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Original Research Article

THE ORF5 VARIATION OF VIETNAMESE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS

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Abstract: Porcine reproductive and respiratory syndrome is a devastating disease that causes heavy losses to the economy and the development of agriculture. In this study, we aimed to assess the genetic variation of the ORF5 gene from 12 Vietnamese porcine reproductive and respiratory syndrome virus (PRRSV) strains. The phylogenetic analysis of the ORF5 sequences of Vietnamese strains and other strains indicated that the Vietnamese strains belong to type II. The Vietnamese strains were also separated into two clusters. Five strains BG/12, TG1/12, TG2/12, TG3/12, and TG4/12 were grouped in cluster 1 with a 98% bootstrap value, while the other seven strains HCM/14, TG5/15, TG6/15, TG7/15, ST1/15, ST2/15, and ST3/15 belonged to cluster 2. The alignment of the deduced amino acid sequences demonstrated that the identity between Vietnamese strains with CH-1a, JXA1, and VR2332 strains were 87–93%, 91–98%, and 83–89%, respectively. The mutation of the N21 glycosylation site (N□S) of the GP5 sequence was observed in five Vietnamese strains from cluster 1. The core sequence of the neutralizing epitopes (including five positions at H25, Q27, I29, Y30, and N31) in GP5 was presented in all Vietnamese strains except strain TG1/12. The hydrophobicity plots of GP5 revealed two different positions of BG/12 strain from CH-1a strain and VR2332 strain. The first difference was the missing of a hydrophilic peak from position amino acid 85 to 95. In this region, the CH-1a and VR2332 strains have 3 hydrophilic peaks. The second difference was the loss of another hydrophilic peak at position amino acid 100.

Key words: genotype; ORF5; phylogenetics; PRRSV; Vietnam

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most dangerous pathogens for the swine industry (1). PRRSV is a member of the *Arteriviridae* family that comprises equine arteritis virus (EAV), simian haemorrhagic fever virus (SHFV), and lactate dehydrogenase-elevating virus (LDV) (2). Genomic sequence comparisons have revealed that PRRSV includes two genotypes: type I and type II (3). These two genotypes share only approximately 60% sequence identity (4). PRRSV is a spherical, enveloped virus containing a single, positive-sense RNA genome. The PRRSV genome is approximately 15 kb in length and contains nine open reading frames (ORFs), ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF6, and ORF7. ORF1a, and ORF1b (~12 kb) encode 12 non-structural proteins (nsp), nsp1–nsp12, which play major roles in viral replication (5). The remaining ORFs encode structural proteins (6).

In Vietnam, the first cases of PRRS were recorded in 1997 (7). Since then, PRRS has quickly spread and seriously affected almost all

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provinces. The PRRS outbreaks have significant economic impacts on the swine industry of Vietnam in 2008, 2010, and 2012. Highly pathogenic PRRSV is the causative agent of porcine high fever syndrome and characterized by high fever and high death rates in pigs of all ages (8). Furthermore, the coding region for ORF5 of PRRSV displays substantial genetic variation (9). Thus, ORF5 has become the regions of choice for monitoring the evolution of PRRSV and for molecular epidemiology research on PRRSV (10). Glycoprotein 5 (GP5) is an envelope glycoprotein of porcine reproductive and respiratory syndrome virus (8). The N-linked glycosylation in GP5 may be associated with the antigenicity of the neutralization epitopes located in the ectodomain (15). GP5 acts as a major inducer of neutralizing antibodies in vivo, containing three putative N-linked glycosylation sites (N34, N44, and N51), where a major neutralization epitope is located (15). The major neutralization epitope of PRRSV is located in the middle of the GP5 ectodomain (aa 36-52) (16). This report describes the investigation of genetic variation of ORF5 gene of Vietnamese PRRSV strains. The phylogenetic analysis was assessed to determine the genotype of Vietnamese PRRSV strains.

Materials and methods

Sample collection

The blood samples (n=12) were collected from the PRRSV-infected pigs with the clinical displaying of PRRS from 2012 to 2015 (Figure 1). All samples were stored in ice boxes and transported to the laboratory. Subsequently, the samples were kept at -80 ºC.

