Original Article

PRRSV genetic variation after the 2010 HP-PRRSV introduction in Thailand

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Abstract

After the first introduction of porcine reproductive and respiratory syndrome virus (PRRSV) into Thailand, Thai type 1 PRRSV has existed in the Thai swine population and caused subclinical diseases while type 2 PRRSV, which most PRRSV isolates belong to, has caused moderate to severe diseases. Highly pathogenic-PRRSV (HP-PRRSV) has recently emerged in Thailand, causing significant damages since its first introduction in 2010. Genetic characterization of Thai PRRSV isolates collected from several clinically affected swine herds distributed in different geographical locations in 2012 was analyzed using a comparative analysis between 2 hyper-variable regions, NSP2 and ORF5. Eleven selected PRRSV clinical samples collected from 11 clinically affected swine herds distributed in 10 provinces located in 4 regions of Thailand were sequenced. Partial NSP2 and ORF5 sequences of the virus revealed that the studied isolates were Thai type 2 PRRSV and could be separated into 2 groups, Thai HP-PRRSV and previous Thai type 2 PRRSV which had been circulating before the HP-PRRSV introduction in 2010. The Thai HP-PRRSV contained 30 deduced amino acids in 2 positions, which is the genetic characteristic of HP-PRRSV prototypes, and might be derived from the isolates introduced in 2010 or introduced later from neighboring countries based on a phylogenetic analysis. However, the classical Thai type 2 PRRSV isolate found in this study was also able to cause severe clinical diseases in the Thai swine herds. Mutations in some positions of both NSP2 and ORF5 genes naturally occurred; NSP2 and ORF5 are often used as genetic markers in terms of PRRSV evolution. In conclusion, after the introduction of HP-PRRSV, major PRRSV outbreaks in Thailand were mostly caused by HP-PRRSV.

Keywords: genetic variation, NSP2, ORF5, porcine reproductive and respiratory syndrome virus (PRRSV)

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the major swine viral diseases having a major impact on the swine industry. The virus gained its entry into Thailand in 1989 and several outbreaks have been reported since then (Oraveerakul, 1995; Damrongwatanapokin, 1996). The virus is divided into 2 types, type 1 (European type) and type 2 (North American type; NA), according to their genetics and origins. Both type 1 and type 2 PRRSV have been isolated and comprehensively investigated previously in Thailand (Thanawongnuwech et al., 2004; Amonsin et al., 2009; Jantafong et al., 2015).

In 2006, the first highly pathogenic PRRSV (HP-PRRSV) outbreak caused severe damages in most swine herds with extremely high morbidity (50-100%) and mortality (20-100%) in China (Tong et al., 2007). More than 2,000,000 pigs were infected and 243,000 pigs died in the outbreak year (Tian et al., 2007; Xiao et al., 2010). Thereafter, HP-PRRSV was transmitted into China's neighboring countries including Vietnam in 2008, affecting up to 65,000 pigs (Feng et al., 2008), and Thailand in 2010 (Nuntawan Na Ayudhya, 2011). Multiple HP-PRRSV outbreaks in Thailand were reported in August 2010 with more than 30% loss in affected herds (Nuntawan Na Ayudhya, 2011). The introduction of the novel PRRSV strains has caused major variation in the genetic characteristics of PRRSV isolates in Thailand. Chaikhumwang et al. (2015) reported that HP-PRRSV influenced the evolution of existed type 2 PRRSV clusters and might also recombine with previous Thai PRRSV isolates circulating before the HP-PRRSV outbreaks.

The first genetic diversity of Thai PRRSV was found in 2004 and it was reported that type 1 PRRSV was more prevalent than type 2 PRRSV (Thanawongnuwech et al., 2004). Thereafter, by studying Thai PRRSV isolates collected between 2000 and 2008, Tun et al. (2010) demonstrated that multiple introductions were originated from at least 3 independent introductions. Co-circulation of the diverse PRRSV strains in some areas, especially in 3 major Thai swine producing provinces including Chonburi, Ratchaburi and Nakhonpathom, was revealed (Tun et al., 2011). According to a phylogenetic tree generated by worldwide type 2 isolates, Thai isolates are divided into 3 clusters within lineage 1, lineage 5, and the orphan cluster related to lineages 6-9 (Shi et al., 2010). Likewise, a recent study has shown that type 2 PRRSV collected from Thai swine population and other Southeast Asian countries is clustered in lineages 1 and 5 (sublineages 5.1, 5.2) and 8.7. The isolates within lineage 8.7 have predominantly been distributed in Thailand after the 2010 HP-PRRSV outbreaks and can be divided into 2 groups, classical North American (NA) and HP-PRRSV (Jantafong et al., 2015).

Since up to four strains of the Chinese HP-PRRSV were introduced into Thailand in 2010 (Tun et al., 2011; Jantafong et al., 2015; Nilubol et al., 2012), the Clinical Practice Guidelines (CPG) for PRRS in Thailand: 3rd Revision (3rd CPG) was revised by the Thai Swine Veterinary Association (TSVA) for swine practitioners to effectively deal with severe outbreaks. According to the guidelines, RT-PCR and specific nucleotide sequencing are used as major diagnostic techniques for HP-PRRSV detection. Genetic information of PRRSV causing clinical disease in swine herds is also useful in disease investigation and control. PRRSV isolates from different geographic distribution frequently contain different genotypic characteristics (Yoshii et al., 2005). Therefore, specific genetic characteristics of novel PRRSV in hyper-variable regions, NSP2 and ORF5, are beneficial for epidemiological investigation.

The first study of NSP2 genetic variation of Thai PRRSV was reported in 2010 and discovered that 90% of the Thai PRRSV isolates were NSP2-truncated viruses that might self-evolve from viruses previously introduced in the past before the introduction of the highly pathogenic PRRSV (HP-PRRSV) from China in 2010 (Kedkovid et al., 2010). A study of 5 selected pig farms in 2012 confirmed the prevalence of HP-PRRSV in Thailand. NSP2 sequences obtained from collected PRRSV isolates represented unique characteristics of HP-PRRSV, 30 deduced amino acids, forming a hypothesis that the virus spread in Thailand was possibly introduced from Vietnam and Lao PDR (Nilubol et al., 2012). Moreover, Jantafong and Lekcharoensuk (2014) reported that one Thai PRRSV isolated from Nong Khai province in 2010 had 30 amino acid deletions in common with the BH58/10 isolate, a HP-PRRSV from Lao PDR, supporting the transboundary transmission hypothesis. However, the genetic characteristics of Thai PRRSV causing clinical problems in Thai swine herds are still unclear. This missing information is useful for comparative analysis of recent Thai PRRSV genetic changes after the introduction of the HP-PRRSV strain.

ORF5 gene, coded GP5 protein, known as the most variable structural protein gene is also the most critical structural protein for development of neutralizing antibodies in infected pigs. Variations of both NSP2 and ORF5 sequences of the Chinese HP-PRRSV isolates were compared and suggested that the 2 genes were the most variable non-structural and structural protein genes of PRRSV (Tong et al., 2007). Therefore, a comparative study of both NSP2 and ORF5 of Thai PRRSV after the HP-PRRSV outbreak would be helpful in terms of molecular epidemiology. The primary objectives of this study were to obtain genetic sequences of both NSP2 and ORF5 genes and to demonstrate genetic characteristics of Thai PRRSV using clinical samples in 2012.

