Virus Genes
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SHORT REPORT

Phylogenetic analysis of porcine reproductive and respiratory syndrome virus in Vietnam, 2021

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Abstract

The porcine reproductive and respiratory syndrome virus (PRRSV) causes more economic losses in the swine industry than any other virus. This study aimed to investigate the genetic diversity of PRRSV to assist in evaluating the effectiveness of PRRS vaccines. Twenty-eight samples from clinical cases were collected from 19 farms in seven provinces of Vietnam in 2021. Full-length PRRSV ORF5 genes from the 19 samples were amplified, sequenced, and compared to the corresponding sequences of referenced PRRSV strains from Genbank. The genetic analysis showed that 12 isolates were the highly pathogenic PRRSV subtype (HP—PRRSV) lineage 8, sublineage 8.7; six isolates were the classical North American PRRSV subtype (US-PRRSV), NADC-like group, lineage 1, sublineage 1.4, which were reported in Vietnam for the first time; and the final isolate was a vaccine-like strain. The field isolates of HP-PRRSV had relatively higher genetic diversity with US-PRRSV vaccine strains (84.0–94.5%) than HP-PRRSV vaccine strains (95.3–98.6%). Meanwhile, the six NADC-like isolates had low nucleotide similarity with US-PRRSV and HP-PRRSV vaccine strains (83.4–85.4% and 83.2–84.0%, respectively). Many amino acid substitutions were found in antigenic regions of GP5 involved in response to early antibody production, neutralizing antibodies, and viral immune evasion between these field strains and PRRSV vaccine strains. These findings provide insights into the molecular characteristics, genetic diversity, antigenicity, and evolution of PRRSV strains in Vietnam and postulate a compelling explanation for the limitations of current vaccination efforts.

Keywords PRRSV · Vaccine · ORF5 · Genetic diversity · phylogenetic analysis

Porcine reproductive and respiratory syndrome virus (PRRSV) causes acute infectious disease, resulting in a significant economic impact on the swine industry. PRRSV is a single-stranded, enveloped RNA virus of the genus *Betaarterivirus* (order *Nidovirales*, family *Arteriviridae*) (Brinton et al., 2021). The PRRSV genome is approximately 15.4 kb with 11 open reading frames (ORFs). ORF5 encodes the GP5 protein, which is the most variable PRRSV structural protein (Murtaugh et al., 1995). Because of the rapidly accumulating variation and large difference in ORF5 between genotypes and between strains within the same genotype, RT-PCR techniques based on ORF5 are often used to differentiate between genotypes 1 and 2 to determine the genetic relationships of PRRSV strains (Murtaugh et al., 1995), and

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distinguish lineages (Shi et al., 2010). Furthermore, GP5 is the most important neutralizing antigen with the strongest ability to induce the neutralizing immune response against PRRSV (Ostrowski et al., 2002; Stoian and Rowland, 2019).

PRRSV strains are categorized as PRRSV-type 1 (European genotype—EU) or PRRSV-type 2 (North American genotype—NA) (Kuhn et al., 2016). Furthermore, PRRSV-type 2 (NA-PRRSV) can be divided into several major subtypes, including classical North American PRRSV (C-PRRSV), highly pathogenic PRRSV (HP-PRRSV), and many others, in which NADC30-like strains (NL-PRRSV) has been identified in many PRRSV studies conducted in China (Gao et al., 2017; Jiang et al., 2020; Tian, 2017; Wei et al., 2019). In Vietnam, all PRRSV field strains isolated from 2007 to 2015 belonged to sub-lineages 8.7 and 5.1 (Do et al., 2016). The study of PRRS cases from 2008 to 2016 showed that the incidence rates in the North of Vietnam usually occur from March to April, and in the South from June to August (Lee et al., 2019). Besides, PRRSV was well controlled by commercial vaccines belonging to two groups: HP-PRRSV (JXA1-R) and US-PRRSV (Ingelvac PRRS ATP, Fostera, Prime Pac, Ingelvac PRRS MLV, BSL-PS100, and Prevacent PRRS). However, recently, especially in 2021, the pigs in many farms that have used the attenuated PRRS vaccine for a long time still showed clinical signs associated with PRRS. This study was to assess genetic diversity based on the ORF5 gene of these field PRRSV strains, clarifying the cause of recent PRRS disease occurrence in these farms.

