Molecular genetic characteristics of the envelope glycoprotein (E2) gene of classical swine fever viruses isolated in Thailand

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Abstract

Thirteen classical swine fever virus (CSFV) isolates collected from field outbreaks in various locations of Thailand during 2001-2009 which had been identified by RT-PCR were genetically analyzed by partial nucleotide sequencing of the envelope glycoprotein (E2) gene. These sequence data were then phylogenetically analyzed along with the sequences previously submitted to GenBank, as well as those from representatives of the different CSFV subgroups. The sequence and phylogenetic analyses of the E2 gene demonstrated that the thirteen isolates of this study were separated into three different groups. The first group consisted of 4 isolates sharing 98.9-100% and 96.8-100% homology among them in nucleotide and amino acid sequences, respectively, and belonged to subgroup 1.1. The second group consisted of 5 isolates sharing 97.3-100% and 93.6-100% homology in nucleotide and amino acid sequences, respectively, and belonged to subgroup 2.1. The last group consisted of 4 isolates sharing 91.5-100% and 92.0-96.8% homology in nucleotide and amino acid sequences, respectively, and belonged to subgroup 2.2. In addition, the isolates in the first group had 97.3-98.4% nucleotide sequence and 93.6-96.8% amino acid sequence similarities with the CSFV C-strain, whereas those in the second group had 88.3-89.9% nucleotide sequence and 95.2-96.8% amino acid sequence similarities with the Italy strain, and those in the last group had 92.0-92.5% nucleotide sequence and 92.0-96.8% amino acid sequence similarities with the GS-JC-strain. These data help to understand the dynamics of Thai CSF virus subgroup evolution and support epidemiological observations.

Keywords: Classical swine fever, E2 glycoprotein, phylogenetic analysis, sequence analysis

Introduction

Classical swine fever (CSF) is a highly contagious and widespread viral disease of swine, which causes important economic losses in swine industry worldwide. The disease may result in an acute, subacute, chronic, late onset or inapparent course. Classical swine fever virus (CSFV), the causative agent of CSF, is an RNA virus in the genus Pestivirus of the family Flaviviridae. This genus also includes bovine viral diarrhoea virus (BVDV), border disease virus (BDV), and unclassified pestiviruses. CSFV is an enveloped virus with a single-stranded, positive-sense RNA genome of 12.5 kb in size. The genome comprises two highly conserved untranslated regions (UTR) at the 5′ and 3′ ends, and one open reading frame (ORF) encoding a 4000-amino acid polyprotein, which is processed by viral and cellular enzymes into four structural (C, E0, E1 and E2) and eight nonstructural (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins (Thiel et al., 1991; Meyers and Thiel, 1996). The structural protein E2 is one of the three envelope glycoproteins and the N-terminal half of E2 is one of the most variable regions in the CSFV genome (Lowings et al., 1996). It plays multiple roles in life cycle of CSFV. Virus mutants with partial or complete deletions of the E2 were found non-viable (van Gennip et al., 2002). The E2 protein is essential for virus attachment and entry into target cells as well as cell tropism (Reimann et al., 2004; Wang et al., 2004). It has been implicated that E2 is one of the virulence determinants (Risatti et al., 2005 and 2006; van Gennip et al., 2004). The glycoprotein E2 is also the most immunogenic among the CSFV proteins, inducing neutralizing antibodies and protection against lethal challenge (Hulst et al., 1993). Phylogenetic analysis based on the 190 nucleotides in this region is extensively used for classification of CSFV into three major groups and their subgroups. Group 1 and its three subgroups (1.1, 1.2 and 1.3) comprise most of the historical strains distributed in most regions of the world (Lowings et al., 1996; Paton et al., 2000). Group 2 containing most of the current viruses, which segregates into subgroup 2.1, 2.2, and 2.3, has increased activity and caused epidemic infection since the 1980s (Paton et al., 2000). The earliest 2.1 strain (VRI2277) was isolated from Malaysia in 1986 (Vilcek et al., 1996; Paton et al., 2000). In the 1990s, the subgroup 2.1 viruses have caused outbreaks in Germany (Oleksiewicz et al., 2003), the Netherlands (Widjojoatmodjo et al., 1999; Stegeman et al., 2000), Switzerland, Austria, Italy, Belgium, Spain (Paton et al., 2000), China (Tu et al., 2001) and Taiwan (Deng et al., 2005). Nevertheless, Group 3 contains disparate viruses distributed in regions such as Taiwan, Korea, Japan, Thailand and the United Kingdom (Sakoda et al., 1999; Paton et al., 2000). In Thailand, the disease was first reported in 1950 (Kongsamak et al., 1980). All groups have been isolated in Thailand, with the major prevalence being in group 3 (Parchariyanon et al., 2000). The aims of this study were to use molecular typing to analyze the relationship between isolates obtained from CSF outbreaks that occurred in different regions of Thailand during 2001 to 2009 and to classify these isolates within the current phylogenetic groups.