Materials and Methods

Sample collection and processing: With the collaboration of 4 veterinary diagnostic laboratories including Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL), Large animal hospital laboratory and Department of Veterinary Pathology, Khon Kaen University-Veterinary Diagnostic Laboratory (KKU-VDL) and allied field veterinarians working in high risk areas, serum, tissue and oral fluid samples from clinical cases of PRRSV-infected pigs were submitted from February to October, 2012. All samples were collected from

suspected PRRS clinical cases and tested by standard RT-PCR to determine PRRSV infection, and also differentiated as type 1 or type 2 PRRSV. Data including farm information, location and severity of the outbreaks of infected herds were recorded.

The serum samples were stored at -80°C until used. The tissue samples of each case were homogenized with beads and minimum essential medium eagle (MEM). Then, the mixture was clarified by 1750 × *g* centrifugation for 20 minutes. Supernatant was collected and cell debris was filtered using a 0.45 µm syringe-driven filter and stored at -80°C until tested. Five submitted samples from the same source were pooled into 1 testing sample as the standard procedure of a diagnostic laboratory.

Sample screening: NucleoSpin® Extract Viral RNA Kit (Macherey-Nagel, Düren, Germany) was used based on the manufacturer's recommended method to extract viral RNA from the processed samples. In brief, 150 µl of each sample was centrifuged. Supernatant was collected and mixed with lysis buffer and carrier RNA. After mixing and incubating, binding solution was added to bind the RNA and washing buffer was added to remove residual elements. Finally, viral RNA was eluted by 70°C nuclease free water for a final volume of 50 µl and the extracted RNA was stored at -80°C until used. The extracted RNA was synthesized to be cDNA by GoScript™ Reverse Transcription System (Promega, USA) as recommended by the manufacturer.

All submitted samples were, then, tested for PRRSV specific nucleotide using standard procedure of veterinary diagnostic laboratories. Positive PRRSV samples with clear clinical information were selected and included in this study.

Reverse transcriptase polymerase chain reaction, amplification of NSP2 gene and ORF5 sequences: Fragments of PRRSV NSP2 and ORF5 genes were amplified by RT-PCR as described elsewhere (Feng et al., 2008; Hao et al., 2011). Five micro-liters of the extracted RNA was mixed with a reaction mixture of 1µM/µl forward and reverse primers, 10µl of 2x AccessQuick® Master Mix (Promega, USA) and 1 µl of AMV Reverse-Transcriptase (Promega, USA). Nuclease-free water was added to make up a final volume of 25 µl.

The RT-PCR products were separated in 1.5% agarose gel electrophoresis. Gels were immersed in Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and visualized under a UV transilluminator. The expected band of PCR product was cut and purified with NucleoSpin® Extract II (Macherey-Nagel, Düren, Germany) following the manufacturer's recommended protocol.

PRRSV nucleotide sequencing and phylogenetic analysis: Sequences of partial NSP2 and ORF5 obtained from different herds in different geographical regions of Thailand were investigated. DNA sequencing was carried out by 1st BASE Company (Singapore) with primers used in the previous RT-PCR reaction. Sequences were edited and analyzed using BioEdit® version 7.1.9 (Ibis Biosciences, Carlsbad, CA). Whole PRRSV genome sequences from the Thai PRRSV isolates were analyzed with other selected sequences available in GenBank (http://www.ncbi.nlm.nih.gov/). Phylogenetic trees were constructed by MEGA5® (Tamura et al., 2007) using the neighbor-joining method with 1500 bootstrapping replicates.

Partial NSP2 sequence (2520 to 2588 of ORF1a) and ORF5 were targeted for NSP2 and GP5 amino acid analysis. The sequences were confirmed as PRRSV NSP2 or ORF 5 gene sequences using Blast in NCBI database. Identical analyses of NSP2 and ORF5 were analyzed by comparing with representative PRRSV isolates of each lineage or sublineage as described elsewhere by Jantafong et al. (2015).

The sequences were aligned, and a partial NSP2 phylogenetic tree was then constructed with other NSP2 genes of important PRRSV prototypes, vaccine isolates, current and past isolates of Thai PRRSV, and HP-PRRSV related isolates (S). A GP5 phylogenetic tree was constructed with other PRRSV sequences as described by Jantafong et al. (2015) to demonstrate allocation of each sequence comparable to previous reports (Supplementary Table 3).

Results

A total of 367 samples from 39 commercial swine farms were submitted to the 4 veterinary diagnostic laboratories and field veterinarians during February to October 2012. All clinical samples including serum and tissue samples were taken from suspected PRRSV-infected herds with significantly severe clinical signs such as reproductive failure and respiratory diseases. The samples from 33 swine farms were type 2 PRRSV positive using PRRSV specific RT-PCR, and none of the studied farms were type 1 PRRSV positive. The remaining 6 farms were affected with non-PRRSV related diseases. Seventeen PRRSV positive samples collected from the 33 farms with known history and in different locations were selected. These swine farms were located in 11 provinces in 4 different regions of Thailand including north-eastern (Surin), western (Tak, Kanchanaburi and Ratchaburi), eastern (Prachinburi and Chantaburi) and central (Lopburi, Singhburi, Nakhonnayok, Nakhonpathom and Suphanburi) regions. Eleven samples were sequenced successfully as 12SURIN01, 12TAK01, 12RB01, 12KB01, 12LB01, 12SHB01, 12NN01, 12NP01, 12NP02, 12PJB01 and 12CHTB01 (Figure 1). The others were not successfully sequenced up to 3 times and were excluded from the study.

As shown in Figure 2, HP-PRRSV characteristic of 30 amino acid deletion at positions 869 and 921 to 949 of NSP2 was found in 10 sequenced samples including 12SURIN01, 12TAK01, 12RB01, 12KB01, 12SHB01, 12NN01, 12NP01, 12NP02, 12PJB01 and 12CHTB01. Only 12LB01 showed full NSP2 amino acid sequence without deletion.

The partial NSP2 phylogenetic tree showed that the PRRSV type 2 isolates were divided into two groups composed of HP-PRRSV related and classical NA-PRRSV isolates (Figure 3). Most of the studied sequences were allocated into the HP-PRRSV isolated group, whereas only 12LB01 was allocated into the

classical NA-PRRSV group.

NSP2 amino acid identity among the studied Thai PRRSV sequences varied between 40.4% and 100%. The studied Thai HP-PRRSV was closely related to the HP-PRRSV in China such as 09HEN1, SX2009 and JXA1, which is HP-PRRSV vaccine isolate (Jantafong et al., 2015). In contrast, 12LB01 was 77.6% related to Ingelvac MLV PRRS vaccine (Key et al., 2003) and shared its genetic similarity of 80.1% to Classical North America PRRSV (Table 1).

Similar to a previous report, the ORF5 phylogenetic tree was divided into 9 lineages with 3 major groups of HP-PRRSV related, vaccine-like and classical NA PRRSV. Most of the studied Thai PRRSV sequences were categorized into the HP-PRRSV related group (lineage 8.7/HP) except for 12LB01 located in the group of local Thai type 2 PRRSV (lineage 1) (Figure 4).

