

Genetic Analysis of the ORF7 Gene in Vietnamese Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) ^[1]

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically devastating diseases for the swine industry worldwide. The 372-bp complete nuclear capsid protein (N-protein) encoding gene (ORF7) of 36 field PRRSV isolates from Vietnam collected during 2008-2012 were sequenced and compared with certain vaccines and published strains of PRRSV. The ORF7 nucleotide sequence (nt) similarity and amino acid (aa) identity among 36 strains showed the highest and ranged from 95.1 to 100%. These isolates shared similarities with VR-2332 (nt 91.9-94.3%, aa 92.6-96.7%) and LV (nt 62.7-65.3%, aa 58.5-60.1%). There were higher level of similarity with QN07 (nt 96.2-99.1%, aa 96.7-99.1%) from the 2007 PRRS outbreak in Quang Nam province, CH-1a (nt 93.0-96.2%, aa 92.6-95.9%) isolated in China in 1995 and JXA1 (nt 96.7-99.7%, aa 97.5-100%), the highly pathogenic strain from China isolated in 2006. Six aa mutations located in both variable and conserved regions of N-protein amino acid sequence were detected in most of the 36 isolates and highly pathogenic PRRSV strains in China in comparison to the prototype strain VR-2332. Results of sequence analyses indicated that PRRSVs isolated in Vietnam during 2008-2012 were classified as North American genotype. Phylogenetic tree also clustered those 36 PRRSV isolates, other recently reported Vietnamese strains, highly pathogenic Chinese strains and JXA1-R vaccine strain in the same cluster and separated from the prototype strain (VR-2332) and Ingelvac MLV/Besta vaccine strains. The result on genetic characterization of ORF7 of circulating PRRSV strains may assist the development of effective strategies for monitoring and controlling PRRS in Vietnam.

Keywords: Genetic variation of ORF7, North American genotype, Phylogenetic analysis, PRRSV

Vietnam Domuz Reprodüktif ve Respiratorik Sendrom Virüsü (DRRSV) ORF7 Geni'nin Genetik Analizi

Özet

Domuz reprodüktif ve respiratorik sendromu (DRRS), dünya çapında domuz endüstrisi için ekonomik olarak en fazla kayba neden olan hastalıklardan birisidir. Vietnam'da, 2008-2012 boyunca 36 sahadaki DRRSV izolatlarının 372-bp komple nükleer kılıf proteinini (N-protein) kodlayan gen (ORF7) dizilimi yapıldı ve DRRSV'nin belli aşları ve yayınlanmış türleri ile karşılaştırıldı. Otuzaltı 36 tür arasında, ORF7'nin nükleotid dizilim benzerliği (nt) ve aminoasit (aa) özdeşliği en yüksek olup, %95.1 ile 100 arasında değişiklik gösterdi. Bu izolatlar, VR2332 (nt %91.9-94.3, aa %92.6-96.7) ve LV (nt %62.7-65.3, aa %58.5-60.1) ile benzerlik taşıyordu. Quang Nam yöresindeki 2007 DRRS salgınında QN07 (nt %96.2-99.1, aa %96.7-99.1) ile 1995'te Çin'de izole edilen CH-1a (nt %93.0-96.2, aa %92.6-95.9) ve Çin'de 2006'da izole edilen yüksek patojenik tür JXA (nt %96.7-99.7, aa %97.5-100) ile daha yüksek düzeyde benzerlikler vardı. Prototip tür VR-2332'ye göre, her iki değişikende ve N-protein amino asit diziliminin korunaklı bölgelerinde yerleşik 6 aa mutasyonları, 36 izolatın çoğunda ve Çin'deki yüksek patojenik DRRSV türlerinde tespit edildi. Dizilim analizleri sonuçları, Vietnam'da 2008-2012 boyunca izole edilen DRRSV'lerin Kuzey Amerikan Genotipi olarak sınıflandırıldığını gösterdi. Filogenetik ağaç, ek olarak 36 DRRSV izolatlarını, diğer yakın zamanda bildirilen Vietnam türlerini, yüksek patojenik Çin türlerini ve JXA1-R aşı türlerini aynı kümede topladı ve prototip tür (VR-2332) ve Ingelvac MLV/Besta aşı türlerinden ayrıldı. Sirkülasyondaki DRRSV türlerindeki ORF7'nin genetik karakterizasyonu sonucu, Vietnam'daki DRRSV'nin etkili izleme ve kontrol stratejilerinin gelişimine yardım edebilir.

Anahtar sözcükler: ORF7'nin genetik varyasyonu, Kuzey Amerikan genotipi, Filogenetik analiz, DRRSV



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INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is one of the most viral diseases caused great economic losses to pig industry. It is characterized by reproductive failure in pregnant sows, and respiratory distress in piglets and fattening pigs. PRRSV is a member of the *Arteriviridae* family, within the order *Nidovirales* [1]. PRRSV is small, an enveloped, positive, single-stranded RNA which is approximately 15 kb in size, composed of at least nine overlapping open reading frame (ORFs) [2]. ORF7 encodes for non-glycosylated nuclear capsid protein (N) with molecular size of 15 kDa, which is composed of 123 or 128 aa in the North American (NA) and European types (EU), respectively. It is the most abundant viral protein in virus-infected cell and contains important immunogenetic epitopes and immunodominant antigen in the pig immune respond to PRRS [3]. Compared to other ORFs, ORF7 is an important target for virological detection using polymerase chain reaction (PCR). Therefore, genetic analysis of ORF7 has been characterized and used for serological detection and diagnosis, genetic variation and phylogenetic analysis among PRRSVs.