Materials and methods

Viruses

Thirteen viruses were isolated from swine samples submitted from field outbreaks in various locations of Thailand to National Institute of Animal Health (NIAH) in Bangkok and Western Veterinary Research and Development Center, Ratchaburi province during 2001 to 2009. The viruses were designated to show their provinces of isolation, NIAH case numbers, and year of isolation. For example, the isolate coded as Chonburi/NIAH132/01 was isolated from Chonburi province, given an NIAH case number as NIAH132/01, and isolated in the year 2001 (Table 1). All isolates were propagated in SK6 (swine kidney) cell line and identified with immunoperoxidase test according to Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2008). Infected cell culture fluids from different isolates were harvested and kept at -70°C until further analysis.

Table 1 Details of 13 CSFV Thai isolates during 2001 to 2009 and their analysis in genogroups

Virus isolate	Region of isolation (Province)	Year	Subgroup	Other viral pathogens		Accession number
				PRRS	PCV2	
Chonburi/NIAH132/01	Chonburi	2001	1.1	-	-	EU935422
Chonburi/NIAH369-4/01	Chonburi	2001	1.1	-	-	EU935423
Saraburi/NIAH669/01	Saraburi	2001	2.2	-	-	EU935424
Kanchanaburi/NIAH690/01	Kanchanaburi	2001	2.2	-	-	EU935425
Nakhonpathom/NIAH818-3/01	Nakhonpathom	2001	1.1	-	-	EU935426
Nakonnayok/NIAH1206/01	Nakonnayok	2001	1.1	-	-	EU935427
Angthong/NIAH1419/03	Angthong	2003	2.1	-	-	EU935428
Nakhonpathom/NIAH108478-1/08	Nakhonpathom	2008	2.1	-	-	EU935429
Nakhonpathom/NIAH108478-2/08	Nakhonpathom	2008	2.1	-	-	EU935430
Nakhonpathom/NIAH108478-3/08	Nakhonpathom	2008	2.1	-	-	EU935431
Pathumthani/NIAH108531/08	Pathumthani	2008	2.2	-	-	EU935432
Chainat/NIAH108587/08	Chainat	2008	2.2	-	-	EU935421
Ratchaburi/NIAH712200 /09	Ratchaburi	2009	2.1	+	-	FJ767710

Reverse transcription-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from infected cell culture fluids using the Qiagen RNA viral kit (Qiagen, Germany). The viral RNA was resuspended in diethylpyrocarbonate (DEPC)-treated water and stored at -70°C. The RNA samples were reverse transcribed and amplified in a one-step RT-PCR assay with the following diagnostic primers of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), and porcine circovirus type 2 (PCV2) described previously by Lowings et al. (1996), Mardassi et al. (1994), and Ellis et al. (2000), respectively. The sequences of primers are indicated in Table 2. Briefly, 5 µL of RNA were added in a 50 µL RT-PCR reaction containing 25 µL of 2x reaction buffer (Invitrogen), 1 µL each (10 µM) of forward and reverse primers, 1 µL of SuperScript III/Platinum Taq High Fidelity enzyme mix (Invitrogen) and 17 µL of DEPC-treated water. Reactions were incubated at 50°C for 30 min, 94°C for 2 min, and 40 cycles of

94°C for 30 sec, 50°C for 30 sec, 72°C for 1.5 min, followed by 72°C for 7 min. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with 0.5 µg/mL ethidium bromide (EtBr), and visualized under ultraviolet transillumination.

