Original Article

Molecular Characterization of Complete Genome of a Thai Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus

Tippawan Jantafong[1] Porntippa Lekcharoensuk[2*]

Abstract

Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) causes severe outbreaks in swine leading to serious economic losses in swine industry in Thailand and other countries in Southeast Asia. However, information regarding whole genome characterization of HP-PRRSV isolate in this region is limited. This report describes sequencing and characterizations of the complete nucleotide sequence of a Thai HP-PRRSV isolate, designated HP/Thailand/19500LL/2010 (TH19500LL/10). The complete genomic sequence consists of 189-nt 5′UTR, 14,982-nt protein-coding region containing 9 ORFs and 150-nt 3′UTR. In addition, the full-length sequence of Thai HP-PRRSV isolate was compared with that of VR2332 and other HP-PRRSV strains. The genomic sequence of TH19500LL/10 is highly similar to that of BH58/10 isolate, a HP-PRRSV from Laos. Nsp2 of TH19500LL/10 has common 30-amino acid discontinuous deletions. Amino acid comparison showed that GP5 of Thai HP-PRRSV was completely identical to that of other HP-PRRSV including JN-HS, BB0907, 09HEN2 and BH58/10 strains. It shares 98.5% and 89.1% similarity at amino acid level to that of the HP-PRRSV prototype (JXA1) and classical NA prototype (VR-2332), respectively. In addition, *in silico* functional analysis of structural proteins of TH19500LL/10 was also performed and discussed. This study provides basic knowledge for further studies of HP-PRRSV related to molecular mechanisms of viral infection and pathogenesis.

Keywords: complete genome, HP-PRRSV, molecular characterization, Thailand

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is classified in the genus *Arterivirus*, family *Arteriviridae* and order *Nidovirales* (Benfield et al., 1992; Plagemann and Moennig, 1992; Cavanagh, 1997). The PRRSV genome is a linear single-stranded RNA with 5′ cap and 3′polyadenylated tail and has approximately 15 kb in length (Wootton et al., 2000). It contains two large open reading frames (ORFs), ORF1a and ORF1ab, and other eight ORFs (ORF2a, ORF2b, and ORF3-7). ORF1a and ORF1ab are translated into replicase and 14 non-structural proteins (Nsp). The remaining ORFs encode structural proteins including glycoprotein (GP) 2, envelop protein (E), GP3, GP4, GP5, membrane protein (M), and neucleoprotein (N) (Conzelmann et al., 1993; Neumann et al., 2005; Firth et al., 2011). Among these proteins, Nsp2 and GP5 are the most variable and have been used for phylogenetic analysis and studies of PRRSV genetic diversity (Meng, 2000; An et al., 2007). PRRSV is divided into two distinct genotypes, Type 1 or European (EU) type and Type 2 or North American (NA) type, and their prototype strains are Lelystad (LV) and VR-2332, respectively (Wensvoort et al., 1991; Collins et al., 1992).

PRRSV is the causative agent of Porcine Reproductive and Respiratory Syndrome (PRRS) characterized by massive reproductive failure and late term abortion in sows and respiratory disorder in piglets (Hill, 1990). After the emergence of PRRS in the United States in 1987 (Keffaber, 1989; Loula, 1991), PRRS has become a devastating disease causing huge losses to swine industry worldwide (Neumann et al., 2005). Up to date, PRRS is endemic in several countries of Asia such as China, Japan, South Korea, Taiwan and Thailand (Cha et al., 2006). In 2006, atypical NA or highly pathogenic (HP) PRRSV emerged in China and then quickly spread to Vietnam and its vicinities such as Cambodia and Laos (Tian et al., 2007; An et al., 2011). Recently, HP-PRRSV has been reported in other Southeast Asian countries including Philippines, Myanmar, Thailand and Singapore (Feng et al., 2008; An et al., 2011) and has become a major problem in these countries. Genomic analysis of HP-PRRSV revealed unique 30-amino acid discontinuous deletions at amino acid positions 481 and 533-561 within its Nsp2 region (Tian et al., 2007). GDQY2, a more recent Chinese HP-PRRSV strain, contains 35-amino acid deletion at positions 470-505 and a continuous 29 amino acid deletion at residues 532-560 (Zhu et al., 2011).

In Thailand, PRRSV was first isolated in 1995 (Damrongwatanapokin et al., 1996); however, retrospective serological survey indicated that PRRSV appeared in Thai swine herd as early as 1989 (Damrongwatanapokin et al., 1996). Since then, PRRS has been the major infectious disease causing high mortality in swine and production loss in swine industry in Thailand. HP-PRRSV was first detected in Thailand in 2008 without notice of clinical outbreak (Jantafong et al., submitted for publication). Thereafter in 2010, the first outbreak caused by HP-PRRSV occurred in Nong Khai, a province located in the northeastern part of Thailand and bordering Lao PDR (Nilubol et al., 2012). This novel isolate has rapidly spread among swine herds in many provinces and currently co-circulates with other strains of PRRSV in Thai swine population (Jantafong et al., submitted for publication). In order to understand the molecular characteristics of HP-PRRSV in Thailand, the complete genome of a Thai HP-PRRSV, HP/Thailand/19500LL/2010, was sequenced and compared to the NA prototype, VR2332, and representative strains of HP-PRRSV.

