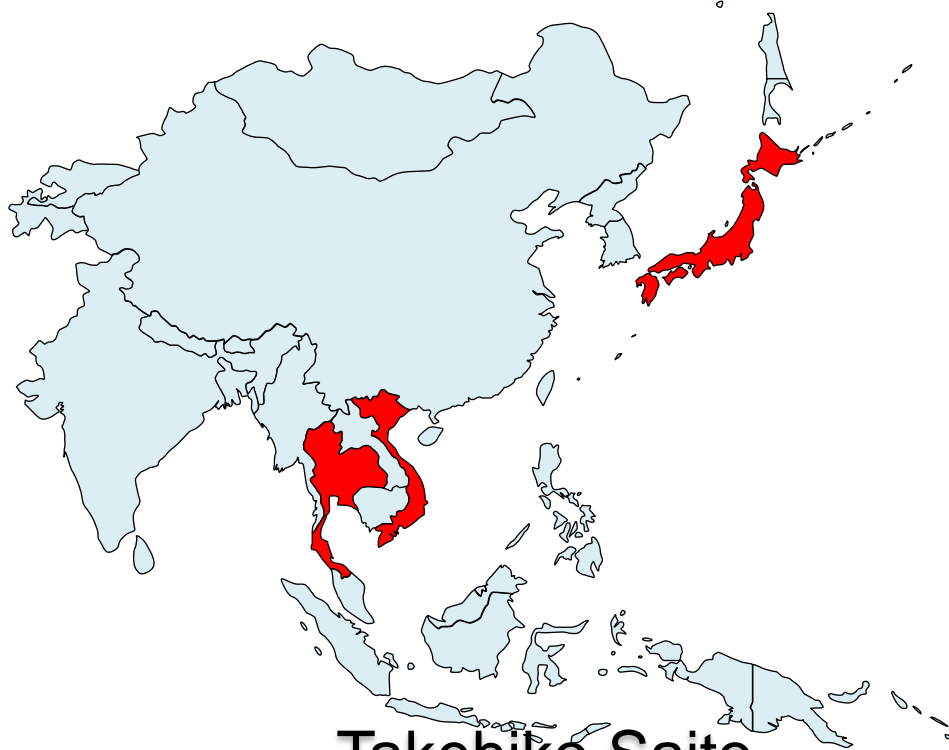


The influenza A viruses of swine in Japan, Thailand, and Vietnam



Takehiko Saito

Division of Transboundary Animal Disease ,
National Institute of Animal Health,
National Agriculture and Food Research Organization, Japan;

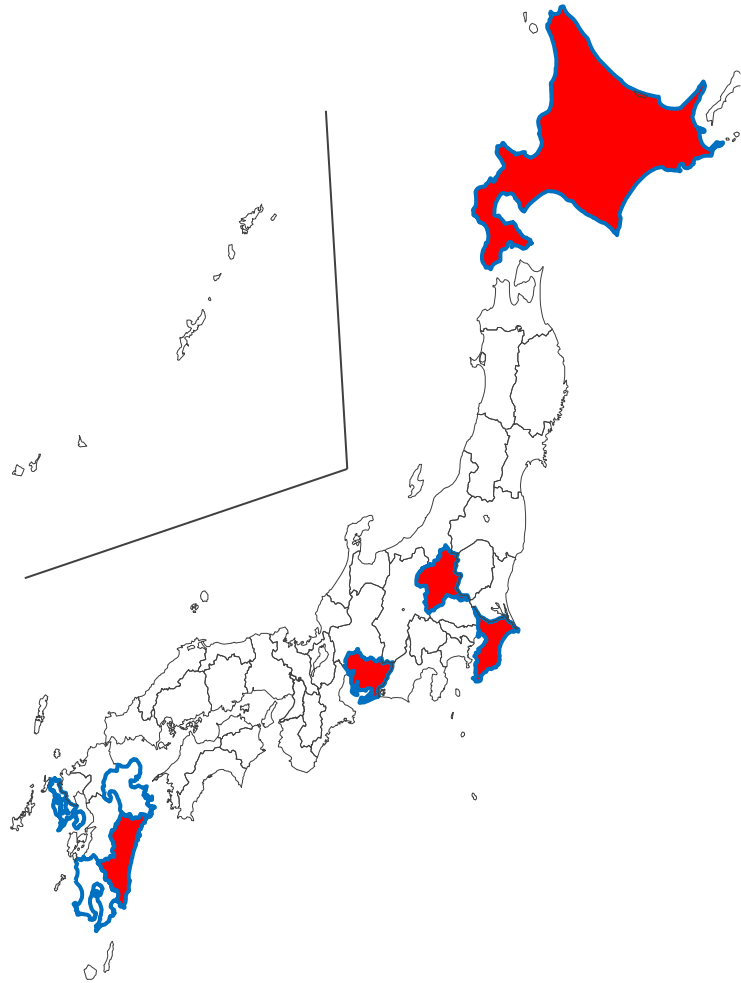
IAV-S surveillance in Japan in 2016/2017

Active surveillance

Prefecture	Number of farm	Number of nasal swab	Virus isolation
Hokkaido	4	432	1
Chiba	1	407	33
Gunma	7	2310	152
Aichi	12	143	3
Kagoshima	3	76	0
Kumamoto	2	30	0
Nagasaki	1	24	0
Miyazaki	1	20	0
Oita	1	25	0
Total	33	3217	189

Passive surveillance








Prefecture	Number of farm	Number of lung homogenate	Virus isolation
Gunma	1	3	3
Miyazaki	1	2	2
Total	2	5	5






 Provinces under survey in 2016

 IAV-S isolation in 2016

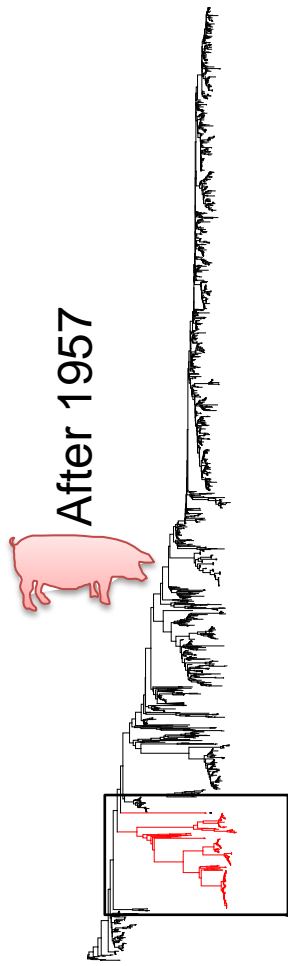
Genetic origin of Japanese IAV-S in 2016/2017

Subtype	Genetic origins of								Province
	HA	NA	PB1	PB2	PA	NP	M	NS	
H1N1	 A(H1N1)pdm09v								Hokkaido Gunma Aichi
H1N1									Gunma Chiba Aichi
H1N2									Gunma Chiba Aichi
H3N2									Chiba

