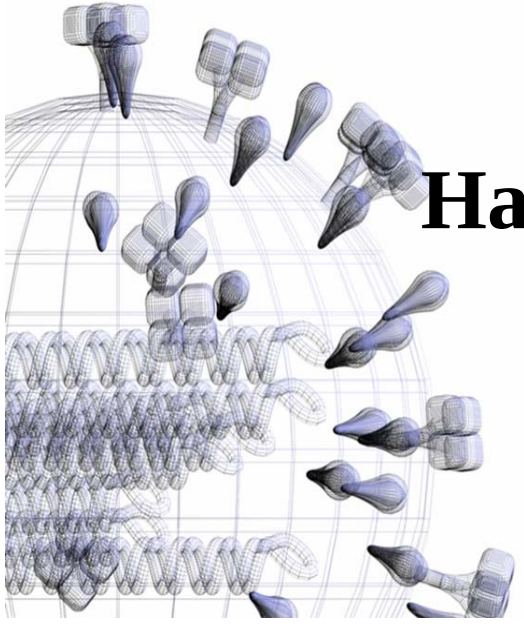
Evolution of H5N1 avian influenza viruses and the vaccines developed in China

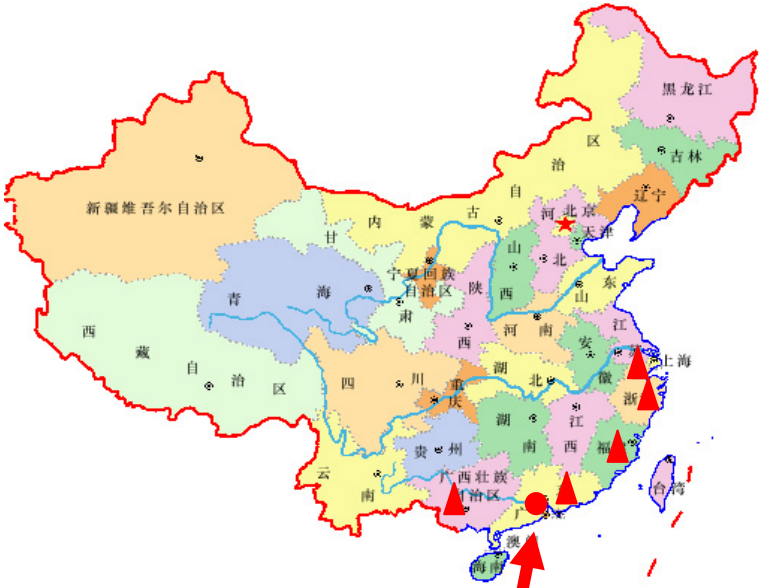


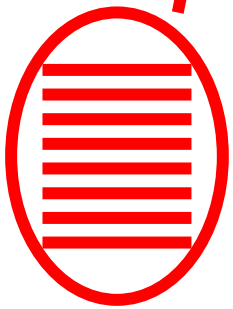
Hualan Chen, Ph.D

Harbin Veterinary Research Institute, CAAS

Report contains

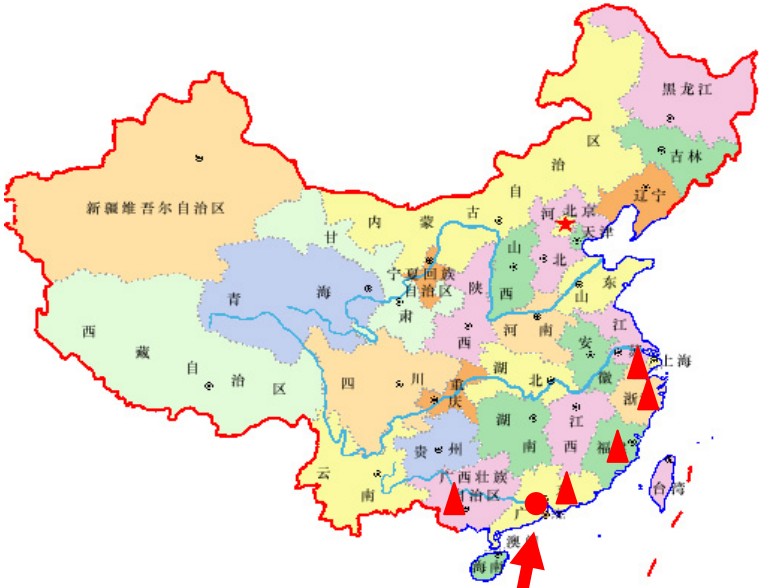
- Evolution of H5N1 avian influenza viruses in China—a whole picture
- What kind of vaccines have been used and why?