PRRS was first detected in the United States in 1987, and then it has spread rapidly throughout the world. In Vietnam, PRRS was first observed in outbreak of PRRS in Vietnam appeared in Hai Duong Province in March 2007 [4]. Then it spreaded to other regions of the country, affecting about 70,577 pigs with more than 20,366 pigs killed [5]. Genetic analysis of PRRS strains collected from outbreaks in China and Vietnam in 2007 indicated high nt identity (99%) among isolates [6].

Currently, both NA and EU types of PRRSV are circulating in the swine population worldwide [7], however, NA type is more common in Vietnam. In the recent years, new NA type PRRSV variants called highly pathogenic strains (HP) has emerged in Vietnam and China causing large-scale outbreaks and destructive clinical syndromes [6,8]. Those HP-PRRSV isolates share high sequence identity and have similar deletions/mutations in difference regions of viral genome such as two deletions in non-structural protein 2, one deletion in the 5'-untranslated region, and one deletion in the 3' untranslated region, and some other point mutations [9]. Recently, several studies on genetic variation and phylogenetic relationship based on major structural genes of PRRSV isolates such as ORF5, ORF7 have been done [10-14]. In this study, the 372-bp complete ORF7 of 36 PRRSV isolated from Vietnam collected during 2008-2012 were sequenced and analyzed with ORF7 sequences of PRRS vaccine viruses as well as its parental virus and other published PRRSV strains.

MATERIAL and METHODS

Total blood ($n = 36$) from PRRSV-infected pigs displaying

clear clinical signs were sampled during 2008-2012 from swine herds in different provinces (Can Tho, Hau Giang, Dong Thap, Dong Nai, Binh Duong, Ho Chi Minh City and Dien Bien). JXA1-R vaccine from China was also sampled for analysis. All samples were stored in ice boxes and transported to the laboratory. A summary of the samples is presented in *Table 1*.

Total RNA was extracted using TRizol reagent (Invitrogen, USA) according to the manufacturer's instructions and used as template of RT-PCR to synthesize cDNA with random hexamers primers. Amplicon with molecular size of 490 bp containing complete ORF7 (372 bp) was amplified using ORF7 specific primer pairs [15]. For DNA sequencing, PCR products were purified using a QIAquick Extraction Kit (Qiagen, Germany) and directly sequenced in both directions (Macrogen, Korea).

Obtained nt sequences were identified with the Basic Local Alignment Search Tool (BLAST) [16]. Multiple nt alignments were carried out with BioEdit version 7.0.9.0 using published PRRSV sequences as references [17]. The phylogenetic tree was constructed with Mega 4.1 [18] using the neighbor-joining (NJ) method [19] and computed with the Kimura 2-parameter method [20]. Boot-strap values were calculated using 1,000 replicates of the alignment. Furthermore, the amino acid sequences deduced from ORF7 of 36 PRRSV isolates were aligned and analyzed the changes in their functional domains.

RESULTS

Sequence Analysis

The complete ORF7 sequences of 36 PRRSV strains isolated from different provinces of country during 2008-2012 have been determined. Out of 36 strains, 32 were submitted to GenBank under the following accession numbers: JQ860392-JQ860423 (*Table 1*). All of the PRRSV sequences were the same length (372 nt), thus encoded 123 aa residues.

Nucleotide (nt) and amino acid (aa) similarities between 36 PRRSV isolates and compared to those of VR-2332, LV, 07QN, CH-1a, and vaccine viruses JXA1-R, Ingelvac MLV, BSL-PS were summarized in *Table 2*. Pairwise comparison revealed that 36 isolates shared 95.1-100% in nt identities with each other and could be separated into 13 haplotypes (*Table 1*) based on the difference in their nt sequences. There were high nt identities (91.9-94.3%) with VR-2332 but only nt 62.7-65.3% and aa 58.5-60.1% similarities with LV. The result implied that our 36 PRRSV strains were of North American genotype (NA). Compared with nt sequence of 07QN (a highly pathogenic Vietnamese strain isolated from PRRS outbreak reported in 2007), the identity was 96.2-99.1% in nt sequence. The higher nt similarity between our 36 PRRSV isolates and high pathogenic JXA1 strains from 2006 (96.7-99.7%) and lower nt identity

Table 1. PRRSV isolates (n = 36) from Vietnam and representative PRRSV strains used for sequencing and phylogenetic analyses**Table 1.** Vietnam'daki DRRSV izolatlari (n = 36) ve dizilim ve filojenik analizlerde kullanılan örnek DRRSV türleri