Materials and Methods

Viral isolation and propagation: Lung tissue was collected from a piglet with clinical signs during the first outbreak of HP-PRRSV in Nong Khai province, Thailand, in 2010. Lung homogenate was inoculated onto a semi-confluent MARC-145 cells. The infected cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) and incubated at 37°C with 5% CO_2 for 3-5 d. Cytopathic effect (CPE) was examined daily. The culture supernatant was collected when 80% CPE was observed and then stored at -80°C as the virus stock until used. The presence of virus was confirmed by RT-PCR using primers specific to the ORF5 gene of HP-PRRSV (Forward; 5′-GGTGGGCAACCGTTTTAGCCTGT-3′ and Reverse; 5′-GTAATGGAAAACGCCAAAAGCACC-3′). After the virus was propagated for three passages, it was purified by plaque purification. The purified virus was designated HP/Thailand/19500LL/2010 (TH19500LL/10) isolate. Titer of the virus was determined as reciprocal of Median Tissue Culture Infective Dose ($TCID_{50}$) per ml using Reed and Muench method.

Primer design for amplification of complete HP-PRRSV genome: To amplify the complete genome of Thai HP-PRRSV isolate, TH19500LL/10, a set of oligonucleotide primers were designed based on the sequences of PRRSV strains BB0907 (HQ315835) and BH5810 (JN626287) available in GenBank database (www.ncbi.nlm.nih.gov). Nucleotide sequences of the 17 primer pairs used in this study will be provided upon request. PCR amplification using these primers generated 17 overlapping DNA fragments utilized for sequencing.

RNA isolation and RT-PCR: Viral RNA was isolated from infected cell supernatant using Viral Nucleic Acid Extraction Kit II (Genaid, Taiwan) according to the manufacturer's protocol. cDNA was synthesized using the isolated RNA as the template with random hexanucleotide primers and SuperScript III reverse transcriptase (Invitrogen, USA) following a protocol provided by the manufacturer. The cDNA was used as the template in subsequent PCR reactions in a final volume of 100 µl containing 10 pM of PRRSV-specific primers, 1 X polymerase buffer, 10 mM dNTP and 2.5 units of Platinum®Taq DNA polymerase (Invitrogen, USA).

DNA cloning and sequence analysis: The amplified PCR products were separated by electrophoresis through 1% agarose gel (Camblex, USA). DNA

fragments were excised from the agarose gel and purified using Gel Extraction Kit (Qiagen, Germany). The purified PCR products were cloned into pGEM-T easy vector (Promega, USA) according to the manufacturer's instructions. Three positive clones from each amplicon were submitted for sequencing at Macrogen (Korea) and the sequencing results were analyzed using Lasergene software (DNASTAR). Thereafter, the 17 contigs were assembled to each other using SegMan and EditSeq programs (DNASTAR). Multiple sequence alignments were performed using Clustal W method (DNASTAR). ORFs encoding structural and non-structural proteins were analyzed and compared with the complete genomes of other PRRSV strains available in GenBank database (www.ncbi.nlm.nih.gov/genbank).

Phylogenetic analysis of the complete PRRSV genome: The complete genomic sequence of TH19500LL/10 was analyzed and compared with 29 representative PRRSV isolates obtained from GenBank database (www.ncbi.nlm.nih.gov/genbank). A phylogenetic tree was generated using Maximum Likelihood method provided in MEGA 5.2 (Tamura et al., 2011). Bootstrap values were calculated based on 1000 replicates of the comparison.

Antigenic and amino acid analysis: To investigate genetic variation at amino acid level, the DNA sequences of GP2, GP3, GP4, GP5 and Nsp2 were translated into amino acid sequences. Subsequently, the deduced amino acid sequences were analyzed and aligned using MegAlign program (DNASTAR), together with those of the representative PRRSV isolates. Besides, some motifs within ectodomain of GP5 such as decoy epitope (DCE) and primary neutralizing epitope (PNE) as well as the signal peptide, transmembrane regions and endodomain were determined as previously described (Ostrowski et al., 2002; Plagemann, 2004). Thereafter, potential glycosylation sites of glycoproteins were determined using Expasy proteomics tool NetNGlyc 1.0.

Results

Full-length genomic sequence analysis of Thai HP-PRRSV: The sequence data of HP-PRRSV strain HP/Thailand/19500LL/2010 was assembled into one contiguous sequence of 15,320 nucleotides, excluding the poly (A) tail. The complete genome of HP19500LL/10 is similar to other PRRSVs consisting of 189-nt 5'UTR, 150-nt 3'UTR and 14,982-nt of the protein-coding region containing 9 ORFs (Table 1). The complete HP-PRRSV genomic sequence from this study was deposited in the GenBank database under the accession number KF735060. The viral genome consists of 9 overlapping ORFs, ORF1a, ORF1b, ORF2a, ORF2b and ORF3-7 as shown in Figure 1A. Polyprotein (pp) 1ab encoded by ORF1a/b is translated and processed into 14 Nsps including 10 Nsp products of pp1a plus Nsp9-12 (Table 1 and Fig 2A). The overlapping region between ORF1a and ORF1b of TH19500LL/10 comprises 22 nucleotides located at nucleotide positions 7590 to 7611 similar to most HP-PRRSVs. This region contains the heptanucleotide slippery sequence (GTTTAAAC) at three nucleotides upstream of ORF1a stop codon (Fig 1B), which is common to type II PRRSVs. The ORFs 2 to 7 encoding for structural proteins of TH19500LL/10 occupy 3,188 bp at C-termini of the viral genome (Fig 2B). This finding indicated that TH19500LL/10 exhibited the genome organization similar to other HP-PRRSVs. However, M protein of TH19500LL/10 contains 522 nucleotides and 172 amino acid residues, which is shorter than that of other HP-PRRSV isolates (Table 2).

