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THE NSP2 VARIATION OF VIETNAMESE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS

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ABSTRACT

The objective of this study is to assess the non-structural protein 2 (NSP2) variation of eight Vietnamese PRRSV strains. The divergent analysis showed that all Vietnamese PRRSV strains were closer to North American genotype (0.081±0.008) than European genotype (0.821±0.054). The deduced amino acid alignment revealed 4 different amino acid substitutions and 37 different amino acid substitutions of Vietnamese PRRSV strains from Henan-1 strain and VR2332 strain, respectively. There were 4 new amino acid substitutions of Vietnamese PRRSV strains at positions 24 (A → V), 63 (V → A), 198 (S → G) and 201 (N → D). The identity of amino acid sequence between Vietnamese PRRSV strains with Henan-1 strain (92-97%) was higher than VR2332 strain (81-84%). Two putative epitopes ^{124}ECVQGCCEHKGLLGSPD^{140} and ^{214}LCQVIEDCCCHQNKTN^{229} were highly conserved in Vietnamese PRRSV strains. The phylogenetic tree demonstrated that all Vietnamese PRRSV strains belonged to North American genotype.

KEY WORDS: Genotype, NSP2, Phylogenetics, PRRSV, Vietnam.

INTRODUCTION

PRRSV is a member of the order Nidovirales, family Arteriviridae, genus Arterivirus (Cavanagh, 1997, Snijder et al., 2005). PRRSV is an enveloped virus composed of a positive-sense, singlestranded RNA genome (Meulenberg et al., 1993). PRRSV genotypes also show considerable genetic variation and contain several phylogenetic clusters. Comparative studies of different European and North American PRRSV isolates showed that the ORF5-encoded glycoprotein is the most heterogeneous structural polypeptide (Suárez et al., 1996). Nucleotide sequence analysis revealed 88 to 99% amino acid (aa) identity among strains from the same continent, and only 52–55% aa identity between North American and European strains (Dea et al., 2000).

The PRRSV genome is approximately 15 kb in length and contains nine open reading frames (ORFs) including ORF1a, 1b, 2a, 2b, 3, 4, 5, 6 and 7 (Stadejek et al., 2002). ORF1a and ORF1ab are translated into two polyproteins, which are cleaved to produce nsp1–nsp8 and nsp9–nsp12, respectively. ORFs 2 to 7 encode viral structural proteins (Bautista et al., 1996, Meulenberg et al., 1995).Non-structural protein 2 (NSP2)was reported to be highly antigenic (de Lima et al., 2006). The nsp2 gene also shows the highest genetic diversity in the viral genome, as well as polymorphisms of considerable sizes. The first cases of PRRS were recorded in Vietnam from 1997 (Nguyen et al., 2015). Since then to present, PRRS has quickly spread and seriously affected on almost provinces of Vietnam.In this study, we aimed to estimate the nsp2 gene variation and genetic relationship between Vietnamese PRRSV strains and other PRRSV strains. The deduced amino acid alignment was analyzed to assess amino acid substitutions and predict the putative linear epitopes of NSP2 from Vietnamese PRRSV strains.

MATERIALS AND METHODS

Sample collection

The blood samples (n=8) were collected from the PRRSV-infected pigs with the clinical displaying of PRRS. All samples were stored in ice boxes and transported to the laboratory. Subsequently, the samples were kept in -80^{o}C. The other PRRSV strains were got from GenBank including European strains and North American strains (Table 1).

Table 1. The PRRSV strains.

No.	Strain	Location - year	Reference	Type
1	CC-1	China-2006	EF153486	North American Genotype
2	GC-2	China-2007	EU255919	
3	GD	China-2008	EU825724	
4	Henan-1	China-2007	EU200962	
5	HM-1	China-2007	EU177102	
6	HQ-5	China-2007	EU255920	
7	HUB2	China-2006	EF112446	
8	HuN	China-2007	EF517962	
9	JSyx	China-2008	EU939312	
10	LMY	South Korea-2006	DQ473474	
11	LN	China-2007	EU109502	
12	SX071226	China-2008	EU595686	
13	VR2332	USA-2007	EF536003	
14	HKEU16	Hong Kong-2007	EU076704	European Genotype
15	Lelystad	Netherlands-2000	M96262	
34	HCM	Vietnam - 2014	This study	
35	TG1	Vietnam - 2015		
36	TG2	Vietnam - 2015		
37	TG3	Vietnam - 2015		
38	TG4	Vietnam - 2015		
39	ST1	Vietnam - 2015		
40	ST2	Vietnam - 2015		
41	ST3	Vietnam - 2015		
42	ST4	Vietnam - 2015		

