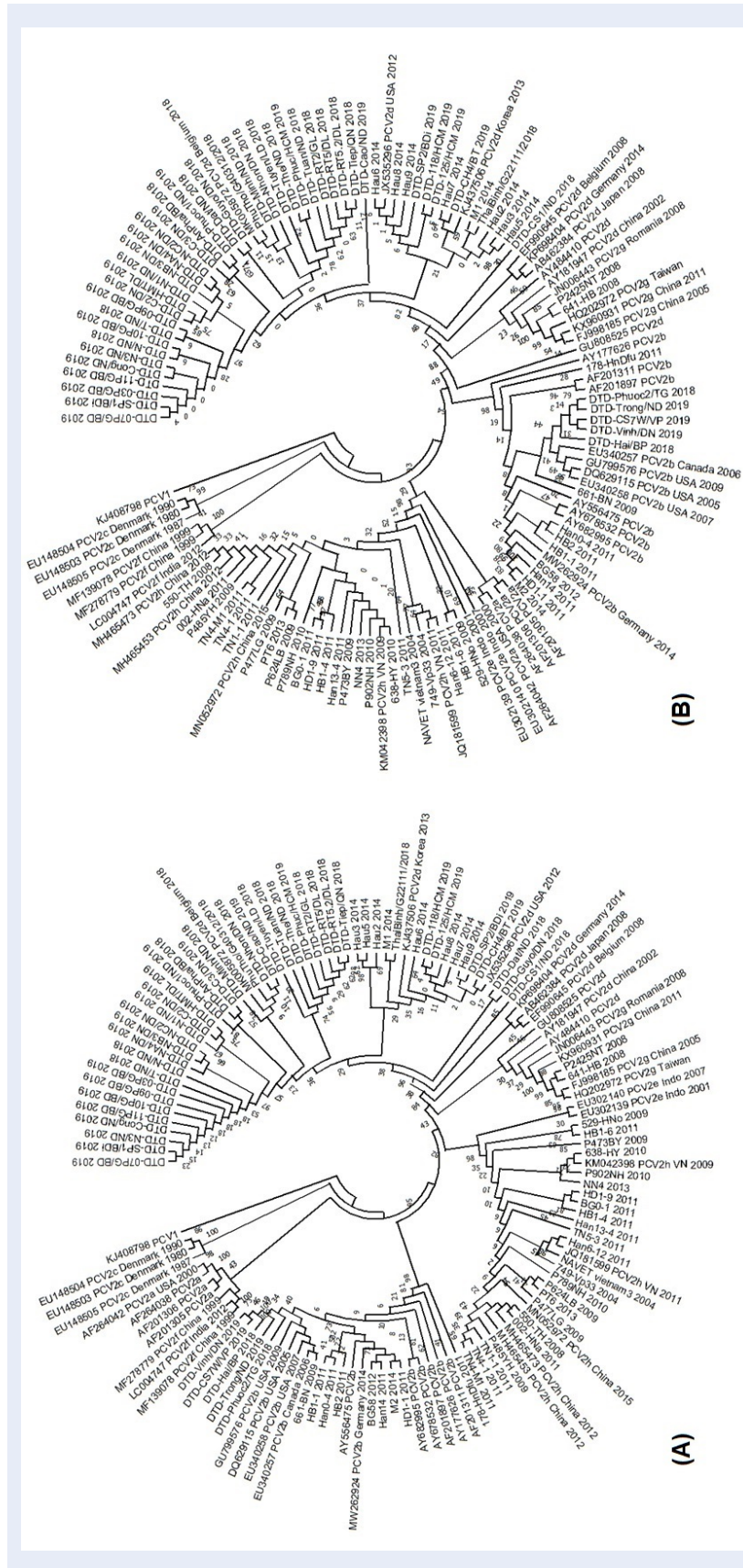
Figure 2: **(A) An NJ phylogenetic tree was built with the MEGA X tool using 87 ORF2 PCV2 sequences.** PCV2 strains were identified based on reference sequences. The implemented bootstrap value is 1000; **(B) An ML phylogenetic tree was built with the MEGA X tool using 87 ORF2 PCV2 sequences.