No.	Strain*	Location-Year	Reference	Haplotype	No.	Strain	Location-Year	Reference
1	DN2008-153	VN-2008	JQ860403	1	37	JXA1-R vaccine	China-2011	-
2	DN2008-444	VN-2008	JQ860392	2	38	CH-1a	China-1996	AY032626
3	DN2008-452	VN-2008	JQ860393	2	39	NB-CH2004	China-2004	FJ536165
4	DN2008-456	VN-2008	JQ860394	3	40	GD3-CH2005	China-2005	GU269541
5	DN2008-460	VN-2008	JQ860395	1	41	JXA1-CH2006	China-2006	EF112445
6	DN2008-499	VN-2008	JQ860396	4	42	HUN4	China-2006	EF635006
7	DN2008-694	VN-2008	JQ860397	2	43	07HEN-CH2007	China-2007	FJ393457
8	DN2009-1107	VN-2009	JQ860406	2	44	EM2007	China-2007	EU262603
9	DN2009-1155	VN-2009	JQ860407	2	45	KP-CH2008	China-2008	GU232735
10	DN2009-292	VN-2009	JQ860404	2	46	HUB7-CH2009	China-2009	GU168567
11	DN2009-339	VN-2009	JQ860405	2	47	09HEN2	China-2009	JF268680
12	DN2009-42	VN-2009	JQ860398	2	48	09HUB1	China-2009	JF268682
13	DN2009-44	VN-2009	JQ860399	2	49	DC-CH2010	China-2010	JF748718
14	DN2009-59	VN-2009	JQ860400	2	50	SD16-CH2012	China-2012	JX087437
15	DN2009-73	VN-2009	JQ860401	2	51	06K805(JB)	Korea-2006	EF441853
16	DN2009-88	VN-2009	JQ860402	2	52	05K205(CN)	Korea-2006	EF441809
17	BD2010-R1	VN-2010	JQ860412	7	53	06K010(CN)	Korea-2006	EF441836
18	BD2010-X13	VN-2010	JQ860413	3	54	EDRD-1	Japan-1995	D45852
19	DN2010-1	VN-2010	JQ860408	1	55	31D.MEX4	Mexico-2003	AY209228
20	DN2010-5.2	VN-2010	JQ860409	2	56	27E.MEX3	Mexico-2003	AY209222
21	HCM2010-CC3	VN-2010	JQ860410	5	57	MN184A	USA-2005	DQ176019
22	HCM2010-D06	VN-2010	JQ860411	6	58	07QN	VN-2007	FJ394029
23	HCM2011-1P	VN-2011	-	8	59	BRVT	VN-2009	GU187019
24	CT2012-C1	VN-2012	JQ860414	9	60	T4SG	VN-2009	GU187020
25	CT2012-C2	VN-2012	JQ860415	9	61	MLV.Bestar	Singapore-2009	GU187018
26	CT2012-HS1	VN-2012	JQ860416	9	62	IngelvacPRRS	USA-1998	AF066183
27	CT2012-HS2	VN-2012	JQ860417	9	63	VR-2332	USA-1990	U87392
28	CT2012-HS3	VN-2012	JQ860418	9	64	Lelystad	Holand-1991	M96262
29	DT2012-DT7	VN-2012	JQ860419	10				
30	DT2012-DT8	VN-2012	JQ860420	10				
31	DT2012-DT9	VN-2012	JQ860421	10				
32	HG2012-RV1	VN-2012	JQ860422	11				
33	HG2012-RV2	VN-2012	JQ860423	12				
34	DB2012-1DB	VN-2012	KM659200	13				
35	DB2012-2DB	VN-2012	KM659201	13				
36	DB2012-9BD	VN-2012	KM659202	13				

*Strains in bold were isolated and used in this study; (-): sequences analysed in this study and not submit in GenBank

(93.0-96.2%) to Chinese strains CH-1a from 1995 were observed. Among the 36 isolates, samples collected from Dien Bien exhibited unique nt changes at positions 183 (T→C), 351 (C→T) (*data not shown*), and both are non-synonymous substitution.

Analysis of Deduced Type II PRRSV ORF7 aa Sequences

The deduced aa sequences for ORF7 of the 36

Vietnamese isolates were aligned with representative PRRSV strains from Vietnam, China, prototype strain VR-2332 and vaccine viruses (*Fig.1*). As shown in *Fig. 1*, all of PRRSV ORF7 sequences were the same length of 372 nt and encoded 123 aa residues. Pair-wise comparison showed that the 36 strains shared 95.1-100% aa identity with each other and high levels of aa identity with 07 QN (96.7-99.1%). The aa identities were of 92.6-96.7% in comparison

Table 2. Nucleotide and amino acid identities (%) for ORF7 among 36 Vietnamese PRRSV isolates, and compared with LV, VR-2332, 07QN, JXA1, CH-1a strains, and MLV-Bestar/ Ingelvac/ JXA1-R vaccines