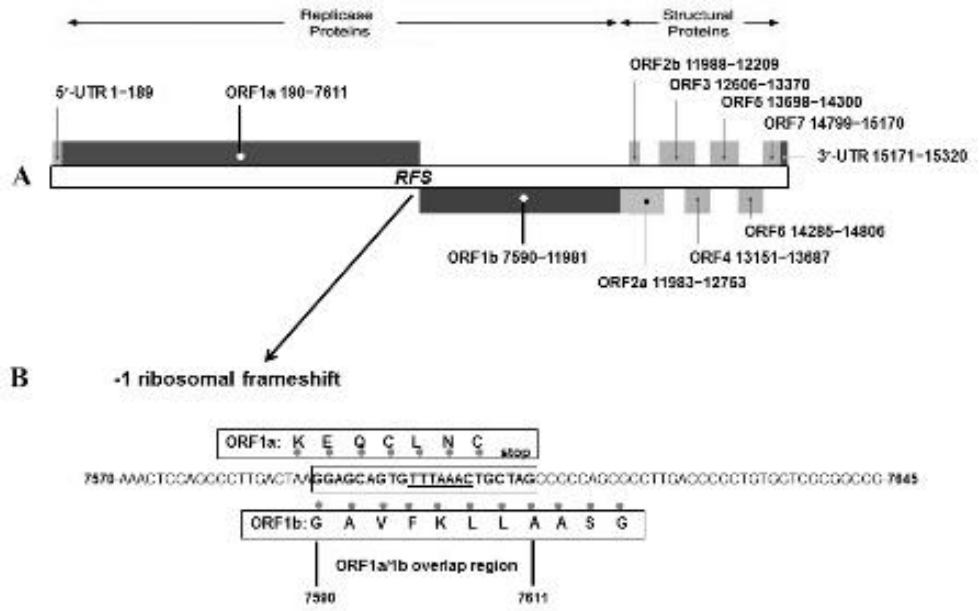

Figure 1 Schematic diagram of genome organization of Thai HP-PRRSV, HP/Thailand/19500LL/2010 isolate. (A) Relative length and nucleotide positions of 5'UTR, each ORF and 3'UTR of TH19500LL/2010. (B) Overlapping region between ORF1a and ORF1b was identified at nucleotide positions 7590 to 7611. The empty box within nucleotide sequences represents 22 nucleotides of -1 ribosomal frameshifting and the underlined letters represent heptanucleotide slippery sequence.

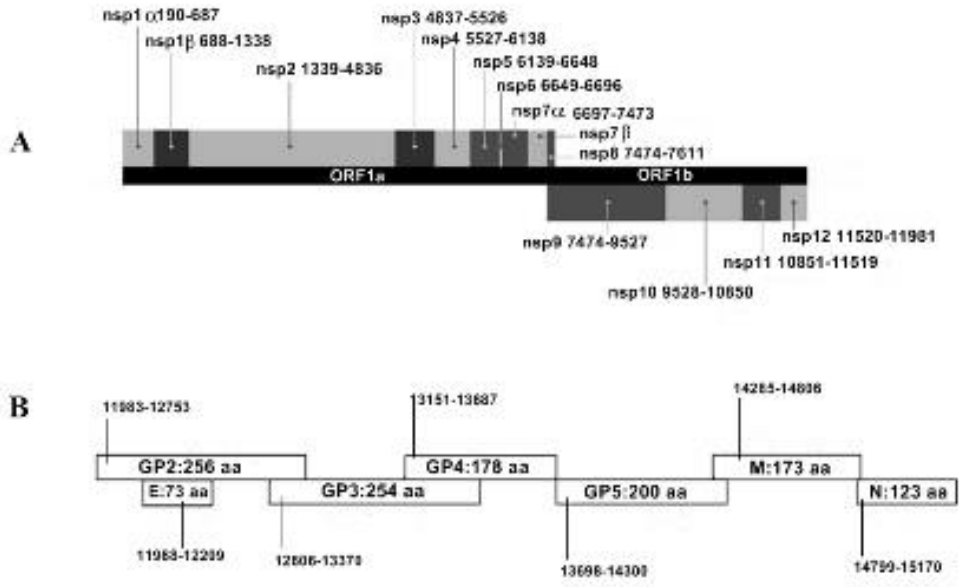

Figure 2 Schematic diagram of non-structural and structural proteins of Thai HP-PRRSV, HP/Thailand/19500LL/2010 isolate. (A) Illustration of 14 putative Nsps including Nsp1α, Nsp1β, Nsp2 to 8, Nsp9 to Nsp12 and their relative length and nucleotide positions related to the genome. (B) Relative length and nucleotide positions of ORFs 2 to 7 within the genome.

Table 1 Genome organization of HP/Thailand/19500LL/2010

mRNA	ORF	Cleavage product	Nucleotide		Amino acid length	Translated proteins
			Position	Length		
	5′UTR		1-189	189	–	Untranslated region
1	ORF1a		190-7611	7422	2473	Replicase
		Nsp1α	190-687	498	166	Papain-like cysteine protease (PCP α)
		Nsp1β	688-1338	651	217	Papain-like cysteine protease (PCP β)
		Nsp2	1339-4836	3498	1166	Chymotrypsin-like cysteine protease (CP)
		Nsp3	4837-5526	690	230	Transmembrane protein
		Nsp4	5527-6138	612	204	Serine protease (SP)
		Nsp5	6139-6648	510	170	Transmembrane protein
		Nsp6	6649-6696	48	16	Unknown functions available
		Nsp7α	6697-7473	777	259	Unknown functions available
		Nsp7β	N/A	N/A	N/A	Unknown functions available
		Nsp8	7474-7611	138	45	Unknown functions available
1	ORF1b		7590-11981	4392	1463	Replicase
		Nsp9	7474-9527	2054	685	RNA-dependent RNA polymerase (RdRp)
		Nsp10	9528-10850	1323	441	Metal binding/Helicase(M/Hel)
		Nsp11	10851-11519	669	223	Endoribonuclease (NendoU)
		Nsp12	11520-11981	462	153	Unknown functions available
2	ORF2a		11983-12753	771	256	Glycoprotein 2 (GP2)
2	ORF2b		11988-12209	222	73	Envelope protein (E)
3	ORF3		12606-13370	765	254	Glycoprotein 3 (GP3)
4	ORF4		13151-13687	537	178	Glycoprotein 4 (GP4)
5	ORF5		13698-14300	603	200	Glycoprotein 5 (GP5)
6	ORF6		14285-14806	522	173	Matrix protein (M)
7	ORF7		14799-15170	372	123	Nucleocapsid protein (N)
	3′UTR		15171-15320	150	–	Untranslated region

