OFFLU Technical Meeting for Head of Avian Influenza Reference Institutions and Swine Influenza Experts

OIE, Paris, 15th and 16th September 2009, Salon Ramon

Activity of the Reference Laboratory, Hokkaido University, on avian influenza

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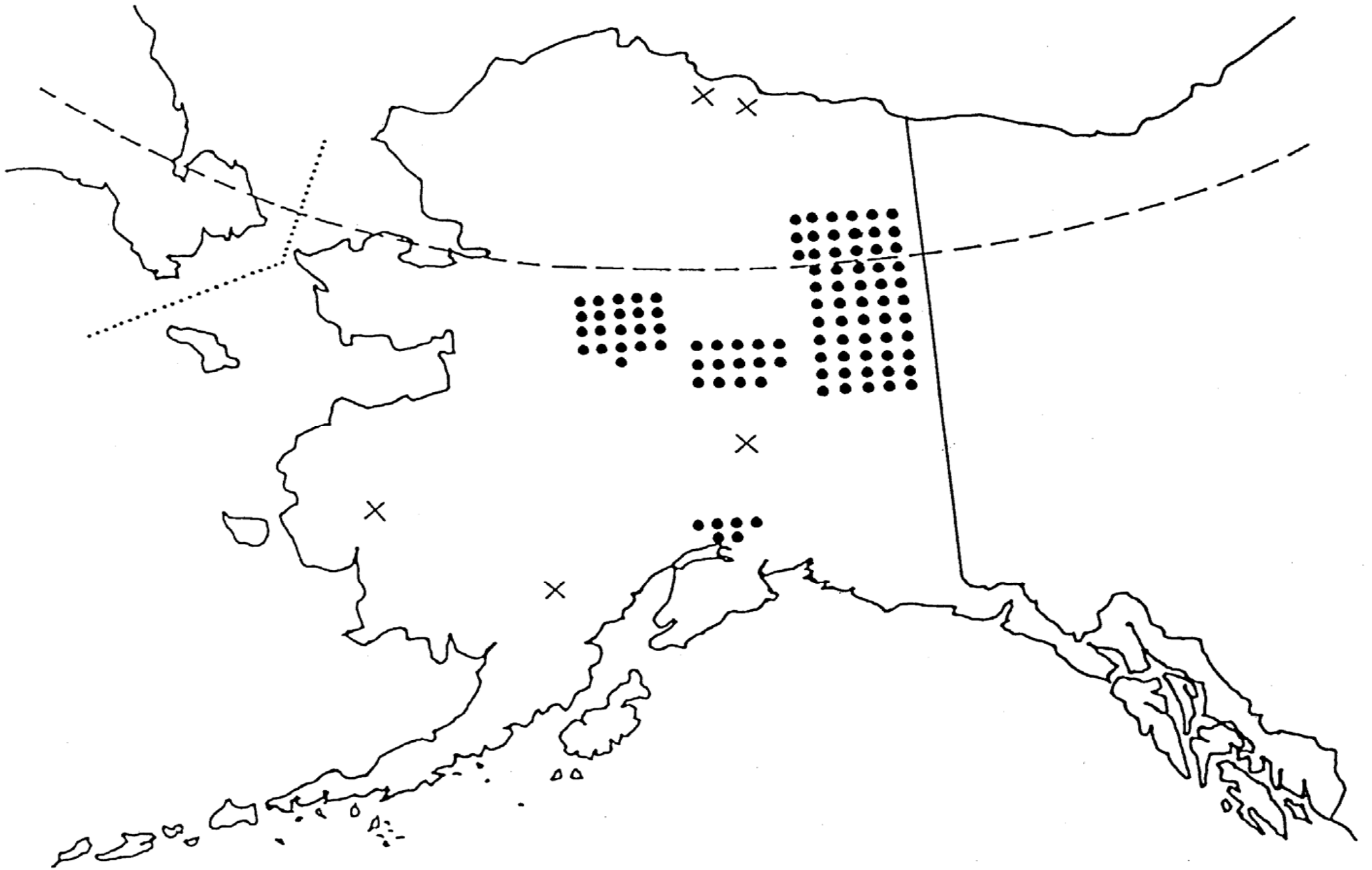
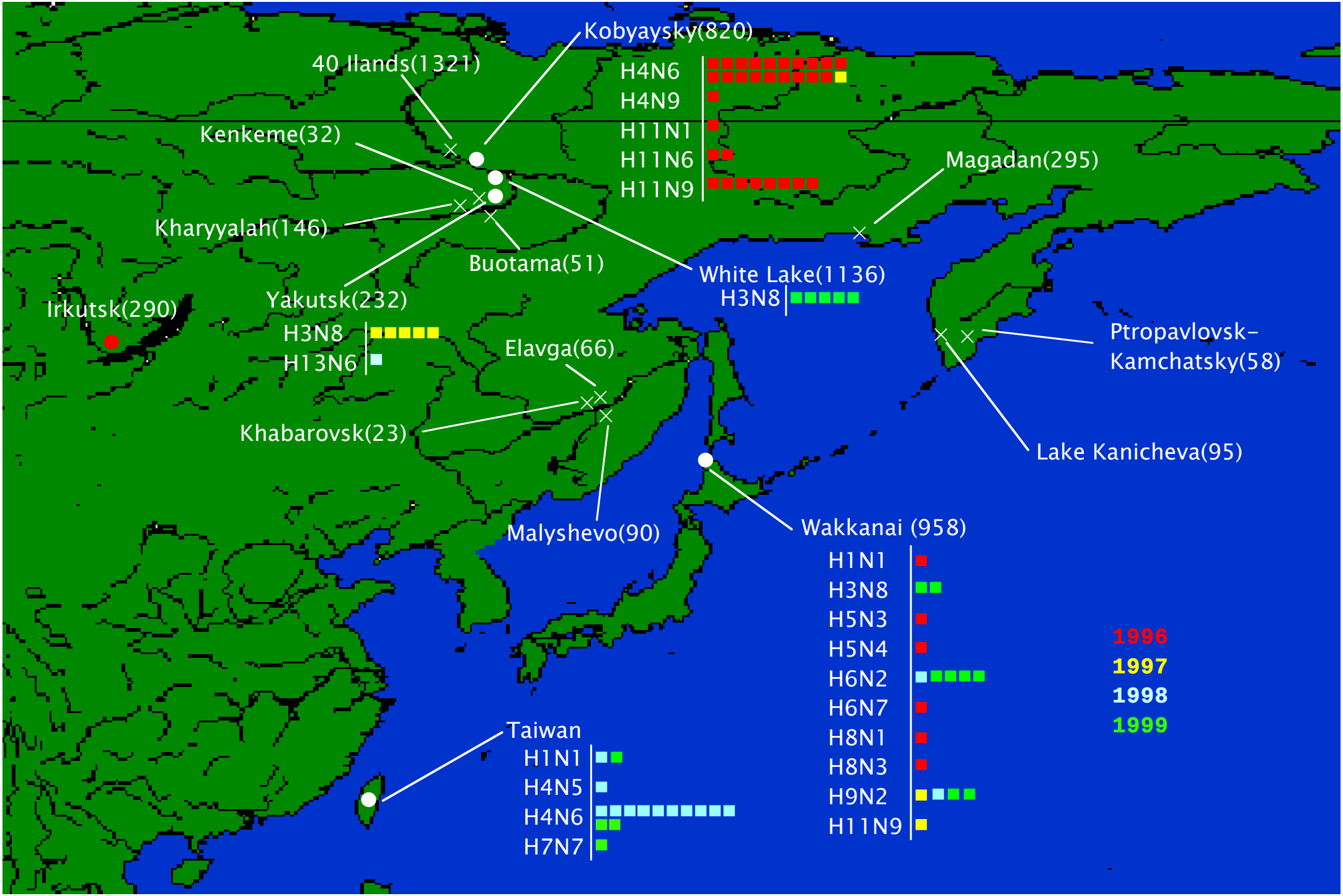
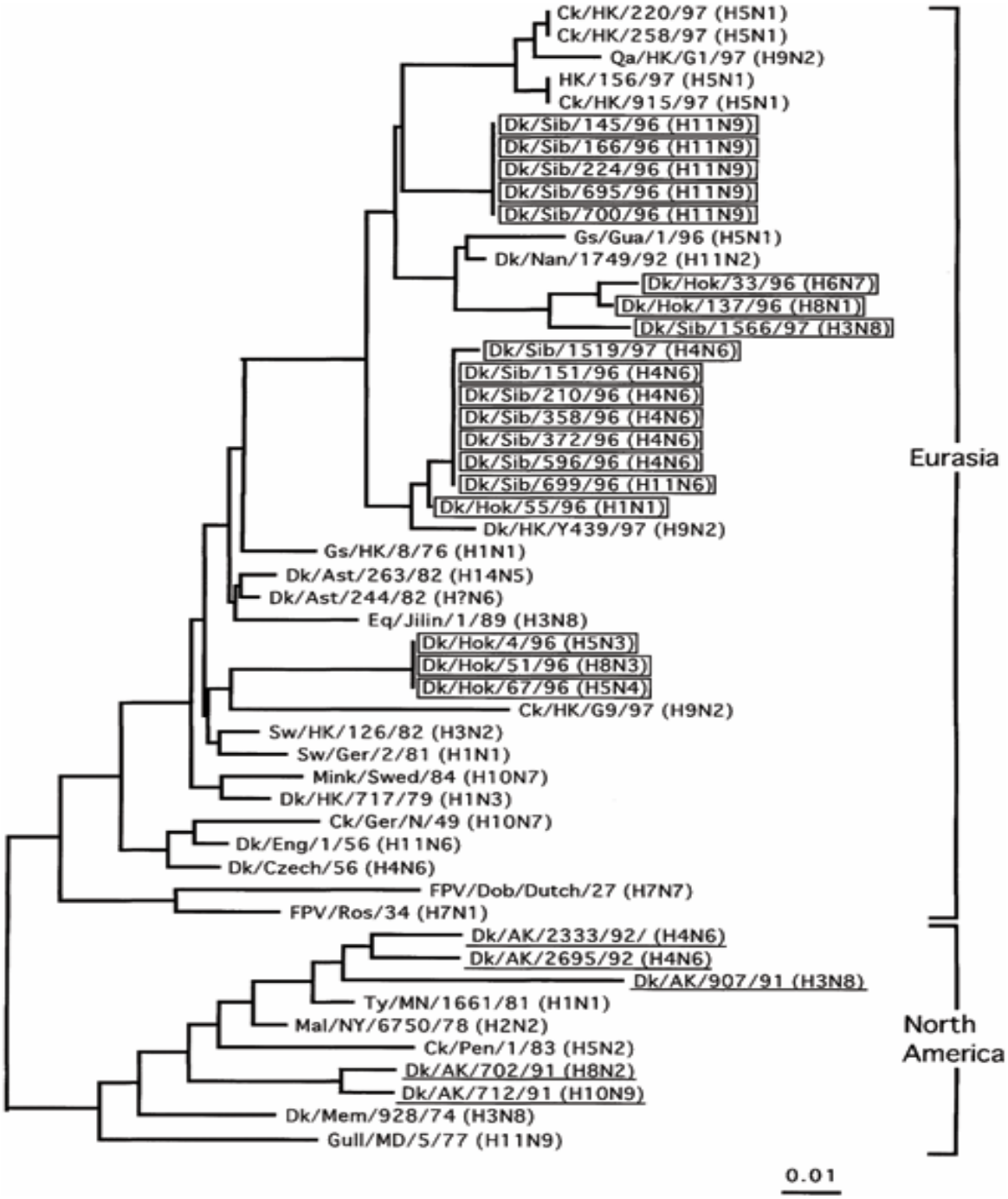
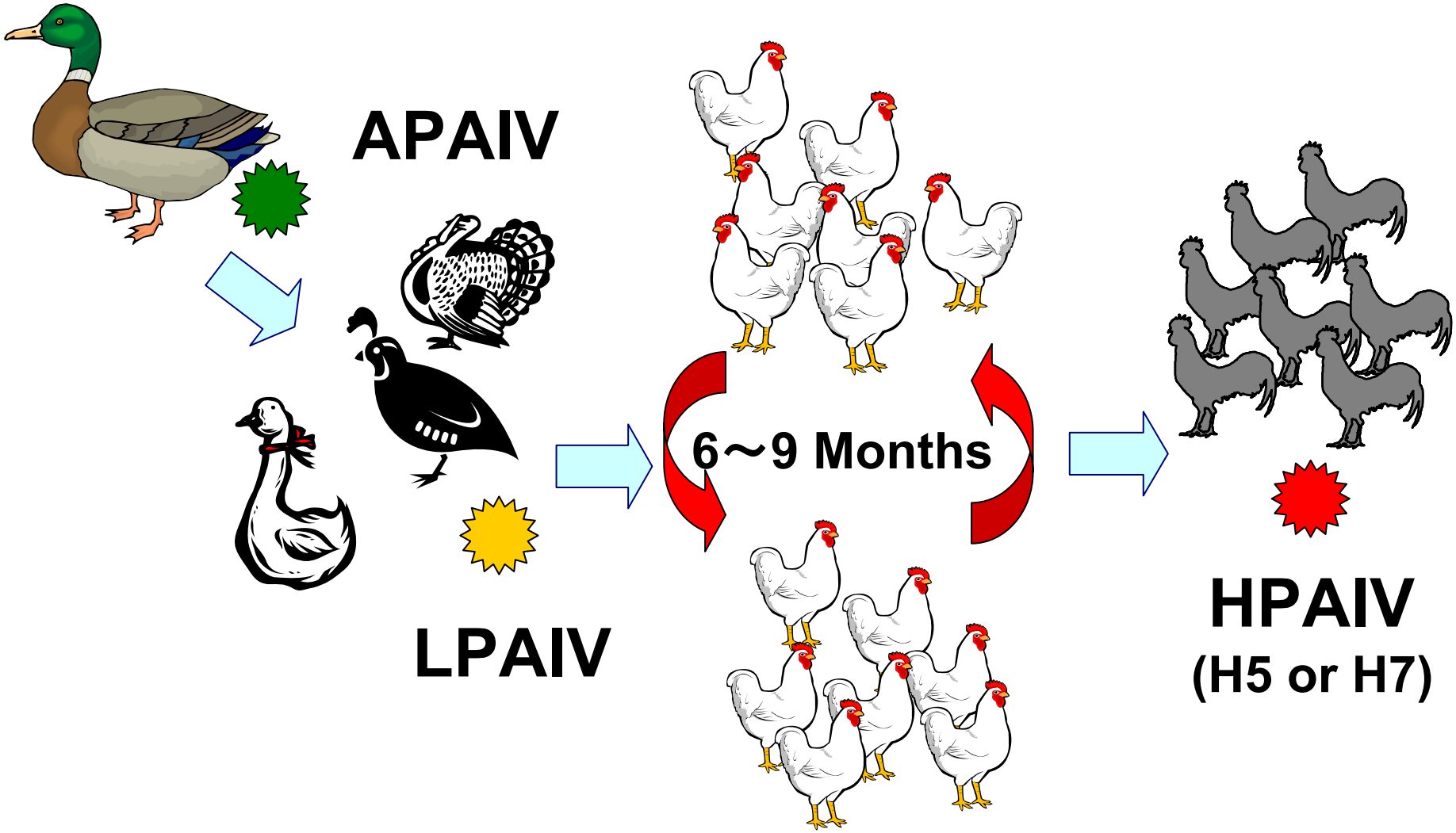