Tablo 2. Otuzaltı Vietnam izolatı arasında ORF7 için nükleotid ve amino asit özdeşlikleri (%), ve LV, VR-2332, 07QN, JXA1, CH-1a türleri, ve MLV-Bestar/ Ingelvac/ JXA1-R aşılarıyla karşılaştırılması

Strains (n)		2008	2009	2010	2011	2012	07QN	VR 2332	LV	CH-1a	JXA1/ JXA1-R Vaccine	MLV-Bestar/ Ingelvac Vaccine
VN-2008 (7)	nt	97.8-100	97.8-100	96.7-100	98.1-99.1	96.2-99.4	97.8-99.1	93.5-94.3	63.8-64.3	94.6-96.2	98.3-99.7	93.2-94.3
	aa	99.1-100	99.1-100	96.7-100	99.1-100	96.7-100	98.3-99.1	94.3-95.1	59.3-60.1	94.3-95.1	99.1-100	94.3-95.1
VN-2009 (9)	nt		100	96.7-100	99.1	96.2-99.4	97.8	93.5	64.3	94.6	98.3	93.2-93.5
	aa		100	97.5-100	100	97.5-100	99.1	95.1	60.1	95.1	100	95.1
VN-2010 (6)	nt			96.5-99.1	96.5-99.4	95.1-99.4	97.3-98.9	92.4-94.0	63.3-65.3	94.6-96.2	97.8-98.4	92.2-94.0
	aa			95.1-100	97.5-100	95.1-100	96.7-99.1	92.6-95.9	59.3-60.1	92.6-95.9	97.5-100	92.6-95.9
VN-2011 (20)	nt				-	96.5-99.1	98.1	93.2	64.3	94.8	98.6	93.0-93.2
	aa				-	97.5-100	99.1	95.1	60.1	95.1	100	95.1
VN-2012 (13)	nt					95.1-100	96.2-97.8	91.9-94.0	62.7-64.0	93.0-95.1	96.7-98.3	91.6-94.0
	aa					97.5-100	96.7-99.1	94.3-96.7	58.5-60.1	92.6-95.1	97.5-100	94.3-96.7