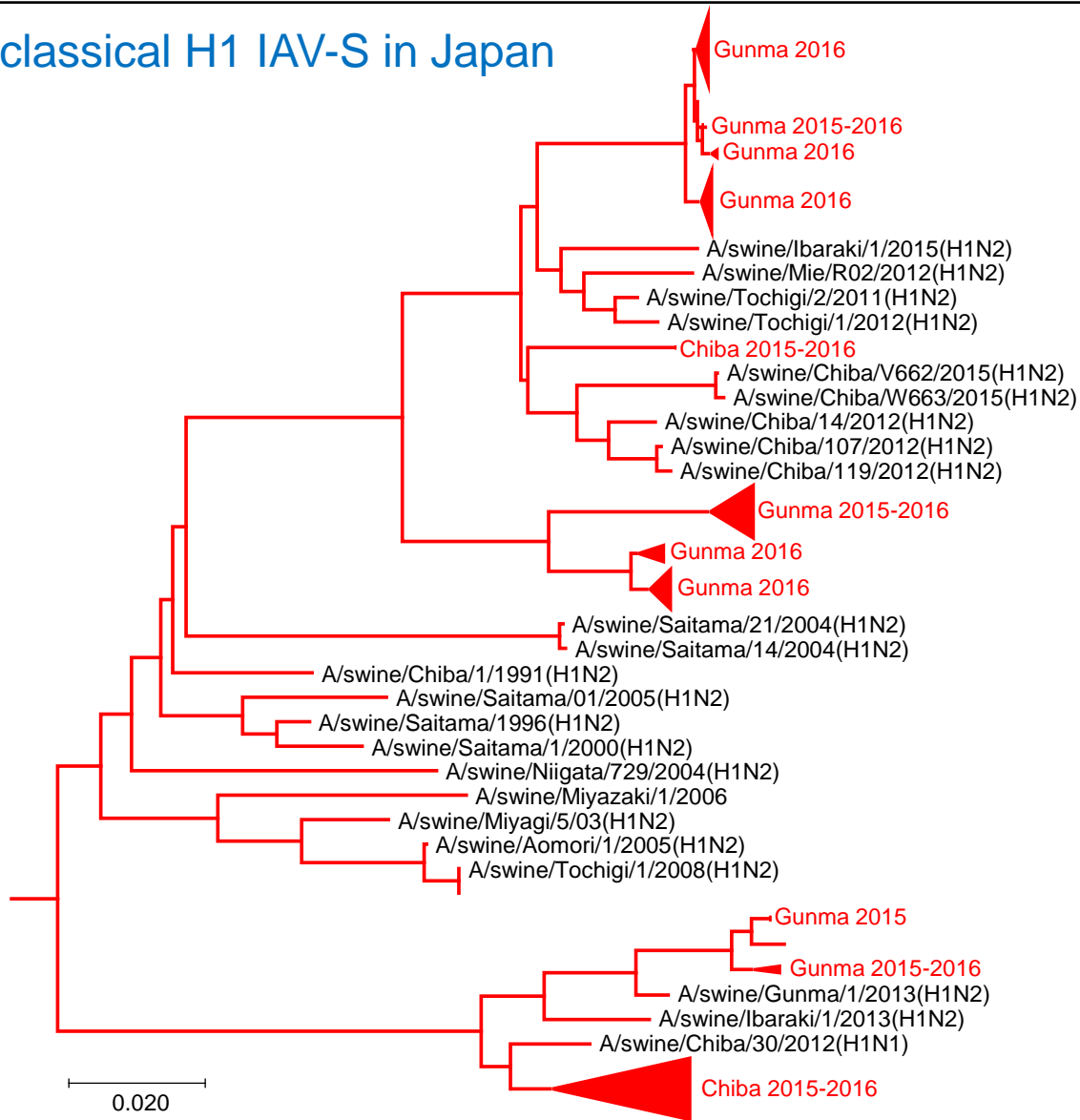
-  A(H1N1)pdm09
-  Classical swine lineage
-  Seasonal A/H3N2 human lineage

Genetic characterization of classical H1 IAV-S in Japan

Phylogenetic trees of classical swine H1 genes (2124 nucleotide sequences)



classical H1 IAV-S in Japan

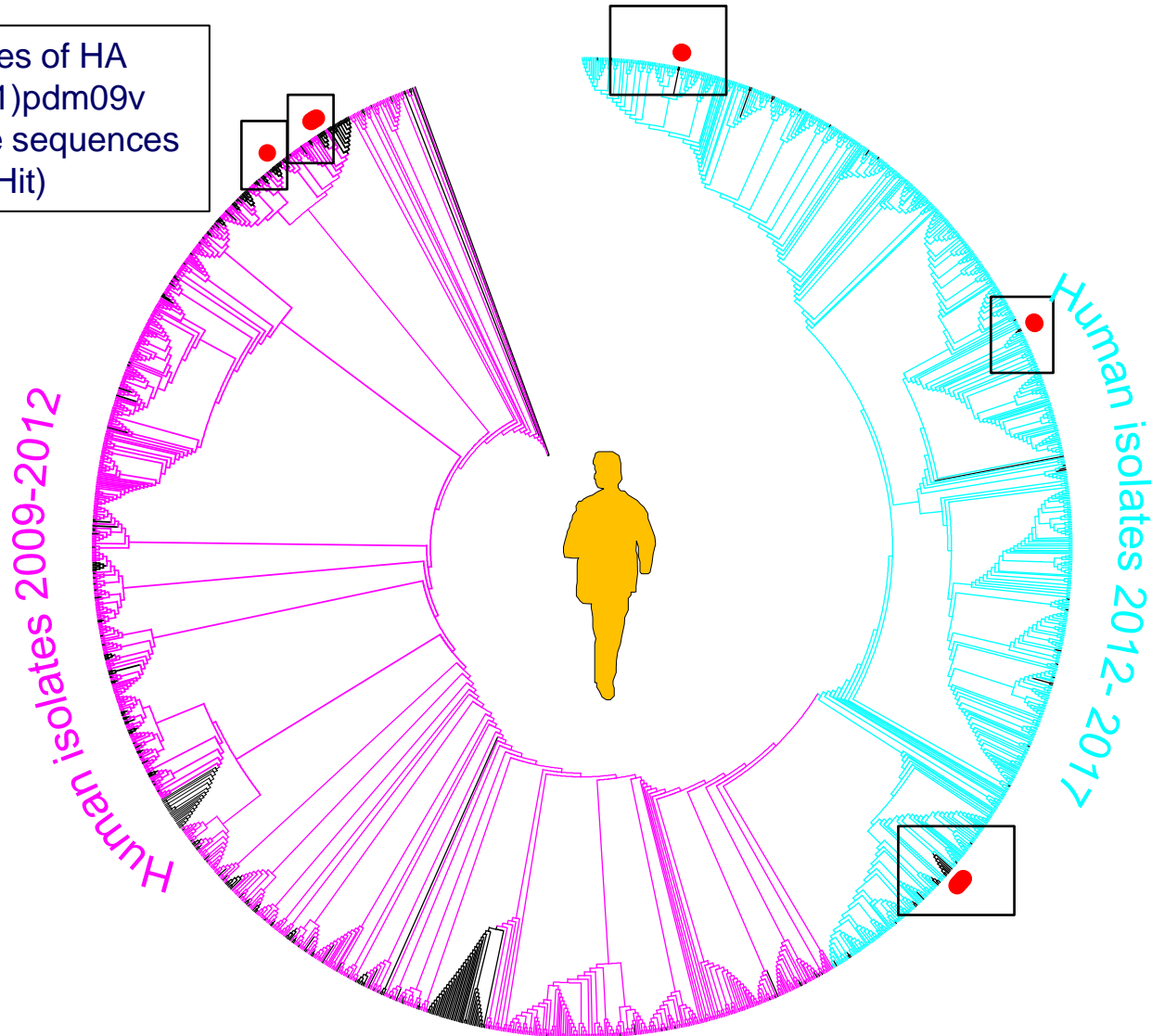


— Japanese IAV-S

Japanese swine isolates in 2015 and 2016 were indicated in red characters

Genetic characterization of the swine A(H1N1)pdm09 viruses in Japan

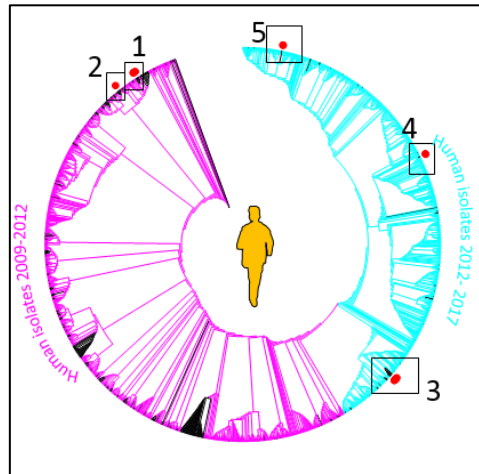
Phylogenetic trees of HA genes of A(H1N1)pdm09v (1901 nucleotide sequences selected by CD-Hit)