Table 2. Primers for diagnosis and sequencing in this study

Primer	Sequences (5 to 3)	Target gene	PCR product (bp)	Reference
CSF E2F	TCRWCAACCAAYGAGATAGG	E2	190 bp	Lowings et al.
CSF E2R	CACAGYCCRAAYCCRAAGTCATC			(1996)
PRRS 1010 PLS	ATGGCCAGCCAGTCAATCA	ORF7	EU* 398 bp	
PRRS 1011 PLR	TCGCCCTAATTGAATAGGTG		NA** 432 bp	Mardassi et al.
PCV2_F	CGGATATTGTAGTCCTGGTCG	ORF2	481 bp	(1994)
PCV2_R	ACTGTCAAGGCTACCACAGTCA			
				Ellis et al. (2000)

* European strain

** North American strain

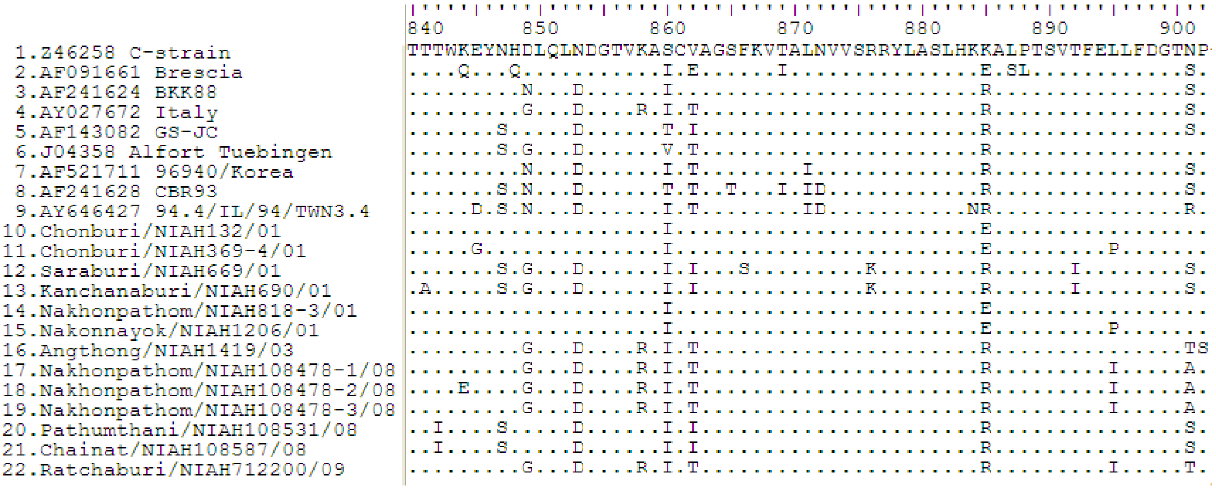
Sequencing and phylogenetic analysis

The PCR products of thirteen virus isolates positive for CSFV were purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, California, USA.) and the concentration of transcripts was determined using an ND-1000 NanoDrop spectrophotometer (NanoDrop® Technologies, USA) at 260 nm, ligated to a pGEM®-T vector system (Promega®, USA), and transferred into *DH5α Escherichia coli* by using Gene Pulser (Bio-RAD, USA). The clones were determined using gene specific primers and Bigdye® Terminator version 3.1 chemistry (Applied Biosystems, USA), according to the manufacturer's instructions. Reactions were run on a 3100 DNA analyser (Applied Biosystems, USA). Partial nucleotide sequencing of the E2 gene of the 13 CSFV isolates was performed from both directions of M13 primers (pGEM®-T vector) in the PCR amplification. Assembly of sequences and removal of low quality sequence data were performed by using SeqScape® software version 2.5 (Applied Biosystems, USA). Nucleotide sequence translation into protein sequence, additional multiple sequence alignments and processing were performed using the Bioedit software version 7.0.4.1 with an engine based on the Clustal W algorithm (Thompson et al., 1994). Phylogenetic analysis was carried out on a 190 nucleotide region of the E2 gene, which corresponds to nucleotide positions 2517 to 2706 encoding amino acid positions 840 to 902. The analysis of the nucleotide and the deduced amino acid sequences of the 13 CSFV isolates along with the sequences previously submitted to GenBank, as well as those from representatives of the different CSFV subgroups were constructed with Molecular Evolutionary Genetics Analysis (MEGA, version 4) (Tamura et al., 2007) using neighbor joining tree inference analysis with the Tamura-Nei model, with 1000 bootstrap replications.