(n): number of strains; nt: nucleotide; aa: amino acid

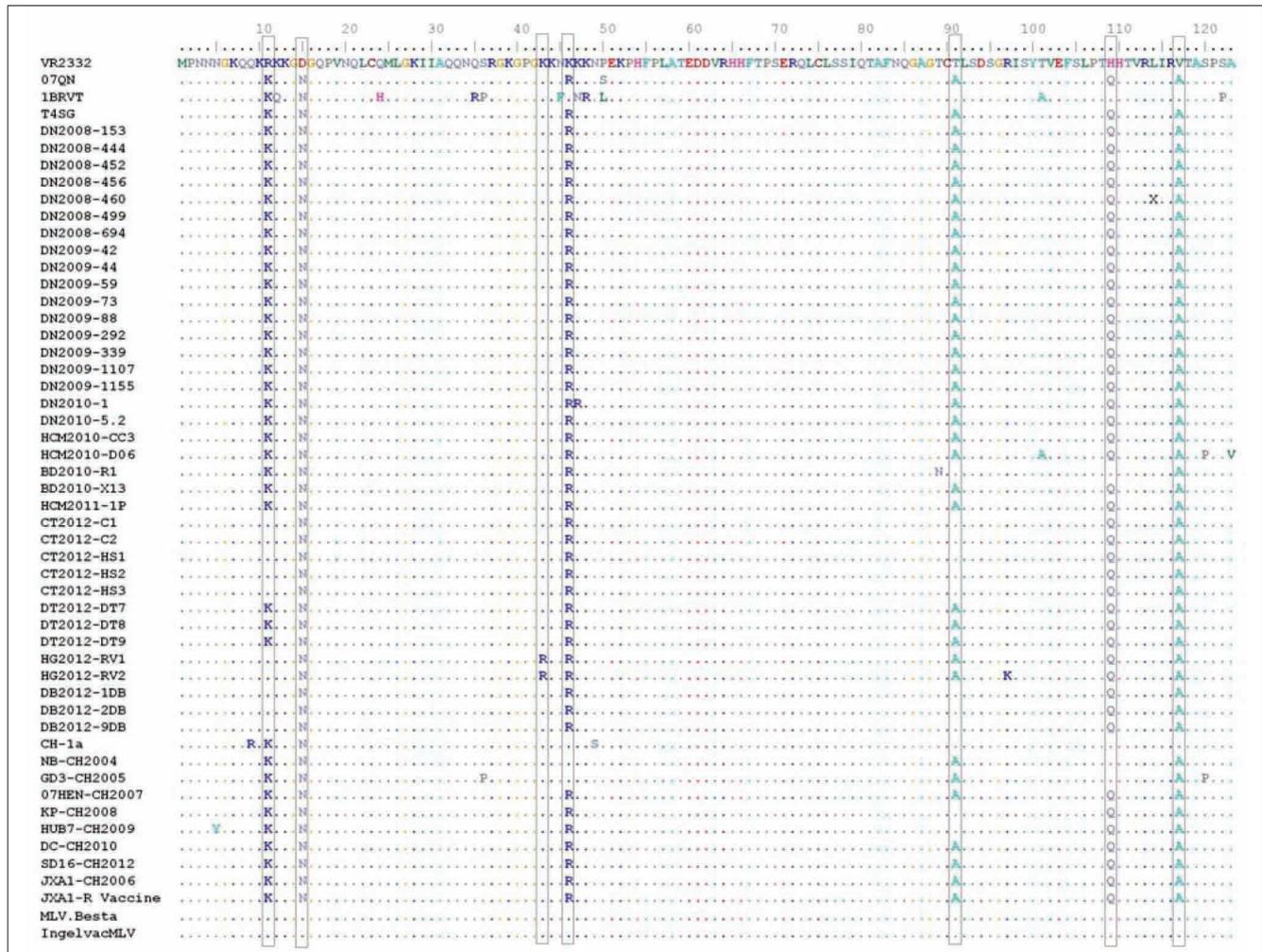


Fig 1. Analysis and comparison of amino acid mutations in N-protein of PRRSV. Mutations are indicated by closed boxes

Şekil 1. DRRSV'nin N-proteinindeki amino asit mutasyonlarının karşılaştırılması ve analizi. Mutasyonlar kapalı kutular tarzında gösterilmektedir

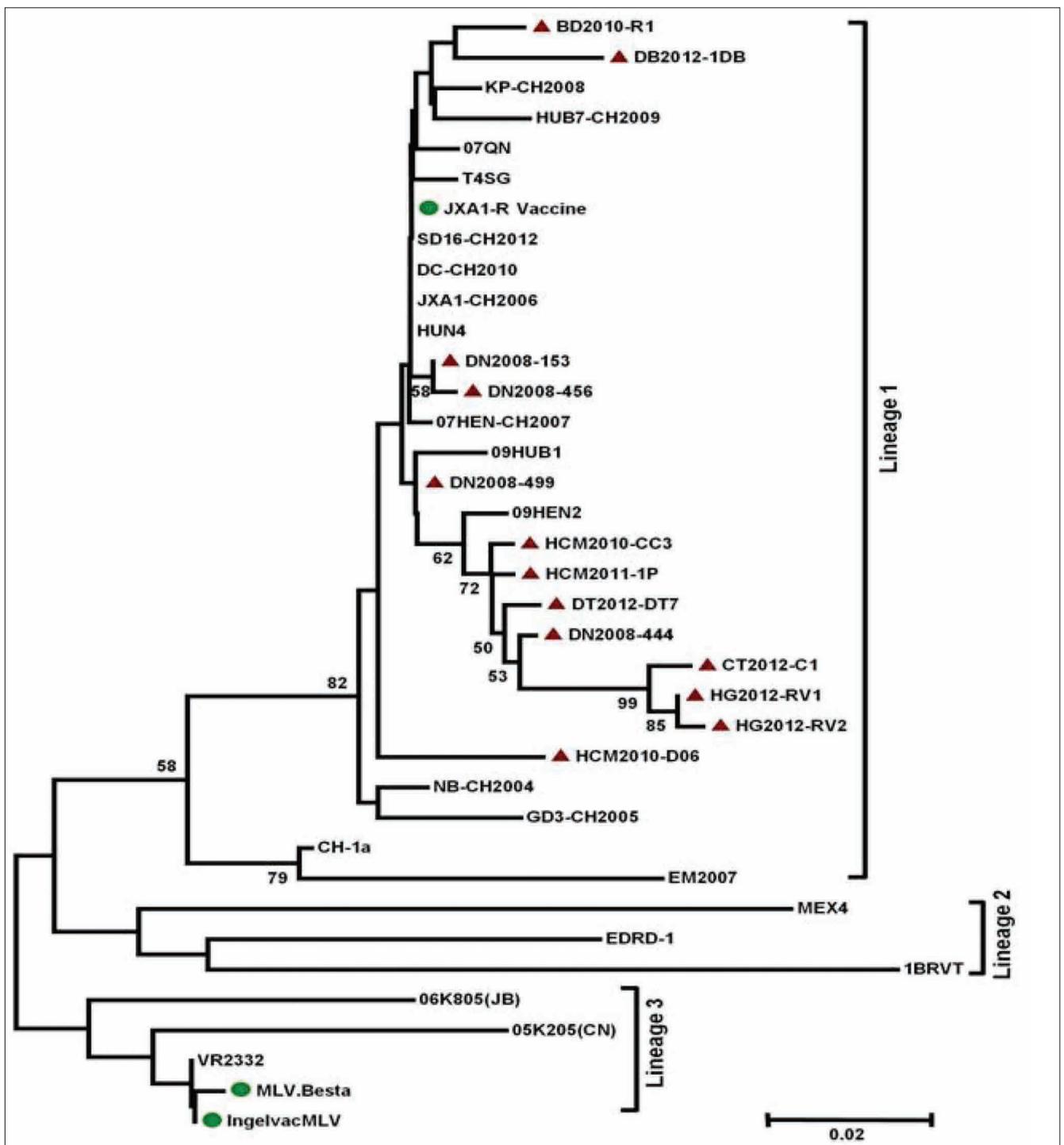


Fig 2. Phylogenetic tree containing 12 Vietnamese isolates and other representative virus strains. The comparison was based upon on the nucleotide sequence of PRRSV ORF7. The phylogenetic tree was generated by the neighbour-joining method using MEGA 4.1 with bootstrap values of 1,000 replicates. Our isolates are marked (▲). Vaccines licensed for use in Vietnam are denoted by (●)