Figure 1: Sample collecting locations: 1: Bac Giang Province (1 sample), 2: Tien Giang Province (7 samples), 3: Ho Chi Minh City (1 sample), 4: Soc Trang Province (3 samples)

RNA isolation and RT-PCR

Total RNA was extracted using Rneasy Mini Kit (74104, Qiagen) according to the manufacturer's instructions. The RT-PCR reaction was carried out with a 1-Step RT-PCR Kit (PB10.52-05, PCR Biosystems) in a total volume of 50 µl containing 25 µl 2x PCRBIO One-Step Mix, 2 µl primers (400 nM), 2.5 µl 20x RTase, 2.5 µl RNA template (1 ng), 18 µl RNase-free H_2O. The RT-PCR was performed in a thermal cycle under the following conditions: the reverse transcription was performed at 42 ºC for 45 min; an initial denaturation at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 45 s, annealing at 58 °C for 45 s, and elongation at 72 °C for 90 s; and the final cycle at 72 °C for 10 min. The primers specific for amplification of the ORF5 gene are ORF5-F: 5'-CAT GAG GTG GGC AAC TGT TT-3' and ORF5-R: 5'-GTC ATG TAC CCG AAG GTG AA-3' (13). Amplified products of ORF5 genes were estimated as 800 bp.

Table 1: PRRS strains

No.	Strain	Location - year	Reference	Type	Subtype
1	01UD6	Thailand - 2003	AY297113	Type 2	
2	02PB1	Thailand - 2003	AY297116		
3	FJ-1	China - 2005	AY881994		
4	GDCZ2	China - 2004	AY857636		
5	Ingelvac	USA - 2004	AY656991		
6	Jis2	Japan - 2004	AB175695		
7	Gu922M	Japan - 2004	AB175721		
8	Jeh1	Japan - 2004	AB175691		
9	CH-1a	China - 2001	AY032626		
10	CH-1R	China - 2008	EU807840		
11	JXA-1	China - 2006	EF112445		
12	VR2332	USA - 2007	EF536003		
13	01CB1	Thailand - 2003	AY297119	Type 1	Subtype 1
14	03RB1	Thailand - 2003	AY297124		
15	361-4	Denmark - 2001	AY035915		
16	Upa-13	Poland - 2005	DQ324688		
17	Amervac	Spain - 2005	DQ324668		
18	Porcillis	Netherlands - 2005	DQ324678		
19	2567/96	Denmark - 2001	AY035932		
20	Bor-41	Belarus - 2005	DQ324671		Subtype 2
21	Bor-54	Belarus - 2005	DQ324672		
22	Sid	Lithuania - 2005	DQ324682		
23	Aus	Lithuania - 2005	DQ324667		
24	Sno-4	Belarus - 2005	DQ324683		
25	Zap-46-50	Belarus - 2005	DQ324697		Subtype 3
26	Soz-6	Belarus - 2007	DQ324686		
27	Soz-8	Belarus - 2005	DQ324687		
28	Zad-1	Belarus - 2007	DQ324694		
29	Zad-14	Belarus - 2005	DQ324695		
30	Yuz-34	Belarus - 2005	DQ324692		
31	Bel-42	Belarus - 2007	DQ324669		Subtype 3/2
32	Bel-43	Belarus - 2005	DQ324670		
33	Okt-35	Belarus - 2005	DQ324677		Subtype 4
34	BG/12	Vietnam - 2012	KY310596	This study (Type 2)	
35	TG1/12	Vietnam - 2012	KY310597		
36	TG2/12	Vietnam - 2012	KY310598		
37	TG3/12	Vietnam - 2012	KY310599		
38	TG4/12	Vietnam - 2012	KY310600		
39	HCM/14	Vietnam - 2014	KY310601		
40	TG5/15	Vietnam - 2015	KY310602		
41	TG6/15	Vietnam - 2015	KY310603		
42	TG7/15	Vietnam - 2015	KY310604		
43	ST1/15	Vietnam - 2015	KY310605		
44	ST2/15	Vietnam - 2015	KY310606		
45	ST3/15	Vietnam - 2015	KY310607		