Table 1 Distance analysis of NSP2 amino acid sequences among the studied Thai PRRSV and the reference PRRSV isolates

Genotype	Type I	Type II						
Lineage/Sublineage /Clade	A	1	5	8.7 Classical NA-PRRSV	8.7 HP-PRRSV			9
Isolates	Lelystad	01NP1.2	VR2332	CH-1R	SX2009	JXA-1	09HEN1	Ingelvac ATP MLV
12CHTB01	0.16	0.41	0.40	0.61	0.91	0.93	0.98	0.54
12KB01	0.16	0.40	0.39	0.60	0.90	0.92	0.98	0.54
12LB01	0.16	0.65	0.64	0.80	0.50	0.51	0.50	0.78
12NN01	0.16	0.38	0.37	0.57	0.86	0.88	0.92	0.50
12NP01	0.16	0.38	0.37	0.57	0.85	0.87	0.91	0.51
12NP02	0.16	0.40	0.39	0.59	0.88	0.90	0.95	0.51
12PJB01	0.16	0.40	0.39	0.60	0.88	0.90	0.95	0.53
12RB01	0.16	0.40	0.39	0.60	0.90	0.92	0.97	0.54
12SHB01	0.16	0.40	0.39	0.60	0.91	0.93	0.89	0.53
12SURIN01	0.16	0.38	0.37	0.58	0.88	0.90	0.95	0.52
12TAK01	0.16	0.40	0.40	0.61	0.92	0.95	0.90	0.55

Table 2 Distance analysis of ORF5 amino acid sequences among the studied Thai PRRSV and the reference PRRSV isolates

Genotype	Type I	Type II						
Lineage/Sublineage /Clade	A	1	5	8.7 Classical NA-PRRSV	8.7 HP-PRRSV			9
Isolates	Lelystad t	01NP1	VR2332	CH-1R	SX2009	JXA-1	09HEN1	Ingelvac ATP MLV
12CHTB01	0.41	0.62	0.63	0.64	0.70	0.69	0.71	0.62
12KB01	0.41	0.58	0.59	0.62	0.66	0.6	0.65	0.61
12LB01	0.41	0.67	0.59	0.61	0.62	0.620	0.62	0.61
12NN01	0.41	0.63	0.64	0.64	0.71	0.70	0.71	0.63
12NP01	0.41	0.63	0.64	0.64	0.71	0. 70	0.71	0.63
12NP02	0.41	0.62	0.63	0.63	0.70	0.69	0.70	0.62
12PJB01	0.41	0.62	0.63	0.63	0.70	0.69	0.70	0.62
12RB01	0.39	0.59	0.61	0.62	0.67	0.68	0.66	0.61
12SHB01	0.40	0.61	0.62	0.64	0.67	0.68	0.67	0.63
12SURIN01	0.41	0.62	0.63	0.64	0.70	0.69	0.70	0.62
12TAK01	0.40	0.60	0.62	0.64	0.68	0.69	0.68	0.62

The GP5 amino acid sequences' identity results were also similar to the results of NSP2 gene sequences. The GP5 amino acid homology among the 11 studied sequences was between 40-100%. All studied HP-PRRSV related sequences showed high genetic similarity (59-63.5%) to VR2332, a type 2 PRRSV prototype, and shared similarity of 61.5-70.5% to SX2009 and 09HEN1. Compared to other PRRSV vaccine strains, GP5 amino acid sequences of the studied Thai PRRSV shared genetic similarity to the commercial type 2 PRRSV vaccine isolates, Ingelvac ATP, between 60.5-62.5%. However, the highest genetic similarity between the studied Thai PRRSV sequences and the Chinese HP-PRRSV vaccine isolates was with JXA1 (66.5-69.5%) (Table 2). In the signal peptide domain, 6 amino acid mutations were found. In the transmembrane region, 1, 2 and 7 amino acid substitutions were found in transmembrane protein 1, transmembrane protein 2 and transmembrane protein 3, respectively. In the ectodomain, 15 amino acid mutations were found with 2 asparagine (N) and changed in positions 44 and 58, and new asparagine substitutions were found separately in 3 positions in this region, which might affect the glycosylation sites of GP5 (Figure 5).

Discussion

A total of 11 PRRSV samples were successfully sequenced from 33 PRRSV RT-PCR positive samples obtained from this study. Due to the instability of RT-PCR and sequencing processes, 22 clinical samples were not able to be sequenced and were excluded from the phylogenetic analyses. The analyses revealed that ten of the studied Thai type 2 PRRSV (12CHTB01, 12KB01, 12NN01, 12NP01, 12NP02, 12PJB01, 12SHB01, 12RB01, 12SURIN01 and 12TAK01) were closely related to the HP-PRRSV isolates found in China, Vietnam, and Thailand in 2010. These 10 studied sequences showed nucleotide

variation and were located in different locations in the phylogenetic tree. Therefore, the studied Thai HP-PRRSV isolates might be introduced at different Thai borders after the first outbreak in 2010 or might have evolved from different HP-PRRSV isolates. In contrast, only one sequence, 12LB01, was closely related to some Thai type 2 isolates collected in 2007-2008 and classical type 2 PRRSV, e.g. VR2332 and 01NP1.2. It should be noted that this particular isolate could cause PRRS clinical signs similar to HP-PRRSV.

Figure 1 Geographic distributions of collected PRRSV in Thailand in this study. The triangle indicates the location of the first isolated Thai HP-PRRSV. The shaded areas represent the provinces where clinical samples were collected and the 2012 PRRSV sequenced. CHTB, Chantaburi; KB, Kanchanaburi; LB, Lopburi; NN, Nakhonnayok; NP, Nakhonpathom; PJB, Prachinburi; SHB, Singhaburi; RB, Ratchaburi; SURIN, Surin; TAK, Tak.

According to the partial NSP2 gene phylogenetic analysis, some Thai type 2 PRRSV might originate from a vaccine virus or its progeny since the primary Thai type 2 PRRSV isolates, 01NP1.2 and 01NP1, were closely related to Ingelvac PRRS strain, which has been used as a commercially available vaccine. The Thai type 2 PRRSV isolates collected between 2007 and 2008 were also grouped in the NA-PRRSV related group, close to the 01NP1.2 isolate, but showed some deduced amino acids in NSP2 genes (Kedkovid et al., 2010). Both Thai type 2 PRRSV isolated before the HP-PRRSV outbreaks and HP-PRRSV groups isolated in 2012 indicate that both groups have dominated and persisted in the Thai swine herds. The results indicate that the introduction of HP-PRRSV into Thailand in 2010 has caused higher NSP2 gene variation of the Thai type 2 PRRSV population similar to previous studies (Jantafong and Lekcharoensuk, 2014; Jantafong et al., 2015).

GP5 is considered as one of the important

targets for analyzing the genetic divergence of PRRSV. The protein size is around 30-aa with N-terminal putative signal sequence containing potential N-glycosylation sites (Mardassi et al., 1995; Meng et al., 1995; Han et al., 2006). The proteins were demonstrated as one of the most variable structural proteins among Thai PRRSV isolates (Nilubol et al., 2013; Nilubol et al., 2014).

