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Genetic diversity of swine influenza viruses in Thai swine farms, 2011–2014

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Abstract The pig is known as a "mixing vessel" for influenza A viruses. The co-circulation of multiple influenza A subtypes in pig populations can lead to novel reassortant strains. For this study, swine influenza surveillance was conducted from September 2011 to February 2014 on 46 swine farms in Thailand. In total, 78 swine influenza viruses were isolated from 2,821 nasal swabs, and 12 were selected for characterization by whole genome sequencing. Our results showed that the co-circulation of swine influenza subtypes H1N1, H3N2, and H1N2 in Thai swine farms was observable throughout the 3 years of surveillance. Furthermore, we repeatedly found reassortant viruses between endemic swine influenza viruses and pandemic H1N1 2009. This observation suggests that there is significant and rapid evolution of swine influenza viruses in swine. Thus, continuous surveillance is critical for monitoring novel reassortant influenza A viruses in Thai swine populations.

Keywords Genetic diversity · Reassortant · Swine influenza · Surveillance · Thailand

Introduction

The influenza A virus (IAV) belongs to the family *Orthomyxoviridae*. IAVs can be classified into subtypes based on two major surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Currently, 18 HA and 11 NA subtypes have been identified [1], while IAV subtypes H1N1, H1N2, and H3N2 have been reported in swine populations worldwide [2]. In Thailand, the endemic swine influenza virus (SIV) subtype H1N1 was first reported in 1991 [3]. The genetic composition of Thai endemic SIV-H1N1 (eH1N1) has been characterized as eH1N1 (6 + 2) and eH1N1 (7 + 1) [4]. The genetic composition of Thai endemic SIV-H3N2 (eH3N2), however, has been

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characterized as of Eurasian swine lineage (PB2, PB1, PA, and M), classical swine lineage (NP and NS), and of seasonal human H3N2 origin (H3 and N2) [5, 6].

In April 2009, pandemic H1N1 (pH1N1) was first reported in humans and quickly spread worldwide. It was first isolated from Thai pigs in November 2009 [7], and subsequent surveillance in Thailand detected a novel reassorted SIV in 2010 [4]. Because of the ongoing circulations of multiple SIV lineages among Thai pigs and the evidence of viral reassortment in swine, SIV surveillance in Thailand should be a priority. This study conducted 3 years of SIV surveillance on Thai swine farms and found SIV subtypes H1N1, H1N2, and H3N2 circulating among pigs. Observations of the reassortant SIVs, rH1N1, rH1N2, and rH3N2, however, were predominant. The genetic diversity of those reassortant viruses is described herein.

Materials and methods

Surveillance of Thai swine farms

Between September 2011 and February 2014, a cross-sectional SIV surveillance program was conducted at Thai swine farms located in 13 high swine density provinces representing all parts of Thailand. In total, 2,821 nasal swab samples were obtained from 46 swine farms. The samples were collected individually from pigs of different ages and transported within 24 h for laboratory analysis. During transport, each sample was kept in a standard viral transport medium encased in ice. Details of samples and farm locations are shown in Table 1.

Identification and isolation of SIVs

All nasal swabs were screened for IAVs through one-step real-time RT-PCR (rRT-PCR). Viral RNAs were extracted from samples with the QIAamp Viral RNA Mini Kit (Qiagen®; Hilden, Germany). rRT-PCR was conducted using a TaqMan probe to detect the IAV matrix (M) gene with some modification [8]. One-step rRT-PCR was performed on a Rotor-Gene 3000 (Corbett Research; Sydney, Australia) utilizing the SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Invitrogen™; California, USA). Data acquisition and analysis of the rRT-PCR assay were done through the Rotor-Gene software, v.6.0.19. Samples exhibiting a Ct value of <36 were interpreted as positive and those with a Ct value of 36–40 as suspect.

The positive rRT-PCR samples were then subjected to IAV isolation using egg inoculation and/or cell culture. For egg inoculations, we inoculated embryonated chicken eggs according to WHO recommendations [9]. After a 72-h incubation period, the allantoic fluid of each egg was collected and tested for hemagglutinin activity with a hemagglutination test (HA test) using a 1 % suspension of chicken red blood cells. For cell culture, Madin Darby Canine Kidney (MDCK) cells were used for viral propagation. During the incubation period of 72 h, we made daily observations for cytopathic effect (CPE) and, after incubation, collected the supernatants of CPE positives. Samples that tested positive by HA test at 4 HA units/50 µl or more and CPE positive cell supernatants were subsequently subjected to IAV confirmation by rRT-PCR for M gene detection as previously described.