Kobyaysky(820)
H4N6
H4N9
H11N1
H11N6
H11N9
40 Ilands(1321)
Kenkeme(32)
Kharyyalah(146)
Buotama(51)
Magadan(295)
White Lake(1136)
H3N8
Irkutsk(290)
Yakutsk(232)
H3N8
H13N6
Elavga(66)
Khabarovsk(23)
Ptropavlovsk-
Kamchatsky(58)
Lake Kanicheva(95)
Malyshevo(90)
Wakkanai (958)
H1N1
H3N8
H5N3
H5N4
H6N2
H6N7
H8N1
H8N3
H9N2
H11N9
Taiwan
H1N1
H4N5
H4N6
H7N7
1996
1997
1998
1999



Phylogenetic tree of the NP genes of influenza A viruses

Acquisition of pathogenicity of avian influenza virus in chicken



Tissue tropism of apathogenic, low pathogenic, and highly pathogenic avian influenza viruses in chicken

Virus strain	Virus infectivity ($logEID_{50}/g$)								
	Trachea	Lungs	Intestine	Kidney	Spleen	Liver	Bone M	Muscle	Blood
Duck/Hok/9/99 (H9N2)	-	–	-	-	-	-	-	-	–
Chicken/Bj/2/97 (H9N2)	**6.3**	**4.5**	–	-	-	-	–	-	–
Tern/SA/61 (H5N3)	**4.7**	**6.7**	**6.3**	**6.3**	**6.7**	**7.5**	-	**6.3**	**3.7**