** PCV2 strains were identified based on reference sequences. The implemented bootstrap value is 1000.
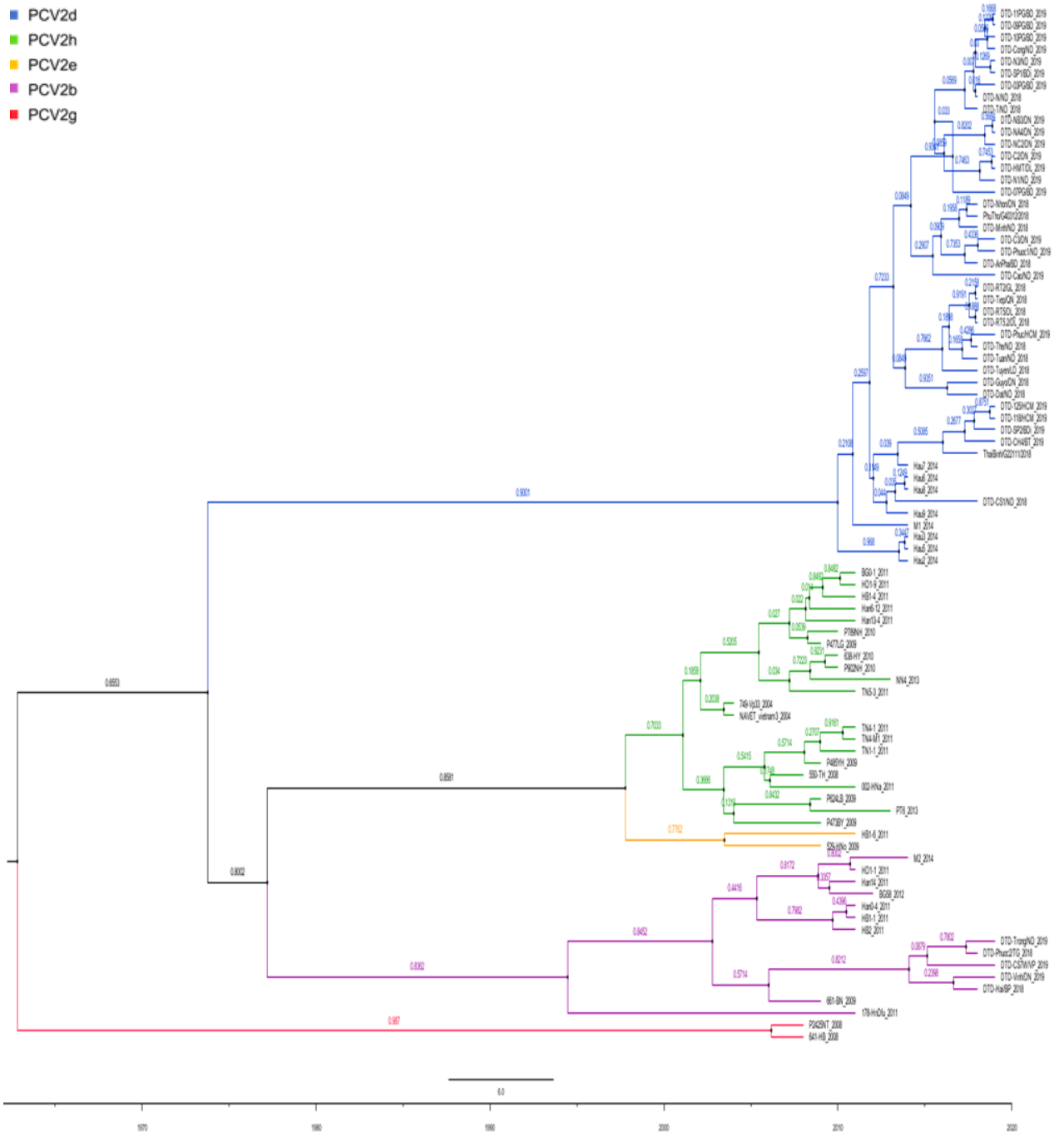
Figure 3: **MCC tree constructed using BEAST (v1.8.4) from 87 Vietnamese PCV2 isolates**. The PCV2 sequences were included for classification over time. The posterior region was displayed along each