Results

The RT-PCR results showed that from 13 CSFV positive samples, there was one sample, (Ratchaburi/NIAH712200/09), positive for both CSFV and PRRS, and none of the isolates was positive for PCV2 (Table 1). The deletion or insertion within the E2 gene of these 13 isolates was not found from the sequencing analysis (Figure 1). The comparisons of nucleotide and deduced amino acid sequences were demonstrated in Table 3. Nucleotide identities varied from 77.7% between the reference strains AY646427_94.4/IL/94/TWN3.4 and AF091661_Brescia to 100% such as between the Thai CSFV isolates Nakhonpathom/NIAH818-3/01 and Chonburi/NIAH132/01. Amino acid identities varied from 76.1% between the reference strains AF091661_Brescia and AY646427_94.4/IL/94/TWN3.4 to 100% such as between the Thai CSFV isolates Nakhonpathom/NIAH818-3/01 and Chonburi/NIAH132/01. The phylogenetic analysis was shown in Figure 2. We found that the 13 Thai CSFV isolates were separated into three groups. The first group consisted of 4 Thai CSFV isolates collected in 2001 including Chonburi/ NIAH132/01, Chonburi/NIAH369-4/01, Nakhonpathom/NIAH818-3/01, and Nakonnayok/ NIAH1206/01 sharing 98.9-100% and 96.8-100% homology among them in nucleotide and amino acid sequences, respectively, and belonged to subgroup 1.1, which was represented by the C-strain (Table 3 and Figure 2). The second group consisted of 5 isolates including Angthong/NIAH1419/03, Nakhonpathom/ NIAH108478-1/08, Nakhonpathom/NIAH108478-2/08, Nakhonpathom/NIAH108478-3/08, and Ratchaburi/ NIAH712200/09) sharing 97.3-100% and 93.6-100% homology in nucleotide and amino acid sequences, respectively, and belonged to subgroup 2.1, which was represented by the Italy strain. The last group consisted of 4 isolates including Saraburi/NIAH669/01, Kanchanaburi/NIAH690/01, Pathumthani/NIAH108531/08, Chainat/NIAH108587/08) sharing 91.5-100% and 92.0-96.8% amino acid homology in nucleotide and amino acid sequences, respectively, and belonged to subgroup 2.2 , which was represented by the the GS-JC strain. In addition, the isolates in the first group had 97.3-98.4% nucleotide sequence and 93.6-96.8% amino acid sequence similarities with the C-strain, whereas those in the second group had 88.3-89.9% nucleotide sequence and 95.2-96.8% amino acid sequence similarities with Italy strain, and those in the last group had 92.0-92.5% nucleotide sequence and 92.0-96.8% amino acid sequence similarities with the GS-JC-strain (Table 3).

Molecular epidemiological data of CSFV isolates during 1988 - 1996 (Parchariyanon et al., 2000) and 2001 - 2009 by their subgroups in Thailand were shown in Table 4.



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                                 |''''|''''|''''|''''|''''|''''|''''|''''|''''|''''|''''|''''|''
                                 840       850       860       870       880       890       900
 1.Z46258 C-strain               TTTWKEYNHDLQLNDGTVKASCVAGSFKVTALNVVSRRYLASLHKKALPTSVTFELLFDGTNP
 2.AF091661 Brescia              ....Q...Q...........I.E......I...............E.SL............S.
 3.AF241624 BKK88                .........N...D......I........................R...............S.
 4.AY027672 Italy                .........G...D....R.I.T......................R...............S.
 5.AF143082 GS-JC                .......S.....D......T.I......................R...............S.
 6.J04358 Alfort Tuebingen       .......S.G...D......V.T......................R.................
 7.AF521711 96940/Korea          .........N...D......I.T........I.............R...............S.
 8.AF241628 CBR93                .......S.N...D......T.T..T...I.ID............R...............S.
 9.AY646427 94.4/IL/94/TWN3.4    .....D.S.N...D......I.T........ID...........NR...............R.
10.Chonburi/NIAH132/01           ....................I........................E.................
11.Chonburi/NIAH369-4/01         .....G..............I........................E.........P.......
12.Saraburi/NIAH669/01           .......S.G...D......I.I...S.........K........R......I........S.
13.Kanchanaburi/NIAH690/01       .A.....S.G...D......I.I.............K........R......I........S.
14.Nakhonpathom/NIAH818-3/01     ....................I........................E.................
15.Nakonnayok/NIAH1206/01        ....................I........................E.........P.......
16.Angthong/NIAH1419/03          .........G...D....R.I.T......................R...............TS
17.Nakhonpathom/NIAH108478-1/08  .........G...D....R.I.T......................R.........I.....A.
18.Nakhonpathom/NIAH108478-2/08  ....E....G...D....R.I.T......................R.........I.....A.
19.Nakhonpathom/NIAH108478-3/08  .........G...D....R.I.T......................R.........I.....A.
20.Pathumthani/NIAH108531/08     ..I....S.....D......I.I......................R...............S.
21.Chainat/NIAH108587/08         ..I....S.....D......I.I......................R...............S.
22.Ratchaburi/NIAH712200/09      .........G...D....R.I.T......................R.........I.....T.
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Figure 1 Alignment of amino acid sequence of CSFV isolates corresponding to positions 840-902 of the vaccine C-strain. Dot (.) indicates sequence identity.