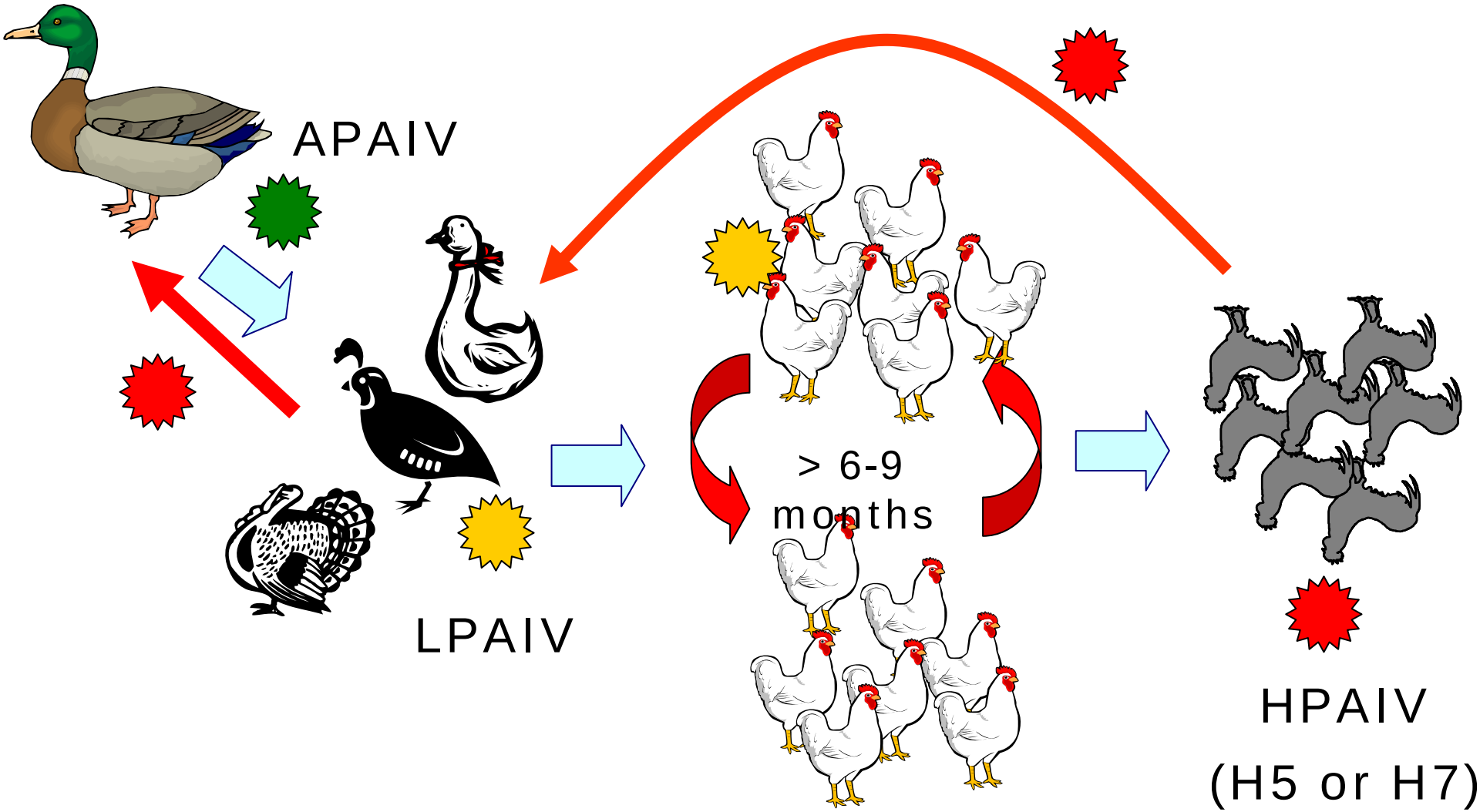
Kishida et al (2004) Arch Virol

Amino acid sequences at the cleavage site of influenza A virus HAs

Subtype	Strains	A A sequences
H1	Dk/Alberta/35/76(H1N1)[b]	IQSR GLF
H2	Mal/MT/Y61(H2N2)[b]	IESR GLF
H3	Dk/Menphis/928/74(H3N8)[b]	KQTR GLF
H4	Dk/Czechoslovakia/56(H4N6)[b]	KASR GLF
H5	Ck/Scotland/59(H5N1)[b]	RKKR GLF
H5	Ty/MN/3/92(H5N2)[a]	RETR GLF
H6	Shw/Australia/1/72(H6N5)[b]	IETR GLF
H7	FPV/Rostock/34(H7N1)[b]	KKRKKR GLF
H7	Mal/Alberta/195/89(H7N3)[a]	KKTR GLF
H8	Ty/Ontario/6118/68(H8N4)[b]	VEPR GLF
H9	Ty/Wisconsin/66(H9N2)[b]	RSSR GLF
H10	Ck/Germany/N/49(H10N7)[b]	VQGR GLF
H11	Dk/England/56(H11N6)[b]	IASR GLF
H12	Dk/Alberta/60/76(H12N5)[b]	VQDR GLF
H13	Gl/Maryland/704/77(H13N6)[b]	ISNR GLF
H14	Mal/Gurjev/263/82(H14N5)[b]	KQAK GLF
H15	Shw/Australia/2576/79(H15N9)[b]	IRTR GLF

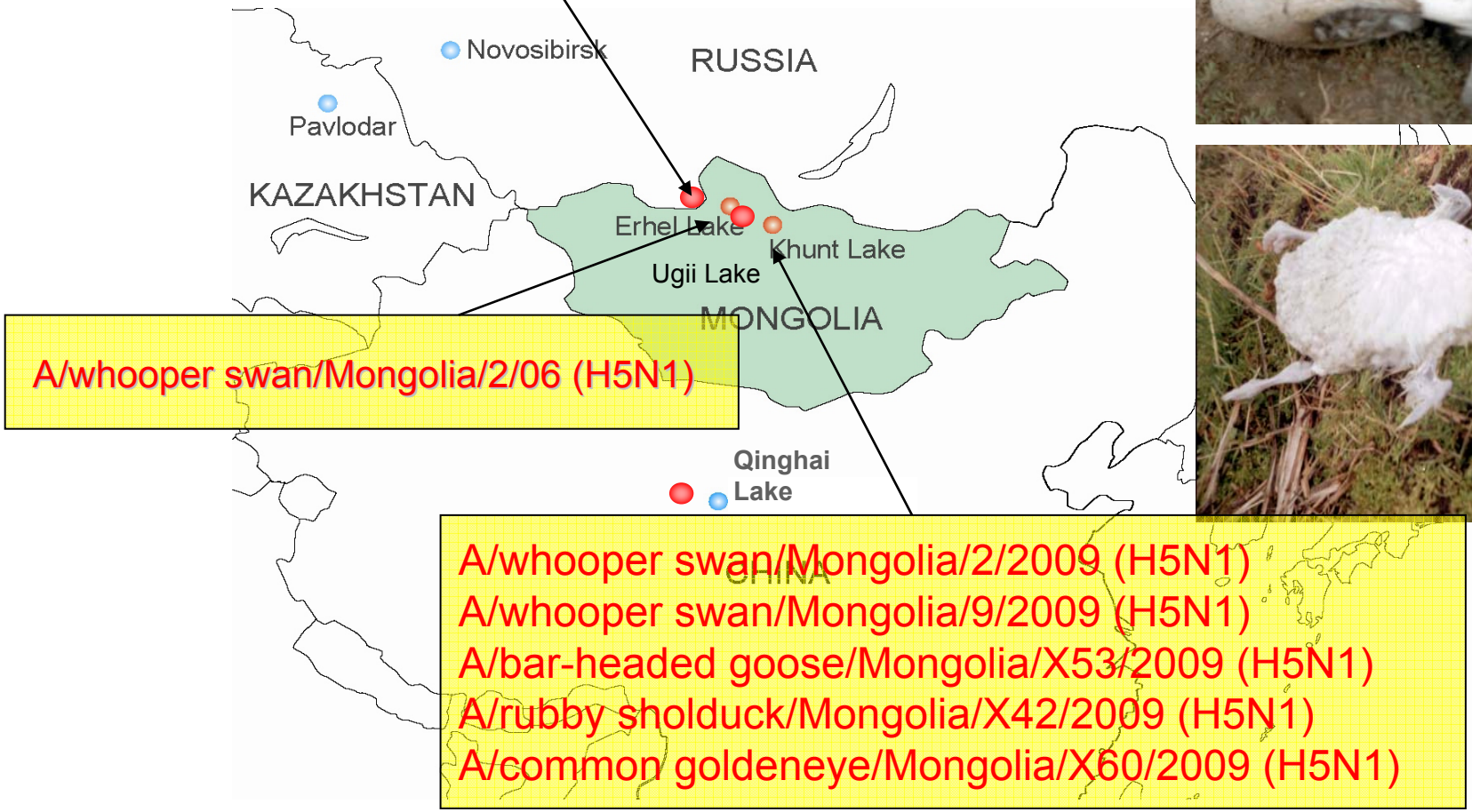
[a] Senne et al, 1996, [b] Kovacova et al, 2002