A total of 28 samples from sick pigs with typical clinical manifestations of PRRS were submitted to Viet Han Veterinary Diagnosis Laboratory, Nong Lam University. These samples were from 19 farms in North, Central, and South Vietnam in 2021 (Figure S1 and Table S1). Samples were delivered to the laboratory in a cool box and processed for RNA isolation within 24 h using a GeneJET Viral DNA/RNA Purification Kit (ThermoFisher, USA) according to the manufacturer's instructions. cDNA was synthesized from the mRNA using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher, USA).

RT-PCR was performed to confirm PRRS using PRRS-P71 and PRRS-P72 primers (Guarino et al., 1999) (Table S2). RT-PCR products were then electrophoresed in 1.5% agarose gel and observed under UV light. RNA of the positive samples was selected to amplify the full-length ORF5 of PRRSV as well as the flanking ORF4 and ORF6 regions (total size 764 bp) by RT-PCR with primer pairs P5F and P5R (Table S2) (Cha et al., 2004). The products of the full-length ORF5 gene were purified using the GenJET Gel Extraction and DNA Cleanup Micro Kit (ThermoFisher, USA), cloned into the pGEM-T vector (Promega, USA), and transformed into *E. coli* DH5α cells (Takara, Japan). Cells containing the ORF5 gene were selected using blue-white screening and colony PCR incorporating T7 and SP6 primers. pGEM-T/ORF5 was purified using a GenJET Plasmid Miniprep Kit (ThermoFisher, USA) and sent to a sequencing laboratory (Nam Khoa Biotek, Vietnam). Both forward and reverse complement sequences were overlapped to obtain a single sequence. A phylogenetic tree was constructed based on the ORF5 gene of 19 PRRSV strains from this study and 43 PRRSV reference strains. The nucleotide sequences of the ORF5 genes from this study were deposited in GenBank under the accession numbers MZ218074-92 (Table S1).

All samples were positive for PRRSV by RT-PCR (Table S1). Phylogenetic analysis based on nucleotide sequences of the ORF5 gene showed that 17/28 isolates belonged to the HP-PRRSV, 10/28 isolates belonged to the US-PRRSV, and 1/28 isolates belonged to a recombinant strain of HP-PRRSV and US-PRRSV (Table S1). These results indicate that HP-PRRSV strains are dominant in PRRS pigs in Vietnam.

We selected 19 PRRSV strains representing 19 farms in this study to build the phylogenetic tree based on the ORF5 nucleotide sequences (Fig. 1A). These isolates belonged to genotype 2 and were divided into three subtypes. Twelve isolates of the HP-PRRSV subtype belonged to lineage 8 and sublineage 8.7, together with the vaccine strain JAX1-R and isolates from Vietnam and China. Meanwhile, six US-PRRSV isolates were clustered into sublineage 1.4, lineage 1 with isolates from Thailand. None of the 6 US-PRRSV strains belonged to lineage 5 with the VR-2332 like isolates collected in China, Thailand, and Vietnam.

Furthermore, despite also belonging to lineage 8 with the HP-PRRSV isolates in the study, the isolate MZ218081 was found not to belong to sublineage 8.7. In particular, this isolate and the Fostera PRRS vaccine strain (AF494042) were classified into a clade belonging to lineage 8 (Fig. 1B). Similar strains derived from PRRSV vaccine isolates have also been detected in outbreaks in Thailand (Tun et al., 2011), the US (Brockmeier et al., 2012), and China (Guo et al., 2019; Jiang et al., 2020).