Nsp: Non-structural protein; GP: Glycoprotein; E: Envelope protein; M: Matrix protein;
N: Nucleocapsid protein; N/A: Not applicable

The ORFs 2 to 7 encoding for structural proteins of TH19500LL/10 occupy 3,188 bp at C-termini of the viral genome. GP2a to GP5 encoded by ORF2a to ORF5, respectively, are envelope proteins that are glycosylated membrane proteins. M and E proteins encoded by ORF6 and ORF2b, respectively, are also viral envelope proteins; however, they are non-glycosylated membrane proteins (Fig 2B). N protein encoded by ORF7 is a non-glycosylated and non-envelope protein. M protein of TH19500LL/10

contains 522 nucleotides and 173 amino acid residues, which is shorter than that of other HP-PRRSV isolates (Table 1).

Whole genomic sequence comparison: The complete genomic sequences of TH19500LL/10 and other PRRSV strains including the EU prototype (LV), the NA prototype (VR-2332), the Thai NA prototype (01NP1.2) (Amonsin et al., 2009), Ingelvac PRRS MLV vaccine, two Chinese Classical-NA strains (CH1a, HB-2(sh)/2002), the HP-PRRSV prototype (JXA1) and five HP-PRRSV isolates (GDQY2, 09HEN2, JN-HS, BB0907 and BH58/10) were compared. Results showed that the genome of Thai HP-PRRSV was most closely related to BH58/10 (JN626287) isolated from Laos in 2010 with 99.7% nucleotide identity and shares 60.7%, 89%, 88.9% and 89% nucleotide identity with LV, VR-2332, 01NP1.2 and Ingelvac PRRS MLV, respectively. In addition, it has 97.6-99.3% nucleotide identity with five Chinese HP-PRRSV strains but has only 91.7% and 94.1% similarity to two Chinese NA strains, HB-2(sh)/2002) and CH1a, respectively (Table 2).

Table 2 Nucleotide homology of complete genome of HP/Thailand/19500LL/2010 and other PRRSV isolates

PRRSV strains	Identity to TH19500LL/10
Lelystad	60.7%
VR-2332	89.0%
Ingelvac PRRS MLV	89.0%
01NP1.2	88.9%
CH1a	94.1%
HB-2(sh)/2002	91.7%
JXA1	97.9%
GDQY2	97.6%
09HEN2	99.2%
JN-HS	99.2%
BB0907	99.3%
BH58/10	99.7%

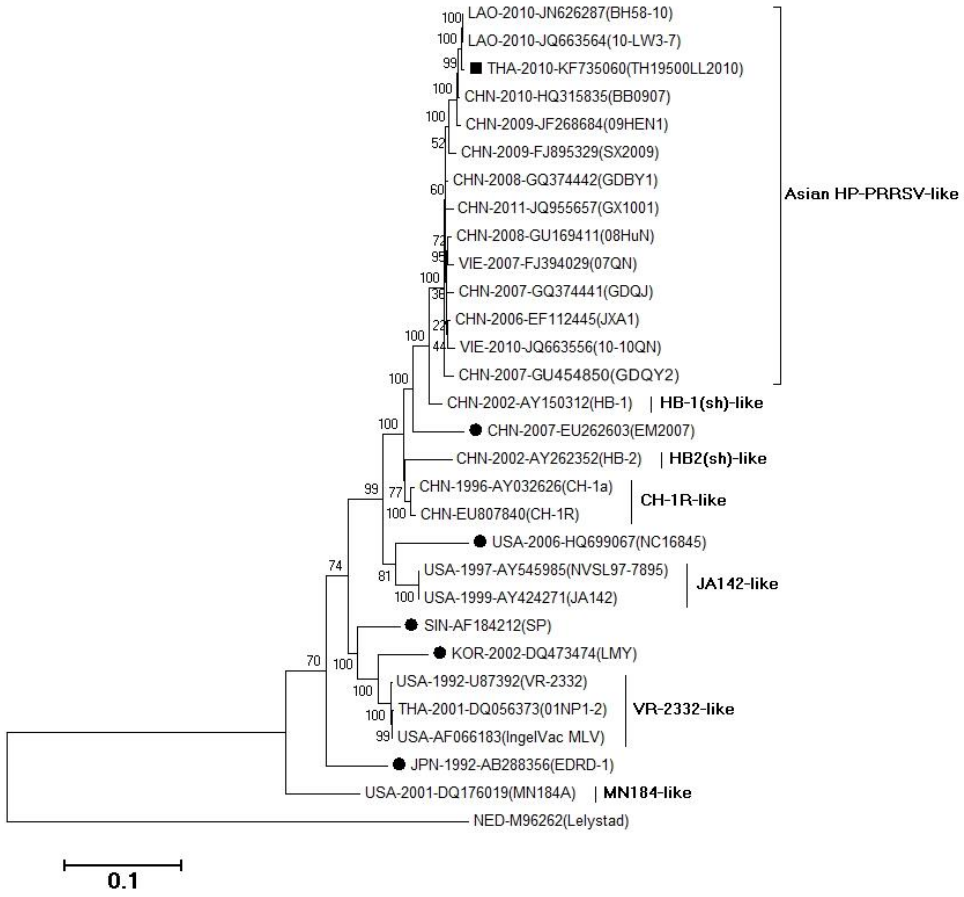

Figure 3 Phylogenetic tree demonstrating genetic relationship between Thai HP-PRRSV and other PRRSV isolates based on complete genomic sequences. A solid square in front of the isolate name indicates position of Thai HP-PRRSV. The solid circles indicate different strains of PRRSVs.