Return of the HPAIV from domestic poultry to migratory water birds



HPAI viruses isolated from wild birds in Mongolia

A/whooper swan/Mongolia/3/05 (H5N1)
A/bar-headed goose/Mongolia/1/05 (H5N1)
A/common goldeneye/Mongolia/12/06 (H5N1)

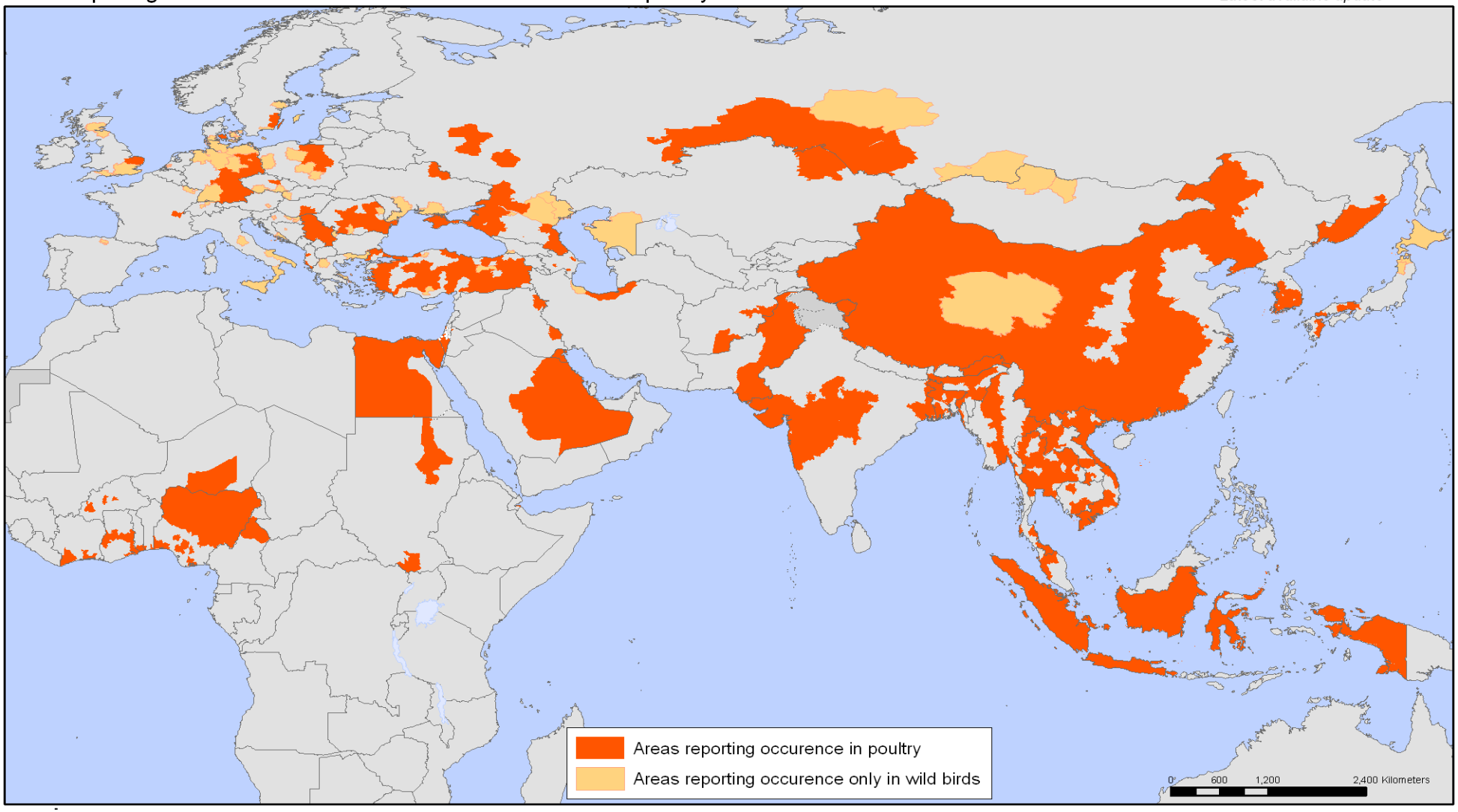


A/whooper swan/Mongolia/2/06 (H5N1)

A/whooper swan/Mongolia/2/2009 (H5N1)
A/whooper swan/Mongolia/9/2009 (H5N1)
A/bar-headed goose/Mongolia/X53/2009 (H5N1)
A/rubby sholduck/Mongolia/X42/2009 (H5N1)
A/common goldeneye/Mongolia/X60/2009 (H5N1)

Areas reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 2003

Status as of 12 December 2008
Latest available update

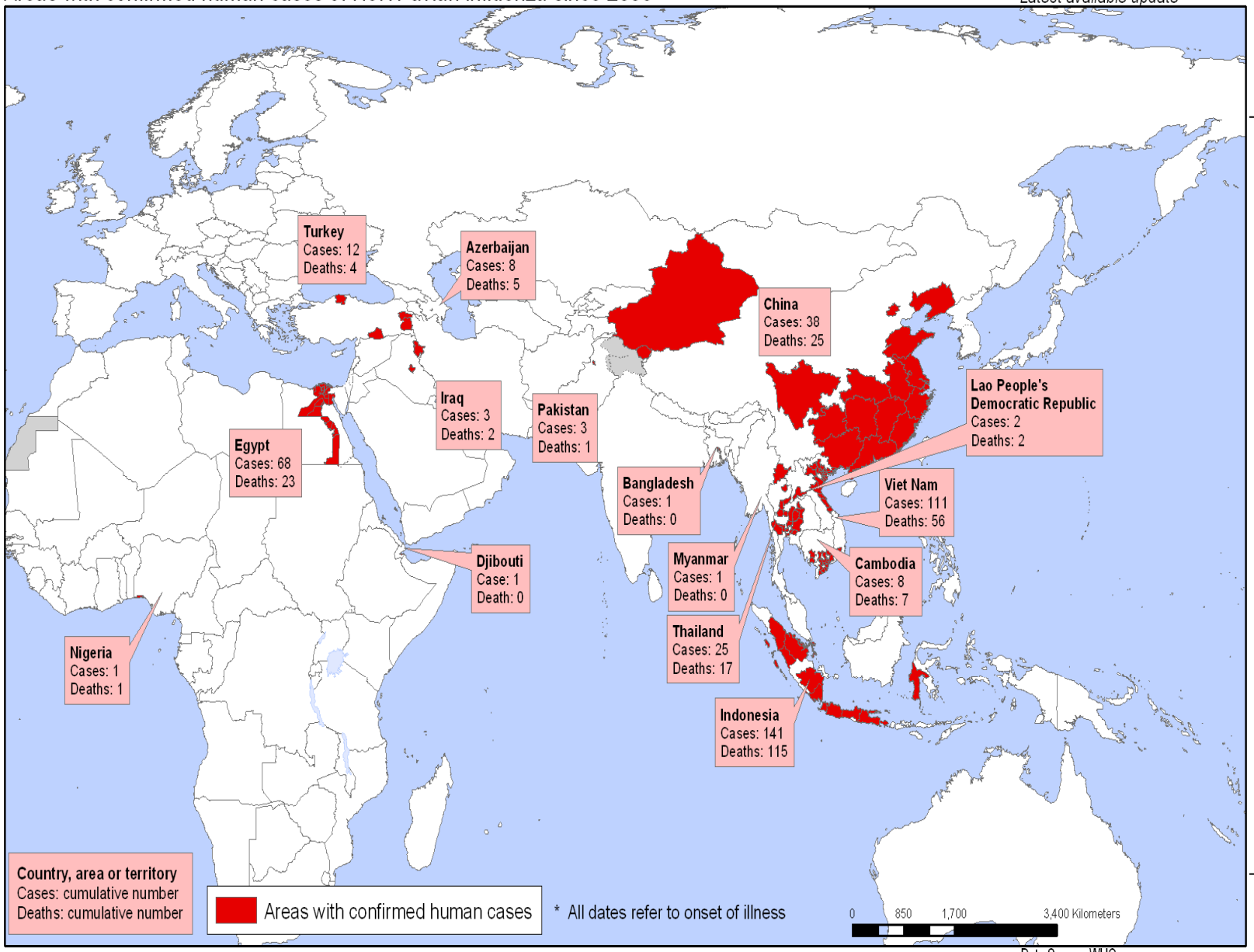


62 Countries where H5N1 HPAIV infections were reported in wild birds, poultry, and both

Japan, Republic of Korea, China, Mongolia, Myanmar, Lao PDR, Thailand, Cambodia, Viet Nam, Malaysia, Indonesia, Bangladesh, India, Pakistan; Afghanistan, Iran, Azerbaijan, Georgia, Iraq, Kuwait, Saudi Arabia, Turkey, Israel; Russian Federation, Kazakhstan, Ukraine, Romania, Bulgaria, Albania, Serbia, Hungary, Slovakia, Czech Republic, Croatia, Poland, Slovenia, Bosnia & Herzegovina; Greece, Switzerland, Austria, France, Italy, Germany, Netherlands, Denmark, Sweden, Spain, England, Ireland; Djibouti, Gaza Strip, Egypt, Sudan, Nigeria, Niger, Cameroon, Burkina Faso, Cote d'Ivoire