The ORF5 sequence of the 19 field PRRSV isolates shared 81–100% genetic identity and 78.5–100% amino acid identity. The 12 HP-PRRSV isolates belonging to lineage 8, sublineage 8.7, shared high similarity at both nucleotide (94.0–100%) and amino acid (90.5–100%) levels. They also had high nucleotide and amino acid similarity with reference HP-PRRSV strains (93.5–99.1% and 90.5–98.5%, respectively) and JAX1 strain (95.6–99.0% and 92.0–98.0%, respectively) (Table S3). They had a lower sequence identity with US-PRRSV vaccine strains. They also had a high nucleotide and amino acid homology with vaccine strains belonging to lineage 8, including the Fostera strain (92.2–94.5% and 88.0–92.0%, respectively) and ATP strain (88.5–90.5% and 86.0–89.5%, respectively). Meanwhile, the similarity of these HP-PRRSV isolates at both nucleotide and amino

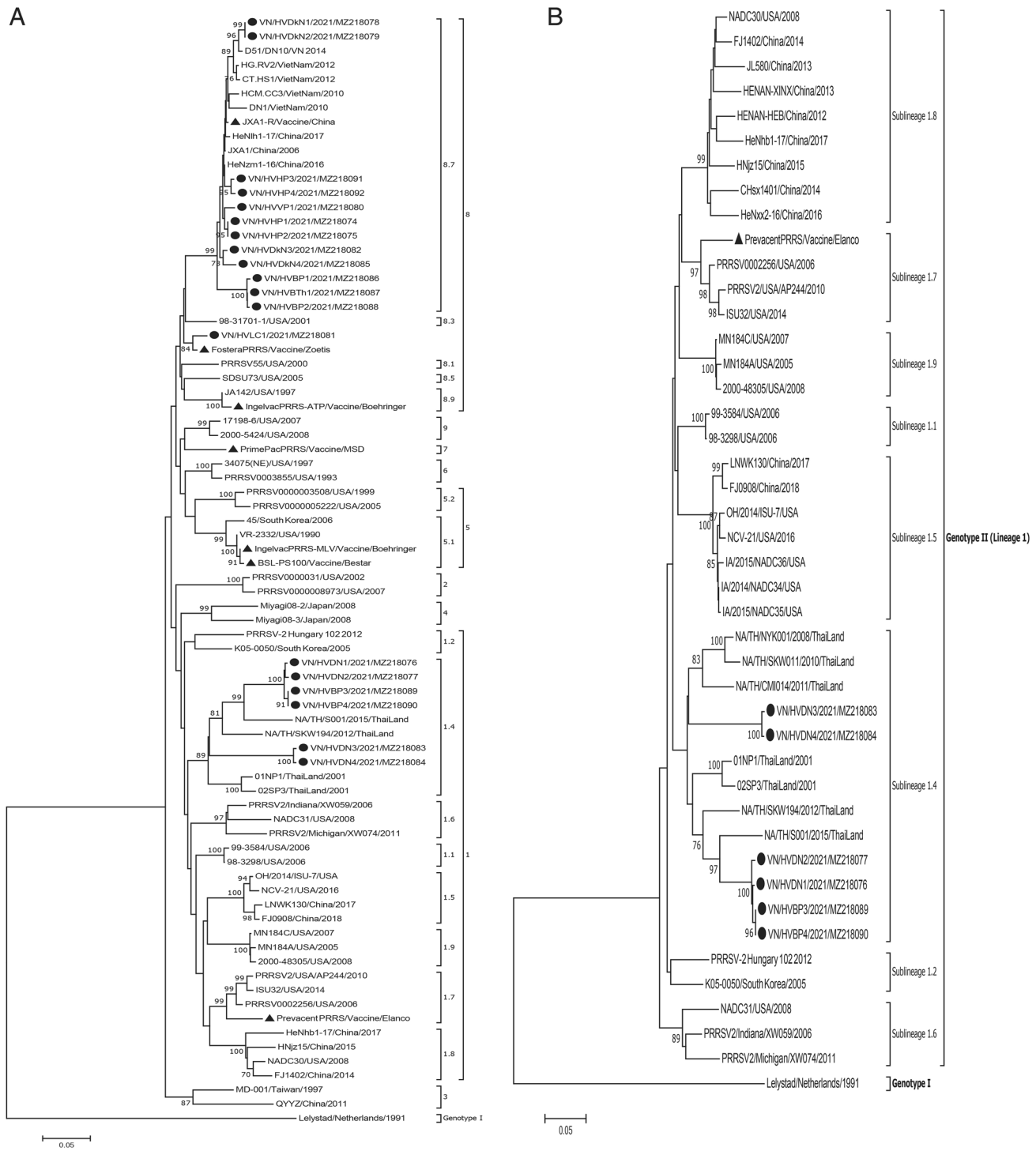

Fig. 1 PRRSV phylogenetic trees based on the nucleotide sequence of full-length gene encoding GP5 of 19 field isolates (**A**) and six sub-lineage 1.4 isolates (**B**) with reference strains. The neighbor-joining method was used to construct phylogenetic trees in MEGA X software (https://www.megasoftware.net/). Numbers along branches indicate bootstrap values > 50% (1000 replicates). The black circles are the PRRSV field isolates from this study. The black triangles are the PRRSV vaccine strains deposited in GenBank