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                  845        855        865        875        885        895
                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
VR2332            PVPAPRRKVG SDCGSPVSLG GDVSNSWEDL AVSSPFDLPT PPEPATPSSE LVIVSSPQCI
12CHTB01          -----?RKVR SDCGSPVLMG DNVPNGSE-? TVGGPLNFPT PSELMTPMSE PVLVPASQFV
12KB01            ------?KVR SDCGSPVLMG DNVPNGSE-E TVGGPLNFPA PSELMTPMSE PALVPASQFV
12NN01            -------?VR SDCGSPVLMG DNVPNGSE-E TVGGPLNFPA PSELMTPMSE SVLMPASQFV
12NP01            ------?KVR SDCGGPVLIG DNVPSGSE-R TVGGPLNFPT PSKLMTPMSE PALVPASQFV
12NP02            -------?VR SDCGSPVLMG DNVPNGSE-E TVGGPLNFPA PSELMTPMSE SVLMPASQFV
12PJB01           -----?RKVR SDCGSPVLMG DNVPNDSE-? TVGGPLNFPT PSELMTPMSG PVLMPASQFV
12RB01            ------?KVR SDCGSPVLMG DNVPNGSE-E TVGGPLNFPA PSELMTPMSE PALVPASQFV
12SHB01           ------?KVR SDCGSPVLMG NNVPNGSE-K TVGGPLNFPT PSEPMTPMSE PVLMPASRRA
12SURIN01         ------RKVR SDCGSPVLMG DNVPNGSE-E TVGGPLNFPA PSELMAPMSE PALVPASQFV
12TAK01           ------?KVR SDCGSPVLMG DNVPNGCE-K TVGGPLNFPT PSEPMTPMSE PVLMPASRRA
12LB01            ------RKIR SDCGSSILLG DNVPNSWEDL TVGGPLDLPA PPEPVTPPRE LAPMPAPQHI

                  905        915        925        935        945        955
                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
VR2332            FRPATPLSEP APIPAPRGTV SRPVTPLSEP IPVPAPRRKF QQVKRLSSAA AIPPYQNEPL
12CHTB01          PTLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12KB01            PTLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12NN01            PKLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTPTHQDEPL
12NP01            PTLITPLIGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12NP02            PKLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTPTHQDEPL
12PJB01           PKLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TALTHQDEPL
12RB01            PTLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12SHB01           PKLMTPLSGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12SURIN01         PTLMTPLIGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12TAK01           PKLMTPLSGS APVPAPRRTV ---------- ---------- ----------T TTLTHQDEPL
12LB01            FRPVTPLSEP APVPAPRRTV FRPMTSLSEP ILVSAPRHKF QQVEKANLAT TTLTHQDEPL

                  965        975        985        995        1005       1015
                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
VR2332            DLSASSQTEH EASPPAPPQS GGVPGVEGHE AEETLSEISD MSGNIKPASV SSSSSLSSVR
12CHTB01          DLSASSQT?- ---------- ---------- ---------- ---------- ----------
12KB01            DLSASSQT?- ---------- ---------- ---------- ---------- ----------
12NN01            DLSASS---- ---------- ---------- ---------- ---------- ----------
12NP01            DLSASSQT-- ---------- ---------- ---------- ---------- ----------
12NP02            DLSASSQT?- ---------- ---------- ---------- ---------- ----------
12PJB01           DLSASSQT-- ---------- ---------- ---------- ---------- ----------
12RB01            DLSASSQT?- ---------- ---------- ---------- ---------- ----------
12SHB01           DLSASS---- ---------- ---------- ---------- ---------- ----------
12SURIN01         DLSASSQ?-- ---------- ---------- ---------- ---------- ----------
12TAK01           DLSASSQ--- ---------- ---------- ---------- ---------- ----------
12LB01            DLSASSQT-- ---------- ---------- ---------- ---------- ----------
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Figure 2 Partial NSP2 gene nucleotide sequence alignment of the studied Thai PRRSV isolates. HP-PRRSV genetic characteristics, 1 and 29 deduced amino acids, labeled in boxes.

The GP5 phylogenetic tree showed the same results as those of the NSP2 phylogenetic tree. Ten of the studied Thai type 2 PRRSV (12CHTB01, 12KB01, 12NN01, 12NP01, 12NP02, 12PJB01, 12SHB01, 12RB01, 12SURIN01 and 12TAK01) were closely related to HP-PRRSV isolates collected in Thailand and neighboring countries including Lao PDR, Cambodia and Vietnam between 2008 to 2012. These 10 sequences were divided into clades A and B of lineage 8.7, closely related to other Thai type 2 PRRSV isolates reported by Jantafong et al. (2015), supporting the hypothesis that pig flows between countries might play a role in the introduction of a novel PRRSV strain into the population (Dietze et al., 2011; Nilubol et al., 2012; Jantafong et al., 2015). Moreover, one sequence, 12LB01, was allocated in lineage 1, closely related to the classical Thai type 2 prototypes, 01NP1.2, and other type 2 isolates collected between 2008 to 2012 (Jantafong et al., 2015).

It should be noted that the samples were pooled (1 to 5) for isolation in the same farm and, then, for gene sequencing to increase the possibility of isolation. Mixed strains from the samples could occur but no evidence of mixed strains was found when analyzing the sequences. Additionally, the samples

collected in this study were the representatives of PRRSV clinical cases affecting swine herds during the studied period. Data of the affected farms revealed that morbidity varied between 30-100%. This supports the previous report by Nilubol et al. (2012) that severity of the disease was relatively milder than the first outbreak due to natural adaptation of the virus. On the other hand, in a Thai NA-PRRSV-infected farm that had 12LB01, the morbidity and mortality rates were as high as 100% and 30%, respectively.