Areas with confirmed human cases of H5N1 avian influenza since 2003 *

Status as of 6 May 2009
Latest available update



Country	Deaths / Cases
China	25/ 38
Viet Nam	56/111
Indonesia	115/141
Egypt	27/ 85
Cambodia	7/ 8
Lao PDR	2/ 2
Thailand	17/25
Iraq	2/ 3
Azerbaijan	5/ 8
Turkey	4/12
Djibouti	0/ 1
Nigeria	1/ 1
Myanmar	0/ 1
Pakistan	1/ 3
Bangladesh	0/ 1
Total	**262/440**

As of 31 August 2009

World Health Organization

Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

Influenza Vaccine for bird flu

- may prevent manifestation of disease signs and decrease the amount of virus shed, but does not confer protection from infection.
- is produced with inactivated virus, not live virus.
- Effect: indivisual, not population.
- “Test and culling or stamping-out policy” is recommended for the control of avian influenza.
- Vaccination is not recommended but one of the options applied only under DIVA (differentiating infected from vaccinated animals) based strategy, and as a tool in addition to, not instead of stamping-out policy.
- Country where vaccine is used is not designated as HPAI-free.

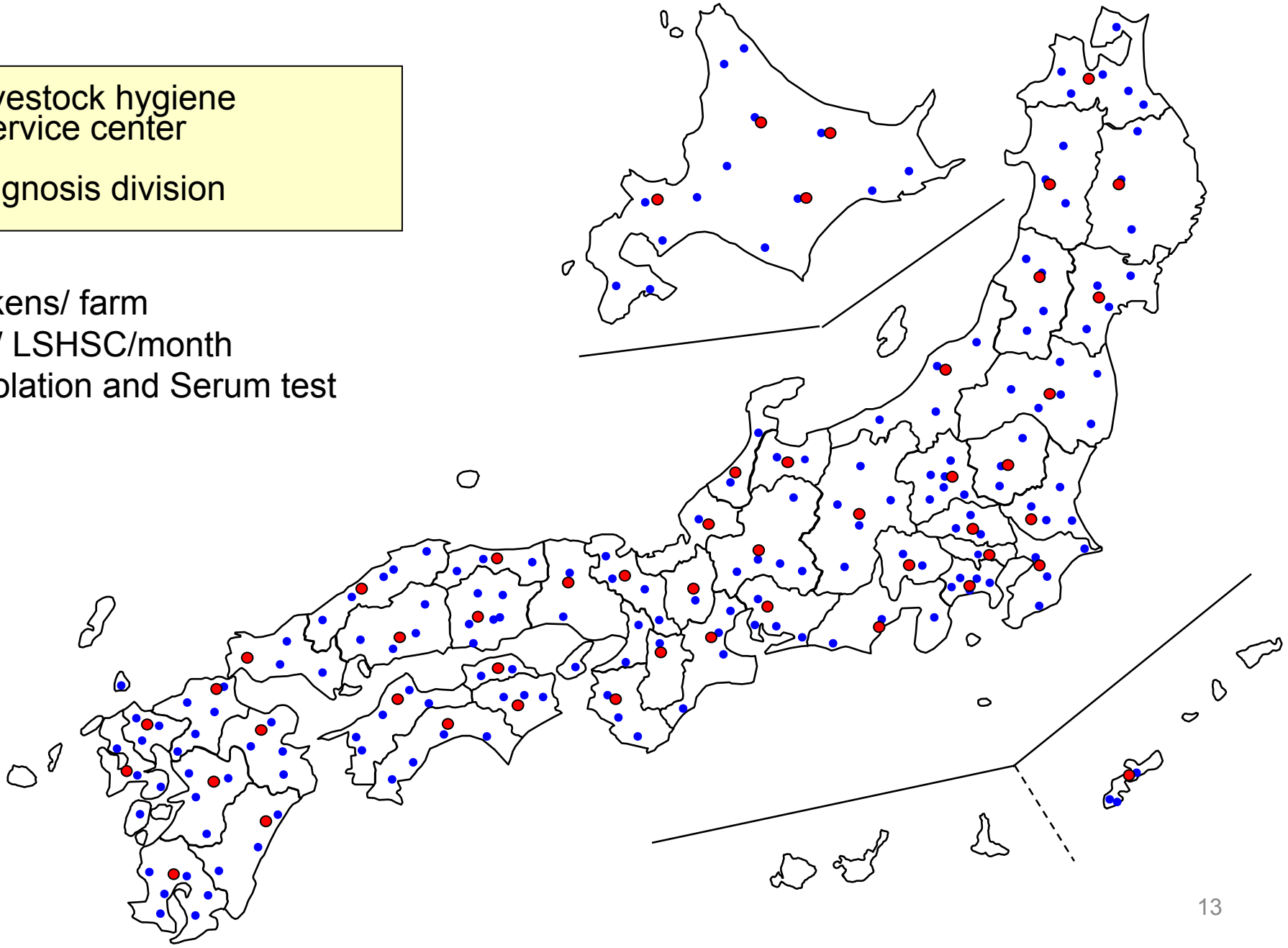
→ must lead silent spread of virus.