Genetic characterization of Thai SIVs

To subtype IAVs, cDNA was synthesized using the influenza universal primer Uni12 [10] and the ImProm-II™ Reverse Transcription System (Promega; Wisconsin, USA). The cDNA served as a template for subtype identification using specific primers in our inventory for the HA and NA genes. In this study, 12 viruses were selected based on epidemiological data as representatives for whole

Table 1 Description of samples, virus identification, virus isolation, and virus subtyping

Year	# of samples	# of farms (provinces)	rRT-PCR (% positive)	SIV isolation (% positive)	SIV subtype (# positive sample)		
					H1N1	H1N2	H3N2
2011[a]	174	9 (4)	28/174 (16.09 %)	4/174 (2.29 %)	0	1	3
2012	1,219	19 (8)	109/1,219 (8.94 %)	28/1,219 (2.30 %)	12	2	14
2013	1,345	32 (10)	42/1,345 (3.12 %)	39/1,345 (2.89 %)	19	0	20
2014[b]	83	3 (3)	9/83 (10.84 %)	7/83 (8.43 %)	5	0	2
	2,821		188/2,821 (6.66 %)	78/2,821 (2.76 %)	36	3	39

[a] 2011: sample collection in September–December

[b] 2014: sample collection in January–February

Table 2 Description of selected SIVs characterized in this study

Virus	Subtype	Year	Age (weeks)	Type of farm[a]	Location	GenBank Accession No.
A/Swine/Chonburi/NIAH9469/04[b]	eH1N1 (6 + 2)	2004	–	–	Chonburi	AB434301-08
A/Swine/Thailand/CU-S3334/12	eH1N1 (6 + 2)	2012	8	C	Chonburi	KJ162027-33, KJ162046
A/Swine/Thailand/CU-S3350/12	eH1N1 (6 + 2)	2012	6	D	Ratchaburi	KJ162034-41
A/Swine/Thailand/CU-S3406/12	eH1N1 (6 + 2)	2012	4	D	Ratchaburi	KJ526053-59, KM355356
A/Swine/Thailand/CU-S3629/12	rH1N1 (TRIG + 2)	2012	4	B	Nakhon Pathom	KJ526067-73, KM355357
A/Swine/Thailand/CU-S3795/13	rH1N1 (TRIG + 2)	2013	4	B	Nakhon Pathom	KJ526034-38, KM355358-60
A/Swine/Saraburi/NIAH13021/05[c]	eH1N2	2005	–	–	Saraburi	AB434333-40
A/Swine/Thailand/CU-S3073/11	rH1N2 (7 + 1)	2011	4	D	Ratchaburi	KJ162042-43, KM355361-66
A/Swine/Thailand/CU-S3631/12	rH1N2 (TRIG + 2)	2012	4	B	Nakhon Pathom	KJ526039-44, KM355367-68
A/Swine/Thailand/KU5.1/04[d]	eH3N2	2004	–	–	–	FJ561057-64
A/Swine/Thailand/CU-S3474/12	rH3N2 (TRIG + 2)	2012	8	C	Chonburi	KM355369-76
A/Swine/Thailand/CU-S3673/12	rH3N2 (TRIG + 2)	2012	4	D	Chonburi	KJ526061-66, KM355377-78
A/Swine/Thailand/CU-S3689/13	rH3N2 (TRIG + 2)	2013	4	A	Chonburi	KJ526048-52 KM355379-81
A/Swine/Thailand/CU-S14129/13	rH3N2 (TRIG + 2)	2013	4	D	Ratchaburi	KM355382-89
A/Swine/Thailand/CU-S14252/14	rH3N2 (TRIG + 2)	2014	4	D	Ratchaburi	KM355390-97

[a] Type of farm abbreviation; A = <50-sow herd, B = 51–200-sow herd, C = 201–500-sow herd, D = >500-sow herd

[b] Reference Thai eH1N1; A/swine/Chonburi/NIAH9469/04 [5] was included in the analysis

[c] Reference Thai eH1N2; A/swine/Saraburi/NIAH13021/05 [5] was included in the analysis

[d] Reference Thai eH3N2; A/swine/Thailand/KU5.1/04 [6] was included in the analysis

genome sequencing. Each viral gene was amplified using specific primers, and then PCR products were subjected to DNA sequencing (1st Base Laboratories Sdn Bhd, Malaysia). The nucleotide sequences of each gene were validated and assembled in SeqMan software v.5.03 (DNASTAR Inc.; Wisconsin, USA).

Phylogenetic and genetic analyses were performed by comparing each viral gene segment with reference SIV sequences available at the GenBank database. The reference nucleotide sequences that were retrieved included all geographical origins (Eurasia and North America) and three host origins (human, swine, and avian) for constructing phylogenetic trees. The nucleotide sequences of each gene were aligned in Muscle v.3.6 [11]. The phylogenetic trees were constructed with two software: MEGA v.6.0, using the neighbor-joining algorithm with the Kimura-2 parameter model applied to 1,000 replications of bootstrap, and BEAST software, using the BMCMC with 1,000,000 generations and an average standard deviation of split frequencies <0.05 [12, 13]. To support tree topology, the bootstrap percentages and posterior probabilities were evaluated. The nucleotide sequences and deduced amino acids of each viral gene were aligned and compared in MegAlign software v.5.03 (DNASTAR Inc.; Wisconsin, USA). The nucleotide sequences of the Thai SIVs were submitted to the GenBank database under the accession numbers shown in Table 2.