IVPI=2.1

A/goose/Guangdong/1/96(H5N1)



From 1999-2002, 21 H5N1 viruses were isolated from apparently healthy ducks from the five provinces

IVPI>1.2

IVPI=2.1

A/goose/Guangdong/1/96(H5N1)

H5N1 duck viruses

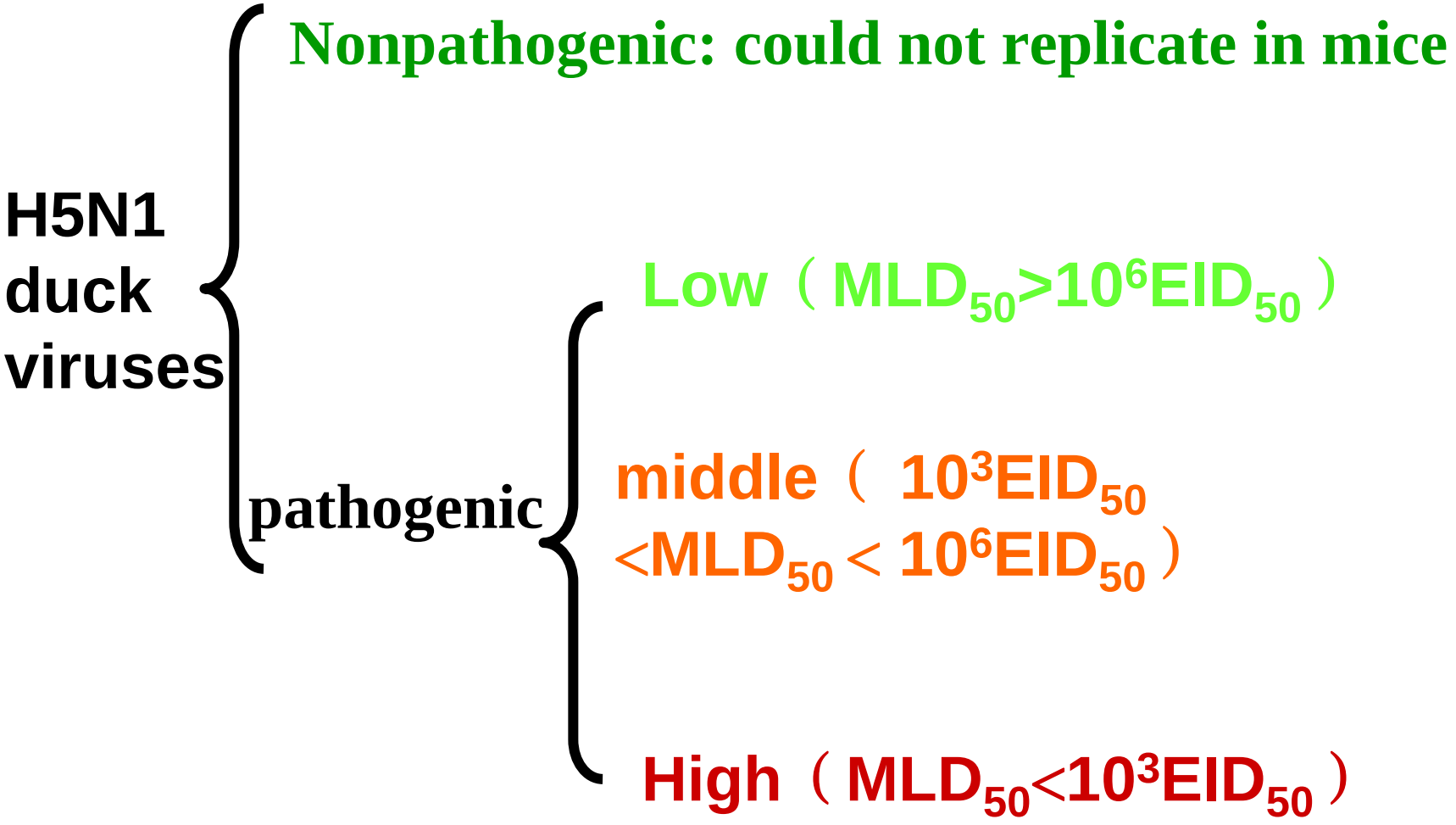
- 21 H5N1 avian influenza viruses were isolated from healthy ducks during routine surveillance
- All of the isolates are highly pathogenic for chickens according to the OIE standard (IVPI>1.2)
- Their replication and lethality were tested in mice