The nucleotide sequences of each ORF of TH19500LL/10 and other HP-PRRSVs were also compared. The homology between 5′UTR, ORF1a, ORF1b, ORF2a, ORF2b, ORF3 to 7 and 3′UTR of Thai HP-PRRSV and those of other HP-PRRSVs range from 97.4-100, 97.7-99.6, 97.8-99.7, 97.5-99.7, 98.2-100, 97.6-99.9, 97.4-100, 98.5-99.0, 98.9-99.6, 97.6-99.5 and 94.7-100%, respectively (Table 3). The putative Nsp1β and Nsp2 regions of TH19500LL/10 and other HP-PRRSVs share 95.9-99.8% and 96.6-99.5% nucleotide identity, respectively. Comparison between TH19500LL/10 and VR-2332, the Classical-NA prototype, revealed that lower nucleotide identity with the homology in 5′UTR, ORF1a, ORF1b, ORF2a, ORF2b, ORF3-7 and 3′UTR regions were 91, 86.9, 90.6, 92.6, 93.7, 88.8, 89.8, 89.1, 94.4, 93.3 and 94.0%, respectively. ORF2a and M gene of both viruses are the most and least variable, respectively.

Phylogenetic analysis of whole genome: To understand the genetic relationship between Thai HP-PRRSV and other PRRSV isolates, a phylogenic tree was constructed using the full-length genomes of 29 Classical-NA representative strains from China, Thailand, Japan, Korea and USA, vaccine strains (SP, CH-1R and Ingelvac PRRS MLV) and HP-PRRSV strains from China, Laos and Vietnam. The phylogenetic analysis revealed that TH19500LL/10 was clustered with other HP-PRRSV strains from Asian countries and most closely related to a HP-PRRSV from Laos. Asian HP-PRRSVs were placed in a separate branch from the Classical-NA isolates and were most similar to HB-1(sh)/2002 like- followed by HB-2(sh)/2002 like-, EM2007- and CH-1R like-Chinese PRRSVs. The results indicated that Thai and other Asian HP-PRRSVs probably originated from the Chinese Classical-NA PRRSV strains. In addition, Thai HP-PRRSV is distant from the NA type from Korea, Japan, Singapore and Thailand (Fig 3).

Table 3 Comparison between each putative ORF and functional region of HP/Thailand/19500LL/2010 and other Asian HP-PRRSV strains

Regions	Nucleotide identity to TH19500LL/10 (%)									
	VR-2332	JXA1	BB0907	SX2009	GDBY1	GDQJ	GDQY2	07QN	10-10QN	BH58-10
5′UTR	91.0	98.4	99.5	98.4	98.4	97.4	98.4	97.4	98.4	100
ORF1a	86.9	97.5	99.2	97.4	97.7	97.1	97.1	97.3	97.8	99.6
Nsp1α	91.4	97.6	99.0	97.2	98.6	97.6	98.2	97.6	98.2	99.6
Nsp1β	86.0	97.4	99.5	97.2	97.5	95.9	96.3	97.4	97.5	99.8
Nsp2	83.9	97.1	99.0	96.8	97.3	96.6	96.7	96.9	97.7	99.5
Nsp3	89.7	98.4	99.3	98.8	98.7	98.1	97.5	98.3	98.8	99.7
Nsp4	90.0	98.9	99.3	98.2	98.9	98.9	98.4	98.4	98.9	99.5
Nsp5	88.8	97.3	99.6	99.2	97.6	97.3	97.3	96.7	97.3	99.8
Nsp6	93.8	97.9	97.9	97.9	97.9	97.9	97.9	97.9	97.9	97.9
Nsp7	89.7	97.4	99.1	97.6	97.6	97.0	97.2	97.2	97.4	99.6
Nsp8	94.9	97.8	99.3	98.6	97.8	99.3	95.7	97.8	97.8	100
ORF1b	90.6	98.1	99.3	97.8	98.2	97.8	98.1	97.8	97.9	99.7
Nsp9	91.9	98.2	99.4	97.7	98.2	98.1	98.1	97.9	98.1	99.8
Nsp10	89.6	97.9	99.1	97.7	98.2	97.5	97.7	97.6	97.6	99.7
Nsp11	89.8	98.4	99.6	98.4	98.4	98.1	98.2	98.2	97.9	99.6
Nsp12	89.6	98.1	99.6	97.8	97.8	97.4	97.8	97.0	97.8	100
ORF2a	92.6	98.3	99.5	98.6	98.6	98.3	98.7	97.6	97.5	99.7
ORF2b	93.7	99.5	99.5	99.5	99.5	99.5	99.5	98.6	98.2	100
ORF3	88.8	98.3	99.3	99.1	98.6	98.3	98.2	97.6	98.0	99.9
ORF4	89.8	97.4	99.6	99.4	99.1	98.3	97.6	98.3	97.4	100
ORF5	89.1	98.7	99.7	99.7	98.5	98.5	98.5	98.5	99.0	99.8
ORF6	94.4	99.4	99.6	99.4	99.0	99.2	98.9	99.4	98.9	99.6
ORF7	93.3	98.1	99.2	98.4	98.4	97.6	97.8	97.6	97.8	99.5
3′UTR	94.0	98.7	100	99.3	98.7	98.7	94.7	99.3	98.7	98.7