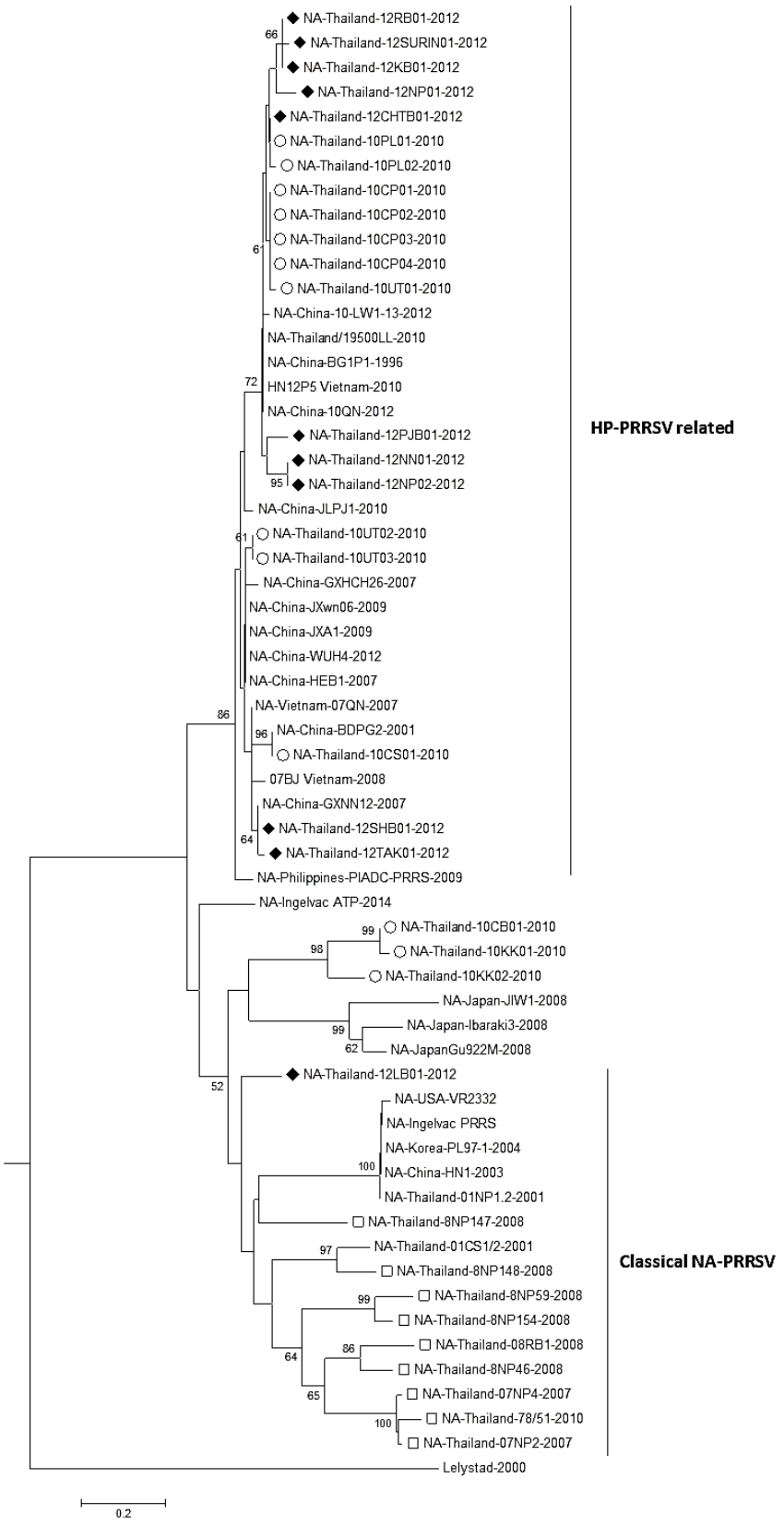

Figure 3 Phylogenetic tree based on partial NSP2 gene sequences. Markers are labeled depending on timing of each sequence; the solid diamonds are the studied Thai PRRSV isolates, the circles are 2010-2011 Thai PRRSV isolates, and the squares are 2007 and 2008 Thai type 2 PRRSV isolates.

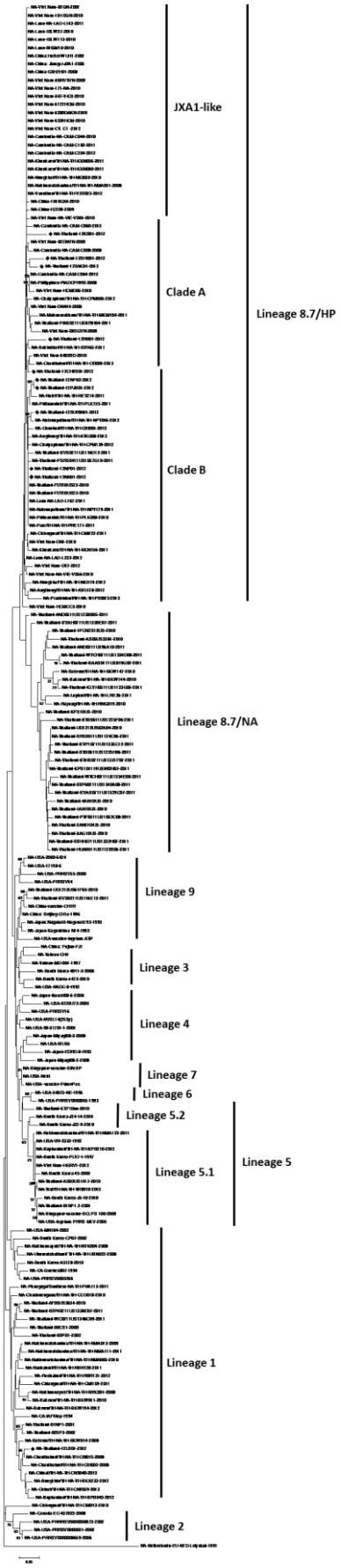

Figure 4 Phylogenetic tree based on partial NSP2 gene sequences. The studied sequenced are labeled with solid diamonds. Classification of PRRSV isolates is based on the phylogenetic tree reported by Jumtafong et al. (2015).

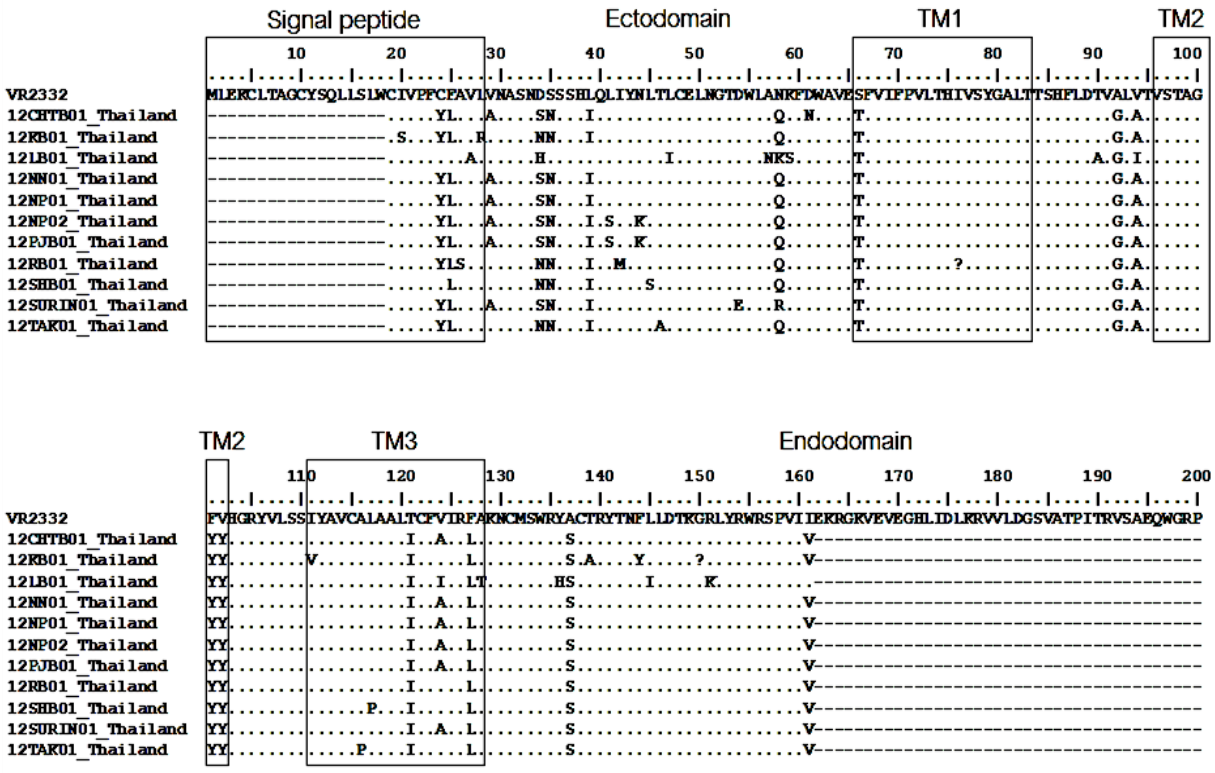

Figure 5 Phylogenetic tree based on partial NSP2 gene sequences. The studied sequenced are labeled with solid diamonds. Classification of PRRSV isolates is based on the phylogenetic tree reported by Jumtafong et al. (2015).

Important correlation between PRRSV isolates, 12NP01 and 12NP02, from 2 farms was revealed. Both farms were located in Nakhonpathom province and the farm having 12NP01 introduced boars from the farm having 12NP02. This might be an important route of the virus transmission among farms. The amino acid sequences of both viruses shared 86.3% and 98.5% similarity in the partial NSP2 and ORF5 genes, respectively.

The PRRSV negative herds where 12CHTB01, 12PJB01, 12SHB01 and 12TAK01 isolates were collected had never used MLV PRRSV vaccines previously. It should be noted that Ingelvac MLV PRRS vaccine was used in the farms having 12NP01 and 12RB01 isolates and also used in sow herds providing growing pigs to the farms having 12LB01 and 12KB01. Based on the outbreaks in these herds, the PRRSV vaccine application incompletely prevented the HP-PRRSV clinical signs during the studied time. Induced specific immunity could only reduce the clinical signs but could not prevent infection of the virus in different genetic backgrounds.