Table 2: Matrix of Tamura & Nei genetic distance among PRRSV strains using deduced amino acid sequence of GP5 protein. Lower triangular matrix values were mean genetic distances; upper triangular matrix values were standard errors

		Type 1					Type 2	Vietnam
		Subtype_1	Subtype_2	Subtype_3	Subtype_3/2	Subtype_4		
Type 1	Subtype_1		0,020	0,016	0,016	0,019	0,035	0,036
	Subtype_2	0,256		0,020	0,021	0,022	0,034	0,035
	Subtype_3	0,198	0,252		0,011	0,019	0,036	0,038
	Subtype_3/2	0,167	0,239	0,107		0,022	0,037	0,039
	Subtype_4	0,212	0,254	0,199	0,199		0,040	0,044
Type 2		0,513	0,510	0,522	0,499	0,535		0,010
Vietnam		0,496	0,490	0,513	0,477	0,547	0,114	

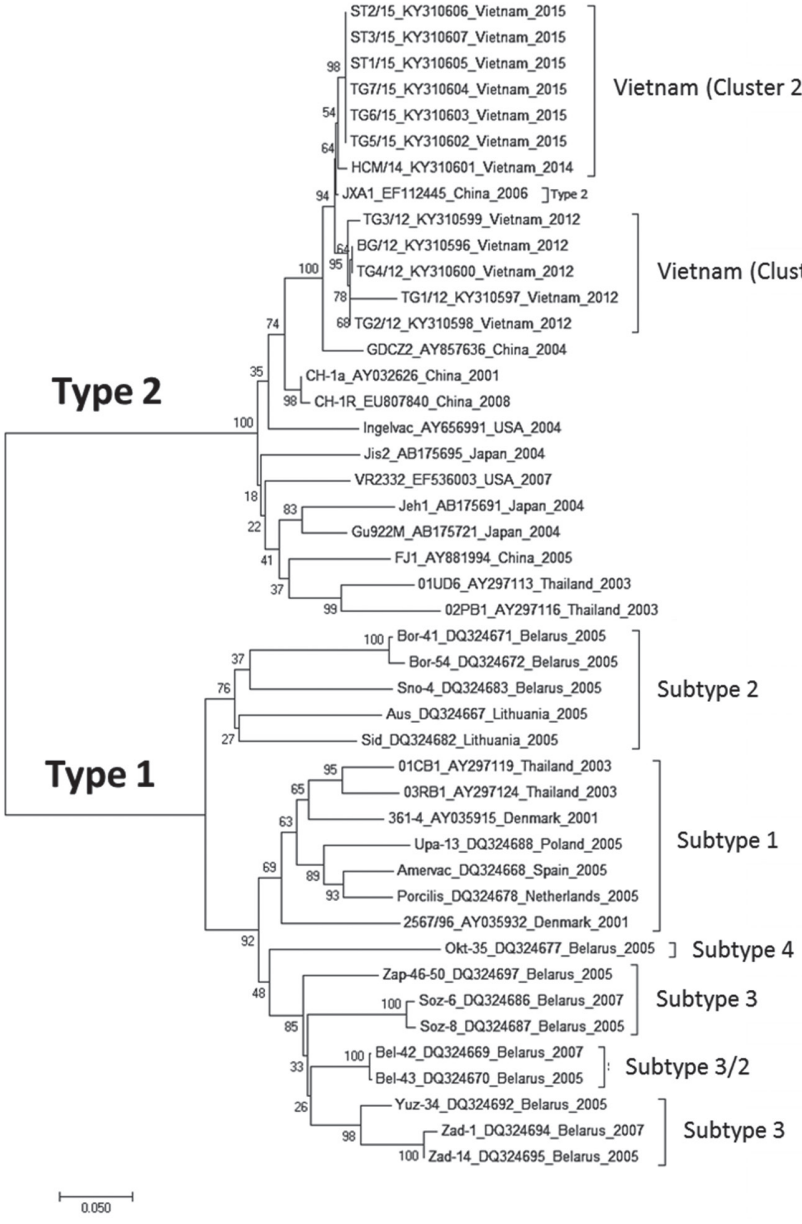

Figure 2: Phylogenetic tree constructed from ORF5 sequences of PRRSV strains using the neighbour-joining analysis method. Bootstrap resampling was done 1000 times, and the resulting bootstrap values are shown on the corresponding branches