● Japanese swine A(H1N1)pdm09v in 2015 and 2016
— Swine A(H1N1)pdm09 viruses

Genetic characterization of the swine A(H1N1)pdm09 viruses in Japan

Introductions of human A(H1N1)pdm09v around 2009-2010 (Group1, 2)

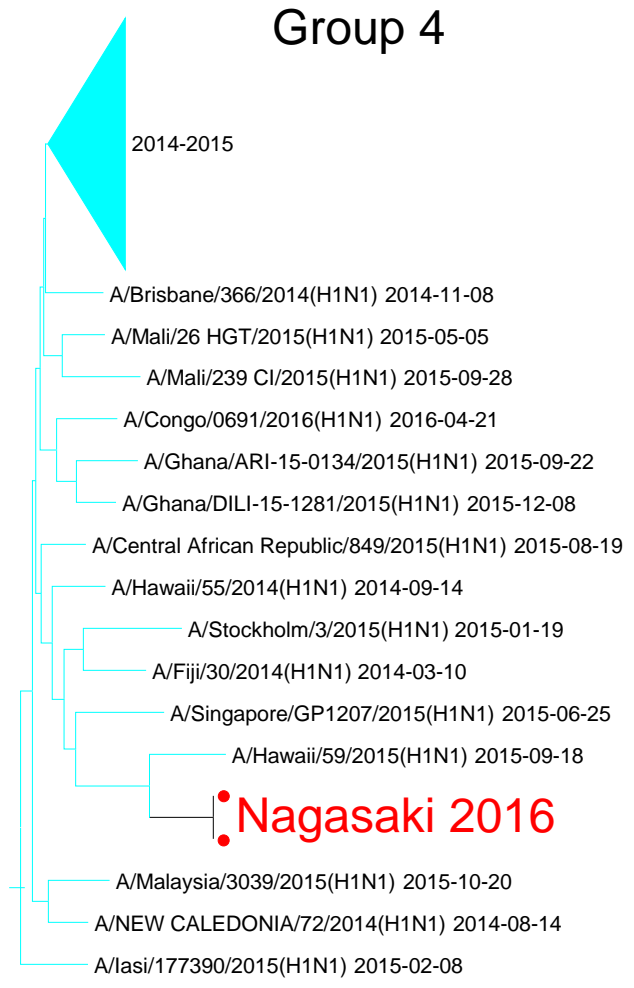
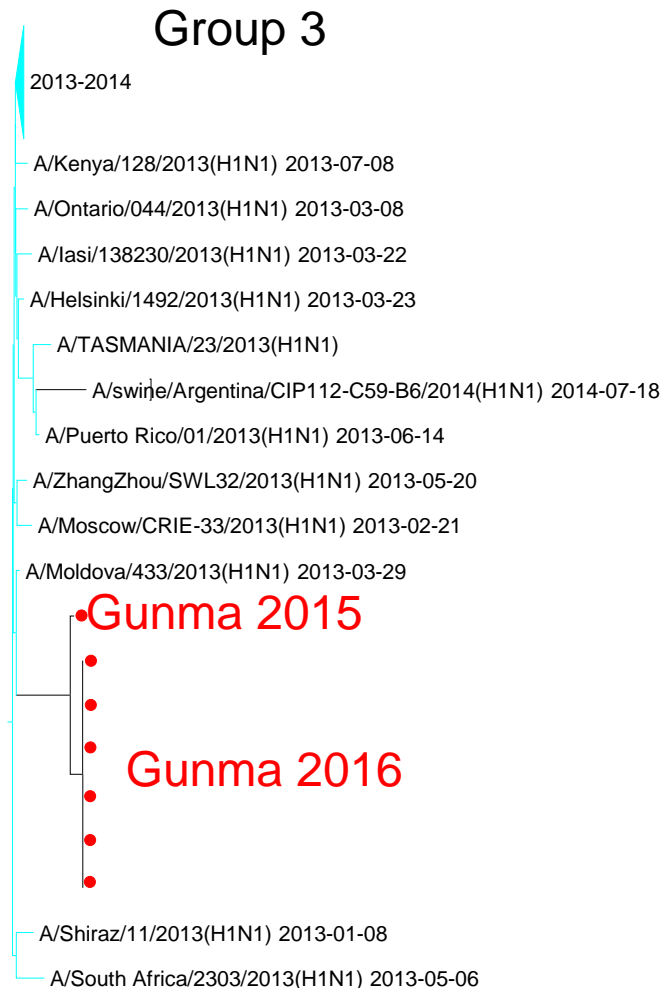
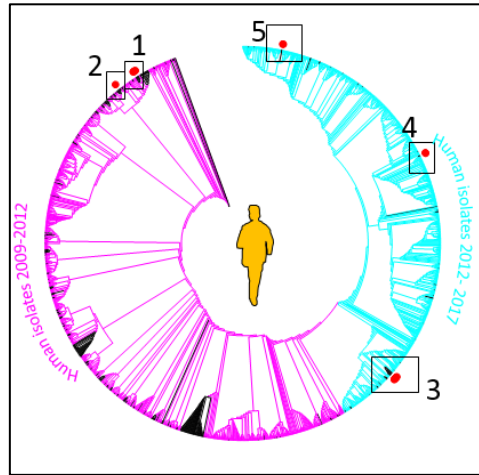


0.002

● Japanese swine
A(H1N1)pdm09v in 2015 and 2016

Genetic characterization of the swine A(H1N1)pdm09 viruses in Japan

Introductions of human A(H1N1)pdm09v around 2013 (Group3), 2015 (Group4)