The amino acid comparison between GP5 amino acid sequences of the studied Thai PRRSV and VR2332 revealed many amino acid substitutions in the signal peptide domain, ectodomain, endodomain and transmembrane domains 1-3. The mutations in the glycosylation site of the ectodomain and the attachment site of the PRRSV specific neutralizing antibody are of interest. Asparagine substitution of the ectodomain was found in position 44(N→K) of 12NP02 and 12PJB02, and position 58 of all sequences (N→Q of 12CHTB01, 12KB01, 12NN01, 12NP01, 12NP02, 12PJB01, 12RB01, 12SHB01 and 12TAK01, and N→R of 12SURIN01). Moreover, Asparagine substitutions were found in position 34(D→N) of 12KB01, 12RB01, 12SHB01 and 12TAK01, and position 35(S→N) of all Thai HP-PRRSV sequences. For 12LB01, one asparagine was changed in position 57(A→N) as well as in position 61(D→N) of 12CHTB01. Similar to the HP-PRRSV related sequences, asparagine substitution of the ectodomain was also found in position 44(N→K) of 12LB01. These mutations in the potential glycosylation sites might affect the neutralizing antibody induced by the infection. Pigs vaccinated with the MLV vaccine may induce PRRSV specific neutralizing antibody not matching the ectodomain of the circulating isolates. However, effectiveness of vaccination is influenced by many factors and unable to predict its genetic sequence. Additional information is needed to evaluate vaccine efficacy since not only Ingelvac PRRS vaccine is authorized in Thailand but other commercial vaccines are also available. Recently, at least 2 type 1 PRRSV MLV vaccines and 3 type 2 PRRSV MLV vaccines have been commercially available in Thailand and the future genetic characteristics of Thai PRRSV could be different from this present study.

In 2013-2014, a novel type 2 PRRSV was again reported in China (NADC30-like strain). The virus caused abortion and stillbirth in sows and highly respiratory disorder to fatality in piglets in 7 intensive farms from five provinces (Zhou et al., 2015). The genome of the virus was the recombination of the NADC30 strain from North America (2008) with the classical HP PRRSV in China via the import of US breeding pigs to China (Zhao et al., 2015). This incidence demonstrates that PRRSV still undergoes

mutation and causes severe disease in naïve pig population. In Thailand, most novel PRRSV strains usually come from the import of pigs from neighboring countries and the usage of modified live vaccines (Nilubol et al., 2012; Jantafong et al., 2015). This novel virus should be monitored and prevented particularly at the Thai borders. In addition, biosecurity should be applied strictly and routine PRRSV surveillance program should be carried on in intensive pig raising areas.

Thai type 1 PRRSV might exist in the subclinical herds in this study, although most of the clinical PRRSV sequences obtained in this study were type 2 PRRSV. This concur with the retrospective study of samples submitted to Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VLD) between 2005 and 2010, which demonstrated that the prevalence of PRRSV in Thailand was 32.6% including type 2, type 1 and mixed type PRRSV (54.5%, 31.0%, and 14.5%, respectively) (Tummaruk et al., 2013). Type 2 PRRSV was found in both MLV-vaccinated herds and non-vaccinated herds. This indicates that vaccine-induced antibody responses might not be sufficient to provide full protection (Thanawongnuwech and Suradhat, 2010).

Ten Thai type 2 PRRSV sequences contained 30 deduced amino acids in 2 positions, which is the genetic characteristic of the Chinese HP-PRRSV prototype, whereas the others contained no deduced amino acid and shared high similarity to other type 2 PRRSV collected from Phitsanulok and Nongkhai in 2010.

In conclusion, based on both partial NSP2 and ORF5 genes, the studied Thai type 2 PRRSV were categorized into 2 groups, the HP-PRRSV related group and the classical Thai type 2 PRRSV group. This indicates that genetic variation of Thai PRRSV might be caused by self-evolution, derivation of the HP-PRRSV isolates, multiple introduction in 2010 or later introduction from neighboring countries. The mutations in several positions of both NSP2 and ORF5 genes might have a negative impact on the vaccine efficacy, especially, after many PRRSV MLV strains have been introduced and heavily used in Thailand.

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Supplementary data

Table 3 Reference PRRSV isolates for partial NSP2 and ORF5 sequence comparison

Gene	References isolates	Location	Year	GenBank accession number
NSP2	12LB01	Thailand	2012	KT289367
	12CHTB01	Thailand	2012	KT289368
	12KB01	Thailand	2012	KT289369
	12NN01	Thailand	2012	KT289370
	12NP01	Thailand	2012	KT289371
	12NP02	Thailand	2012	KT289372
	12PJB01	Thailand	2012	KT289373
	12RB01	Thailand	2012	KT289374
	12SHB01	Thailand	2012	KT289375
	12SURIN01	Thailand	2012	KT289376
	12TAK01	Thailand	2012	KT289377
	HN1	China	2003	AY457635.1
	JXA1	China	2007	EF112445.1
	HEB1	China	2007	EF112447.1
	GXNN12	China	2007	JX046237.1
	07BJ	China	2007	FJ393459.1
	GXHCH26-2007	China	2007	JX046226.1
	JXwn06	China	2009	EF641008.1
	JLPJ1	China	2010	HM232822.1
	WUH4	China	2011	JQ326271.1
	Gu922M	Japan	1992	AB288111.1
	Ibaraki3	Japan	1993	AB288113.1
	JIW1	Japan	2000	AB288126.1
	PIADC-PRRS	Philippines	2008	FJ641193.1
	PL97-1	South Korea	1997	AY585241.1
	01NP1.2	Thailand	2001	EF153486.1
	07NP4	Thailand	2007	HM134183.1
	78/51	Thailand	2007	HM134186.1
	07NP2	Thailand	2007	HM134182.1
	8NP148	Thailand	2008	HM134189.1
	8NP59	Thailand	2008	HM134187.1
	8NP154	Thailand	2008	HM134185.1
	08RB1	Thailand	2008	HM134184.1
	8NP46	Thailand	2008	HM134191.1
	8NP147	Thailand	2008	HM134190.1
	01CS1/2	Thailand	2010	HM134188.1
	10PL01	Thailand	2010	NA
	10PL02	Thailand	2010	NA
	10CP01	Thailand	2010	NA
	10CP02	Thailand	2010	NA
	10CP03	Thailand	2010	NA
	10CP04	Thailand	2010	NA
	10UT01	Thailand	2010	NA