Results

Prevalence and subtypes of SIVs in Thai swine farms

During our 3 years of SIV surveillance in Thai swine farms, 2,821 nasal swab samples were collected and examined. 188 (6.66 %) were identified as IAV positive through rRT-PCR. Subsequently, 78 SIV isolates (41.5 %) were successfully recovered from IAV-positive samples by egg inoculation and/or MDCK cell culture (Table 2). Further identification of the 78 SIV isolates revealed the subtypes H1N1 (36; 46.15 %), H1N2 (3; 3.85 %), and H3N2 (39; 50.00 %). These results suggest that SIV subtypes H1N1 and H3N2 were the predominant subtypes in Thai swine populations (Table 1).

Genetic characteristics of Thai SIV

Out of the 78 SIV isolates, 12 were selected for whole genome sequencing based on epidemiological data such as location, influenza subtype, year of isolation, age of pig, and type of swine farm (Table 2). The twelve SIVs characterized in this study were of the subtypes H1N1 ($n = 5$), H1N2 ($n = 2$), and H3N2 ($n = 5$).

In general, the H1 gene of SIVs can be phylogenetically grouped into two major lineages: classical and Eurasian. The classical lineage can be further divided into four

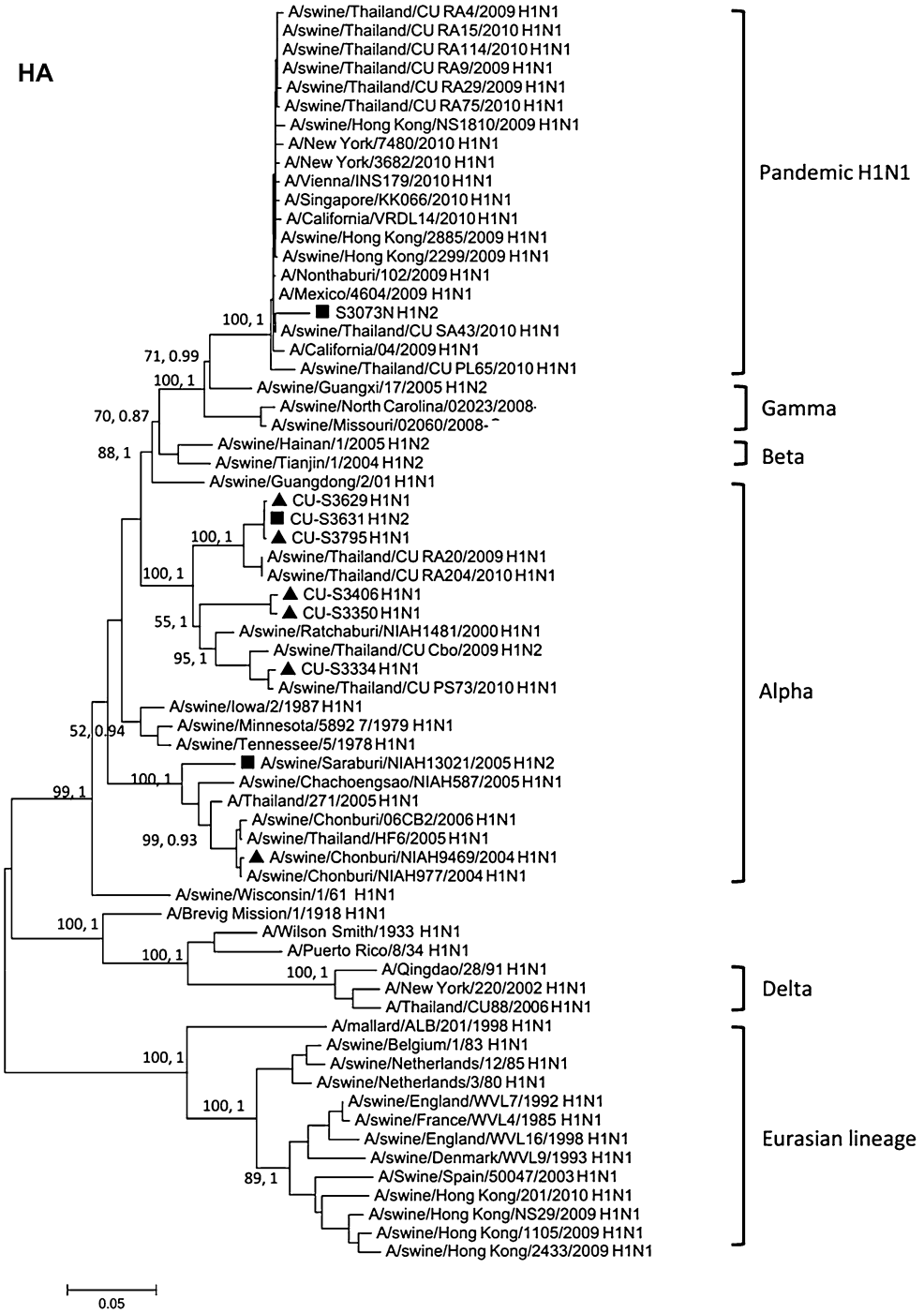

Fig. 1 Phylogenetic analysis of the H1. The phylogenetic tree was constructed with the neighbor-joining algorithm and the Kimura-2 parameter model applied to 1,000 replications of bootstrap and with the BMCMC. Node label shows the bootstrap percentage and posterior probabilities in *parenthesis* (bootstrap percentage, posterior probability). *Triangle* and *quadrilateral* indicate SIV-H1N1 and SIV-H1N2, respectively