Framework of avian influenza surveillance in Japan

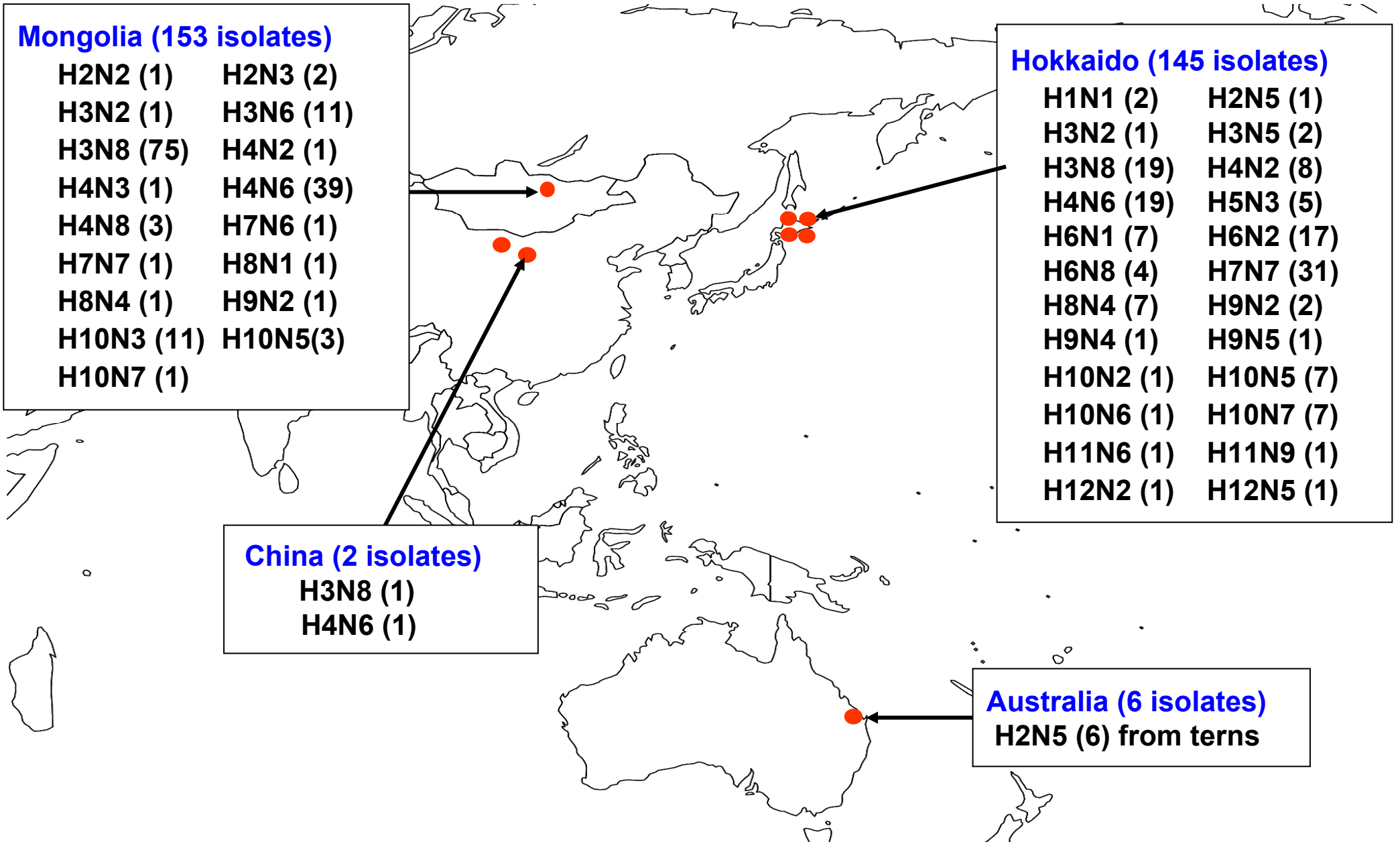
: Livestock hygiene service center

: Diagnosis division

10 chickens/ farm
3 farms/ LSHSC/month
Virus isolation and Serum test



Surveillance of avian influenza in migratory ducks in 2004-2008



Virus recovery from swabs and tissues of chickens intranasally inoculated with Ck/Yamaguchi/7/04 (H5N1)

No.	Days p.i.	Virus titers[a]							
		Swabs		Tissue homogenates					
		Trachea	Cloaca	Trachea	Lung	Liver	Spleen	Kidney	Colon
1	1	-	-	3.1	3.1	3.1	2.6	3.1	-
2		-	-	3.3	3.1	3.1	-	-	-
3		-	-	3.1	2.9	3.1	3.1	3.4	-
4	2	3.9	-	3.1	2.9	2.9	3.4	3.1	3.1
5		-	-	2.9	3.1	3.9	3.9	4.1	3.9
6†		8.1	8.1	7.9	7.9	8.0	7.6	7.6	7.2
7	3	8.6	7.9	8.1	7.6	8.1	7.6	7.8	7.6
8		-	-	3.1	3.1	4.1	3.1	3.6	3.9
9†	4	8.3	7.9	7.8	7.6	8.1	7.6	7.6	6.9
10†		8.6	8.1	7.8	7.6	7.6	7.2	7.6	6.9

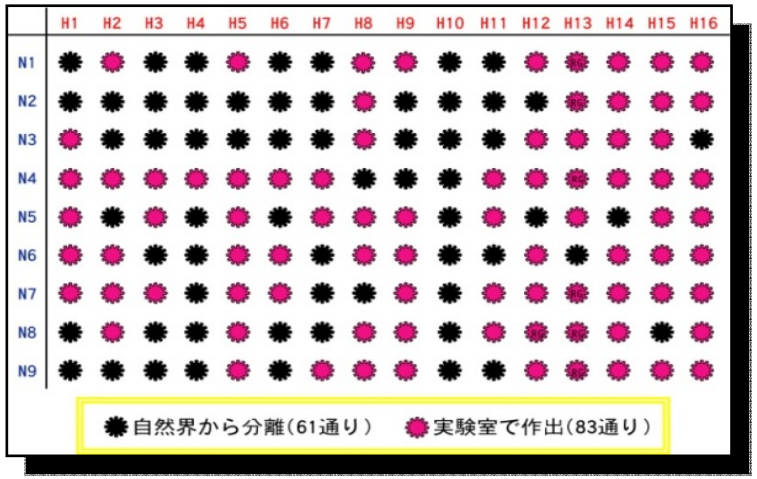
[a]Virus titers（$\log_{10}TCID_{50}$/ml for swabs and $\log_{10}TCID_{50}$/g for organs）in MDCK cells.
†Dead

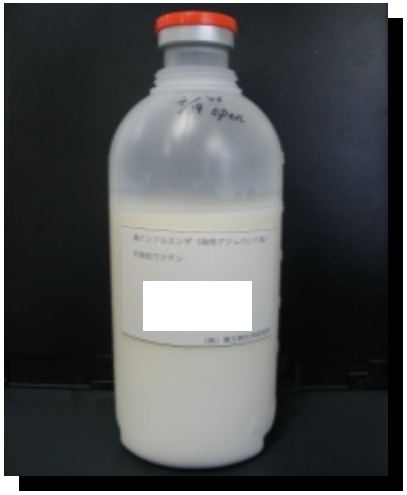
Virus recovery from swabs and tissues of chickens experimentally infected with Ck/Yokohama/aq55/01 (H9N2)

No.	Days p.i.	Virus titers							
		Swabs		Tissue homogenates					
		Trachea	Cloaca	Trachea	Lung	Liver	Spleen	Kidney	Colon
1	1	3.6[a]	-	3.6	3.1	3.1	3.2	3.2	3.1
2		3.6	-	3.6	3.1	3.1	3.2	3.2	3.2
3	2	3.8	-	3.6	3.1	-	3.1	3.2	3.1
4		3.8	-	3.6	2.6	2.6	2.6	3.1	3.1
5	3	3.4	-	3.4	-	-	-	-	-
6		3.4	-	3.4	-	-	-	-	-
7	4	3.4	-	-	-	-	-	-	-
8		3.4	-	-	-	-	-	-	-
9	5	-	-	-	-	-	-	-	-
10		-	-	-	-	-	-	-	-

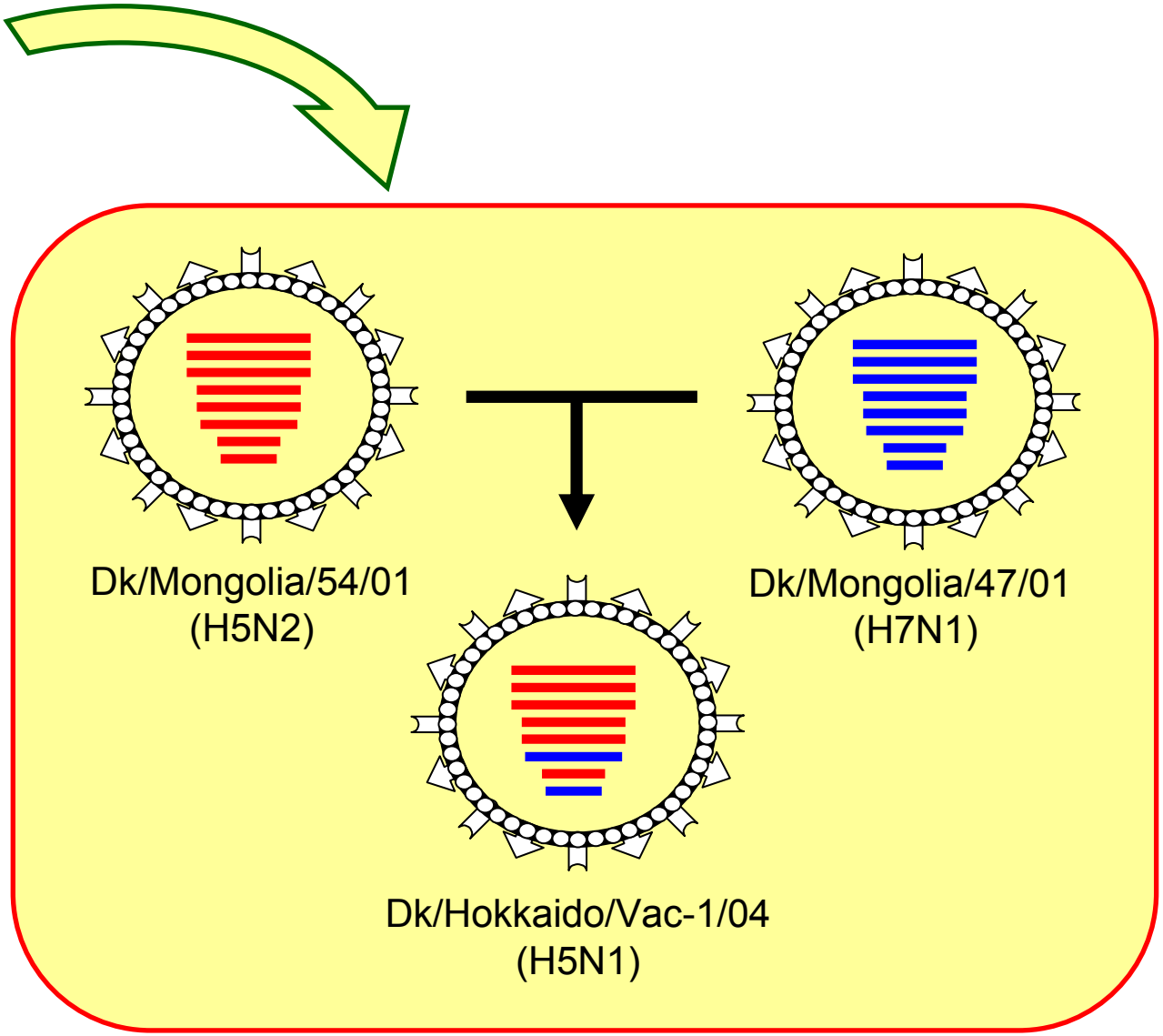
[a]Virus titers（$\log_{10}TCID_{50}$/ml for swabs and $\log_{10}TCID_{50}$/g for organs）in MDCK cells.
No clinical signs were exhibited