Şekil 2. Oniki Vietnam izolatu ve diğer örnek virus türlerini kapsayan filogenetik ağaç. Karşılaştırma DRRSV ORF7'nin nükleotid dizilimi esasına göre yapıldı. Filogenetik ağaç, 1.000 kopyanın bootstrap (çekme) değerleri komşu-katılım metoduyla MEGA 4.1 kullanılarak oluşturuldu. İzolatlarımız (▲) ile işaretlendi. Vietnam'da kullanım için lisanslı aşilar (●) ile sembolize edildi

with VR-2332, 58.5-60.1% with LV, 92.6-95.9% with CH-1a, and 97.5-100% with JXA1-R. The 36 Vietnamese isolates had high aa similarity with JXA1-R (97.5-100%), the Chinese isolate used in PRRS vaccines, than that of Ingelvac MLV/BSL-PS (94.3-96.7%); (Table 2).

There were six common aa mutations located in both variable and conserved regions of N-protein amino acid sequence of the 36 PRRSV isolates compared to prototype strain LV-2332 (R11K, D15N, K46R, T91A, H109Q and V117A). In which, four mutations were observed in all strains at

position 15, 46, 109 and 117 (except isolate BD2010-R1). Out of six mutations, two aa changes at positions 11 (R→K) and 91 (T→A) were not detected in eight strains sampled from Dien Bien and Can Tho in 2012. There was a mutation at position 43 (K→R), which detected in two strains from Hau Giang only (HG2012-RV1 and HG2012-RV2). These mutations were also found in other Chinese HP-PRRSV strains^[16]. Only one strain (HCM2010-D06) showed identical mutations at C-terminal (S120P and A123V).

Phylogenetic Analysis

Several genotyping studies based on ORF7 sequences have been conducted on type II PRRSVs, however, a lack of a reference sequence set, no satisfactory classification system was available. In the phylogenetic tree (Fig. 2), the 36 Vietnamese PRRSV isolates were belonged to the type II (NA genotype) and were grouped closely to each others and to representative Chinese HP strains, such as HUN4, JXA1, 09HEN2, 09HUB1,... (Lineage 1). They were divided into two subgroups, which is corresponding to subgroup IV and V of the previous classification^[11]. The first subgroup contained 24 of our isolates belonged to eight haplotypes (2,4,5,8,9,10,11,12) and two Chinese HP reference strains (09HEN2 and 09HUB1). Other isolates belonged to haplotype 1,3,7 and 13 and representative HP strains (HUN4, HUB7 and JXA1-R) clustered in separate subgroup.

Isolates collected from Dien Bien and Binh Duong were closely grouped to HP strain 07QN, which was collected from PRRS outbreak in Quang Nam province in 2006 and Chinese vaccine JXA1-R. Seven isolates sampled from Can Tho and Hau Giang were grouped together into one branch and showed a very closely genetic relationship.

DISCUSSION

Since HP-PRRSV outbreak was first reported in 2007 in Vietnam, the virus has spread widely and is always accompanied with pig farms throughout the country. This study was to describe the genetic variation of PRRSV field isolates collected from infected pigs in different regions of Vietnam during 2008-2012 based on ORF7 sequence. Result of ORF7 sequencing confirmed that all of isolates were of NA genotype with high identity to prototype strain VR-2332 (91.4-94.3%). Within Vietnamese isolates, a highly pathogenic strain isolated from PRRS outbreak reported in 2007 (07QN) showed the the lower nt similarity with the PRRSV strains isolated in 2012 (96.2-97.8%) in compared to that of 2008 (97.8-99.1%). It implied that PRRSV isolates showed the certain genetic variation regarding the time. The nucleotide similarity of ORF7 among isolates ranged from 95.1 to 100% which was similar to these of 59 Chinese PRRSV strains (91.9-100%)^[21]. Nucleotide homology analysis revealed that all isolates were closely related to the Chinese HP-PRRSV and also Chinese PRRSV vaccine circulating in Vietnam (JXA1-R). High nt/aa similarities among Vietnamese

and Chinese HP-PRRSV isolates (Table 2) correspond with findings from Feng et al.^[5]. They observed 99% identity at the genomic level for Vietnamese and Chinese PRRSV isolates. With respect to ORF7 sequence, PRRSV strains circulating in Vietnam showed a greater nu/aa similarity to JXA1-R than to virus strains used in other vaccines (Ingelvac MLV and Besta-PS). It provides useful information for vaccine selection and renewal. The similar result was also obtained for ORF5 sequence of those strains^[13,14].