sub-lineages: alpha, beta, gamma, and delta [14]. Phylogenetic analysis of the H1 gene revealed that six Thai SIVs (CU-S3334, CU-S3350, CU-S3406, CU-S3629, CU-S3795, and CU-S3631) were clustered into the alpha group of the classical lineage, while only one (CU-S3073) was grouped into the pandemic cluster, which is a member of the gamma group of the classical lineage (Fig. 1). Phylogenetic analysis of the N1 gene showed that all five Thai H1N1 SIVs belonged to the Eurasian lineage.

In general, the H3 gene of SIVs can be grouped into Ha and Hb subgroups in which H3 can be evolved from either a human H3N2 strain circulating in late 1990s or human-like H3N2 swine strain circulating in early 1970s [5]. In this study, the H3 genes of five H3N2 SIVs (CU-S3474, CU-S3673, CU-S3689, CU-S14129, and CU-S14252) were clustered into the Ha subgroup of human H3N2 lineage (Fig. 2). Similarly, seven of the N2 genes of H1N2 (CU-S3073 and CU-S3631) and H3N2 SIVs (CU-S3474,

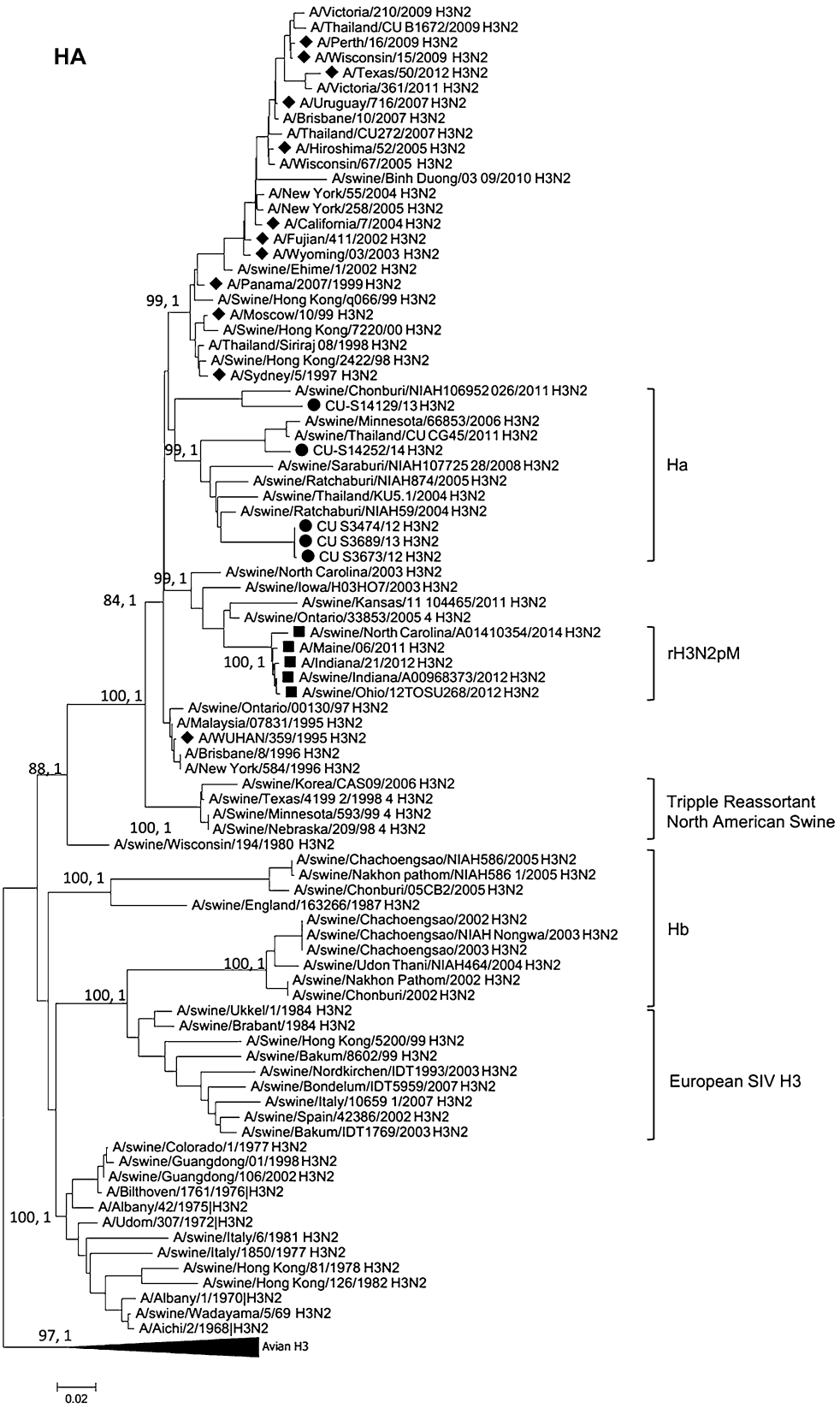

Fig. 2 Phylogenetic analysis of the H3. The phylogenetic tree was constructed with the neighbor-joining algorithm and the Kimura-2 parameter model applied to 1,000 replications of bootstrap and with the BMCMC. Node label shows the bootstrap percentage and posterior probabilities in *parenthesis* (bootstrap percentage, posterior probability). *Diamond*, *circle,* and *squares* indicate seasonal human vaccine strains H3N2, SIV-H3N2 in this study, and H3N2pM, respectively

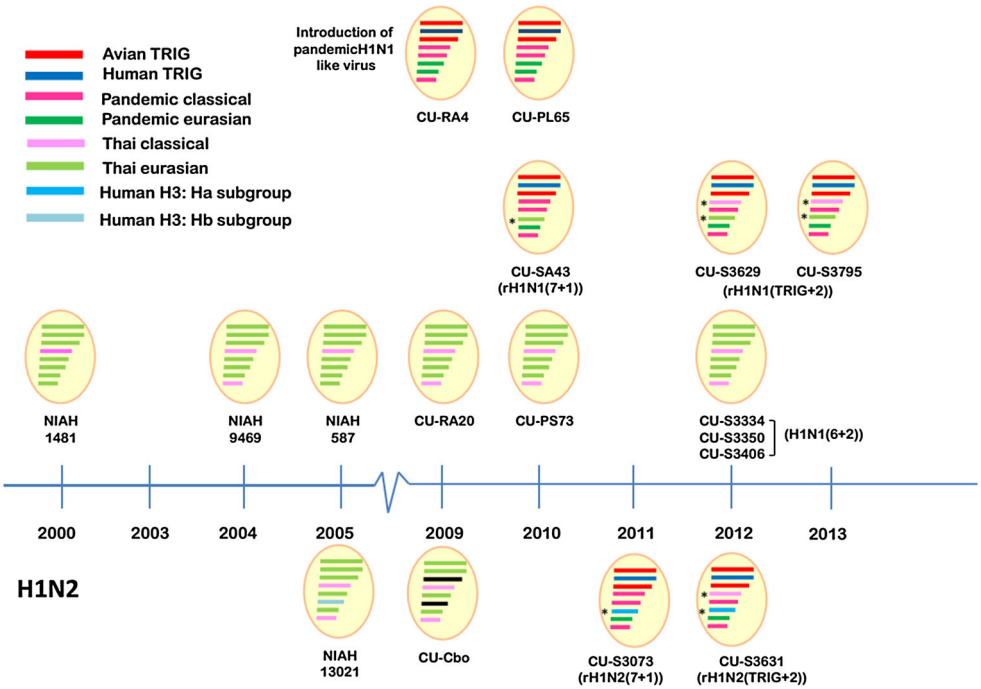