	10UT02	Thailand	2010	NA
	10UT03	Thailand	2010	NA
	10CS01	Thailand	2010	NA
	10CB01	Thailand	2010	NA
	10KK01	Thailand	2010	NA
	10KK02	Thailand	2010	NA
	Lelystad	Netherlands	2001	M96262
	VR2332	USA	1992	EF536003
	Ingelvac PRRS	USA	2001	AF303357.1
	Ingelvac ATP	USA	2006	EF532801.1
	07QN	Vietnam	2007	FJ394029
	BG1P1	Vietnam	2010	HQ538597.1
	HN12P5	Vietnam	2010	HQ538598.1
	BDPG2	Vietnam	2010	HQ538611.1
ORF5[a]	12CHTB01	Thailand	2012	KT289356
	12LB01	Thailand	2012	KT289357
	12SURIN01	Thailand	2012	KT289358
	12NP02	Thailand	2012	KT289359
	12NP01	Thailand	2012	KT289360
	12NN01	Thailand	2012	KT289361
	12PJB01	Thailand	2012	KT289362
	12SHB01	Thailand	2012	KT289363
	12TAK01	Thailand	2012	KT289364
	12RB01	Thailand	2012	KT289365
	12KB01	Thailand	2012	KT289366
	Quebec807	Canada	1994	Z82995
	IAFKlop	Canada	1994	U64928
	PRRSV0003766	USA	-	DQ477786
	MN184	USA	2002	EF442777
	CP07	South Korea	2007	FJ972727
	A5128	South Korea	2010	JF681193
	00CS1	Thailand	2000	AY297111
	01NP1	Thailand	2001	AY297112
	02SP2	Thailand	2002	AY297117
	02PB1	Thailand	2002	AY297116
	AF05US3634	Thailand	2010	JQ040794
	WC0711US1346C09	Thailand	2011	JN848710
	STP40711US1338E07	Thailand	2011	JN848701
	NA-TH-CBI002-2008	Chanthaburi/TH	2008	KF698675
	NA-TH-CBI015-2008	Chanthaburi/TH	2008	KF698676
	NA-TH-CMI013-2010	Chiangmai/TH	2010	KF698677
	NA-TH-PNA113-2011	Phangnga/Southern	2011	KF698678
	NA-TH-SKW014-2008	Sakeow/TH	2008	KF698679
	NA-TH-NMA005-2010	Nakhonratchasima/TH	2010	KF698680
	NA-TH-NMA013-2009	Nakhonratchasima/TH	2009	KF698681
	NA-TH-NMA111-2011	Nakhonratchasima/TH	2011	KF698682

NA-TH-RBR128-2011	Ratchaburi/TH	2011	KF698683
NA-TH-RBR131-2012	Ratchaburi/TH	2012	KF698684
NA-TH-SKW194-2012	Sakeow/TH	2012	KF698685
NA-TH-CCO018-2010	Chachoengsao/TH	2010	KF698686
NA-TH-CMI109-2011	Chiangmai/TH	2011	KF698687
NA-TH-CNT039-2013	Chinat/TH	2013	KF698688
NA-TH-CNT040-2013	Chinat/TH	2013	KF698689
NA-TH-NYK001-2008	Nakhonnayok/TH	2008	KF698692
NA-TH-NYK004-2008	Nakhonnayok/TH	2008	KF698693
NA-TH-SKA233-2012	Songkhla/TH	2012	KF698695
NA-TH-SKW011-2010	Sakeow/TH	2010	KF698696
NA-TH-SPB043-2012	Suphanburi/TH	2012	KF698699
NA-TH-UBN022-2008	Ubonratchathani/ TH	2008	KF698702
PRRSV0000031	USA	2002	DQ474791
PRRSV0000008659	USA	2006	EU758687
PRRRSV0000008973	USA	2007	EU758940
EC-437022	Canada	2008	JQ691600
FJ1	China: Fujian	-	AY881994
CH4	Taiwan	-	EU273692
MD-001	Taiwan	1997	AF121131
e472	South Korea	2010	JF681191
4011-3	South Korea	2008	FJ972731
Ibaraki08-5	Japan	2008	AB546113
Miyagi08-2	Japan	2008	AB546105
Miyagi08-3	Japan	2008	AB546106
EDRD-8	Japan	1993	AB175720
01NP1.2	Thailand	2001	DQ056373
AS03US1417	Thailand	2010	JQ040792
BCLPS 100-2009	Singapore, vaccine	2009	GU187014
HGRV1-2012	Vietnam	2012	JQ860390
45	South Korea	2003	DQ473455
J5-10	South Korea	2010	JF681199
PL97-1	South Korea	1997	AY585241
Ingelvac PRRS MLV	USA	2005	AF066183
VR-2332	USA	1992	U87392
NA-TH-NMA122-2011	Nakhonratchasima/TH	2011	KF698691
NA-TH-SPB210-2012	Suphanburi/TH	2012	KF698700
NA-TH-TRT018-2013	Trat/TH	2013	KF698701
KTF10us	Thailand	2010	JN002319
ISU55	USA	-	U34299
NADC-8	USA	1992	U66394
J24-14	South Korea	2010	JF681215
J37-9	South Korea	2010	JF681217
34075-NE	USA	1996	U66380
NVSL14(252p)	USA	-	AF396841
PRRSV0003855	USA	1993	DQ477856

SIN-SP	Singapore, vaccine	-	AF184212
Neb1	USA	-	EU755263
PrimePac	USA, vaccine	-	AF066384
PRRSV55	USA	2000	AF176476
98□-31701□-1	USA	2001	AF339494
SDSU73	USA	2005	AY656993
Ingelvac ATP	USA, vaccine	-	AY656991
CH1R	China, vaccine	-	EU807840
CH1a	China: Beijing	1996	AY032626
UD1210US61F03	Thailand	2010	JN255828
SVS0211US116E12	Thailand	2011	JN848651
FDT10US22	Thailand	2010	JN255835
FDT10US23	Thailand	2010	JN255836
PSRS0411US1057G10	Thailand	2011	JN848663
SVS0211US116D12	Thailand	2011	JN848650
PINS0211US879H04	Thailand	2011	JN848647
1010GX4	China	2010	JQ663561
HZ209	China	2009	JQ828967
GXHZH01	China	2008	JX046317
JXA1	China: Jiangxi	2006	EF112445
WUH1	China: HuBei	2007	EU187484
BH58/10	Laos	2010	JN626287
10LW113	Laos	2010	JQ663557
10LW37	Laos	2010	JQ663564
NA-LAO-L142-2011	Laos	2011	KF698647
NA-LAO-L167-2011	Laos	2011	KF698648
NA-LAO-L232-2012	Laos	2012	KF698649
28SGVN-2009	Vietnam	2009	GU187016
5402BD-2010	Vietnam	2010	HQ700886
DN444-2008	Vietnam	2008	JQ860362
DN1-2010	Vietnam	2010	JQ860375
HCMCC3-2010	Vietnam	2010	JQ860379
HCMD06-2010	Vietnam	2010	JQ860380
DT7-2012	Vietnam	2012	JQ860387
07QN-2007	Vietnam	2007	FJ394029
10-10QN	Vietnam	2010	JQ663556
NA-VIE-V001-2010	Vietnam	2010	KF698650
NA-VIE-V056-2010	Vietnam	2010	KF698651
3BRVTVN	Vietnam	2009	GU187017
82DNVN	Vietnam	2009	GU187015
171-NA	Vietnam	2010	AB588638
347-T-KS	Vietnam	2010	AB588636
5172HCM	Vietnam	2010	HQ700879
5280DAKN	Vietnam	2010	HQ700882
5339HCM	Vietnam	2010	HQ700884
CT. C1	Vietnam	2012	JQ860382