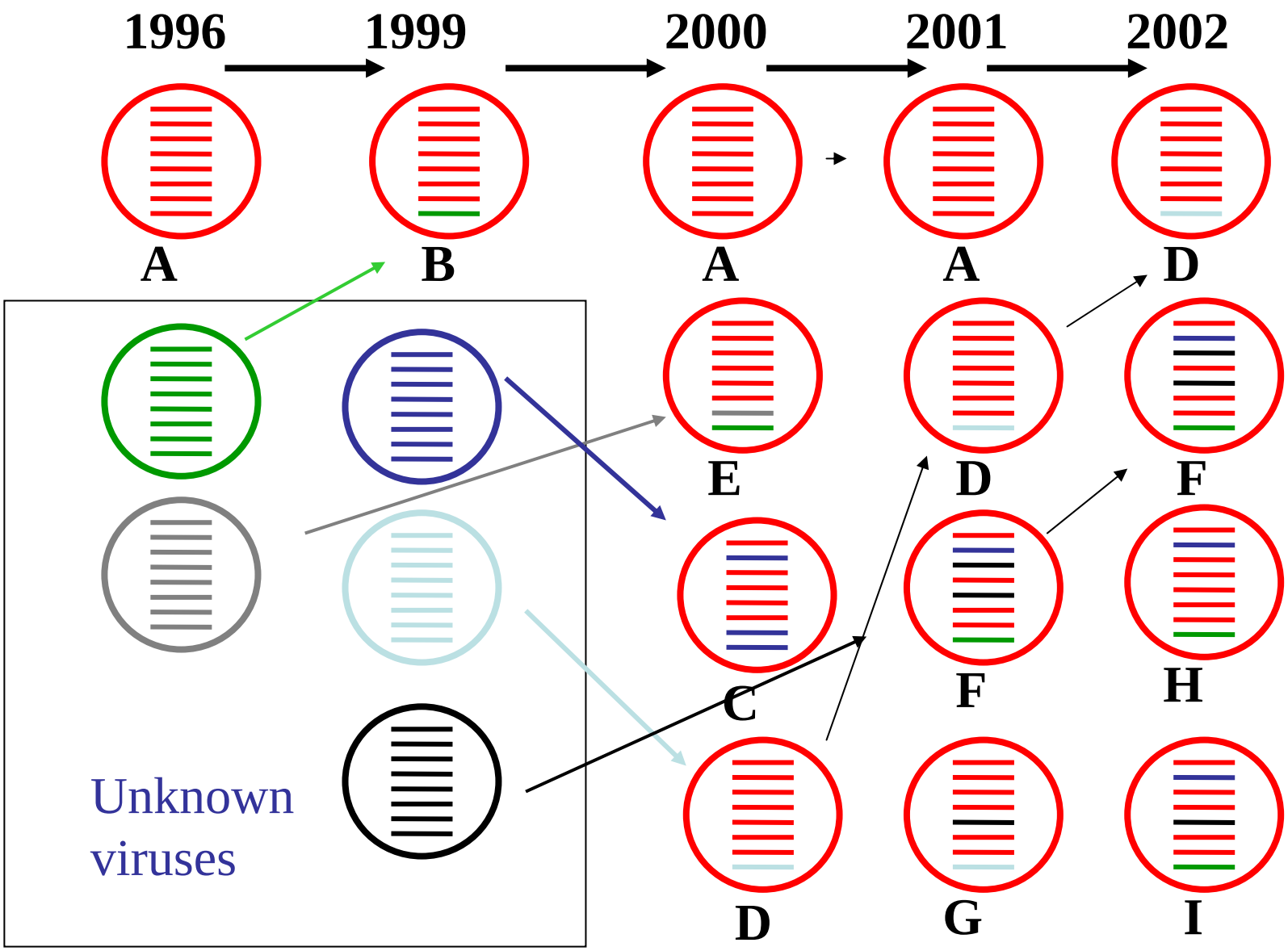
Pathogenicity of H5N1 duck virus in mice 1