Fig. 3 Schematic representation of the genetic constellation of Thai SIV-H1N1 and SIV-H1N2 during 2000–2013. The *oval* represents viral particle, and each *line* represents each gene segment ascending from segment 1 to segment 8, respectively. The lineages of gene segment present in different *colors*. The reassortant segments are emphasized by shape outline (Color figure online)

CU-S3673, CU-S3689, CU-S14129, and CU-S14252) were clustered into the human H3N2 lineage.

Overall, five, distinct genetic constellations of Thai SIVs were observed in this study: eH1N1 (6 + 2), rH1N1 (TRIG + 2), rH1N2 (7 + 1), rH1N2 (TRIG + 2), and rH3N2 (TRIG + 2). Based on previous reports from Thailand, Thai endemic SIV-H1N1 (eH1N1) has only two genetic constellations. The first genetic constellation is eH1N1 (7 + 1), comprised the H1 gene from the classical lineage and seven other genes from the Eurasian lineage. The second genetic constellation is eH1N1 (6 + 2), comprised the H1 and NS genes of the classical lineage and six other genes from the Eurasian lineage [4]. Both eH1N1 (7 + 1) and eH1N1 (6 + 2) were circulating among Thai swine populations until 2005. Subsequently, eH1N1 (7 + 1) disappeared, while eH1N1 (6 + 2) was continuously observed until 2012. In this study, we observed both eH1N1 (6 + 2) (CU-S3334, CU-S3350, and CU-S3406) and reassortant H1N1 viruses (rH1N1) (CU-S3629 and CU-S3795) (Fig. 3). The rH1N1 viruses contained the TRIG cassette of pH1N1 as well as the H1 and N1 genes of Thai endemic SIVs (TRIG + 2) (Fig. 3).