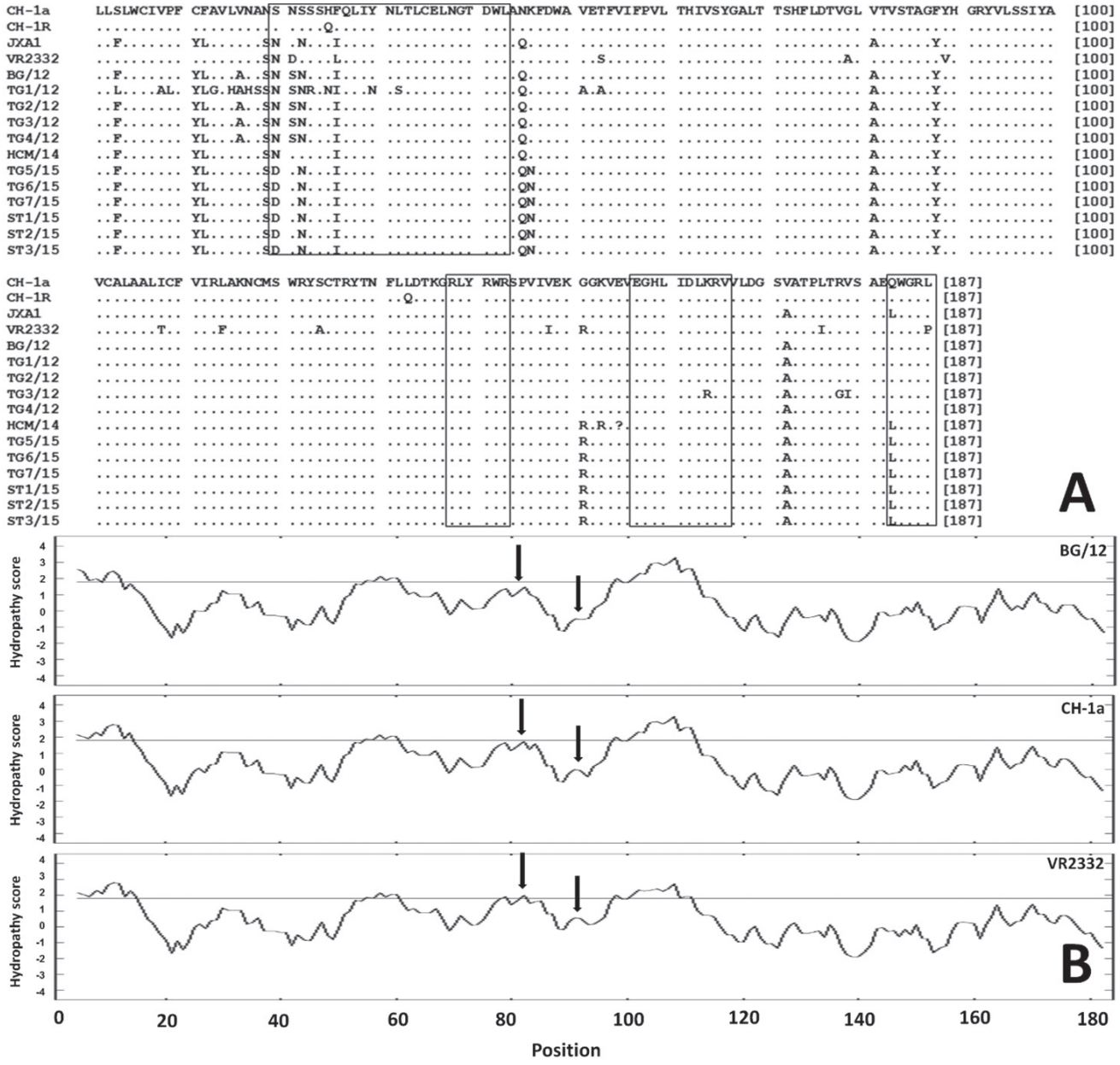

Figure 3: The variable positions of deduced amino acid sequences from PRRSV strains. A. Alignment of the deduced amino acid sequences of glycoprotein GP5 of 12 Vietnamese strains in comparison with strains CH-1a and VR2332. Dots indicate identical amino acids, and deletions are indicated by dashes (–). Black box indicates epitope. B. Hydrophobicity plots of ORF5 generated using the Kyte and Doolittle method. Major areas of difference are indicated by black arrows