Viruses	Virus replication in organs (log EID_{50}/ml)				MLD_{50} (log EID_{50})
	Lung	Spleen	Kidney	Brain	
GSGD/1/96	-	-	-	-	>6.5
DKGX/07/99	-	-	-	-	>6.5
DKFJ/19/00	-	-	-	-	>6.5
DKGD/12/00	-	-	-	-	>6.5
DKZJ/11/00	-	-	-	-	>6.5
DKZJ/52/00	-	-	-	-	>6.5
DKGX/22/01	-	-	-	-	>6.5
DKGD/07/00	+	-	-	-	>6.5
DKGD/1/01	+	-	-	-	>6.5
DKFJ/17/01	+	-	-	-	>6.5
DKGX/53/02	+	-	-	-	>6.5

Pathogenicity of H5N1 duck virus in mice 2

Viruses	Virus replication in organs (log EID_{50}/ml)				MLD_{50} (log EID_{50})
	Lung	Spleen	Kidney	Brain	
DKGD/40/00	+	+	-	-	6.4
DKGX/50/01	+	+	-	-	6.4
DKSH/13/01	+	-	-	-	5.0
DKSH/38/01	+	+	-	-	5.8
DKSH/8/01	+	+	+	+	4.7
DKGD/22/02	+	-	-	-	4.8
DKSH/37/02	+	+	-	-	5.3
DKGX/35/01	+	+	+	+	1.5
DKSH/35/02	+	+	+	+	2.3
DKFJ01/02	+	+	+	+	<0.5
DKFJ/13/02	+	+	+	+	<0.5


H5N1 duck viruses
Nonpathogenic: could not replicate in mice
pathogenic
Low（MLD50>10^6EID50）
middle（ 10^3EID50 <MLD50 < 10^6EID50）
High（MLD50<10^3EID50）


1996
1999
2000
2001
2002
A
B
A
A
D
E
D
F
C
F
H
D
G
I
Unknown viruses

The relationship of genotype and the pathotype?

Virus	pathotype	genotype	Virus	pathotype	genotype
GSGD/1/96	non	A	DKGD/40/00	middle	E
DKGX/07/99	non	B	DKGX/50/01	middle	G
DKFJ/19/00	non	C	DKSH/13/01	middle	D
DKGD/12/00	non	A	DKSH/38/01	middle	F
DKZJ/11/00	non	A	DKSH/8/01	middle	A
DKZJ/52/00	non	D	DKGD/22/02	middle	D
DKGX/22/01	non	D	DKSH/37/02	middle	H
DKGD/07/00	low	A	DKGX/35/01	high	D
DKGD/1/01	low	D	DKSH/35/02	high	F
DKFJ/17/01	low	D	DKFJ01/02	high	I
DKGX/53/02	low	I	DKFJ/13/02	high	H