The genetic constellation of Thai endemic SIV-H1N2 (eH1N2) in 2005 (NIAH13021) had five genes of the Eurasian lineage (PB2, PB1, PA, NP, and M), two genes of the classical lineage (H1 and NS), and an N2 gene of human origin. In this study, we observed two types of rH1N2 during 2011 and 2012. The first rH1N2 (CU-S3073) contained seven genes from pH1N1 with an N2 gene from eH1N2 and was designated as 7 + 1. The second rH1N2 (CU-S3631), containing the TRIG cassette and the H1 and N2 genes from eH1N2, was designated as TRIG +2 (Fig. 3).

Thai endemic SIV-H3N2 (eH3N2) in 2004 and 2005 had two distinct genetic constellations. The first constellation comprised PB2, PB1, PA and M of the Eurasian lineage, NP and NS of the classical lineage and H3 and N2 of human H3N2 origin (KU5.1). The second constellation comprised PB2, PB1, PA, M, and NS of the Eurasian lineage, NP of the classical lineage, and H3 and N2 of human H3N2 origin (NIAH586-1). In this study, we found that rH3N2 (TRIG + 2) was predominant in Thai pigs from 2011 to 2014. The viruses (CU-S3474, CU-S3673, CU-S3689, CU-S14129, and CU-S14252) contained the TRIG cassette with H3 and N2 genes from eH3N2 (Fig. 4).

Genetic analyses of SIVs characterized in this study are shown in Tables 3 and 4. The seven SIV H1 genes characterized in this study were compared with four reference viruses: eH1N1 (NIAH9469), pH1N1 (CA/04 and CU-RA4), and eH1N2 (NIAH13021). Our results showed that the H1 viruses were divided into either pandemic (P) or alpha (α) clusters. H1 SIVs of the alpha cluster exhibit high amino acid diversity at five antigenic sites: Sa, Sb, Ca1, Ca2, and Cb. In contrast, only one amino acid change at the Sa antigenic site (G158E) was observed in H1 SIVs of the pandemic cluster. Analysis of the receptor binding site showed that recent (2011–2014) Thai H1 SIVs contained aspartic acid (D) at positions 190 and 225, indicating preferential binding to the SA α2,6 receptor. In contrast, older (2004–2005) Thai H1 SIVs contained glycine (G) at position 225 (Table 3).

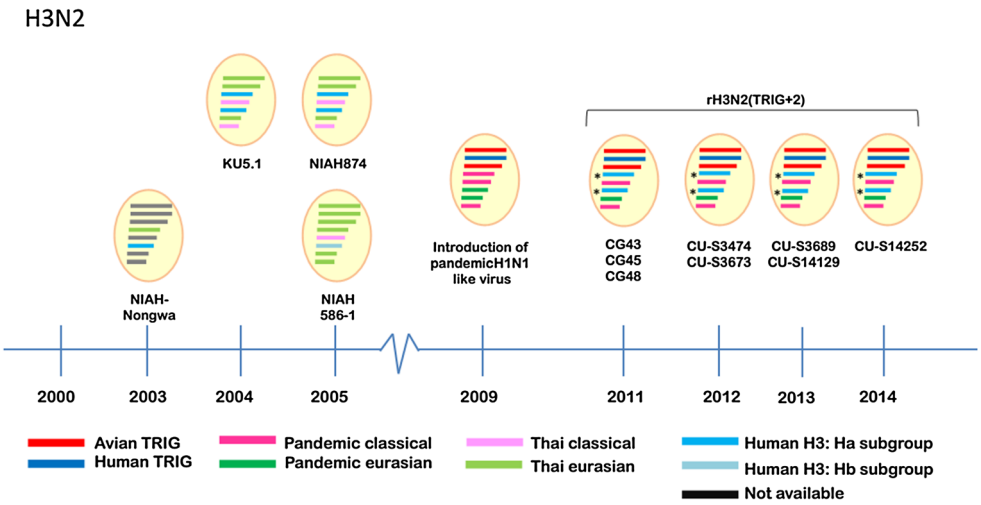

Fig. 4 Schematic representation of the genetic constellation of SIV-H3N2 during 2003–2014. The *oval* represents viral particle and each *line* in *oval* represents each gene segment ascending from segment 1 to segment 8. The lineages of gene segment present in different *colors*. The reassortant segments are emphasized by shape outline (Color figure online)

Five H3 SIVs were compared with two reference viruses: eH3N2 (KU5.1) and the human H3N2 vaccine strain (Wuhan/359). Three (CU-S3474, CU-S3673, and CU-S3689) had no amino acid changes at five antigenic sites, although the viruses were isolated from different farms. One H3 SIV (CU-S14129) had amino acid changes at four antigenic sites: A, B, C, and E. This observation corresponded well with the phylogenetic analysis result in which CU-S14129 was clustered away from the main group. In analyses of the receptor binding site, all H3 SIVs had isoleucine (I) at position 226 and serine (S) at position 228, which is similar to the reference H3 viruses and indicates Thai H3 SIVs prefer to bind to the SA α2,6 receptor.