branch. The different strains are represented by different colors, as displayed in the figures. The scale bar represents time (in years).
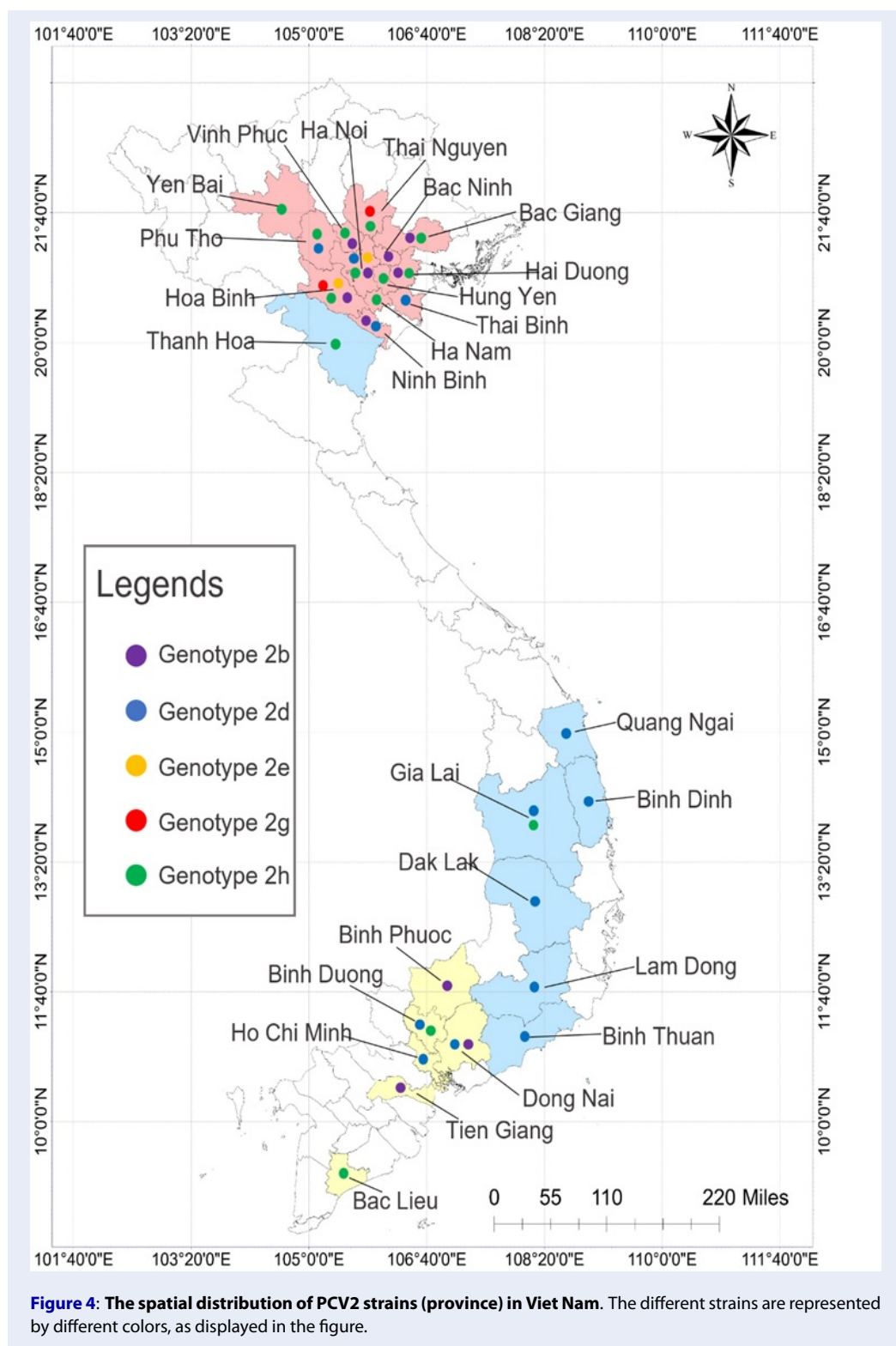
Figure 4: **The spatial distribution of PCV2 strains (province) in Viet Nam**. The different strains are represented by different colors, as displayed in the figure.