Table 3 Comparison of specific nucleotide and deduced amino acid of E2 gene of CSF viruses

Sequence Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1.Z46258_C-strain*	ID	85.7	92	88.8	90.4	90.4	88.8	82.5	82.5	96.8	93.6	84.1	84.1	96.8	95.2	87.3	87.3	85.7	87.3	88.8	88.8	87.3
2.AF091661_Brescia*	93.1	ID	85.7	84.1	84.1	80.9	84.1	79.3	76.1	88.8	85.7	79.3	79.3	88.8	87.3	80.9	80.9	80.9	80.9	84.1	84.1	80.9
3.AF241624_BKK88*	90.4	89.4	ID	95.2	93.6	92	96.8	88.8	88.8	93.6	90.4	90.4	90.4	93.6	92	92	92	90.4	92	93.6	93.6	92
4.AY027672_Italy*	84.1	83.5	86.2	ID	92	93.6	95.2	87.3	87.3	90.4	87.3	90.4	90.4	90.4	88.8	96.8	96.8	95.2	96.8	92	92	96.8
5.AF143082_GS-JC*	84.6	83	86.7	86.7	ID	93.6	92	90.4	87.3	90.4	87.3	92	92	90.4	88.8	88.8	88.8	87.3	88.8	96.8	96.8	88.8
6.J04358_Alfort_Tuebingen*	85.7	81.4	84.6	87.3	88.8	ID	93.6	92	90.4	87.3	90.4	87.3	92	92	90.4	88.8	88.8	88.8	87.3	88.8	96.8	96.8
7.AF521711_96940/Korea*	87.3	84.1	88.3	82	82	83.5	ID	92	92	90.4	87.3	88.8	88.8	90.4	88.8	92	92	90.4	92	92	92	92
8.AF241628_CBR93*	84.1	82.5	87.3	78.3	83	82	86.2	ID	90.4	82.5	79.3	84.1	84.1	82.5	80.9	84.1	84.1	82.5	84.1	87.3	87.3	84.1
9.AY646427_94.4/IL/94/TWN*	80.9	77.7	80.9	81.4	80.9	80.9	85.1	83	ID	84.1	82.5	84.1	84.1	84.1	82.5	85.7	85.7	84.1	85.7	87.3	87.3	85.7
10.Chonburi/NIAH132/01	98.4	94.7	91	84.6	84.6	86.2	86.7	83	80.4	ID	96.8	85.7	85.7	100	98.4	88.8	88.8	87.3	88.8	90.4	90.4	88.8
11.Chonburi/NIAH369-4/01	97.3	93.6	89.9	83.5	83.5	85.1	85.7	82	79.3	98.9	ID	82.5	82.5	96.8	98.4	85.7	87.3	85.7	87.3	87.3	87.3	87.3
12.Saraburi/NIAH669/01	82	80.4	84.1	85.1	92.5	88.8	82	80.4	79.3	82.5	81.4	ID	96.8	85.7	84.1	87.3	87.3	85.7	87.3	92	92	87.3
13.Kanchanaburi/NIAH690/01	81.4	79.8	83.5	84.6	92	88.3	82.5	79.8	78.8	82	80.9	98.4	ID	85.7	84.1	87.3	87.3	85.7	87.3	92	92	87.3
14.Nakhonpathom/NIAH818-3/01	98.4	94.7	91	84.6	84.6	86.2	86.7	83	80.4	100	98.9	82.5	82	ID	98.4	88.8	88.8	87.3	88.8	90.4	90.4	88.8
15.Nakonnayok/NIAH1206/01	97.8	94.1	90.4	84.1	84.1	85.7	86.2	82.5	79.8	99.4	99.4	82	81.4	99.4	ID	87.3	88.8	87.3	88.8	88.8	88.8	88.8
16.Angthong/NIAH1419/03	81.4	80.9	83	89.9	88.8	86.2	79.8	81.4	82.5	82	80.9	86.2	85.7	82	81.4	ID	95.2	93.6	95.2	88.8	88.8	96.8
17.Nakhonpathom/NIAH108478-1/08	80.4	79.8	82.5	88.8	87.8	85.7	78.8	80.4	82	80.9	79.8	85.1	84.6	80.9	80.4	97.8	ID	98.4	100	88.8	88.8	98.4
18.Nakhonpathom/NIAH108478-2/08	79.8	79.8	82	88.3	87.3	85.1	78.3	79.8	81.4	80.4	79.3	84.6	84.1	80.4	79.8	97.3	99.4	ID	98.4	87.3	87.3	96.8
19.Nakhonpathom/NIAH108478-3/08	80.4	79.8	82.5	88.8	87.8	85.7	78.8	80.4	82	80.9	79.8	85.1	84.6	80.9	80.4	97.8	100	99.4	ID	88.8	88.8	98.4
20.Pathumthani/NIAH108531/08	84.1	82.5	85.7	84.6	92	87.8	84.1	82	82.5	84.6	83.5	92	91.5	84.6	84.1	85.7	84.6	84.1	84.6	ID	100	88.8
21.Chainat/NIAH108587/08	84.1	82.5	85.7	84.6	92	87.8	84.1	82	82.5	84.6	83.5	92	91.5	84.6	84.1	85.7	84.6	84.1	84.6	100	ID	88.8
22.Ratchaburi/NIAH712200/09	80.4	79.8	82.5	88.8	87.8	85.7	78.8	81.4	81.4	80.9	79.8	85.1	84.6	80.9	80.4	97.8	98.9	98.4	98.9	84.6	84.6	ID