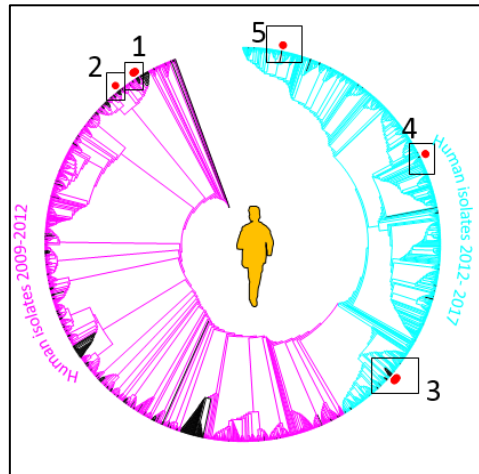
H
0.001

H
0.00050

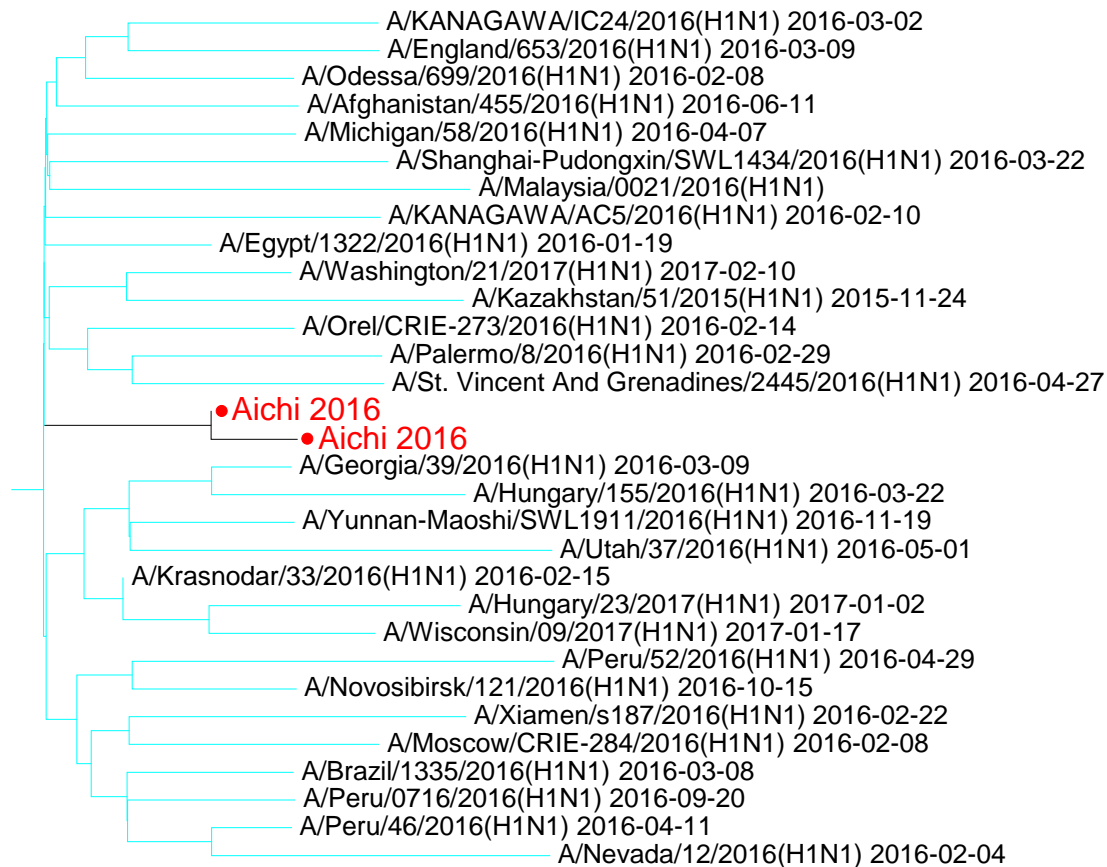
● Japanese swine
A(H1N1)pdm09v in 2015 and 2016

Genetic characterization of the swine A(H1N1)pdm09 viruses in Japan

Introduction of human A(H1N1)pdm09v around 2016 (Group5)



Group 5



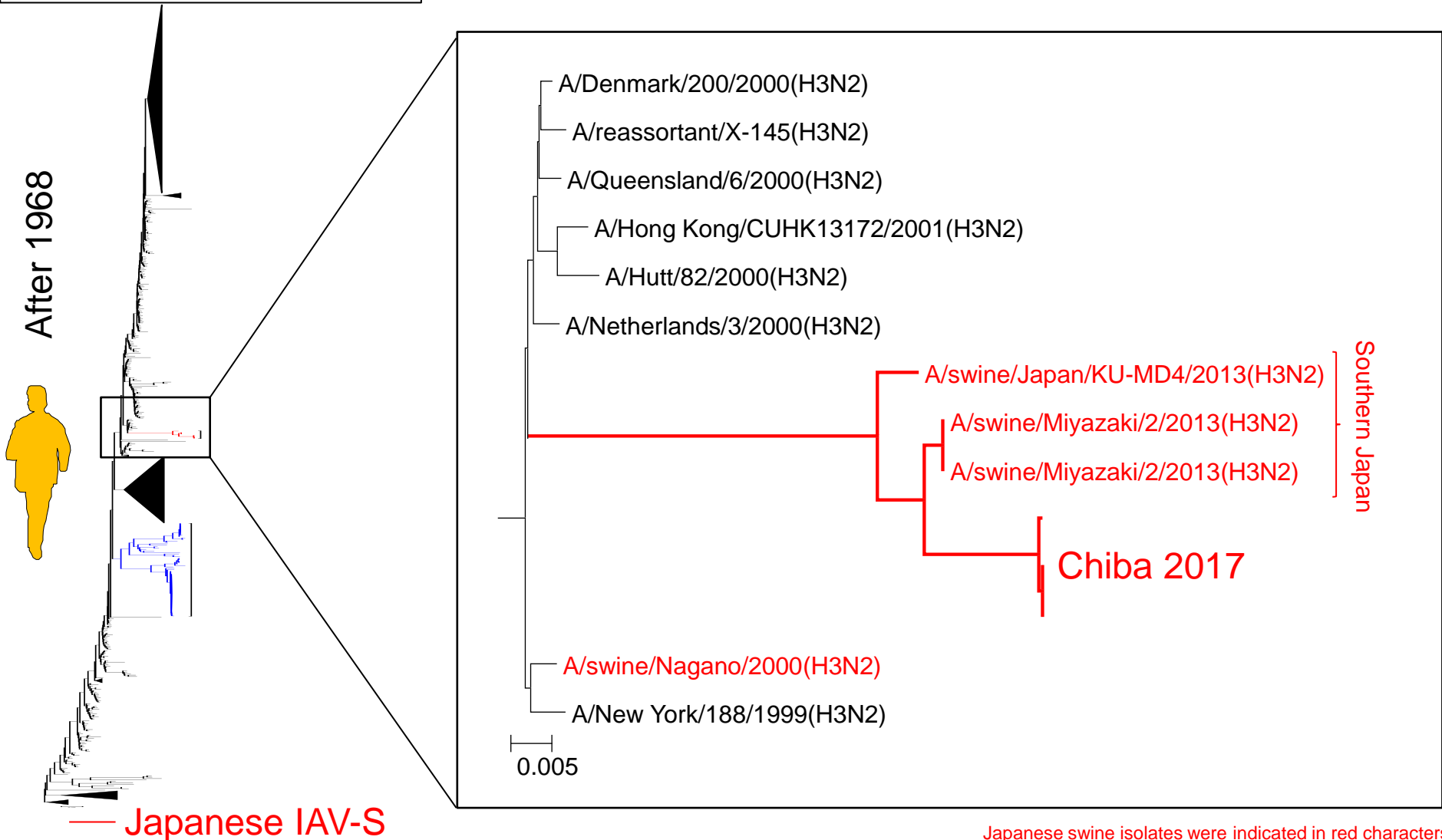
0.00050

● Japanese swine
A(H1N1)pdm09v in 2015 and 2016

Genetic characterization of human-like H3 IAV-S in Japan

Phylogenetic trees of seasonal human H3 genes (2676 nucleotide sequences selected by CD-Hit)

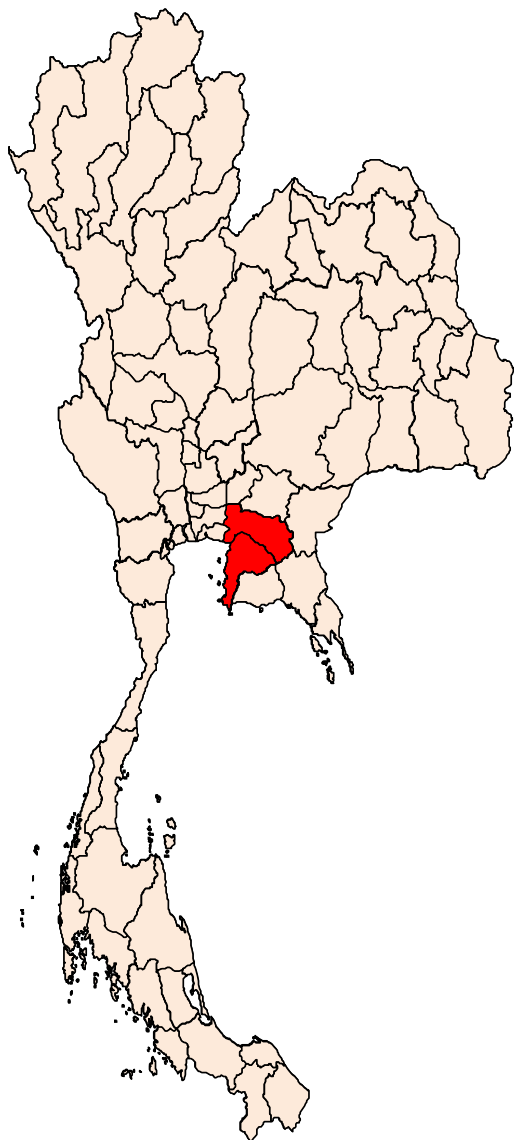
Introduction of human H3N2 virus around 2000