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 outwith 1-Step RT-PCR Kit (PB10.52-05, PCR Biosystems) in a total volume of 50µl containing 25µl 2x PCRBIO One-Step

Mix, 2µl primers (400 nM), 2.5µl 20x RTase, 2.5µl 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 of the NSP2 gene are NSP2-F: 5'-GGT TTG GCA GTC ATA AGT GGT-3' and NSP2-R: 5'-TCC TCC TCC ATC TGG TCT TTA-3' (Han et al, 2009).

Sequence analysis

The RT-PCR products were purified and used as sequencing templates. The nucleotide sequences were directly sequenced (Macrogen, Seoul, Korea). The comparison NSP2 sequences wereanalyzed for 8 Vietnamese PRRSV strains and other strains from GenBank. The sequences alignment was performed with CLUSTAL W (Tamura, 2011). Tamura & Nei model was used as a genetic distance model.Neighbor-joining method was applied for phylogenetic construction (Saitou and Nei, 1987). Bootstrap analysis (using 1000 replications) was used to assess the confidence in branching order.The DNA sequences of GP5 and NSP2 weretranslated in to amino acid sequences to investigate genetic variation at amino acid level.The amino acid sequences of Henan-1 and VR2332 were used as reference PRRSV strains.

RESULTS AND DISCUSSION

In this study, the alignment of deduced amino acid sequences indicated that the identity between Vietnamese PRRSV strains with Henan-1 and VR2332 strains were 92-97%, 81-84%, respectively. The genetic distance between VietnamesePRRSV strains with North American strains andEuropean strains were 0.081±0.008and 0.821±0.054, respectively (Table 2), suggesting that the Vietnamese PRRSV strains were closer to North American genotype than European genotype. The deduced amino acid sequences of NSP2 from 2 European PRRSV strains, 13 North American PRRSV strains and 8 Vietnamese PRRSV strains were used to build phylogentic tree. The result showed that all Vietnamese PRRSV strains belonged to North American genotype with the 97% bootstrap value (Figure 2).

NSP2 protein has 5 putative linear B epitopes including ^{3}RALSARETRQA^{13} (3-13), epitope ^{14}KKHEG^{18} (14-18), epitope ^{19}ADANKAEHLKR^{29} (19-29), epitope ^{124}ECVQGCCEHKGLLGSPD^{140} (124-140), and epitope ^{214}LCQVIEDCCCHQNKTN^{229} (214-

229) (Wang et al., 2010). Two putative epitopes ^{124}ECVQGCCEHKGLLGSPD^{140} and ^{214}LCQVIEDCCCHQNKTN^{229} were highly conserved in Vietnamese PRRSV strains. Four substituion mutations were found in Vietnamese strains at positions 24 (A → V), 63 (V → A), 198 (S → G) and 201 (N → D) (Figure 1). The mutation at position 24 (A → V) located in the ^{19}ADANKAEHLKR^{29} is the highest frequence of immunogenic epitopes (Chengmin Wang, 2010).

Table 2. Matrix of Tamura & Nei genetic distance among PRRSV strains using deduced amino acid sequence of NSP2 protein. Lower triangular matrix values were mean genetic distances, Upper triangular matrix values were standard errors.