Table 2: Summary of the spatial genotype information for 87 ORF2 PCV2 sequences subjected to epidemiological analysis (the sequences are colored North, Central, and South) over time

Year/Loc	2004	2008	2009	2010	2011	2012	2013	2014	2018	2019
YB			2 h							
HB		2 g			2 h - 2e - 2b					
TNg		2 g			2 h					
PT							2 h		2d	
BG					2 h	2b				
BN			2b							
HN				2 h	2 h					
Han			2e		2 h - 2b		2 h	2d		
HD					2 h - 2b					
HY			2 h	2 h						
NB								2b-2d		
TB									2d	
VP	2 h									2b
TH		2 h								
QNg									2d	
BDi										2d
BTh									2d	2d
GL			2 h						2d	
DL									2d	2d
LD									2d	
BP									2b	
BD	2 h								2d	2d
DN									2d	2b-2d
HCM										2d
TG									2b	
BL			2 h							

Table 3: Selection analysis of PCV2 ORF2 (cap) sequences from PCV2b and PCV2d

Site	FEL (0 positive site)		(2 positive sites) MEME		(0 positive site) SLAC		(1 positive site) FUBAR	
	dN-dS (a=b)	P<0.05	W+ (b+)	P<0.05	dN-dS	P<0.1	dN-dS (b-a)	Post.pro>0.9 (pro a<b)
59	1.619	0.98	87.52	0.02	0.131	0.448	0.153	0.563
63	1.955	0.22	2.49	0.24	6.78	1.00	5.092	0.908
68	1.736	0.665	18.86	0.04	2.54	0.62	2.594	0.751

(The bold line represents p < 0.05 and p and posterior probability > 0.9, indicating a significant difference).

FUBAR algorithm (post.pro > 0.9) identified 63 sites (post.pro = 0.908). Position 63 was common with respect to the amino acid ARG, while the other 2 positions were different. Position 59 of PCV2b was an ARG, while PCV2d was an LYS. In PCV2b, position 68 was ALA, but it was ASN in PCV2d.

DISCUSSION

Phylogenetic tree analyses (NJ, ML, and MCC) were conducted based on the obtained ORF2 sequences. The PCV2 strains identified in Viet Nam included PCV2b, PCV2d, PCV2e, PCV2h, and PCV2g. PCV2d was the most prevalent strain, consistent with the findings of previous studies[49,50]. It appears that PCV2d is increasingly dominant in causing disease in Vietnamese individuals and worldwide swine[51], consistent with what was shown in this study. Similarly, in another study in Viet Nam, Nguyen *et al.* collected samples and clearly identified 8/13 as the PCV2d strain in southern Viet Nam[52]. The second most common genotype was PCV2h (25.29%), and the third was PCV2b (16.09%). However, the PCV2h genotype was not considered and was included in the follow-up analysis (positive model selection) because it ceased to occur after 2013 based on the results of the phylogenetic tree analysis and the records. Therefore, the genotypes PCV2b and PCV2d were the focus of this study. In comparison, with the findings of previous studies, three new strains were discovered in our study (i.e., PCV2h, PCV2g, and PCV2e), which were previously identified as PCV2b and PCV2d strains[49,50,52]. However, further studies with larger sample sizes are needed to confirm these findings, considering the influence of the analysis method and reference strains on the overall conclusion.