Summary of Japanese IAV-S in 2016

- Active surveillance of the IAV-S in Japan has been conducted since September 2015.
- IAV-S possessing either Classical H1 HA or human-like H3 HA with most of the human A(H1N1)pdm09 virus derived internal genes have co-circulated in Japanese pig populations.
- H1 genes of swine A(H1N1)pdm09 viruses isolated from 2015 to 2017 in Japan were derived from 5 distinct human isolates.
- H3 genes of H3N2 IAV-S in Japan were derived from a human ancestral virus circulating around 2000




IAV-S surveillance in Thailand in 2016





 Provinces under survey

Province	Number of farm	Number of nasal swab	Virus isolation
Chonburi	2	404	17
Chachoengsao	2	404	18
Total	4	808	35

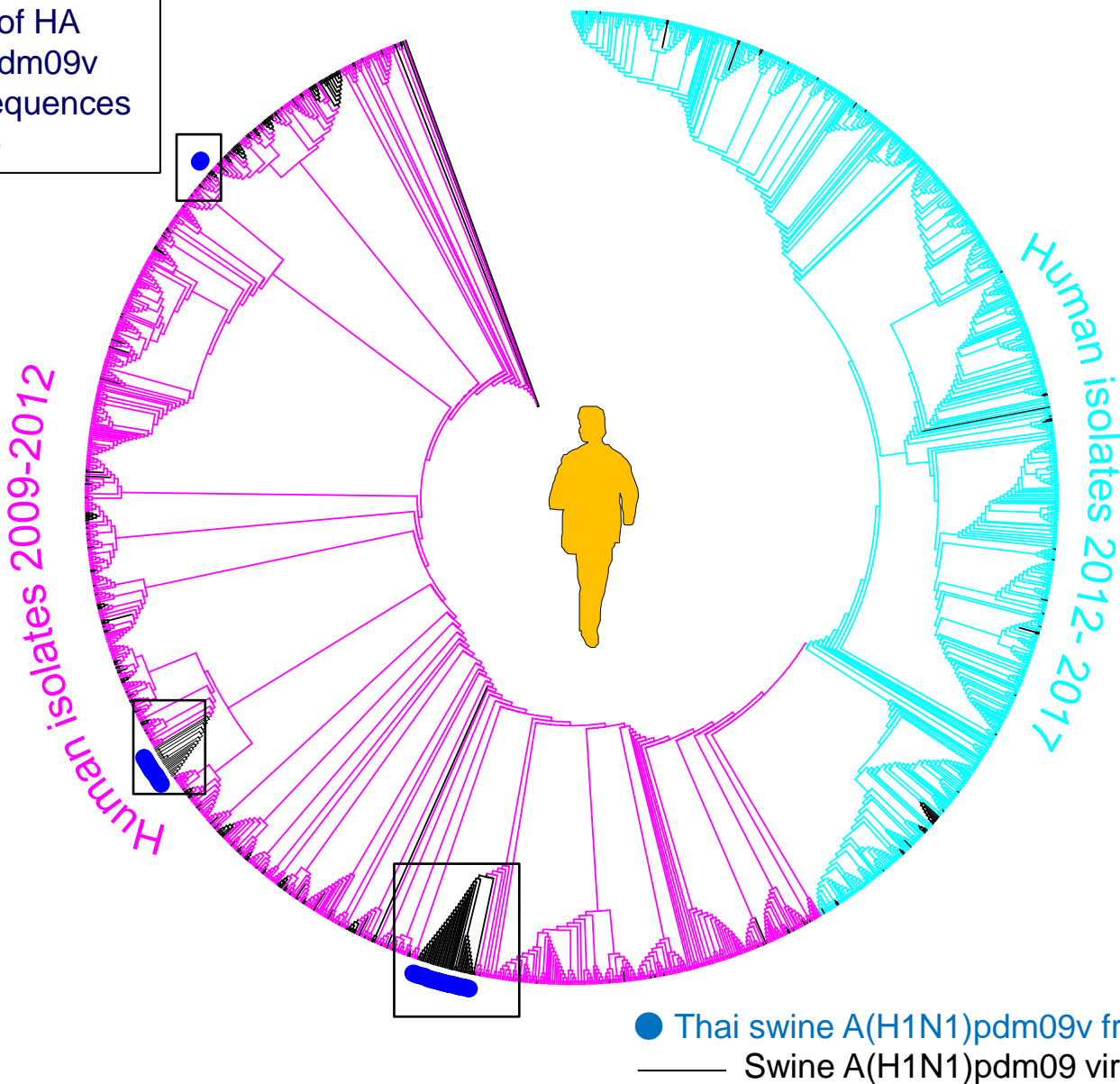
Genetic origin of Thai IAV-S in 2016

Subtype	Genetic origins of								Province
	HA	NA	PB1	PB2	PA	NP	M	NS	
H1N1	 A(H1N1)pdm09v								Chonburi
H3N2									Chachoengsao

-  A(H1N1)pdm09
-  Seasonal A/H3N2 human lineage

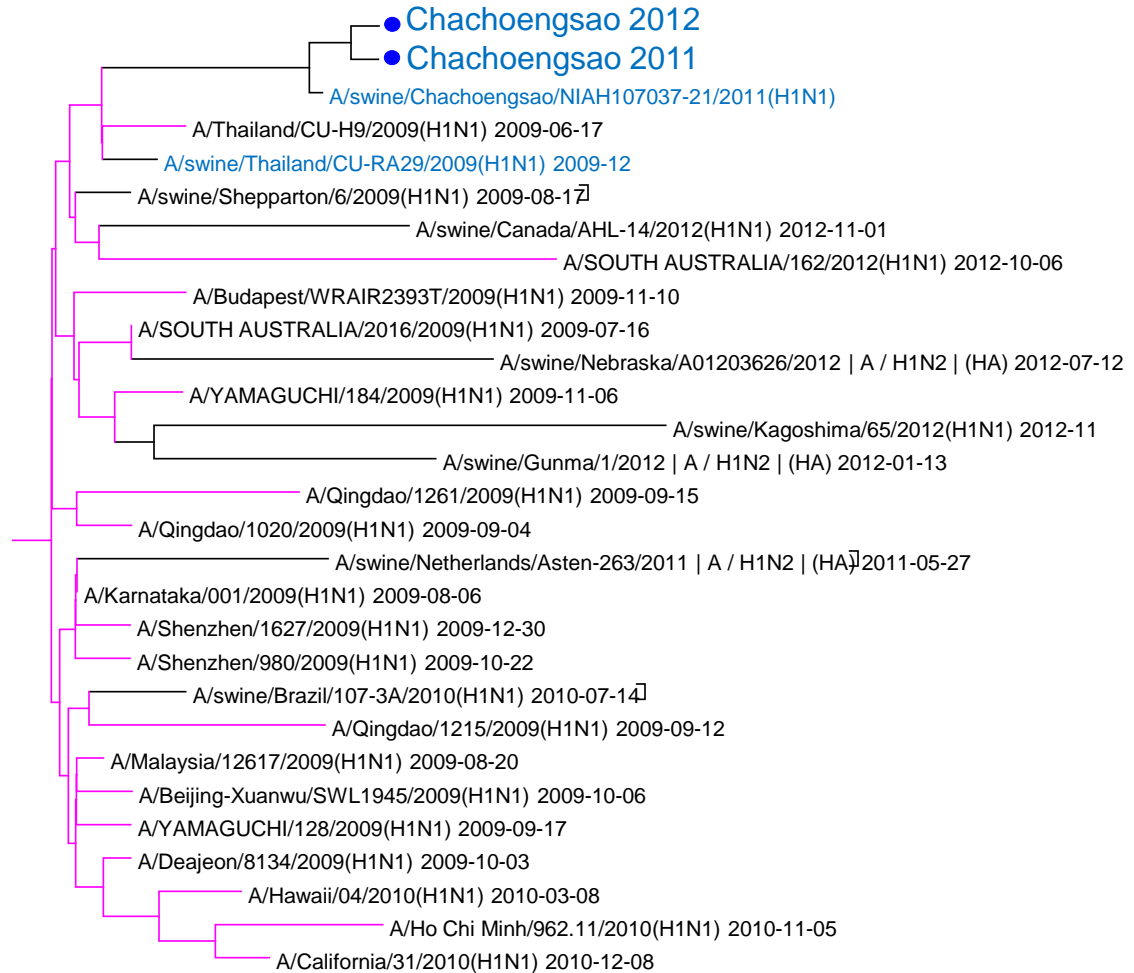
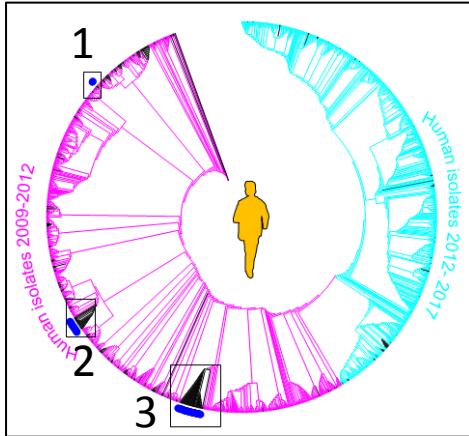
Genetic characterization of the swine A(H1N1)pdm09 viruses in Japan