Table 4 Glycosylation sites on each glycoprotein of HP/Thailand/19500LL/2010

Glycoprotein	Glycosylation site							
	Number	Position						
GP2a	2	N178	N184					
GP3	7	N29	N42	N50	N131	N152	N160	N195
GP4	4	N37	N84	N120	N130			
GP5	4	N30	N35	N44	N51			

Genetic diversity of Nsp2: The Nsp2 coding region of HP-PRRSV contains unique discontinuous 30 amino acid deletions compared to other PRRSV strains. Comparison of amino acid sequence in the Nsp2 region of the TH19500LL/10, 01NP1.2, VR-2332, CH-1a, HB-2(sh)/2002, JXA1 and other HP-PRRSV isolates showed that all HP-PRRSV isolates have common 30-amino acid discontinuous deletions comprising 1-amino acid deletion at position 481(L) and another 29-amino acid deletion at positions 533-561 (RPVTPLSEPIPVPAPRRKFQQVKRLSSAA) (Fig 4).

Amino acid analysis of GP5: To gain more understanding of the genetic diversity and evolution of Thai HP-PRRSV, the deduced amino acid sequences of GP5 were analyzed and compared with those of NA representative strains (VR-2332, IngelVac MLV and NVSL97-7895) and HP-PRRSV isolates (JXA1, GDQJ, GDQY2, GDBY1, SX2009, BB0907, 07QN, 10-10QN and BH58-10). Results revealed that GP5 of all isolates contains 200 amino acids. GP5 of TH19500LL/10 and JXA1, the HP-PRRSV prototype collected in 2006 from China, has 98.5% identity. Amino acid similarity between GP5 of TH19500LL/10 and that of NVSL97-7895, VR-2332 and IngelVac MLV is only 91%, 89.1%

and 81.1%, respectively. The homology among GP5 of these HP-PRRSV strains is high and ranges from 98-100%; TH19500LL/10 is highly similar to SX2009, BB0907 and BH58-10.

Furthermore, some crucial motifs within GP5 including DCE, PNE, signal peptide, transmembrane regions and endodomain were determined as described previously (Ostrowski et al., 2002; Plagemann, 2004; Han et al., 2006). Multiple alignment of GP5 amino acids showed that the signal peptide and ectodomain were the most variable regions while the sequences at C-termini were more conserved. The signal peptide and ectodomain of TH19500LL/10 GP5 span residues 1-26 and 27-64, respectively (Fig 5). GP5 of TH19500LL/10 has three transmembrane domains, TM 1 (aa 65-82), TM 2 (aa 96-102) and TM 3 (aa 110-127). Cysteine (Cys, C) residue, which implicates in heterodimer formation with M protein, was detected at residue 48 and it is strongly conserved in all isolates. The DCE within GP5 of TH19500LL/10 comprises four amino acids, VLAN, resigning from residues 27 to 30. In previous reports, PNE of other PRRSV strains which play a crucial role in viral neutralization has a consensus $S^{37}H(F/L)QLIYN$ (Ostrowski et al., Plagemann et al., 2002; Plagemann, 2004). PNE within GP5 of TH19500LL/10 and other HP-PRRSV resigns at amino acid positions 37-44 similar to other PRRSV strains. However, it has isoleucine (I) at position 39 and thus its PNE contains amino acid sequence $S^{37}HIQLIYN$ (Fig 5).

Analysis of potential glycosylation sites: The potential glycosylation sites containing the conserved motif Asn-X-Ser/Thr (N-X-S/T) within glycoproteins of TH19500LL/10 were identified and compared with those of VR-2332. Results revealed that the N-linked glycosylation sites within GP5 of TH19500LL/10 were diverse from those of VR-2332. However, the glycosylations are more conserved in GP2a, GP3 and GP4. The potential N-linked glycosylation sites on GP2a, GP3, GP4 and GP5 of TH19500LL/10 are shown in Table 4.

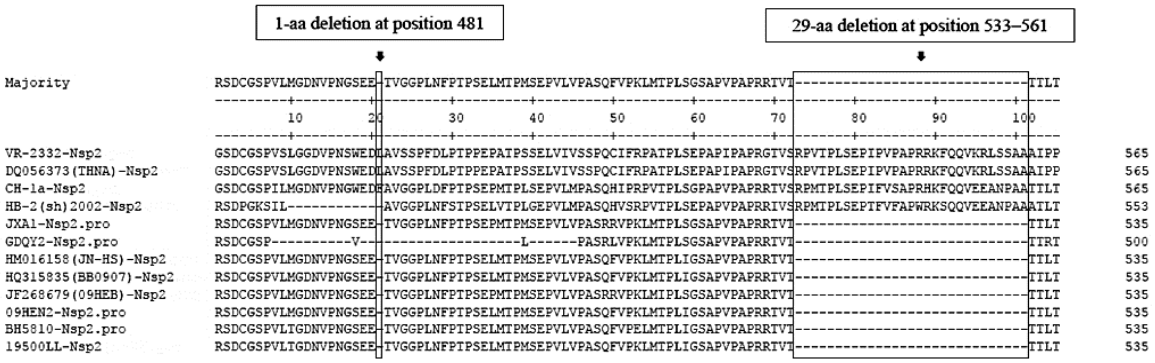

Figure 4 Multiple alignments of partial amino acid sequences in Nsp2 region of HP-PRRSVs. The black-line boxes represent 30-amino acid discontinuous deletion regions.