Inactivated avian influenza vaccine prepared from a non-pathogenic H5N1 reassortant virus

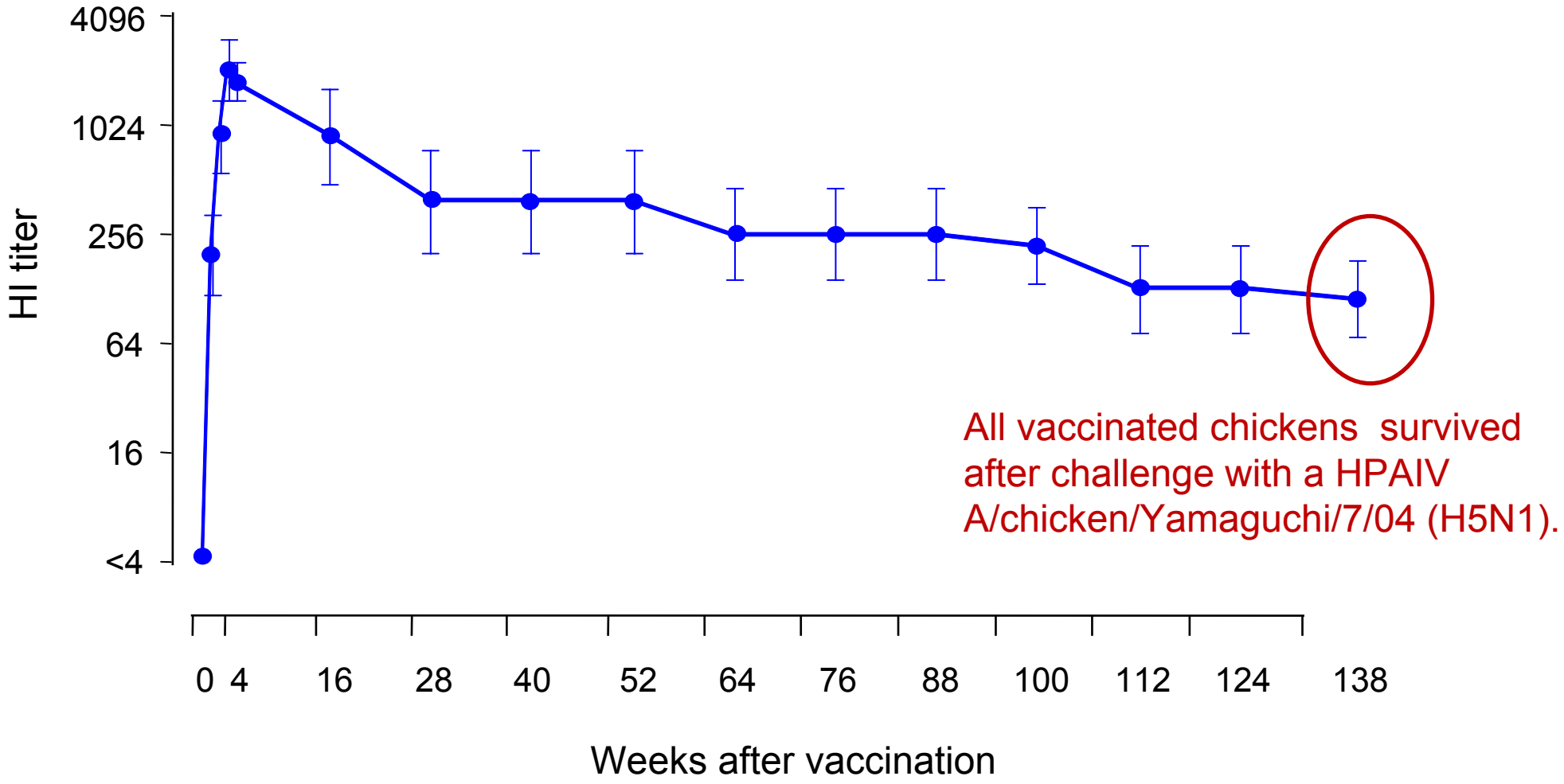




Inactivated whole virion vaccine



Serum HI antibody titers in chickens during 138 weeks following Dk/Hok/Vac-1/04 (H5N1) vaccination



Sasaki et al., Vaccine, 2009

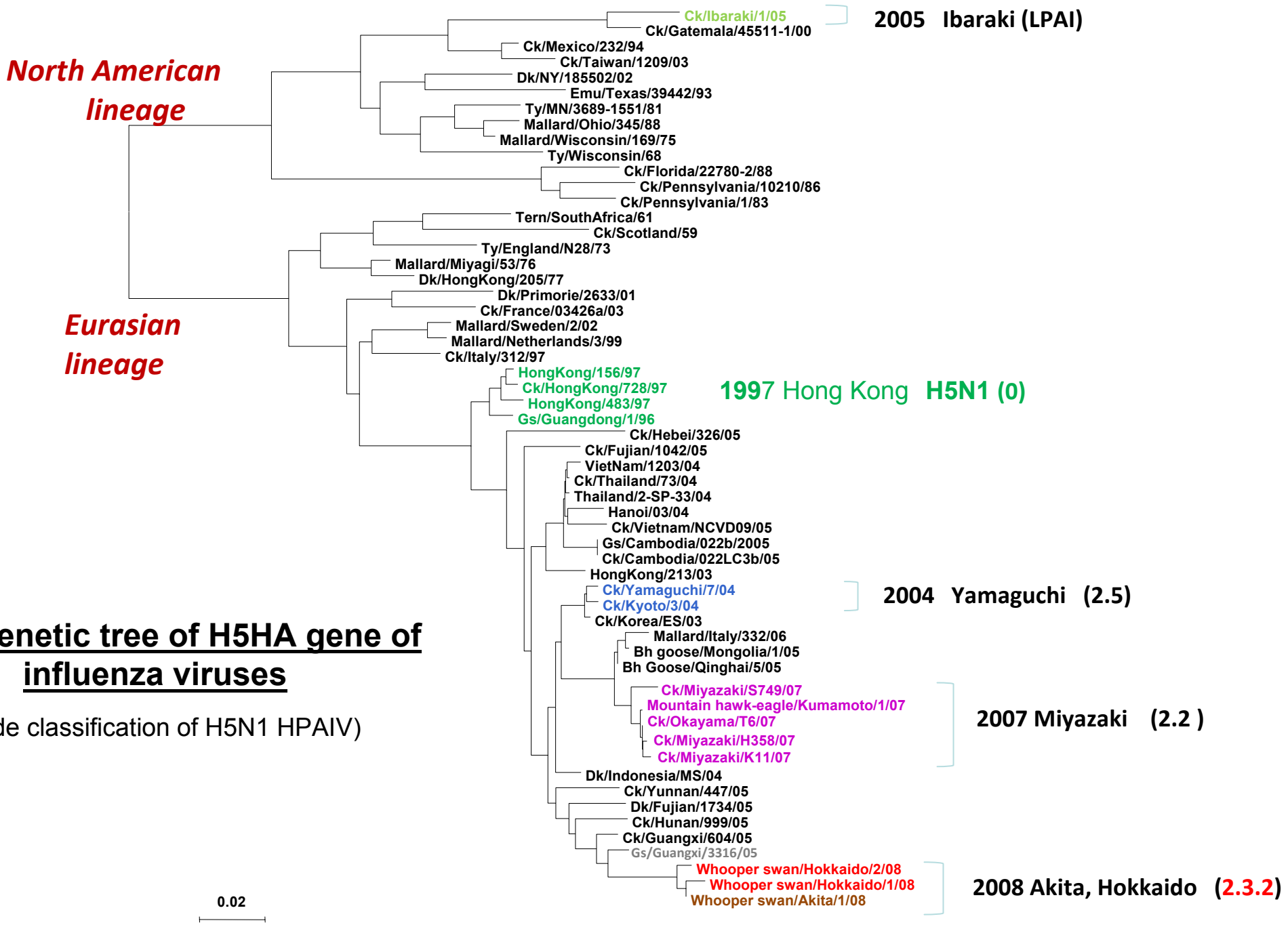
Virus recovery from vaccinated chickens after challenge with A/whooper/Mongolia/3/2005 (H5N1)