acid levels was high with the Prime Pac strain of lineage 7 (87.8–90.3% and 86.5–92.0%, respectively) but lower with three vaccine strains belonging to a different lineage (84.0–89.2% and 81.0–87.5% respectively) (Table S5).

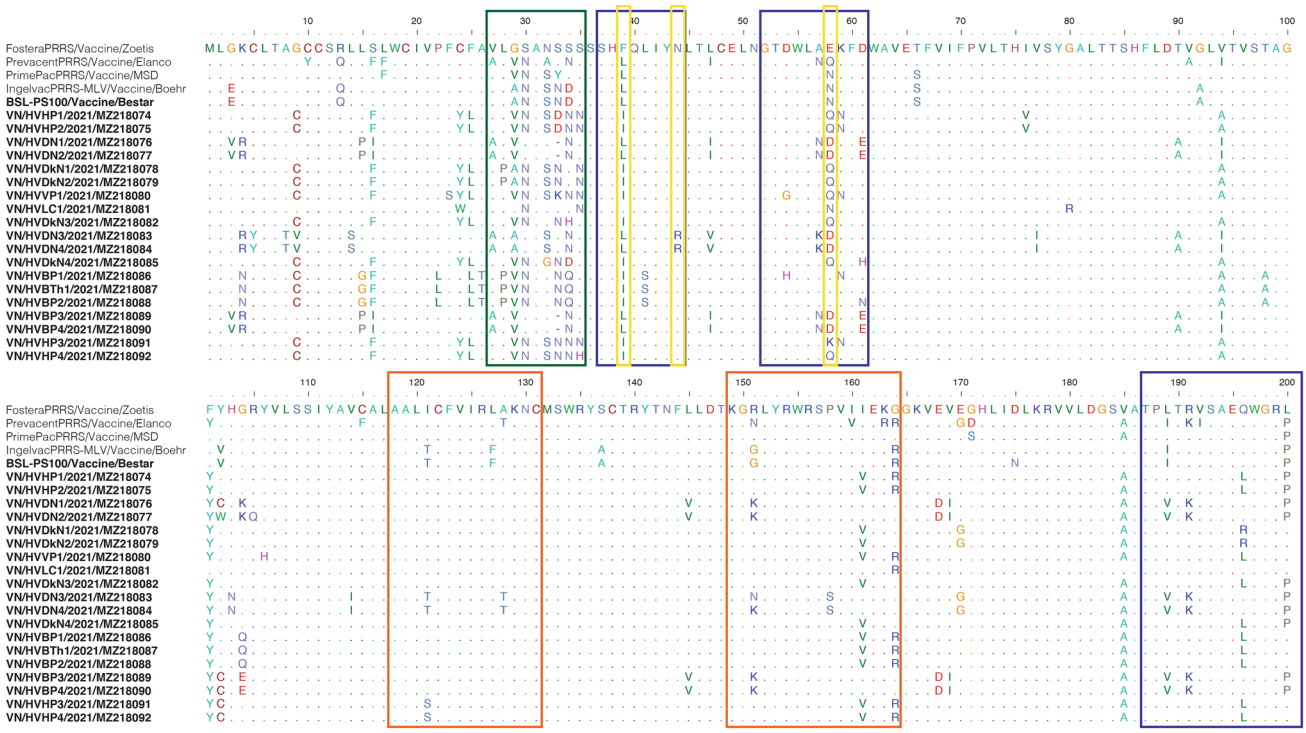

Fig. 2 Amino acid sequences of antigenic regions in GP5 of PRRSV; antigen regions are framed. Amino acid sequence alignments of GP5 from the 19 field isolates and five reference vaccine isolates; dots indicate the amino acid position, letters indicate amino acid changes. The green, blue, and orange boxes indicate the amino acid positions of non-neutralizing, neutralizing and T-cell epitopes, respectively. The yellow box demarcates the position of the important linear epitope