NA-CAM-C008-2008	Cambodia	2008	KF698641
NA-CAM-C044-2010	Cambodia	2010	KF698642
NA-CAM-C060-2013	Cambodia	2013	KF698643
NA-CAM-C064-2013	Cambodia	2013	KF698644
NA-CAM-C182-2011	Cambodia	2011	KF698645
NA-CAM-C204-2012	Cambodia	2012	KF698646
PIADCPRRS	Philippines	2008	FJ641194
NA-TH-ATG008-2013	Angthong/TH	2013	KF698652
NA-TH-ATG128-2012	Angthong/TH	2012	KF698653
NA-TH-CBI001-2013	Chonburi/TH	2013	KF698654
NA-TH-CMI222-2011	Chiangmai/TH	2011	KF698655
NA-TH-CPM005-2012	Chaiyaphum/TH	2012	KF698656
NA-TH-CPM139-2012	Chaiyaphum/TH	2012	KF698657
NA-TH-CTI009-2013	Chanthaburi/TH	2013	KF698658
NA-TH-KKN036-2011	Khonkaen/TH	2011	KF698659
NA-TH-KKN080-2011	Khonkaen/TH	2011	KF698660
NA-TH-KKN156-2011	Khonkaen/TH	2011	KF698661
NA-TH-MKM104-2011	Mahasarakham/TH	2011	KF698662
NA-TH-NKI030-2010	Nongkhai/TH	2010	KF698663
NA-TH-NKI119-2012	Nongkhai/TH	2012	KF698664
NA-TH-NMA031-2008	Nakhonratchasima/TH	2008	KF698665
NA-TH-NPT016-2012	Nakhonpathom/TH	2012	KF698666
NA-TH-NPT179-2011	Nakhonpathom/TH	2011	KF698667
NA-TH-PBI073-2013	Prachinburi/TH	2013	KF698668
NA-TH-PLK098-2010	Phitsanulok/TH	2010	KF698669
NA-TH-PLK155-2011	Phitsanulok/TH	2011	KF698670
NA-TH-PRE171-2011	Prae/TH	2011	KF698671
NA-TH-RET214-2011	Roiet/TH	2011	KF698672
NA-TH-STI165-2011	Sukhothai/TH	2011	KF698673
NA-TH-YST025-2012	Yasothon/TH	2012	KF698674
NA-TH-LRI126-2011	Lopburi/TH	2011	KF698690
NA-TH-RNG019-2010	Rayong/TH	2010	KF698694
NA-TH-SKW144-2010	Sakeow/TH	2010	KF698697
NA-TH-SKW147-2010	Sakeow/TH	2010	KF698698
KPS10US	Thailand	2010	JN002309
4AN10US	Thailand	2010	JN002307
3AG10US	Thailand	2010	JN002300
2AND10US	Thailand	2010	JN002298
1AN10US	Thailand	2010	JN002284
UD1210US62A04	Thailand	2010	JN255831
WTCH0711US1334E08	Thailand	2011	JN848713
WTCH0711US1334D08	Thailand	2011	JN848712
SYAS0711US1329C07	Thailand	2011	JN848709
STP60711US1340A08	Thailand	2011	JN848705
STP10711US1335C12	Thailand	2011	JN848695
STHS0711US1331F07	Thailand	2011	JN848691

STHH0711US1332H07	Thailand	2011	JN848689
STS0611US1225H06	Thailand	2011	JN848677
STS0611US1225F06	Thailand	2011	JN848675
SRS0511US1124C06	Thailand	2011	JN848668
HUA0511US1122B06	Thailand	2011	JN848665
PTF0311US1052C08	Thailand	2011	JN848657
SAAS0111US819G02	Thailand	2011	JN848643
KPS101119US892H07	Thailand	2011	JN848639
1PCN2S10US	Thailand	2010	JN002310
AS05US3381	Thailand	2010	JQ040787
SYAH0711US1330E07	Thailand	2011	JN848708
AND0611US1238B05	Thailand	2011	JN848671
KLYH0511US1123G06	Thailand	2011	JN848667
AND0311US96A10	Thailand	2011	JN848652
Nagasaki 93	Japan: Nagasaki	1993	AB175725
Kagoshima N14	Japan	1993	AB175723
PRRSV16	USA	-	AF176438
PRRSV44	USA	-	AF176466
2000-5424	USA	2008	EU556160
17198-6	USA	-	AY656989
EU-NED-Lelystad-1991	Netherlands	1991	M96262

[a] ORF5 sequences were selected to represent each lineage/sublineage according to the study by Juntafong et al. (2015).

บทคัดย่อ

การเปลี่ยนแปลงลักษณะทางพันธุกรรมของเชื้อไวรัสพีอาร์อาร์เอสในประเทศไทยภายหลังการระบาดของเชื้อไวรัสสายพันธุ์รุนแรงในประเทศไทยในปี 2554

กรกฤต พูนสุข[1] จิรภัทธ อรุโณรัตน์[1] นนทิยา เอี่ยมประภาส[2] คมกริช เทียนคำ[1] รุ่งโรจน์ ธนาวงษ์นุเวช[1,2]*

ภายหลังการระบาดของเชื้อไวรัสพีอาร์อาร์เอส (PRRSV) ในประเทศไทย พบว่าเชื้อไวรัสพีอาร์อาร์เอสชนิดที่ 1 ยังคงมีการติดเชื้อวนเวียนอยู่ในประเทศไทยโดยไม่แสดงอาการ ส่วนเชื้อไวรัสพีอาร์อาร์เอสชนิดที่ 2 จะแสดงอาการในระดับปานกลางจนถึงรุนแรง ตั้งแต่ช่วงปี 2553 เชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์รุนแรง (HP-PRRS) ได้มีการระบาดและสร้างความสูญเสียเป็นอย่างมากในประเทศไทย การศึกษานี้ได้เก็บตัวอย่างเชื้อไวรัสพีอาร์อาร์เอสในช่วงปี 2555 จากฟาร์มที่มีอาการของการติดเชื้อจำนวน 11 ฟาร์ม จาก 10 จังหวัด ใน 4 ภาคของประเทศไทย และนำมาวิเคราะห์ในส่วนของยีน NSP2 และ ORF5 ซึ่งเป็นส่วนที่มีการเปลี่ยนแปลงทางพันธุกรรมค่อนข้างมาก จากการวิเคราะห์รหัสพันธุกรรมบางส่วนของ NSP2 และ ORF5 พบว่าตัวอย่างไวรัสทั้งหมดเป็นไวรัสพีอาร์อาร์เอสชนิดที่ 2 ซึ่งแบ่งได้เป็น 2 กลุ่ม ได้แก่ กลุ่มที่เป็นเชื้อไวรัสสายพันธุ์รุนแรงที่มีการหายไปของกรดอะมิโนจำนวน 30 ตัวใน 2 ตำแหน่ง เช่นเดียวกับเชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์รุนแรงอื่น ๆ กับกลุ่มที่เป็นเชื้อไวรัสพีอาร์อาร์เอสท้องถิ่นชนิดที่ 2 ที่มีการติดเชื้อวนเวียนตั้งแต่ก่อนปี 2553 ซึ่งการวิเคราะห์ทางสายวิวัฒนาการพบว่า เชื้อไวรัสสายพันธุ์รุนแรงอาจเป็นเชื้อที่กลายพันธุ์มาจากเชื้อไวรัสท้องถิ่นในช่วงปี 2553 หรือเชื้อไวรัสที่เข้ามาจากประเทศเพื่อนบ้าน อย่างไรก็ตาม พบว่าเชื้อไวรัสพีอาร์อาร์เอสท้องถิ่นสามารถก่อให้เกิดการติดเชื้อที่มีอาการรุนแรงในฝูงสุกรในประเทศไทยได้ และยังพบการกลายพันธุ์ของยีน NSP2 และ ORF5 ในบางตำแหน่ง ซึ่งสามารถใช้เป็น marker ทางพันธุกรรมของเชื้อไวรัสได้ กล่าวโดยสรุป ภายหลังจากการพบการระบาดของเชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์รุนแรง การติดเชื้อไวรัสพีอาร์อาร์เอสในประเทศไทยส่วนมากมักเกิดจากเชื้อไวรัสพีอาร์อาร์เอสสายพันธุ์นี้

คำสำคัญ: การกลายพันธุ์ NSP2 ORF5 เชื้อไวรัสพีอาร์อาร์เอส

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