Nucleotide identity (%) in lower triangle; Deduced amino acid identity (%) in upper triangle

* References strains

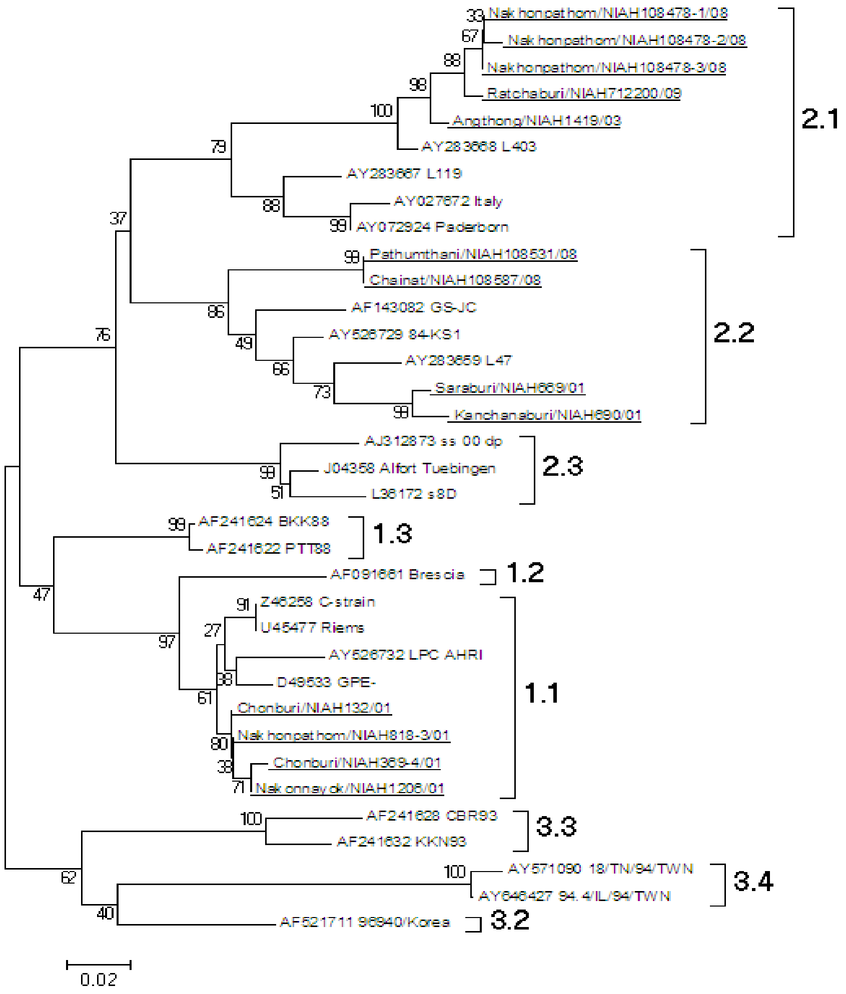

Figure 2 Phylogenetic relationships of the E2 gene of representative CSF viruses isolated in Thailand and compared with other CSFV sequences in GenBank. Tree was generated by using MEGA 4 program (neighbor-joining analysis method and with Tamura -Nei model). Numbers below branches indicate bootstrap values from 1000 replicates. Analysis was based on nucleotides 2517 to 2706 (190 nt).

Table 4 Number of CSFV isolates in each subgroup isolated in Thailand during 1988-1996* and 2001-2009

Subgroup	Year		Total
	1988-1996	2001-2009	
1.1	4	4	8
1.2	1	0	1
1.3	6	0	6
2.1	0	5	5
2.2	1	4	5
3.3	18	0	18
Total	30	13	43

* Parchariyanon et al. (2000)

Discussion

According to the sequence and phylogenetic analyses results of the 13 Thai CSFV isolates collected during 2001-2009, we found that the virus isolates in this study were segregated into three subgroups belonged to subgroups 1.1, 2.1, and 2.2. Comparing to the previous study of Parchariyanon et al. (2000), which indicated that the Thai CSF viruses isolated between 1988 and 1996 fell into subgroups 1.1, 1.2, 1.3, 2.2, and 3.3, none of the 13 Thai CSF viruses isolated during 2001-2009 in this study fell into subgroup 1.2, 1.3, and 3.3 (Table 4).

Subgroup 1.1 was represented by the CSFV C-strain and included most vaccine strains, which was recognized as a historical group. From 1988 to 2001, a total of eight Thai CSFV isolates of this subgroup were found including one isolate in 1988, two in 1993, one in 1994 (Parchariyanon et al., 2000) and four in 2001 (Table 1). However, we found five virus isolates falling into subgroup 2.1 represented by the Italy strain, which was not found in the previous study. The first strain (VRI 2277) of CSFV subgroup 2.1 was isolated from Malaysia in 1986 (Vilcek et al., 1996; Paton et al., 2000). In Thailand, the first isolate of CSFV subgroup 2.1 was found in 2003 in Angthong province. Five years later, it was isolated from Nakhonpathom province in 2008 and then from Ratchaburi province in 2009 (Table 1 and Table 4). Subgroup 2.2 included the reference strain, GS-JC-strain. From 1988 to 2009, a total of five Thai CSFV isolates of this subgroup were found including one isolate in 1996 (Parchariyanon et al., 2000), two in 2001, and two in 2008 (Table 1 and Table 4).