In addition, the six US-PRRSV strains belonged to the NADC-like subtype, lineage 1, sublineage 1.4, sharing 87.5–100% similarity at the nucleotide and 86.0–100% similarity at the amino acid level; and had 84.2–91.5% nucleotide identity and 86.0–91.9% amino acid identity with reference strains belonging to the same sublineage 1.4. Besides, these 6 US-PRRSV strains showed low genetic and amino acid similarity with US-PRRSV isolates belonging to lineage 1 NADC-like subtype (83.7–86.0%, 83.5–91%), MN184-like subtype (84.5–85.7%, 84.5–88.5%), lineage 5 VR-2332 (84.2–84.7%, 83.5–84.0%), and JXA1 strain (83.2–83.9%, 83.5–85.0%) (Table S4). Moreover, they share low sequence identity with the HP-PRRSV vaccine strain (83.2–84.0% and 84.0–85.5% at nucleotide and amino acid levels, respectively) and the six US-PRRSV vaccine strains (83.9–85.4% and 82.5–87.5% at nucleotide and amino acid levels, respectively) (Table S5).

Several substitutions were identified in the important antigenic regions involved in response to early antibody production (amino acid positions 27–35) and neutralizing antibody (amino acid positions 37–45, 52–61, and 187–200) (de Lima et al., 2006; Guo et al., 2019; Ostrowski et al., 2002). Eight amino acid substitutions were found at positions 27–35 (epitope A), which can induce rapid and potent non-neutralizing antibodies. Three amino acid substitutions occurred at positions 37–45 (epitope B), including the antigenic determinant site of this region (amino acid positions 39, 41, and 44). The epitope B is highly conserved and related to broadly neutralizing antibodies (Kwon et al., 2008). The substitution of amino acid 39 in GP5 helps PRRSV field strains escape from the neutralizing immunity of the vaccines (Vashisht et al., 2008). Two changes were found at glycosylation sites (amino acid positions 34 and 44), which play a key role in viral immune evasion (Ansari et al., 2006). Furthermore, five amino acids were substituted at positions 52–61 (epitope C), including amino acid positions 54, 57, 58, 59, and 61. Amino acid substitutions at positions 57 in GP5 resulted in an escape from neutralizing antibodies (Guo et al., 2019). Five amino acids were substituted at positions 187–200, particularly 189, 191, 192, 196, and 200. Amino acid changes were also observed in two T-cell epitopes with five amino acid sites at 117–131 and four amino acid sites at 149–163 (Fig. 2). The amino acid positions 187–200 of GP5 play an important role in the cross-neutralizing response to antibodies formed by both genotypes 1 and 2 (de Lima et al., 2006). Amino acid changes at epitopes A, B, and C in GP5 of field strains may reduce the protective effect of commercial PRRS vaccines against field NADC30-like strains (Guo et al., 2019).

PRRS outbreaks were found in farms implementing routine vaccinations against PRRS. The new PRRSV strains carry new genetic variations and antigenic alterations that may lead to escaping PRRS vaccination-induced immunity. The antigenic changes in the GP5 of the PRRRS strains in Vietnam may provide a compelling explanation for the limitations of current vaccination efforts. Therefore, it will be critical for novel vaccine development to account for these factors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-022-01912-w.

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Author contributions MNN and NHN designed the study. HATT, BNTP, THTL, and TQN performed experiments. MNN, BNTP, DCL, and NHN analyzed the data. MNN, NHN, and DCL wrote the manuscript.

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Data availability The ORF5 sequences of 19 PRRSV strains identified in this study have been deposited in GenBank under the accession numbers MZ869026—MZ869046.

Declarations

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

Ethical approval The study was conducted in compliance with the institutional rules for the care and use of laboratory animals and using a protocol approved by the Ministry of Agriculture and Rural Development (MARD) Vietnam (TCVN 8402:2010).

Consent for publication Not applicable.

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