2004 H5N1 outbreaks in China

83% of the 50 outbreaks occurred in southern China

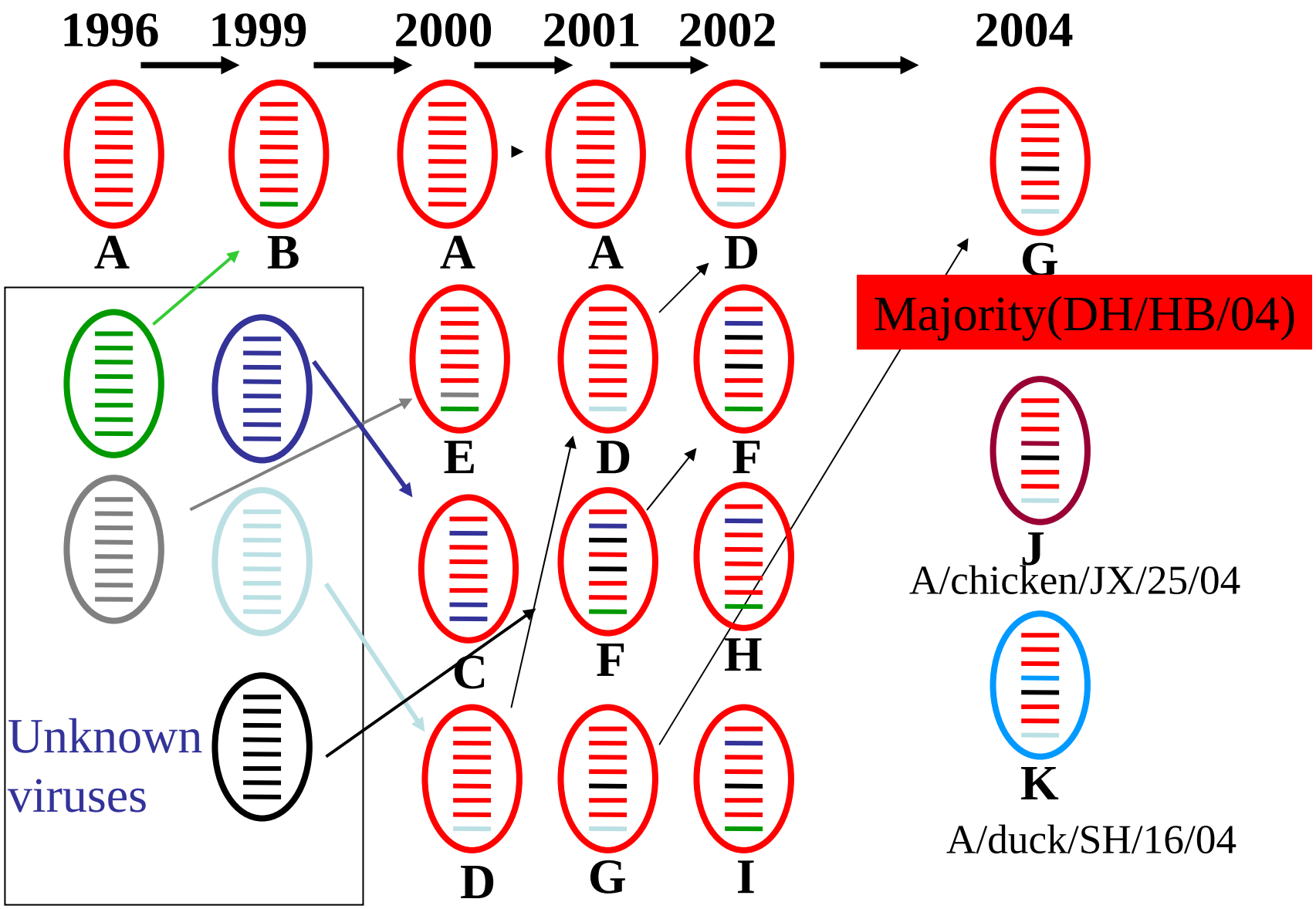
Provinces	Outbreak
Guangxi	6
Guangdong	9
Zhejiang	1
Shanghai	1
Jiangxi	3
Hunan	1
Hubei	10
Henan	5
Anhui	5
Yunnan	6
Tibet	1
Gansu	1
Xinjiang	1
Shanxi	2
Tianjin	1
Jllin	1
Total	50

Total birds that were affected

- About 150, 000 birds infected
- About 120, 000 birds died
- 7.9 millions were slaughtered
- 11.34 millions birds were vaccinated