Genetic analysis of the NA gene on Oseltamivir resistance related to E119V, H275Y, R293K, and N295S on N1 and N146K, S219T, A272V, and 245–248 deletion on N2 [15]. The results showed that all SIVs in this study had an amino acid referred to Oseltamivir susceptibility. Genetic analysis of the N2 showed that all five Thai H3N2 SIVs contained valine (V) at position 275 (Data not shown) that may increase SA α2,6 receptor specificity [16]. Moreover, analysis of virulence determinants on PB2 (E627K and N701D) and NS1 (E92D) [17] showed no significant amino acid changes in particular positions.

Discussion

From 2011 to 2014, our SIV surveillance revealed that reassortant SIVs are a dominant subtype circulating among Thai pigs. Previous studies, however, reported that pH1N1 was a major SIV subtype between 2010 and 2011 [18, 19]. In Thailand, the first reassortant SIV between pH1N1 and Thai SIVs was reported in 2010 (CU-SA43) with a genetic constellation of seven genes from pH1N1 and an N1 gene from eH1N1 [4]. In this study, we reported that novel rH1N1 (TRIG + 2) has become a dominant variant of SIV-H1N1. We identified rH1N2 (7 + 1) (CU-S3073) in October 2011 and rH1N2 (TRIG + 2) (CU-S3631) in December 2012. Similar results have been reported from Argentina and Japan. In Argentina, rH1N1 and rH1N2 containing the TRIG cassette and human-like H1 and N1/N2 were reported between 2009 and 2010 [20]. In Japan, rH1N2 containing seven genes from pH1N1 and an N2 from Japanese SIV was reported in 2012 [21]. For SIV-H3N2, rH3N2 (TRIG + 2) was a dominant SIV subtype in late 2011 in Thailand [18]. This corresponds with our finding that eH3N2 disappeared from Thai swine populations in 2011, and rH3N2 (TRIG + 2) has dominated since. These observations indicate that Thai SIVs, after the introduction of pH1N1, have evolved by maintaining the TRIG cassette and retrieving other genes from endemic SIVs in their virus gene pools. This confirms the hypothesis that the TRIG cassette has a very high potential for viral infection, replication, and transmission within pig populations. The TRIG cassette in the virus particle is very compatible, and the virus changes its surface proteins for escaping host immunity [22]. In contrast, rH3N2pM was reported in the USA with a different constellation: PB2, PB1, PA, HA, NP, NA, and NS relate to TRIG SIV-H3N2 and carry M from pH1N1 [23].

It should be noted that SIV-H1N1 and SIV-H1N2 took approximately 3 years (November 2009 to December 2012) for adaptation to the TRIG + 2 constellation. During this period, we observed both rH1N1 (7 + 1) and rH1N2 (7 + 1) constellations. In contrast, SIV-H3N2 took a shorter time (15 months; November 2009 to February 2011) to settle its genetic constellation. This evidence supports the theory that the pig is a mixing vessel for novel viruses which could potentially exhibit high virulence or cause pandemics. Strict biosecurity is therefore an

Table 3 Genetic analysis of the H1 gene of Thai SIVs in this study

Viruses	Subtype	HA cluster	Amino acid sequence alignment of H1 gene													GenBank Accession No.
			Antigenic site										Receptor binding site		HA cleavage site	
			Sa			Sb	Ca1			Ca2		Cb	190[a]	225[a]	325–333[a]	
			128–129[a]	156–160[a]	162–167[a]	187–198[a]	169–173[a]	206–208[a]	238–240[a]	140–145[a]	224–225[a]	78-83[a]				
A/Swine/Chonburi/NIAH9469/04	eH1N1	α[b]	PN	KKGNS	PKLRKA	TNTDQQSLYQNA	VNNKK	GSS	EPG	PYAGTN	RG	LFAVNS	D	G	PSIQSRGLF	AB434304
A/California/04/09	pH1N1	P[c]	PN	KKGNS	PKLSKS	TSADQQSIYQNA	INDKG	GSS	EPG	PHAGAK	RD	LSTASS	D	D	PSIQSRGLF	GQ280797
A/Swine/Thailand/CU-RA4/09	pH1N1	P	PN	KKGNS	PKLSKS	TSADQQSLYQNA	INDKG	GTS	EPG	PHAGAK	RD	LSTASS	D	D	PSIQSRGLF	CY062308
A/Swine/Thailand/CU-S3334/12	eH1N1	α	PN	KKGNS	PKLSKS	TSTDQQSLYQNA	VNNKK	SSS	EPG	HHAGAK	RD	LFKANS	D	D	PSIQSRGLF	KJ162030
A/Swine/Thailand/CU-S3350/12	eH1N1	α	PN	KKANS	PKLSKS	TITDQQSLYQNT	LNNKK	SSS	EPG	PHAGAN	RD	LFRANS	D	D	PSIQSRGLF	KJ162037
A/Swine/Thailand/CU-S3406/12	eH1N1	α	PN	KKANS	PKLSKS	TITDQQSLYQNT	LNNKK	SSS	EPG	PHAGAN	RD	LFRANS	D	D	PSIQSRGLF	KM355356
A/Swine/Thailand/CU-S3629/12	rH1N1	α	PN	KKENS	PKISKS	TSNDQQSLYQNA	FNNKG	SSS	KPG	PYAGAN	RD	LFNANS	D	D	PSIQSRGLF	KJ526069
A/Swine/Thailand/CU-S3795/13	rH1N1	α	PN	KKENS	PKISKS	TSNDQQSLYQNA	FNNKG	SSS	KPG	PYAGAN	RD	LFNANS	D	D	PSIQSRGLF	KM355360
A/Swine/Saraburi/						NIAH13021/05	eH1N2	α	PD	KKGNS	PKLSKS					
						TDTDQQSLYQNV	VNDKK	GSS	EPG	PYAGTN	RG	LFEVNS	D	G	PSIQSRGLF	AB434336
A/Swine/Thailand/CU-S3073/11	rH1N2	P	PN	KKENS	PKLSKS	TSADQQSIYQNA	INDKG	GSS	EPG	PHAGAK	RD	LSTASS	D	D	PSIQSRGLF	KJ162042