Among viral protein, nucleocapsid protein (N-protein), encoded by ORF7, is the most abundant protein in the virion, accounting for 20-40% of the total virion protein content^[22]. Because of more conserved property, N-protein has been used as target of several diagnostic tests. As shown in Fig. 1, analysis of the complete deduced amino acid sequence of N-protein from the 36 PRRSV isolates shared common six mutations with Chinese HP-PRRSV strains. These mutations were also detected in other HP-PRRSV strains isolated in China^[11,12]. Compared to classical strain CH-1a, four mutations at position 46, 91, 109 and 117 were just observed in HP strains. Two identical mutations at position 11 and 91, which were not found in most of strains isolated in 2012. These unique variations in ORF7 gene should be considered in the development of diagnostic RT-PCR for PRRSV.

The N-terminus is presumed to play a role in the interaction with genomic viral RNA because of high composition of basic aa, such as Lysine and Arginine^[23]. Therefore, the variations of R11K and D15N found in most of our strains and Chinese HP-PRRSV should be noted. N protein contains conserved nucleotide determinants such as nuclear localization signal (NLS), nuclear export signal (NES). Previous study had demonstrated that mutations at 43 and 44 within the NLS weaken viral replication^[24], NLS motif such as "Pat7" located at position 41-47 (PGKKNKK) might block the recognition of the epitopes^[11]. In our study, the substitution K46R observed in 36 isolates and also in Chinese HP-PRRSV strains might influence to its function. The final 11 residues at the C-terminus have been shown to be an important intermolecular reactions mediated via N-N interactions, thus is necessary for maintaining proper tertiary structure of this protein^[25,26]. Two mutations at this region (positions P120S and A123V) were also detected in isolate HCM2010-D06. Conserved mutation at position 117 (V→A) was also found in our 36 strains and other HP-PRRSV strains in compared to reference as classical strains of CH-1a and VR-2332.

Based on ORF7 sequences, Chinese HP-PRRSVs were mostly concentrated in sub-group IV and V^[6]. As shown in Fig. 2, all of 36 isolates was also classified in those sub-groups (appeared in Fig. 2 as Lineage 1) because they share numerous common point mutations (nu/aa). According to sequence comparison and phylogenetic tree analysis, it demonstrates that a Vietnamese PRRSV strains are genetically closely related to HP-PRRSV strains that were

circulating in China at the same time. Therefore, it is very important to have effective strategy for controlling PRRS transboundary disease and monitoring pig movements.

Because of identical mutations at C-terminal (P120S and A123V) observed in HCM2010-D06 strain only, it was clustered separately and suggested the new genetic variants. Thus, molecular epidemiological studies of PRRSV should be carried out to provide annual genetic information for development of reliable PRRS diagnosis and disease control.

All of the 36 PRRSVs isolated during 2008-2012 in Vietnam belonged to the NA genotype. To our knowledge, this is the first report describing the genetic characterization of nucleoprotein encoding gene ORF7 of PRRSV circulating in different provinces of Vietnam collected during 2008-2012. Based on ORF7 sequence, the Vietnamese PRRSV strains were genetically closely related to each others and HP-PRRSV strains circulating in China.