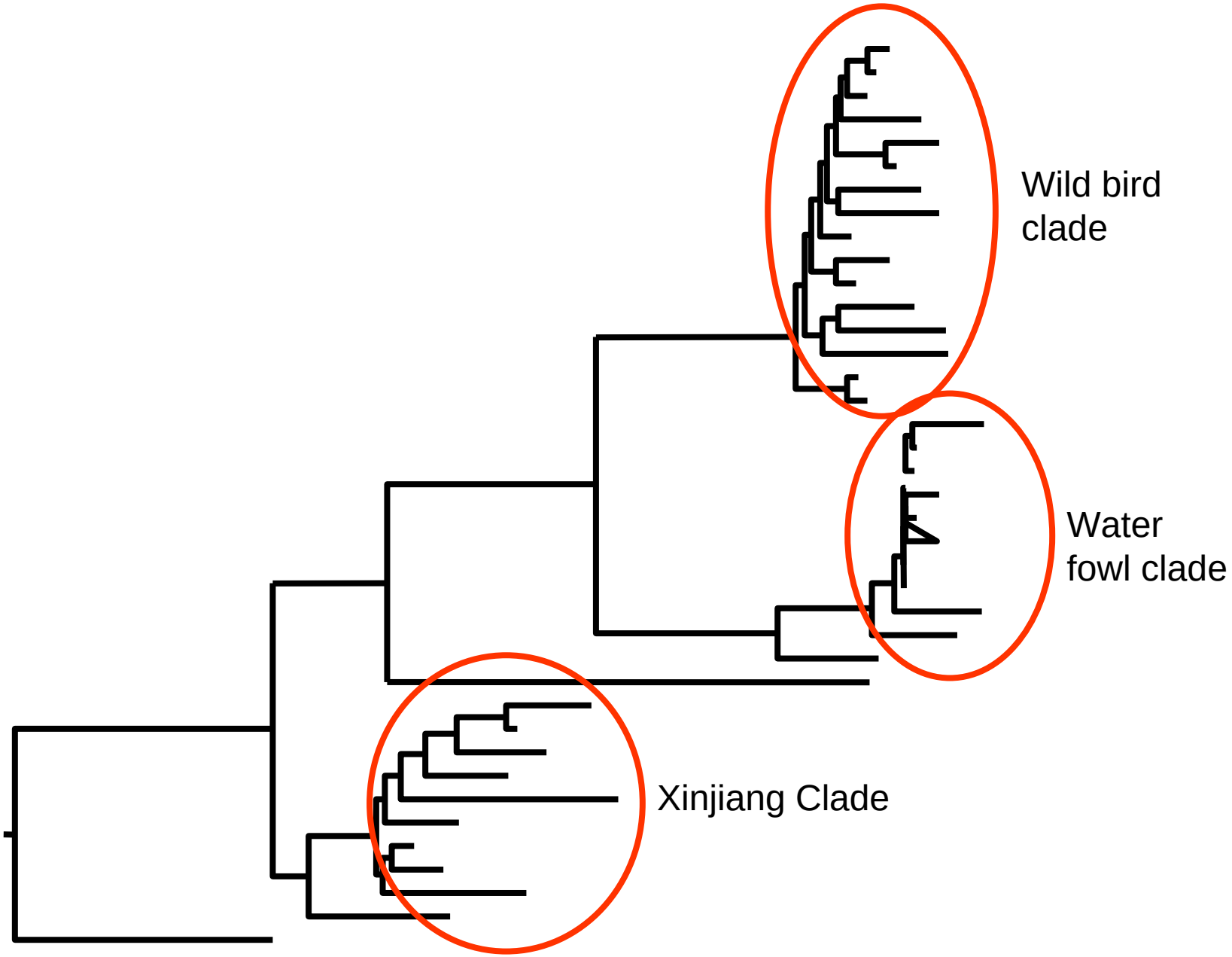
Virus isolations

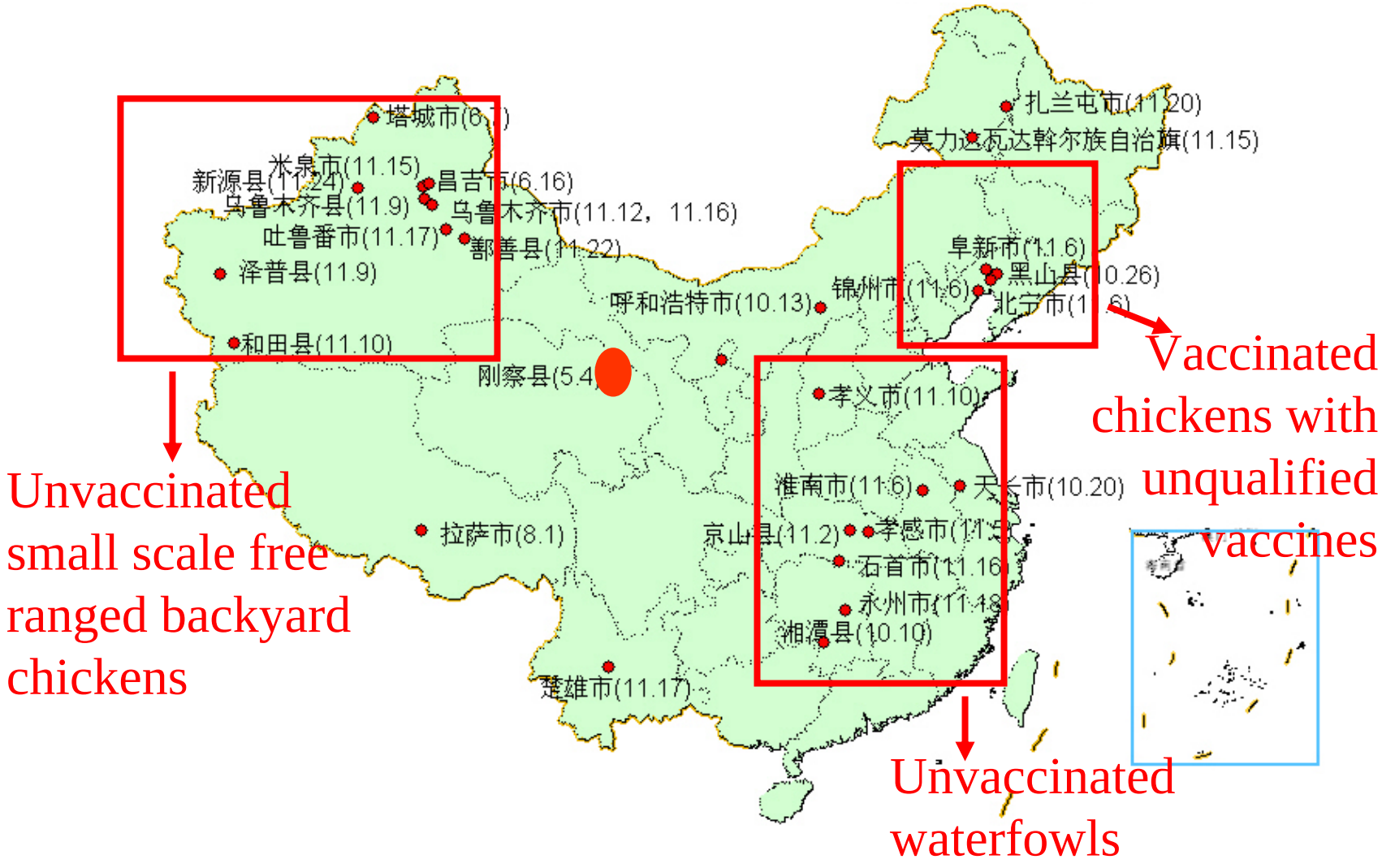
- Over than 500 samples were sent to the reference laboratory for the final confirmation
- 60 H5N1 avian influenza viruses were isolated from chickens, ducks, geese, black swans, quails, pigeons and pheasants
- All of the viruses have been fully sequenced


1996
1999
2000
2001
2002
2004
A
B
A
A
D
G
Majority(DH/HB/04)
E
D
F
J
A/chicken/JX/25/04
C
F
H
K
A/duck/SH/16/04
Unknown viruses
D
G
I

H5N1 outbreaks in China in 2005


塔城市(6.7)
扎兰屯市(11.20)
莫力达瓦达斡尔族自治旗(11.15)
米泉市(11.15)
新源县(11.24)
昌吉市(6.16)
乌鲁木齐县(11.9)
乌鲁木齐市(11.12，11.16)
吐鲁番市(11.17)
鄯善县(11.22)
阜新市(11.6)
泽普县(11.9)
黑山县(10.26)
锦州市(11.6)
呼和浩特市(10.13)
北宁市(11.6)
和田县(11.10)
刚察县(5.4)
孝义市(11.10)
淮南市(11.6)
天长市(10.20)
拉萨市(8.1)
京山县(11.2)
孝感市(11.5)
石首市(11.16)
永州市(11.18)
湘潭县(10.10)
楚雄市(11.17)