PCV2 has been recognized as the primary cause of swine diseases worldwide since the late 1990s[32]. Genotypes such as PCV2a, PCV2b, and PCV2c were classified based on nucleotide variations in ORF2[34]. PCV2b, present in Europe and Asia since 1997[36] and in North America since 2005[40], is one of the known genotypes. PCV2c was identified in Denmark in 1980[36]. PCV2d and PCV2e, two new genotypes, were reported in China in 2009[53]. PCV2d likely emerges as a mutation of PCV2b[33,43,54]. Mutant PCV2b (mPCV2b) strains were also detected in North and South America in 2012[39,55]. Pham *et al.* reported that PCV2d strains in Viet Nam originated from China[50]. PCV2d represents a notable example of a genotype substitution, such as when PCV2b evolves into PCV2d, possibly driven by natural selection or vaccine-related factors.

This study investigated the molecular epidemiology of PCV2 strains based on geography and time, highlighting the diversity and dominance of the strains. Geographically, PCV2h, PCV2b, and PCV2d are distributed across provinces in Viet Nam, with PCV2d being the most prevalent. PCV2b and PCV2d strains were recently identified. The next section will focus on analyzing the positive positions of these two genotypes. The cooccurrence of PCV2b and PCV2d in the same locality indicates variability and potential vaccine inefficiencies. Attention should be given to this coinfection for the development of an effective vaccine in Viet Nam. Furthermore, strict management methods are necessary to prevent interlocality infections and reduce the diversity of PCV2 genotypes causing diseases.

Selection analysis revealed that three amino acid sites could be considered potential immune system components, indicating that these sites are markers of positive selection during the specific evolution of PCV2b and PCV2d[11,56–60]. In other words, these amino acid sites could shift PCV2b to PCV2d. These results indicate that the antigenicity of PCV2b differs from that of PCV2d. Codon sites with potential for positive selection are shown on the basis of the conformational prediction of the PCV2 cap protein (**Figure 5**). These results and phylogenetic analysis might provide insight into the genotypic classification of PCV2.

Selected positions relevant to functional activities, including epitopes in the capsid protein, were identified in previous research. For example, codon 59 is crucial for conformational neutralizing epitopes, and changes in amino acids at this position can strongly affect the immunogenicity of a PCV2 genotype[56]. Residue region 165-200 interacts with residues 58-63 to form conformational neutralizing epitopes[57]. Codons 63 and 68 are immune-related epitopes[58–60] potentially involved in evading the host immune system. Understanding these positive selections helps predict virulence changes and aids in PCV2 research. The 3D structure of the capsid protein is also important because it reveals the impact of amino acids on virion expression and the environment[61]. The capsid protein can mutate to evade the host immune system or enhance binding to receptors[62].

Various PCV2 variants have been identified in Viet Nam, posing challenges for the development of an effective PCV2 vaccine. Inactivated vaccines, including those targeting the PCV2a and PCV2b genotypes, have shown promising results in improving pig weight and reducing mortality[63,64]. Recombinant vaccines, such as Suvaxyn PCV2® One doseTM, have been developed by integrating PCV2a ORF2 into PCV1[65].
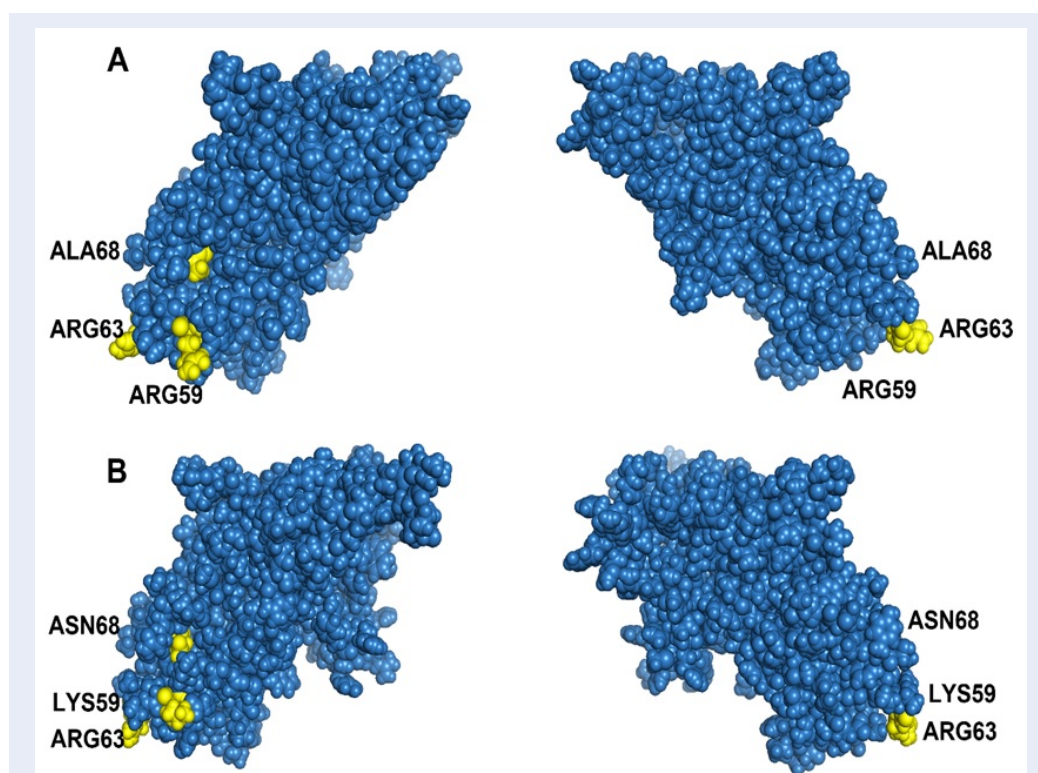
Figure 5: **The structure of the PCV2 Cap protein was predicted by using I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/).** The green filled circles indicate the epitopes of the PCV2 cap protein, and the yellow filled circles indicate the amino acids selected based on positive selection. (**A**) PCV2b and (**B**) PCV2d.