Sequence analysis

RT-PCR products were purified and used as sequencing templates. The nucleotide sequences were directly sequenced (Macrogen, Seoul, Korea). The sequence trimming was used to remove misleading data from the ends of sequencing fragments. After trimming, the size of the ORF5 sequences was 561 bp (deduced amino acid sequences are 187 aa). The comparisons of the ORF5 sequences were analysed for 12 Vietnamese PRRSV strains and other strains from Genbank. The sequences alignment was performed with CLUSTAL W (14). The Tamura & Nei model was used as a genetic distance model. A neighbour-joining method was applied for phylogenetic construction (15). Bootstrap analysis (using 1000 replications) was used to assess the confidence in

branching order. The DNA sequences of ORF5 were translated into amino acid sequences to investigate genetic variation in the amino acid level. The amino acid sequences of CH-1a, CH-1R, JXA1, and VR2332 were used as reference PRRSV strains. The hydrophobicity plots of GP5 were generated using the Kyte and Doolittle method (16).

Results

The alignment of deduced amino acid sequences indicated that the identity between Vietnamese strains with CH-1a, JXA1, and VR2332 strains were 87–93%, 91–98%, and 83–89%, respectively. The genetic distance between Vietnamese strains with type II strains (0.114) was lower than type I strains (0.477 - 0.547) (Table 2).

A phylogenetic tree was constructed based on the nucleotide sequences from the ORF5 region of 12 Vietnamese strains and other type I and type II strains. All Vietnamese PRRSV strains were located in the type II group. These strains were also separated into two clusters. Cluster 1 included strains BG/12, TG1/12, TG2/12, TG3/12, and TG4/12 with 98% bootstrap value, the other strains HCM/12, TG5/15, TG6/15, TG7/15, ST1/15, ST2/15, and ST3/15 were belonged to cluster 2. The genetic divergence between Vietnamese strain cluster 1 and Vietnamese strain cluster 2 is 0.03±0.005.

In this study, two N-linked glycosylation sites (N31 and N38) were conserved in all Vietnamese PRRSV strains (Figure 3). The mutation of the N21 glycosylation site (N→S) was observed in all Vietnamese strains of cluster 1. The core sequence of the neutralizing epitopes (H25, Q27, I29, Y30 and N31) of GP5 was presented in Vietnamese strains except the TG1/12 strain. This strain revealed two mutations at position 25 (H→N) and position 30 (Y → N). The C terminus of GP5 protein contains three minimal epitopes including RLYRWR (aa 138 → aa 143), EGHLIDLKRV (aa 157 → aa 166), and QWGRL (aa 183 → aa 187) (17). These regions are highly conserved in Vietnamese strains. The mutation at position 183 (Q→L) occurred in all Vietnamese strains from cluster 2.

The Figure 3 demonstrated the GP5 hydrophobicity plots of strains BG/12, CH-1a, and VR2332. The strain BG/12 had a profile similar to strains CH-1a and VR2332. However, the hydrophobicity plots also revealed two distinct positions of the profile of strain BG/12 from strains CH-1a and VR2332. One of the differences was located between amino acids 75 and 85, where a hydrophilic peak was absent. Another variable area was hydrophilic peak loss at position amino acid 91. Each of two differences resulted from a single amino acid substitution, V to A (amino acid 81) and F to Y (amino acid 88), respectively. There were 2 amino acid substitutions in Vietnamese strains from cluster 1 (V → A at position 16, N → S at position 21). Two other amino acid substitutions were observed in Vietnamese strains from cluster 2 (G → R at position 151, and Q → L at position 183).