Our previous studies revealed that subgroup 3.3 was the main genogroup that contributed to the epidemic of CSFV during 1988-1996 in Thailand (Parchariyanon *et al.,* 2000; Paton *et al.,* 2000). The subgroup 3.3 was the dominant genogroup prior to 1996 in Thailand and could not be isolated from the field thereafter. Molecular epidemiology data from this study showed that genogroup 2 had replaced subgroup 3.3, similar to the studies in Taiwan and China. In Taiwan, there was evidence that subgroup 2.1a viruses had replaced subgroup 3.4 strains, which was the dominant genogroup prior to 1996 (Pan *et al.,* 2004), whereas genogroup 2 strains had replaced genogroup 1 viruses in China (Chen *et al.,* 2008; Zhu *et al.,* 2009).

There is only one serotype of CSFV and the live attenuated commercial C-strain vaccines against CSFV are available, which have been used prophylactically for many years, however, some questions remain, whether the replacement of the dominant subgroup affected the efficacy of the vaccines. The question was solved when the vaccine-challenge studies of the dominant subgroup 3.3 strain and the replacement genogroup 2 strain isolated in Thailand were reported including the study of Parchariyanon *et al*. (2001), which demonstrated the ability of C-strain to protect against clinical signs upon challenge with subgroup 3.3 (CBR/94/2) two weeks after vaccination and the experiment conducted by Damrongwatanapokin *et al.* (2002), which showed that the C-strain immunized pigs were fully protected from challenge with subgroup 2.2. Similar results were also observed by Taiwanese researchers with subgroups 2.1a and 2.1b (Pan et al., 2004). These study results were supported by the recent investigation of Graham et al. (2012a), which showed that pigs vaccinated with C-strain vaccine at 1, 3 and 5 days prior to challenge with genogroup 2.1 (UK 2000/7.1) and 3.3 (CBR/93) viruses, protection was complete at 5 days post-vaccination and even by one day post-vaccination approximately half the animals were partially protected and were able to control the infection. The rapid protection conferred by the C-strain vaccine involves broadly cross-reactive T cell IFN-γ responses. These informations may help the development of the next generation of CSFV vaccines (Graham et al., 2012b).