Table 3 continued

Viruses	Subtype	HA cluster	Amino acid sequence alignment of H1 gene													GenBank Accession No.
			Antigenic site										Receptor binding site		HA cleavage site	
			Sa			Sb	Ca1			Ca2		Cb	190[a]	225[a]	325–333[a]	
			128–129[a]	156–160[a]	162–167[a]	187–198[a]	169–173[a]	206–208[a]	238–240[a]	140–145[a]	224–225[a]	78-83[a]				
A/Swine/Thailand/CU-S3631/12	rH1N2	α	PN	KKENS	PKISKS	TSNDQQSLYQNA	FNNKG	SSS	KPG	PYAGAN	RD	LFNANS	D	D	PSIQSRGLF	KM355368

[a] H3 numbering

[b] Alpha group of classical lineage

[c] Pandemic cluster of classical lineage

Table 4 Genetic analysis of the H3 gene of Thai SIVs in this study

Virus	Subtype	Amino acid sequence alignment of H3 gene									Genbank Accession No.
		Antigenic site							Receptor binding site		
		A	B		C	D	E				
		140–146[a]	156–161[a]	189–199[a]	277–282[a]	205–221[a]	171–175[a]	243–249[a]	226[a]	228[a]	
A/Wuhan/359/1995	hH3N2	KRGSVKS	KLEYKY	SDQTSIYVQAS	CNSECI	STKRSQQTVIPNIGSRP	NDKFD	LLINSYG	I	S	JX518888
A/Swine/Thailand/KU5.1/2004	eH3N2	KRGSVKS	KLDYKY	SDQTNLYVQAS	CNSECI	STKRSQQTVIPNIGSRP	NDKFD	LLINSTG	I	S	FJ561060
A/Swine/Thailand/CU-S3474/12	rH3N2	KRGSVKS	KLDYKY	NNQTNLYVQAS	CNYGCI	STKRSQQTVIPNIGSRP	NDKFN	LLINSTG	I	S	KJ526029
A/Swine/Thailand/CU-S3673/12	rH3N2	KRGSVKS	KLDYKY	NNQTNLYVQAS	CNYGCI	STKRSQQTVIPNIGSRP	NDKFN	LLINSTG	I	S	KJ526062
A/Swine/Thailand/CU-S3689/13	rH3N2	KRGSVKS	KLDYKY	NNQTNLYVQAS	CNYGCI	STKRSQQTVIPNIGSRP	NDKFN	LLINSTG	I	S	KJ526048
A/Swine/Thailand/CU-S14129/13	rH3N2	KRGYVNS	QSGHKY	SDQTSLYVQAS	CNSECV	STKRSQQTVIPNIGSRP	NEKFD	LLINSTG	I	S	KM355385
A/Swine/Thailand/CU-S14252/13	rH3N2	KRGSVKS	KLDYKY	SDQTNLYVQAS	CNSECI	STKRSQQTVIPNIGFRP	NDKFD	LLINSTG	I	S	KM355393

[a] H3 numbering

important measure to reduce the chance of new genetic material being introduced into swine populations.

Genetic analyses of all Thai SIV subtypes showed amino acids with preferential binding to the SA α2,6 receptor. All Thai SIV-H3N2 isolates had isoleucine (I226) instead of leucine (L226) in the HA1 region. This unique amino acid residue was observed in human H3N2 from China and Japan in 1994 and 1995, indicating the potential risk for human infection with Thai SIV-H3N2 [24]. This observation supports the idea that the Ha subgroup of H3 originated during the late 1990s from human H3N2 to become a dominant cluster. Based on our observation that no significant amino acid mutations occurred, it should be noted that influenza vaccination was incomprehensive practices in Thai swine farms.

In summary, the reassortant SIVs have become predominant among SIVs circulating in Thai pig populations since the introduction of pH1N1 2009. This observation suggests that there was a significant diversity and rapid evolution of Thai SIVs during the past 3–4 years. Further swine influenza surveillance is critical for monitoring the novel reassortant SIVs in Thai swine populations and their potential to spread to humans.

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Conflict of interest The author(s) declare that they have no competing interests.

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