Discussion

In this study, the genetic variation of the Vietnamese PRRSV strains using ORF5 sequences were assessed. The phylogenetic analysis indicated that all Vietnamese strains belong to type II. The previous study showed that some Vietnamese PRRSV strains, collected from Northern areas (Ha Noi City, Quang Ninh Province, Nghe An Province) or Southern areas (Dong Nai Province, Tay Ninh Province) belonged to type II (18). Another study demonstrated that the Vietnamese PRRSV strains collected from 2008 to 2012 in Northern areas (Dien Bien Province) and Southern areas (Can Tho City, Dong Thap Province, Dong Nai Province) also belonged to type II (19). These results revealed the large distribution PRRSV strains of type II in Vietnam. The Vietnamese cluster 2 strains showed 4 different variable positions from Vietnamese cluster 1 strains (A → V at position 16, S → N at position 21, G → R at position 151, and L → Q at position 183). The PRRS virus could be transmitted between farms from various areas. The shipment of semen for artificial insemination may be an important mode of transmission of PRRSV between farms (20). Moreover, PRRSV-infected waterfowl carry and shed live infectious virus, implying that PRRSV may travel between farms in animal vectors (21, 22). It also has been suggested that airborne transmission is important for the spread of PRRSV between nearby farms (23). In Vietnam, households accounted for about 90% of pig stocks (24), and pigs were transported by personal vehicles or trucks between different regions and from Northern areas to Southern areas for consumption. Thus, this transport caused the PRRSV transmission between different areas.

Nucleotide sequence analysis revealed 88 to 99% aa identity among strains from the same continent, and only 52 to 55% aa identity between type I and type II strains (25). Vietnam and China are located in the same continent. Thus, most of the amino acid substitutions observed amongst strains are clustered in a hypervariable region (between aa 26 and 39) adjacent to the amino-terminal signal sequence, which also involves N-linked glycosylation sites varying from none to three (25). In this study, five amino acid substitutions were found in the variable region in Vietnamese strains of cluster 1 and CH-1a, CH-1R. One of the above amino acid substitutions is located at position 21 of GP5 protein, which is an N-linked glycosylation site (N → S). These results revealed that the Vietnamese PRRSV strains and Chinese PRRSV strain have a close genetic relationship.

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RAZNOLIKOST GENA ORF5 V SEVIH VIRUSA VIETNAMSKEGA PRAŠIČJEGA RESPIRATORNEGA IN REPRODUKTIVNEGA SINDROMA

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Povzetek: Prašičji respiratorni in reproduktivni sindrom je huda bolezen, ki povzroča velike izgube v gospodarstvu in vpliva na razvoj kmetijstva. V raziskavi smo želeli oceniti gensko raznolikost gena ORF5 v 12 sevih virusa vietnamskega prašičjega respiratornega in reproduktivnega sindroma (PRRSV). Filogenetska analiza baznih zaporedij ORF5 vietnamskih in drugih sevov je pokazala, da vietnamski sevi pripadajo tipu II. Tudi vietnamski sevi so bili ločeni v dve skupini. Pet sevov (BG/12, TG/12, TG2/12, TG3/12 in TG /12) je bilo združenih v skupino 1 z 98-odstotno vrednostjo bootstrap, medtem ko je bilo ostalih sedem sevov (HCM/14, TG5/15, TG6/15, TG7/15, ST1/15, ST2/15 in ST3/15) uvrščenih v drugo skupino. Poravnava zaporedij aminokislin je pokazala, da je podobnost med vietnamskimi sevi in sevi CH-1a 87–93-odstotna, JXA1 91–98-odstotna in VR2332 83–89-odstotna. Pri petih vietnamskih sevih iz skupine 1 smo opazili mutacijo mesta z glikozilacijo N21 (N S) zaporedja GP5. Glavno zaporedje nevtralizacijskih epitopov (vključno s petimi položaji pri H25, Q27, I29, Y30 in N31) v GP5 je bilo opaženo pri vseh vietnamskih sevih, razen v sevu TG1/12. Grafikoni hidrofobnosti GP5 so pokazali dva različna položaja BG/12 iz CH-1a in VR2332 sevov. Prva razlika je bila odsotnost hidrofilnega vrha pri položaju aminokislin 85 do 95. V tem področju so imeli sevi CH-1a in VR2332 tri hidrofilne vrhove. Druga razlika je bila izguba drugega hidrofilnega vrha na položaju aminokisline 100.

Ključne besede: genotip; ORF5; filogenetika; PRRSV; Vietnam