Phylogenetic trees of HA genes of A(H1N1)pdm09v (1901 nucleotide sequences selected by CD-Hit)



Genetic characterization of the swine pdm09 viruses in Thailand

Maintenance of A(H1N1)pdm09v among pigs (Group1)

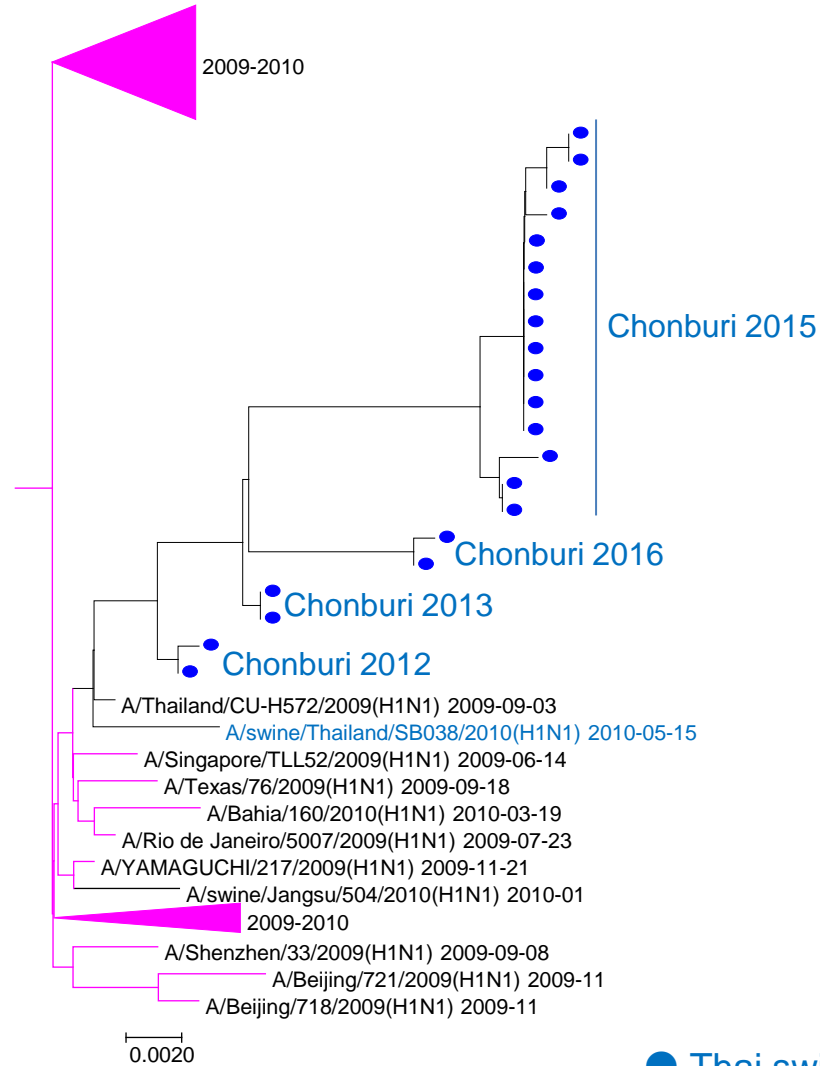
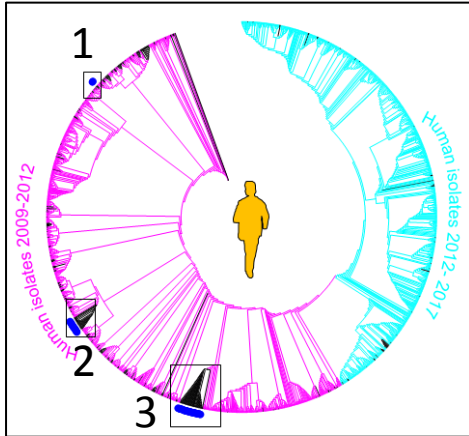


0.001

● Thai swine A(H1N1)pdm09v from 2011

Genetic characterization of the swine pdm09 viruses in Thailand

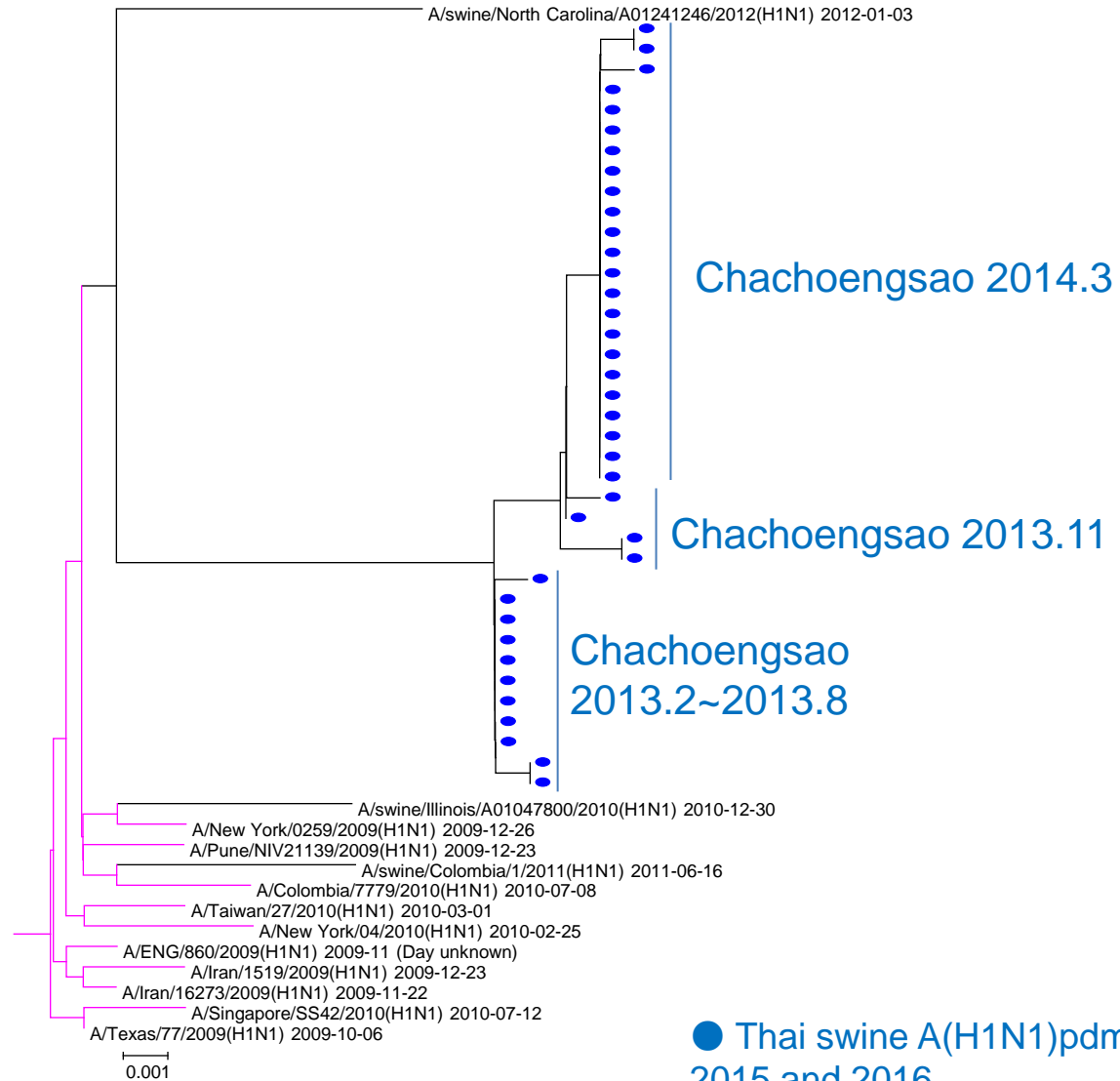
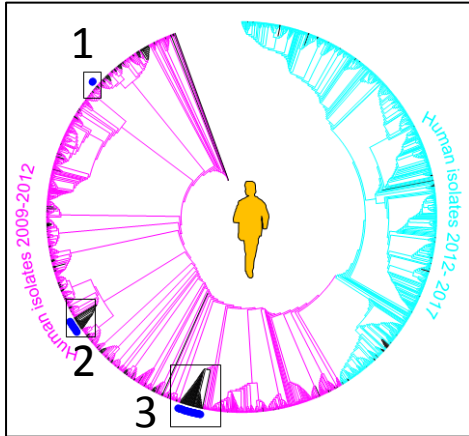
Maintenance of A(H1N1)pdm09v among pigs (Group2)