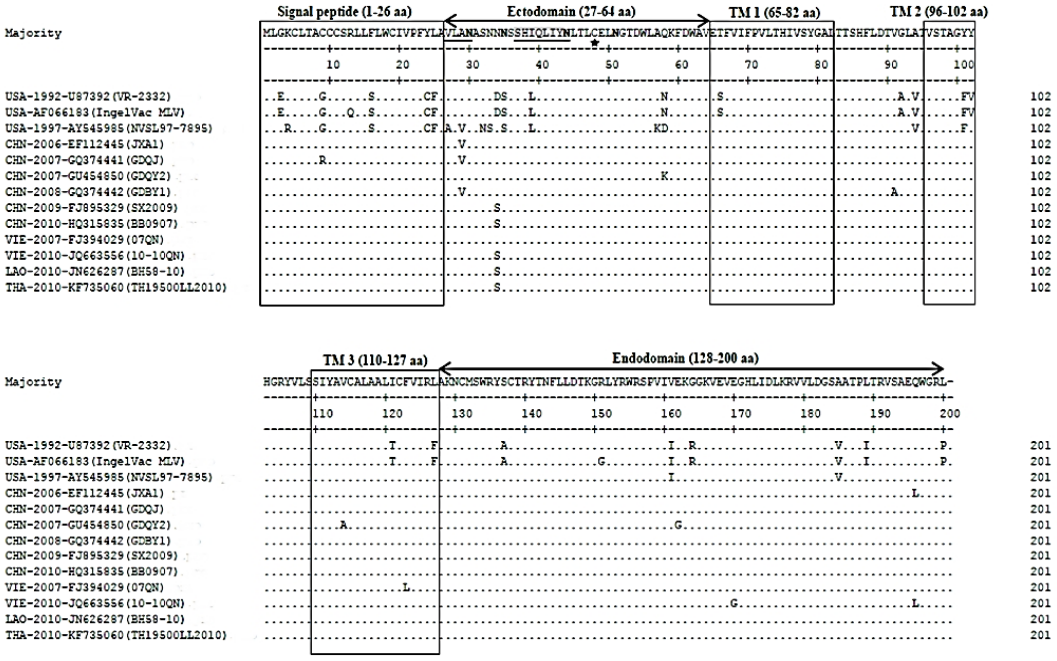

Figure 5 Multiple alignments of GP5-deduced amino acids. Functional domains are marked by the black-line boxes and a star under letter C indicates a cysteine residue. The underlined letters and gray shade indicate DCE and PNE, respectively. The bold-face letter N indicates glycosylation sites.

Non-glycosylated structural proteins: M protein of TH19500LL/10 has a nucleotide substitution at position 520 (A520T), resulting in a stop codon at amino acid position 173 compared to JXA1; thus, it contains 173 instead of 174 residues. Nucleocapsid (N) of TH19500LL/10 is a non-envelope protein and comprises 123 residues. It is the most genetically and antigenically conserved protein.

Discussion

HP-PRRSV has become a major problem worldwide including Thailand and HP-PRRSV infection has continually increased very year. However, information regarding the full-length sequences of Thai HP-PRRSV has never been reported. This is the first study of characterization and *in silico* analysis of the complete genome of a Thai HP-PRRSV, designated HP/Thailand/19500LL/2010 isolate. This virus strain was isolated in 2010 from a piglet with clinical signs of PRRS during the first outbreak of HP-PRRSV in Nong Khai province. Thus, TH19500LL/10 was the representative of Thai HP-PRRSV field strains. The pairwise nucleotide comparison between Thai HP-PRRSV and Ingelvac PRRS MLV vaccine based on complete genomic sequence indicated that TH19500LL/10 were distant from the vaccine strain. However, genomic sequence of Thai HP-PRRSV showed the highest similarity to that of HP-PRRSV isolate from Laos, which is closely related to HP-PRRSV strains from China. Therefore, it is possible that Thai HP-PRRSV may have originated from the Chinese HP-PRRSV, which was introduced into Thailand via Laos. Therefore, PRRSV monitoring and surveillance along the boundary should be strengthen to prevent the spreading of the virus across the countries.

This is the first report on the full length genomic analysis of Thai HP-PRRSV, THIL19500LL/10. Genome organization of THIL19500LL/10 is similar to that of classical NA genotype, which contains two large overlapping ORFs, ORF1a and 1b, and six structural protein-coding ORFs at 3′ end. ORF 1a/b of PRRSV occupies approximately 75% of the viral genome and code for 14 putative Nsps by -1 ribosomal frameshifting mechanism (Snijder and Meulenberg, 1998). The polyprotein (pp) 1a encoded by ORF1a is co- and post-translational processed into 10 Nsps, designated Nsp1α, Nsp1β, and Nsp2-6, Nsp7α, Nsp7β and Nsp8 (Snijder and Meulenberg, 1998; van Akenet al., 2006). The structural proteins consist of six envelope and one non-envelope proteins encoded by ORF2 to 7 (Meulenberg et al., 1995; Snijder and Meulenberg, 1998; Dea et al., 2000; Wu et al., 2001). All envelope proteins of PRRSV are essential for viral infectivity (Wissink et al., 2005).

The N-linked glycosylation on the surface glycoprotein assists in proper protein folding and is involved in biological activity and antigenicity of the virus which may relate to viral virulence and survival (Ansari et al., 2006). N-linked glycans of PRRSV glycoprotein mask the antigenic epitopes from the recognition of neutralizing antibodies and hence facilitate viral escape during persistent infection (Ansari et al., 2006). GP2a of the Thai HP-PRRSV contains 256 residues and has two conserved N-linked glycosylation sites at amino acid positions N178 and N184 similar to that of VR-2332. A recent study found that the glycosylation at N178 of GP2a was indispensable for viral growth in cells line but at N184 was essential for infectious virus production (Das et al., 2011). Additionally, GP2a has a strong interaction with CD163 molecule, which is one of the receptors for PRRSV entry (Das et al., 2010). The non-glycosylated E (GP2b) protein is encoded by an overlapping ORF within the ORF2a and composed of 73 residues.