	European Group	North American Group	Vietnamese Group
European Group		0.051	0.054
North American Group	0.808		0.008
Vietnamese Group	0.821	0.081	

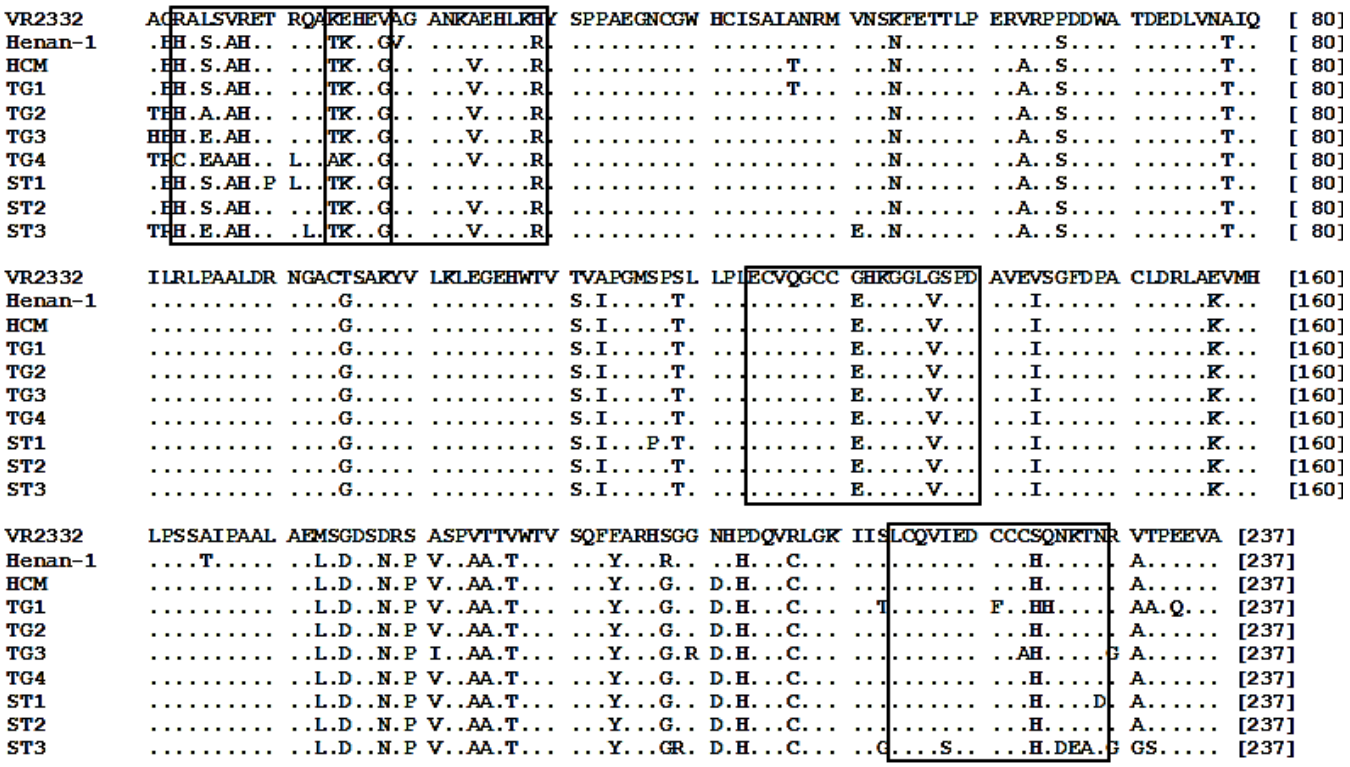

Figure 1. Alignment of the deduced amino acid sequences of NSP2 of 8 Vietnamese strains in comparison with strains Henan-1 and VR2332. Dots indicate amino acids identical. Black box indicates putative linear B epitopes.

In Vietnam, the households accounted for about 90% of pig stocks (Tisdell, 2009) and pigs were transported by personal vehicles or trucks between different regions and from Northern areas to Southern areas for consumption. These transportsmake the PRRSV transmission between different areas. PRRSV-infected transport carry and shed live infectious virus, implying that PRRSV may travel between farms in animal vectors (Zimmerman et al. (1993, 1997*b*)). The previous study showed that some Vietnamese PRRSV strains, which collected from Northernareas and Southern areas, were belonged to North American genotype (Van et al., 2013; Thuy et al., 2013). In this study, the Vietnamese PRRSV strains were clustered in North American group. Thus, our resultssupported to the large distribution of North American genotype in Vietnam.