● Thai swine A(H1N1)pdm09v in 2015 and 2016

Genetic characterization of the swine pdm09 viruses in Thailand

Introductions of human A(H1N1)pdm09v around 2009-2010 (Group3)



● Thai swine A(H1N1)pdm09v in 2015 and 2016

Genetic characterization of the H3 IAV-S in Thailand

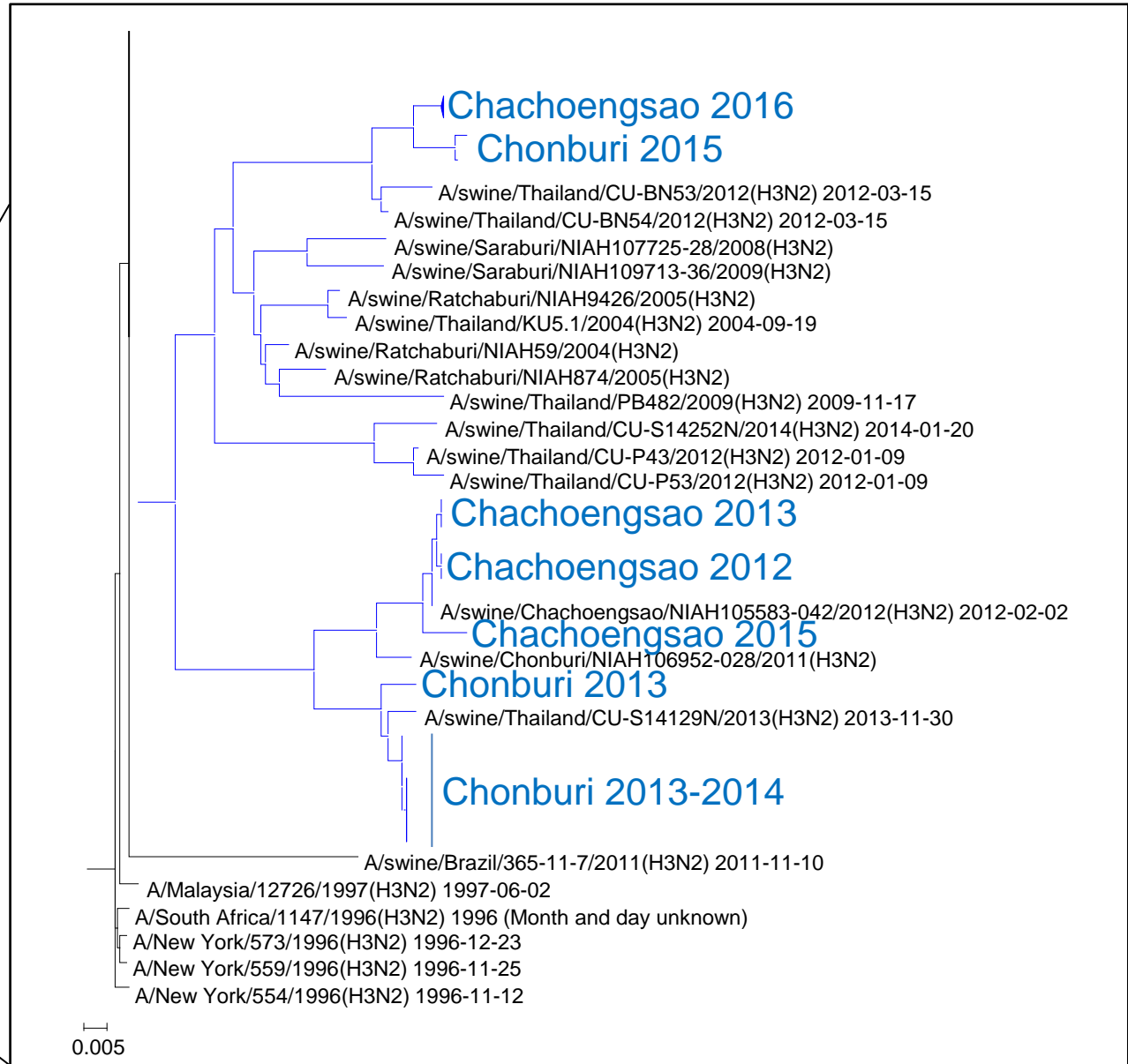
Phylogenetic trees of seasonal human viruses (2676 nucleotide sequences selected by CD-Hit)