	Serum HI titer with Vac-1 (0day)	Days p.c.	Virus titer				
			Swabs ($logEID_{50}$/ml)		Tissue homogenates ($logEID_{50}$/g)		
			Trachea	Cloaca	Trachea	Kidney	Colon
Vaccinated chickens	512	2†	–	–	–	–	–
	256	2†	–	–	–	–	–
	256	2†	1.3	–	1.8	–	–
	512	2†	–	–	–	–	–
	256	2†	1.3	–	1.8	–	–
	512	2†	–	–	–	–	–
	512	4†	0.8	–	1.8	–	–
	256	4†	–	–	–	–	–
	512	4†	–	–	1.8	–	–
	512	4†	–	–	–	–	–
	128	4†	0.8	–	–	–	–
	256	4†	–	–	–	–	–

Chickens were challenged on 3 weeks after vaccination

† Sacrificed

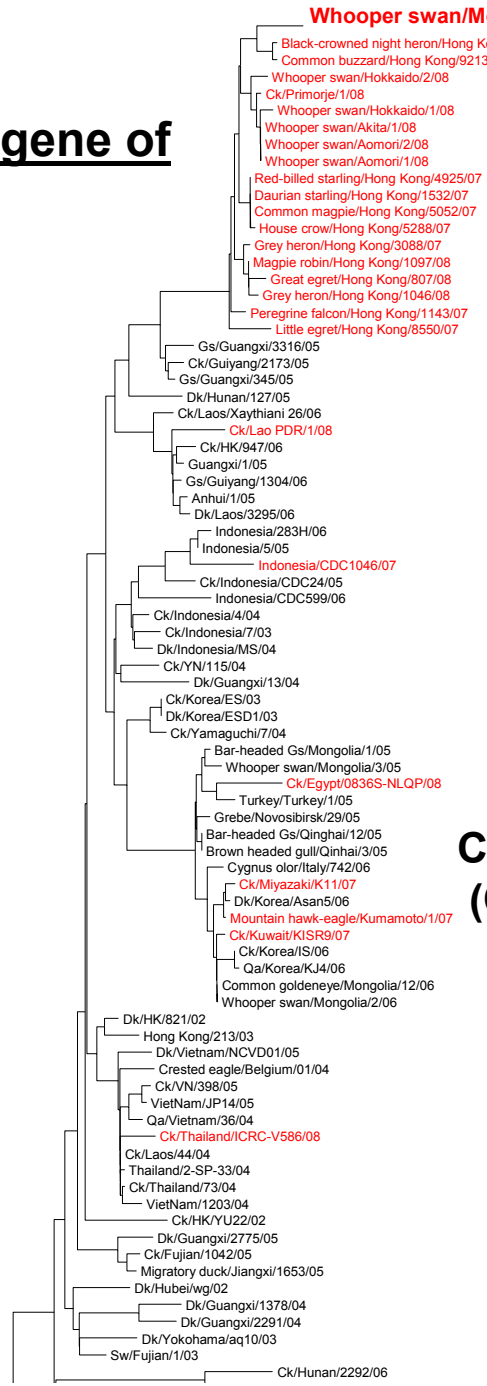
Isoda et al., Arch Virol, 2008



Phylogenetic tree of H5HA gene of influenza viruses

(Clade classification of H5N1 HPAIV)

Phylogenetic tree of H5HA gene of influenza viruses

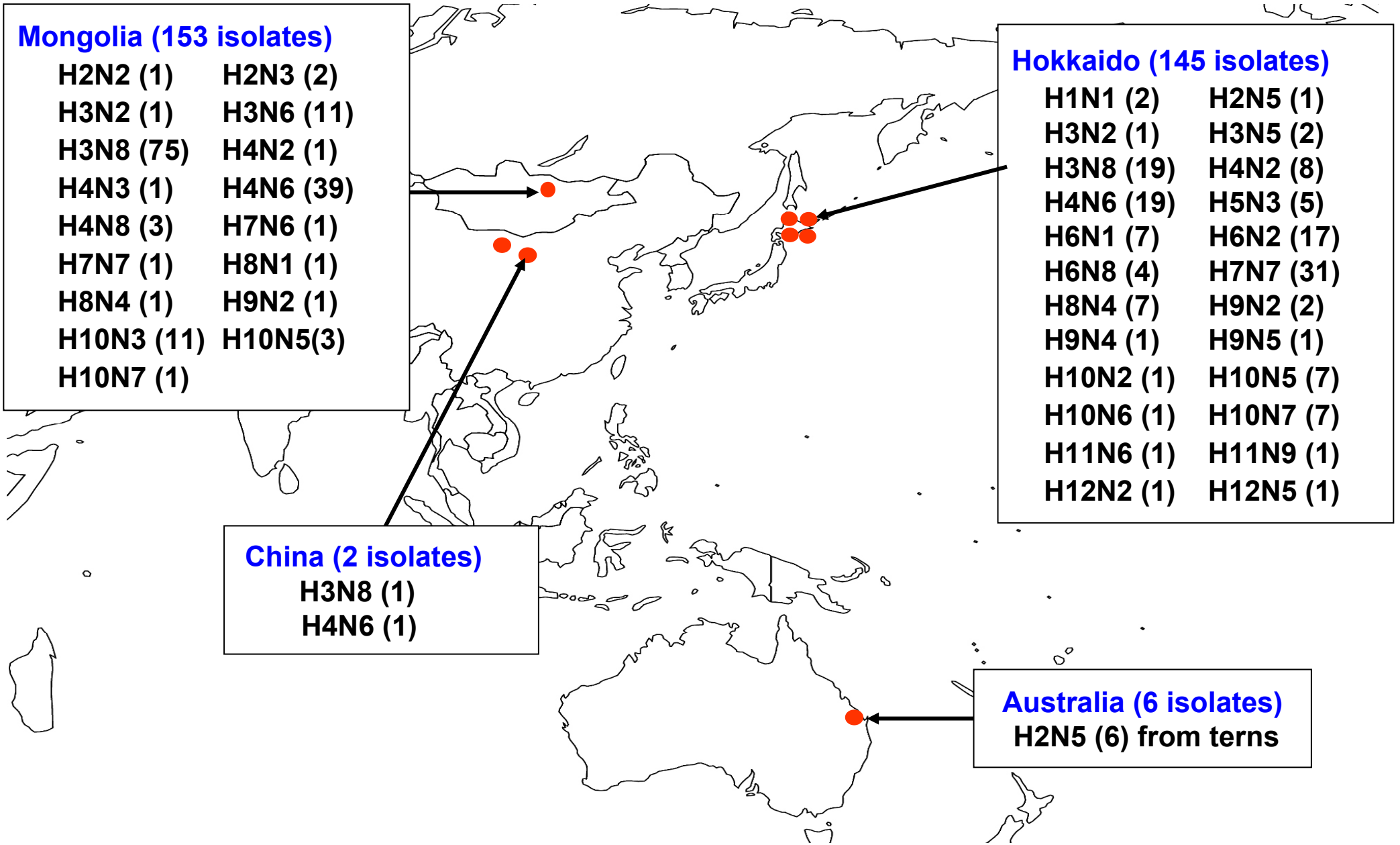


Clade 2.3.2

Clade 2.2
(Qinghai-like viruses)

* H5N1 viruses isolated from migratory water birds in 2007-2009.
* Antigenically different from other H5N1 viruses
* Highly pathogenic against water birds

Surveillance of avian influenza in migratory ducks in 2004-2008



How should avian influenza be controlled?

* Why do the H5N1 HPAIV strains have persisted in poultry for 12 years?
* Why do these strains show antigenic variation? Misuse of Vaccine
* Is H5N1 HPAIV alone as a pandemic strain candidate?
* Will the HPAIV strains that returned to migratory birds persist in nature?
* How should avian influenza be controlled in poultry?

Stamping-out without misuse of vaccine is only way, so far.

Eradication of HPAIV from poultry birds by stamping-out policy.