Interestingly, we also found that the Ratchaburi/NIAH712200/09 virus isolate co-infected with PRRSV that might be one reason for CSFV vaccination failure in endemic area (Suradhat *et al.,* 2006). Besides PRRSV, other pathogens could also cause CSFV escape from immune system such as infected of PCV2 may render the host more susceptible to CSFV infection (Vincent *et al.,* 2007). Therefore, identification of multiple causative agents of the diseases including PRRSV and PCV2 should be considered in the investigation of suspected CSF outbreak.

In conclusions, the results of sequence and phylogenetic analyses help to understand the dynamics of Thai CSF virus subgroup evolution and support epidemiological observations.

Acknowledgments

We thank Ms. Patcharin Muensa and our colleagues of Virology Section, National Institute of Animal Health for laboratory assistance and Western Veterinary Research and Development Center, Ratchaburi province for providing us a sample, Ratchaburi/NIAH712200/09.

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ลักษณะทางพันธุกรรมระดับโมเลกุลของยีน E2 ไกลโคโปรตีนของเชื้อไวรัสอหิวาต์สุกรในประเทศไทย

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บทคัดย่อ

การศึกษาลักษณะทางพันธุกรรมระดับโมเลกุลของเชื้อไวรัสอหิวาต์สุกร จำนวน 13 ตัวอย่าง ที่พบระบาดในประเทศไทย ระหว่างปี พ.ศ. 2544 ถึง 2552 และวินิจฉัยโดย RT-PCR นำมาศึกษาการเรียงลำดับเบสของรหัสพันธุกรรมและ การวิเคราะห์ทาง phylogenetics ของยีน E2 ไกลโคโปรตีน โดยเปรียบเทียบกับเชื้อที่รายงานใน GenBank และเชื้ออ้างอิง พบว่าเชื้อที่ศึกษาทั้งหมดแบ่งออกเป็น 3 กลุ่ม โดยกลุ่มที่ 1 จัดอยู่ใน subgroup 1.1 มี 4 ตัวอย่าง ซึ่งยีน E2 มีลำดับนิวคลีโอไทด์ และกรดอะมิโนเหมือนกัน 98.9-100% และ 96.8-100% ตามลำดับ และมีความเหมือนกับเชื้อสายพันธุ์อ้างอิง C-Strain 97.3-98.4% และ 93.6-96.8% ตามลำดับ กลุ่มที่ 2 จัดอยู่ใน subgroup 2.1 มี 5 ตัวอย่าง ซึ่งยีน E2 มีลำดับนิวคลีโอไทด์ และกรดอะมิโนเหมือนกัน 97.3-100% และ 93.6-100% ตามลำดับ และมีความเหมือนกับเชื้อสายพันธุ์อ้างอิงในประเทศอิตาลี 88.3-89.9% และ 95.2-96.8% ตามลำดับ และกลุ่มที่ 3 จัดอยู่ใน subgroup 2.2 มี 4 ตัวอย่าง ซึ่งยีน E2 ของกลุ่มนี้มีลำดับนิวคลีโอไทด์ และกรดอะมิโนเหมือนกัน 91.5-100% และ 92.0-96.8% และมีความเหมือนกับเชื้อสายพันธุ์อ้างอิง GS-JC 92.0-92.5% และ 92.0-96.8 ตามลำดับ ข้อมูลเหล่านี้ช่วยให้เข้าใจการเปลี่ยนแปลงอย่างต่อเนื่องของวิวัฒนาการของเชื้อไวรัสอหิวาต์สุกรกลุ่มย่อยต่างๆ และช่วยในการสังเกตการณ์ทางระบาดวิทยา

คำสำคัญ: เชื้อไวรัสอหิวาต์สุกร　E2 ไกลโคโปรตีน　การวิเคราะห์ทาง phylogenetics
การวิเคราะห์การเรียงลำดับเบสของรหัสพันธุกรรม