After 1968



Triple reassortant

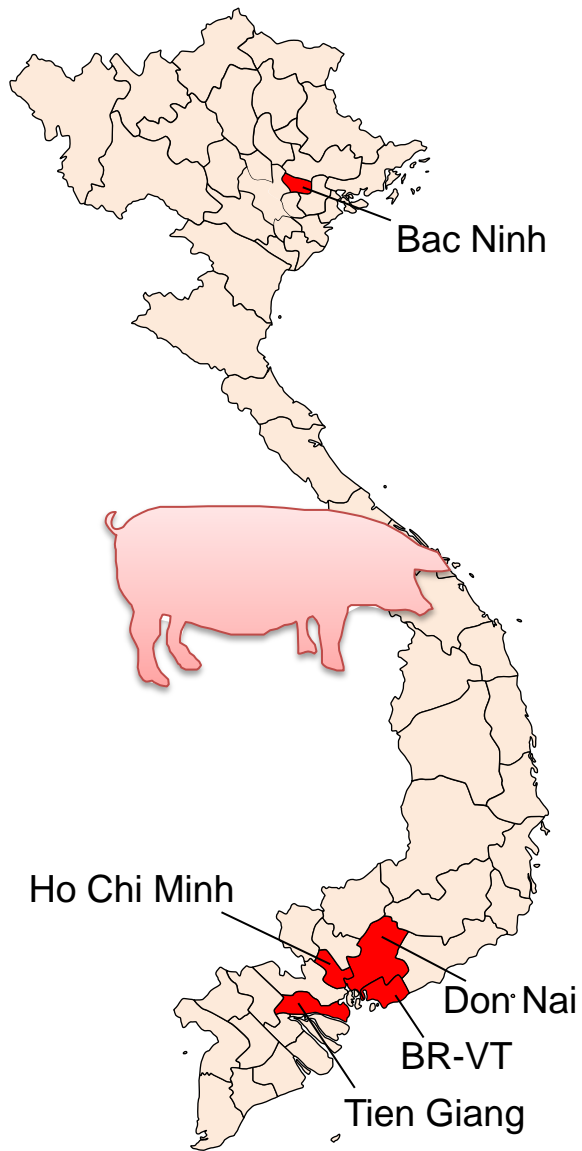
— Thai IAV-S



Summary of Thai IAV-S in 2016

- At least 3 introductions of human A(H1N1)pdm09 viruses had established swine A(H1N1)pdm09 lineages in Thai pig population.
- Thai H3N2 lineage possessing seasonal human-like surface antigens has been circulating in Thai pig population since 2005.
- H3 genes of Thai H3N2 IAV-S are derived from a human ancestral virus circulating in the late '90s.
- Genotypes of H3N2 IAV-S in Thailand and Japan are same but all of the origins of each segment are different.

IAV-S surveillance in Vietnam in 2016





Province	Number of farm	Number of nasal swab	Virus isolation
Bac Nihn	14	840	34
Tien Giang	4	240	8
Ho Chi Minh	3	180	22
BR-VT	3	180	2
Dong Nai	4	240	17
Total	28	1680	83

 Provinces under survey


Genetic origin of Vietnamese IAV-S in 2016

Subtype	Genetic origins of								Province
	HA	NA	PB1	PB2	PA	NP	M	NS	
H1N1	A(H1N1)pdm09v								North, South
H1N2	Pre-2009 H1 human-origin	Seasonal H3N2 human-origin	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	South
H1N2	H1 triple reassortant-origin (C-TR cluster)	Seasonal H3N2 human-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	South
H3N2	Seasonal H3N2 human-origin	Seasonal H3N2 human-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	A(H1N1)pdm09v-origin	North, South
H3N2	Seasonal H3N2 human-origin	Seasonal H3N2 human-origin	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	H1 triple reassortant-origin (C-TR cluster)	A(H1N1)pdm09v-origin	H1 triple reassortant-origin (C-TR cluster)	North

 A(H1N1)pdm09v-origin
















 Seasonal H3N2 human-origin

 H1 triple reassortant-origin (C-TR cluster)


 Pre-2009 H1 human-origin

Antigenic analysis of Vietnamese IAV-S

HI titers against H1 IAV-S

Virus	HI titers of sera from chickens and ferret infected with†									
	Classical IAV-S		A(H1N1)pdm09 virus		Pre-2009 human-lineage virus					
	VN 2011	VN 2015	Cal04	VN 2015	Human vaccine strains			Vietnamese IAV-S		
					'01~ '07	'07~ '08	'09~ '10	VN 2010	VN 2012	VN 2015
Classical IAV-S										
VN 2011(H1N2) 	640	1280	640	<20	<20	<20	<20	40	<20	20
VN 2015(H1N2) 	640	1280	640	20	<20	<20	<20	40	<20	<20
VN 2016(H1N2) 	160	320	320	20	<20	<20	20	40	<20	<20
A(H1N1)pdm09 virus										
Vaccine strain '09~ 	40	20	1280	1280	<20	<20	<20	20	<20	<20
VN 2010 (pH1N1) 	<20	<20	1280	1280	<20	<20	<20	<20	<20	<20
VN 2014 (pH1N1) 	80	20	1280	640	<20	<20	<20	20	20	<20
VN 2015 (pH1N1) 	20	20	1280	1280	<20	<20	<20	<20	<20	<20
VN 2016 (pH1N1) 	160	40	5120	2560	40	80	80	80	40	80
Pre-2009 human-lineage virus										
VN 2010 (H1N2) 	40	20	40	40	160	20	20	1280	80	80
VN 2012 (H1N2) 	80	40	<20	80	<20	20	<20	160	160	320
VN 2015 (H1N2) 	80	40	20	80	80	<20	<20	160	80	640
VN 2016 (H1N2) 	20	40	20	20	20	20	20	160	80	320
Vaccine strain '01~ '07 	40	40	40	40	640	80	80	160	20	20
Vaccine strain '07~ '08 	<20	20	<20	<20	160	640	320	640	20	<20
Vaccine strain '09~ '10 	<20	20	<20	<20	20	160	640	80	<20	<20

† Homologous HI titers are indicated in bold and underlined.

 Antigenic drift among same lineages

Antigenic analysis of Vietnamese IAV-S

HI titers against H3 IAV-S

Virus	HI titers of sera from chickens and ferrets infected with†												
	Human-like IAV-S			Human vaccine strains								TR IAV-S	
	VN 2010	VN 2011	VN 2015	'96~ '98	'98~ '00	'00~ '04	'04~ '05	'05~ '06	'08~ '10	'13~ '14	VN 2012	VN 2015	
<i>Seasonal human lineage</i>													
VN 2010 (H3N2)	<u>640</u>	640	160	160	80	160	320	160	20	20	320	160	
VN 2011 (H3N2)	160	<u>640</u>	160	80	20	40	160	40	<20	<20	160	80	
VN 2015 (H3N2)	40	640	<u>160</u>	40	20	<20	160	20	<20	<20	640	160	
VN 2016 (H3N2)	40	320	160	80	<20	40	160	20	<20	<20	320	160	
Vaccine strain '96~ '98	<20	<20	<20	<u>320</u>	80	80	40	<20	<20	<20	<20	<20	
Vaccine strain '98~ '00	<20	<20	<20	320	<u>640</u>	320	640	20	40	<20	<20	<20	
Vaccine strain '00~ '04	<20	<20	<20	80	40	<u>320</u>	160	<20	20	<20	<20	<20	
Vaccine strain '04~ '05	20	40	<20	160	80	160	<u>1280</u>	640	160	<20	<20	<20	
Vaccine strain '05~ '06	<20	<20	<20	<20	<20	<20	320	<u>1280</u>	320	<20	<20	<20	
Vaccine strain '08~ '10	<20	<20	<20	<20	<20	<20	80	640	<u>640</u>	20	<20	<20	
Vaccine strain '13~ '14	<20	40	<20	<20	<20	<20	<20	20	40	<u>160</u>	40	<20	
<i>Triple reassortant</i>													
VN 2012 (H3N2)	<20	160	80	<20	<20	<20	20	<20	<20	<20	<u>640</u>	640	
VN 2014 (H3N2)	<20	320	160	<20	<20	<20	40	<20	<20	<20	1280	1280	
VN 2015 (H3N2)	20	80	40	<20	<20	<20	80	20	<20	<20	1280	<u>1280</u>	

† Homologous HI titers are indicated in bold and underlined.

 Antigenic drift among same lineages

Summary of Vietnamese IAV-S in 2016

- Continuing genetic reassortments generate the novel combination of gene segments.
- Unlike human population, pre-2009 human-like H1 viruses still have circulated in Vietnamese pig population.
- Antigenic drifts of Vietnamese classical and pre-2009 human-like H1, and human-like H3 IAV-S are evident.

Acknowledgements

- Department of Animal Health, Vietnam
- National Institute of Animal Health, Thailand
- Dr. Takato Odagiri, National Institute of Infectious Diseases, Influenza Virus Research Center, Japan
- Sub-Department of Animal Health of Bac Ninh, Ba Ria Vung Tau, Binh Duong, Dong nai, Hanoi, Ho Chi Minh City, Long An, Nam Dinh, Soc Trang, and Tien Giang provinces in Vietnam

This work was supported in part by a program of the Japan Racing Association (JRA), and Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) from Ministry of Education, Culture, Sport, Science & Technology in Japan, and Japan Agency for Medical Research and Development (AMED)