REFERENCES

- Cavanagh D:** Nidovirales: A new order comprising Coronaviridae and Arteriviridae. *Arch Virol*, 142, 629-633, 1997.
- Meulenber JJ, Hulst MM, de Meijer EJ, Moonen PL, den Besten A, de Kluyver EP, Wensvoort G, Moormann RJ:** Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology*, 192, 62-72, 1993. DOI: 10.1006/viro.1993.1008
- Murtaugh MP, Xiao Z, Zuckermann F:** Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. *Viral Immunol*, 15, 533-547, 2002. DOI: 10.1089/088282402320914485
- Thanh TL:** Porcine reproductive and respiratory syndrome. *Vet Sci Techn*, 3, 81-87, 2007.
- Tien NN:** Status of PRRS in pigs in Vietnam and disease prevention. *Vet Sci Techn*, 18, 12-20, 2011.
- Feng Y, Zhao T, Nguyen T, Inui K, Ma Y, Nguyen TH, Nguyen VC, Liu D, Bui QA, To LT, Wang C, Tian K, Gao GF:** Porcine respiratory and reproductive syndrome virus variants, Vietnam and China. *Emerg Infect Dis*, 14, 1774-1776, 2008. DOI: 10.3201/eid1411.071676
- Kim HK, Yang JS, Moon HJ, Park SJ, Luo Y, Lee CS, Song DS, Kang BK, Ann SK, Jun CH, Park BK:** Genetic analysis of ORF5 of recent Korean porcine reproductive and respiratory syndrome viruses (PRRSVs) in viremic sera collected from MLV-vaccinating or non-vaccinating farms. *J Vet Sci*, 10, 121-130, 2009. DOI: 10.4142/jvs.2009.10.2.121
- Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, Hu Y, Chen X, Hu D, Tian X, Liu D, Zhang S, Deng X, Ding Y, Yang L, Zhang Y, Xiao H, Qiao M, Wang B, Hou L, Wang X, Yang X, Kang L, Sun M, Jin P, Wang S, Kitamura Y, Yan J, Gao GF:** Emergence of fatal PRRSV variants: Unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *PLoS One*, 2, e526, 2007. DOI: 10.1371/journal.pone.0000526
- An TQ, Tian ZJ, Zhou YJ, Xiao Y, Peng JM, Chen J, Jiang YF, Hao XF, Tong GZ:** Comparative genomic analysis of five pairs of virulent parental/attenuated vaccine strains of PRRSV. *Vet Microbiol*, 149, 104-112, 2011. DOI: 10.1016/j.vetmic.2010.11.001
- Shi M, Lam TT, Hon CC, Murtaugh MP, Davies PR, Hui RK, Li J, Wong LT, Yip CW, Jiang JW, Leugh FC:** Phylogeny-based evolutionary, demographical, and geographical dissection of North American type 2 porcine reproductive and respiratory syndrome viruses. *J Virol*, 84, 8700-8711, 2010. DOI: 10.1128/JVI.02551-09
- Hao X, Lu Z, Kuang W, Sun P, Fu Y, Wu L, Zhao Q, Bao H, Fu Y, Cao Y, Li P, Bai X, Li D, Liu Z:** Polymorphic genetic characterization of the ORF7 gene of porcine reproductive and respiratory syndrome virus (PRRSV) in China. *Virology*, 8, 73, 2011. DOI: 10.1186/1743-422X-8-73
- Li J, Yin Y, Guo B, Zhou S, Zhang Y, Liu X, Sun T:** Sequence analysis of the NSP2, ORF5, and ORF7 genes of 11 PRRS virus isolates from China. *Virus Genes*, 45, 256-264, 2012.
- Thuy NTD, Thu NT, Son NG, Ha LTT, Hung VK, Nguyen NT, Khoa DVA:** Genetic analysis of ORF5 porcine reproductive and respiratory syndrome virus isolated in Vietnam. *Microbiol Immunol*, 57, 518-526, 2013. DOI: 10.1111/1348-0421.12067
- Jantafong T, Sangtong P, Saenglub W, Mungkundar C, Romlamduan N, Lekchareonsuk C, Lekchareonsuk P:** Genetic diversity of porcine reproductive and respiratory syndrome virus in Thailand and Southeast Asia from 2008 to 2013. *Vet Microbiol*, 176, 229-238, 2015. DOI: 10.1016/j.vetmic.2015.01.017
- Zhu L, Zhang G, Ma J, He X, Xie Q, Bee Y, Gong SZ:** Complete genomic characterization of a Chinese isolate of porcine reproductive and respiratory syndrome virus. *Vet Microbiol*, 147, 274-282, 2011. DOI: 10.1016/j.vetmic.2010.07.001
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ:** Basic local alignment search tool. *J Mol Biol*, 215, 403-410, 1990.
- Hall T:** BioEdit: A user-friendly biological sequences alignment editor and analysis program for Window 95/98/NT. *Nucl Acids Symp Ser*, 41, 95-98, 1999. DOI: 10.1016/j.jembe.2015.02.006
- Tamura K, Dudley J, Nei M, Kumar S:** MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol*, 24, 1596-1599, 2007. DOI: 10.1093/molbev/msm092
- Saitou N, Nei M:** The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4, 406-425, 1987.
- Kimura M:** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, 16, 111-120, 1980.
- Zhou L, Chen S, Zhang J, Zeng J, Guo X, Ge X, Zhang D, Yang H:** Molecular variation analysis of porcine reproductive and respiratory syndrome virus in China. *Virus Res*, 145, 97-105, 2009. DOI: 10.1016/j.virusres.2009.06.014
- Mardassi H, Mounir S, Dea S:** Molecular analysis of the ORFs 3 to 7 of porcine reproductive and respiratory syndrome virus, Quebec reference strain. *Arch Virol*, 140, 1405-1418, 1995. DOI: 10.1007/BF01322667
- Murtaugh MP, Elam MR, Kakach LT:** Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus. *Arch Virol*, 140, 1451-1460, 1995. DOI: 10.1007/BF01322671
- Lee C, Hodgins D, Calvert JG, Welch SK, Jolie R, Yoo D:** Mutations within the nuclear localization signal of the porcine reproductive and respiratory syndrome virus nucleocapsid protein attenuate virus replication. *Virology* 346, 238-250, 2006. DOI: 10.1016/j.virol.2005.11.005
- Wootton SK, Koljesar G, Yang L, Yoon KJ, Yoo D:** Antigenic importance of the carboxy-terminal beta-strand of the porcine reproductive and respiratory syndrome virus nucleocapsid protein. *Clin Diagn Lab Immunol*, 8, 598-603, 2001. DOI: 10.1128/CDLI.8.3.598-603.2001
- Wootton SK, Nelson EA, Yoo D:** Antigenic structure of the nucleocapsid protein of porcine reproductive and respiratory syndrome virus. *Clin Diagn Lab Immunol*, 5, 773-779, 1998.