However, the detection of recombinant viruses in pig herds led to the discontinuation of these vaccines, indicating incomplete virus inactivation. Recombinant DNA technology has allowed the production of PCV2 Cap protein-expressing vaccines, such as those based on the PCV2a and PCV2b genotypes[66–68]. These vaccines have demonstrated effectiveness in pigs. Currently, imported commercial vaccines circulating in Viet Nam primarily target the PCV2a genotype[37]. Vaccines such as Cirovac, circo pigvac, and Fostera PCV2 conjugate, which are used in Viet Nam, are also based on the PCV2a genotype[69].

Effective vaccines for PCV2 should consider the prevailing strains in Viet Nam, particularly PCV2b and PCV2d[49,50]. Similarities between the vaccine strain and local strains are crucial for vaccine efficacy. The presence of PCV2, especially PCV2d, poses significant risks to Viet Nam's economy and global health. Therefore, the development of dedicated vaccines targeting novel PCV2 subtypes is essential for timely prevention and mitigation of the severe consequences associated with PCV2.

CONCLUSIONS

This study identified diverse PCV2 genotypes in Viet Nam, including PCV2b, PCV2d, PCV2e, PCV2g, and PCV2h. PCV2d was the most common genotype (47/87). The coexistence of multiple genotypes at the same location suggests potential challenges in vaccine effectiveness. Phylogenetic analysis revealed that the PCV2g, PCV2h, and PCV2e genotypes form distinct branches. These findings provide valuable insights into the distribution and diversity of PCV2 strains in Viet Nam. These findings can guide the optimal allocation of vaccine resources and the development of region-specific vaccines in Viet Nam.

ABBREVIATIONS

AA: Amino acid
bp: Base pair
Cap: Capsid
DNA: Deoxyribonucleic acid
MCC: Maximum clade credibility
ML: Maximum likelihood
NJ: Neighbor-joining trees
nt: Nucleotide

ORF: Open reading frame

PCR: Polymerase chain reaction

PCV2: Porcine circovirus type 2

PMWS: Postweaning multisystemic wasting syndrome

PRCD: Porcine respiratory disease complex

Rep: Replicase

RNA: Ribonucleic acid

AUTHOR CONTRIBUTIONS

MPNN, MNN, and DTD designed the study. MPNN, MNN, and DTD performed the experiments. MPNN, MNN, TTN and THN analyzed the data. MPNN, TNL, and MNN wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

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

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