Non-structural protein 2 (NSP2) is the largest protein of porcine reproductive and respiratory syndrome virus (Chen et al., 2010). This nsp2 gene is the most genetically variable region of PRRSV. There were 37 different amino acid substitutions between Vietnamese strains and VR2332 strains which is originated in United State. Four different amino acid substitutions between Vietnamese strains and Henan-1 strain also were found. The identity of deduced amino acid sequences between Vietnamese PRRSV strains with Henan-1 strain (92-97%) was higher than VR2332 strains (81-84%). It suggested that the Vietnamese PRRSV strains had closely genetic relationship with Chinese PRRSV strains.

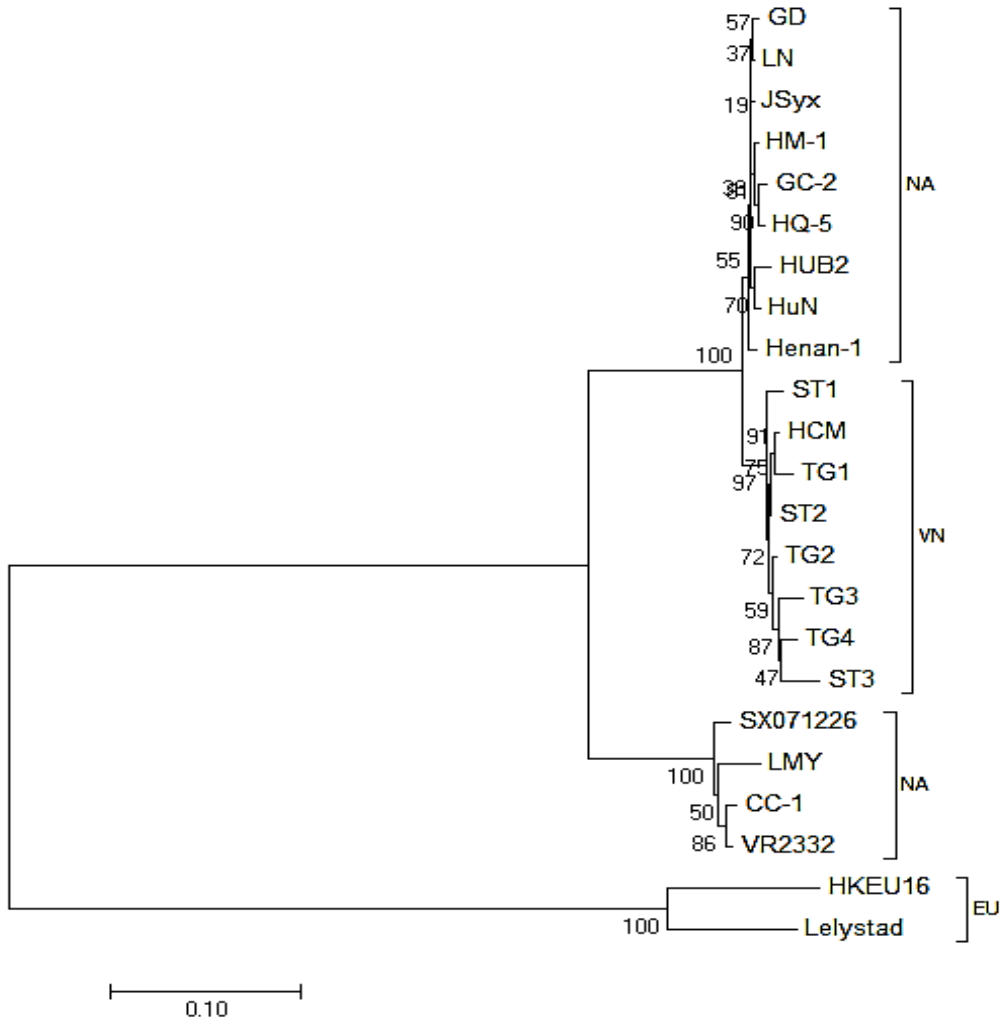

Figure 2. Phylogenetic tree constructed from NSP2 sequences of PRRSV strains by the neighbor-joining analysis method. Bootstrap resampling wasdone 1000 times, and resulting bootstrap values are shown on the corresponding branches.

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