The envelope protein GP3 of TH19500LL comprises 254 residues and is heavily glycosylated. There are seven N-linked glycosylation sites present on TH19500LL GP3 consisting of N29, N42, N50, N131, N152, N160 and N195, respectively. The glycosylation sites at positions N42, N50 and N131 of GP3 are essential for virus production (Das et al., 2011) while at position N131 may involve in induction of neutralizing antibody. N-linked glycosylation of GP3 associates with the process of virus escape from neutralizing antibody (Vu et al., 2011). The GP4 of the Thai HP-PRRSV contains 178 residues and has four potential glycosylation sites at residues 37, 84, 120 and 130. GP4 is important for virus infection and has a strong interaction with CD163 (Wissink et al., 2005; Das et al., 2010).

M protein of PRRSV encoded by ORF6 is non-glycosylated and composed of 173 and 174 residues for Type 1 and Type 2 PRRSV, respectively (Dea et al., 2000). M is one of the major envelope proteins that forms disulfide-linked heterodimers with GP5 (Meulenberg et al., 1995; Wissink et al., 2005). In the viral particle, both M and GP5 are composed of at least half of the viral protein components and are essential for virus assembly and infectivity (Wissink et al., 2005). This basic knowledge is important for future research on various aspects of PRRSV molecular biology.

The whole genome sequencing of Thai HP-PRRSV demonstrates that HP/Thailand/19500LL/2010 is genetically diverse from JXA1, the HP-PRRSV prototype from China, but most closely related to the HP-PRRSV isolate from Laos, the neighboring country of Thailand. This suggests that distribution of HP-PRRSV across countries is possible. Therefore, PRRSV surveillance near border area should be performed regularly to prevent PRRSV cross transmission between countries. This study provides insight information for further studies of molecular mechanisms of viral and pathogenesis.

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บทคัดย่อ

คุณสมบัติทางอณูชีววิทยาของจีโนมเต็มสายของไวรัสพีอาร์อาร์เอส สายพันธุ์ HP-PRRSV ในประเทศไทย

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ไวรัสพีอาร์อาร์เอส สายพันธุ์ HP-PRRSV เป็นสาเหตุของการแพร่ระบาดอย่างรุนแรงในสุกรซึ่งนำไปสู่การสูญเสียทางเศรษฐกิจที่รุนแรงในอุตสาหกรรมการผลิตสุกรของประเทศไทยและประเทศอื่น ๆ ในเอเชียตะวันออกเฉียงใต้ อย่างไรก็ตามข้อมูลที่เกี่ยวกับจีโนมเต็มสายของ HP-PRRSV ที่แยกในภูมิภาคนี้มีจำนวนจำกัด รายงานนี้จึงอธิบายถึงการวิเคราะห์หาลำดับเบสและคุณลักษณะของสายพันธุกรรมหรือจีโนมที่สมบูรณ์ของไวรัส HP-PRRSV สายพันธุ์ไทย HP/Thailand/19500LL /2010 (TH19500LL /10) โดยจีโนมแบบเต็มสายของไวรัสประกอบด้วยส่วนที่ไม่ถูกแปลรหัสที่ปลาย 5’ (5’UTR) ขนาด 189 นิวคลีโอไทด์, บริเวณที่เป็นรหัสของโปรตีนขนาด 14,982 นิวคลีโอไทด์ ซึ่งประกอบด้วย 9 กรอบการอ่านรหัส (ORFs) และ 3’UTR ขนาด 150 นิวคลีโอไทด์ เมื่อเปรียบเทียบจีโนมแบบเต็มสายของไวรัส HP-PRRSV สายพันธุ์ไทย กับ VR-2332 ซึ่งเป็นสายพันธุ์ต้นแบบจีโนไทป์อเมริกาเหนือ (Classical NA) และ HP-PRRSV สายพันธุ์อื่นๆ พบว่า HP-PRRSV สายพันธุ์ไทยมีความใกล้ชิดในระดับพันธุกรรมกับ HP-PRRSV สายพันธุ์ BH58/10 จากประเทศลาวมากที่สุด ซึ่งยีน Nsp2 ของ TH19500LL/10 มีการขาดหายไปจำนวน 30 กรดอมิโนในตำแหน่งที่ไม่ติดต่อกัน ซึ่งเป็นลักษณะจำเพาะสำหรับ HP-PRRSV การเปรียบเทียบลำดับกรดอะมิโนของยีน GP5 ของ TH19500LL /10 พบว่ามีความเหมือนร้อยละ 100 กับไวรัส HP-PRRSV สายพันธุ์ JN-HS, BB0907, 09HEN2 และ BH58/10 และมีความเหมือนกับสายพันธุ์ JXA1 ซึ่งเป็นสายพันธุ์ต้นแบบของ HP-PRRSV ร้อยละ 98.5 และ VR-2332 ซึ่งเป็นสายพันธุ์ต้นแบบของ classical NA ร้อยละ 89.1 ตามลำดับ นอกจากนี้ทำการวิเคราะห์ตำแหน่งและหน้าที่ของโปรตีนของ HP-PRRSV สายพันธุ์ไทย โดยใช้การวิเคราะห์ทางคอมพิวเตอร์ ซึ่งข้อมูลที่ได้จากการศึกษาในครั้งนี้จะเป็นประโยชน์ต่อการศึกษาที่เกี่ยวข้องกับกลไกทางอณูชีววิทยาของการติดเชื้อและก่อโรคของไวรัส

คำสำคัญ: จีโนมเต็มสาย HP-PRRSV การศึกษาคุณสมบัติทางอณูชีววิทยา ประเทศไทย

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