Wild bird clade
Water fowl clade
Xinjiang Clade


塔城市(6.7)
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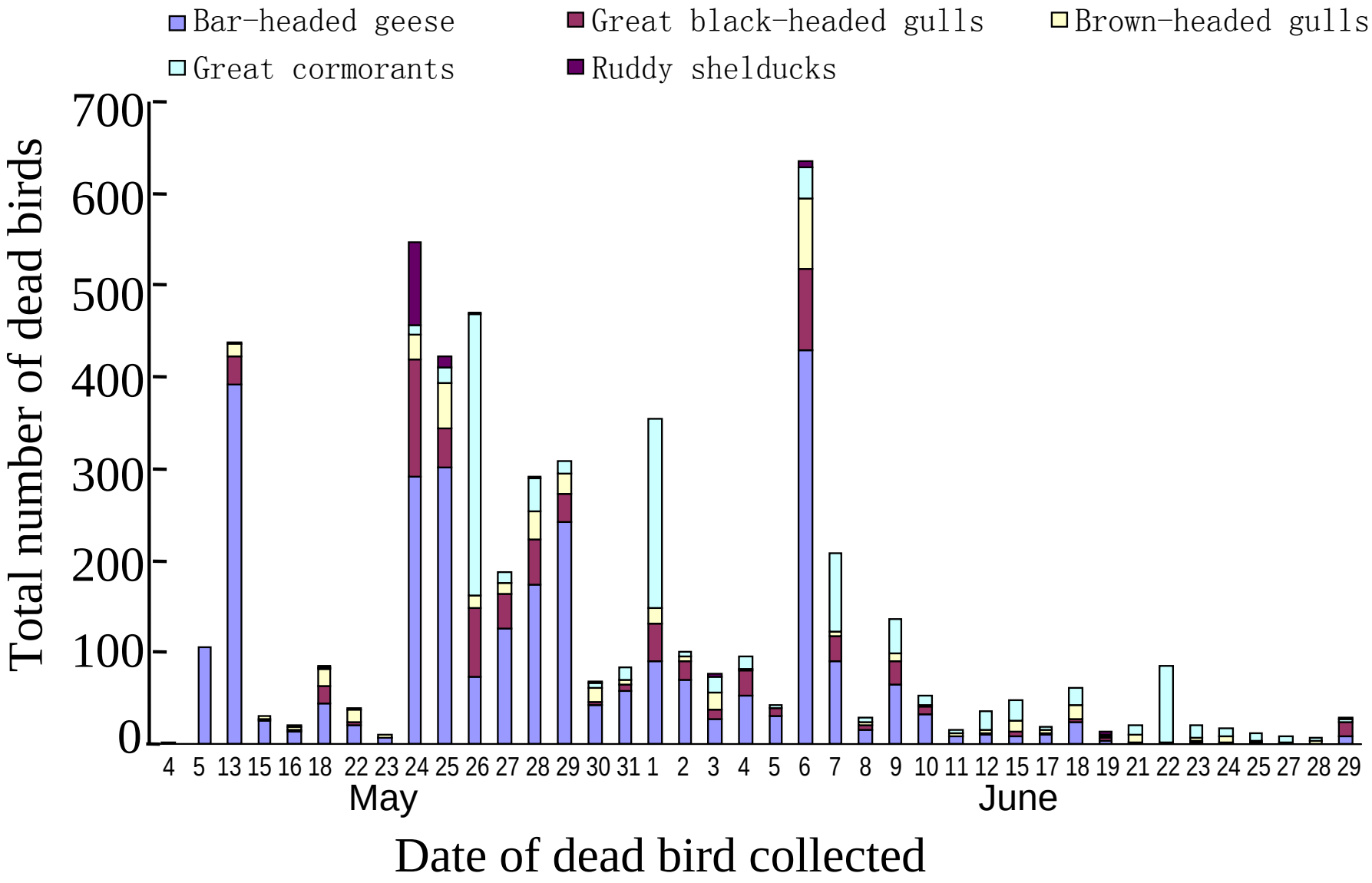
京山县(11.2)
孝感市(11.5)
石首市(11.16)
永州市(11.18)
湘潭县(10.10)
楚雄市(11.17)
Unvaccinated small scale free ranged backyard chickens
Vaccinated chickens with unqualified vaccines
Unvaccinated waterfowls


21 12:37PM


6 7:21 AM


Bar-headed geese
Great black-headed gulls
Brown-headed gulls
Great cormorants
Ruddy shelducks
Total number of dead birds
0
100
200
300
400
500
600
700
4 5 13 15 16 18 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 15 17 18 19 21 22 23 24 25 27 28 29
May
June
Date of dead bird collected

3282

Bar-headed geese
Great black-headed gulls
Brown-headed gulls
Great cormorants
Ruddy shelducks

Total number of dead birds

700
600
500
400
300
200
100
0

4 5 13 15 16 18 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 15 17 18 19 21 22 23 24 25 27 28 29

May June

Date of dead bird collected

3282

929

570

Bar-headed geese

Great black-headed gulls

Brown-headed gulls

Great cormorants

Ruddy shelducks

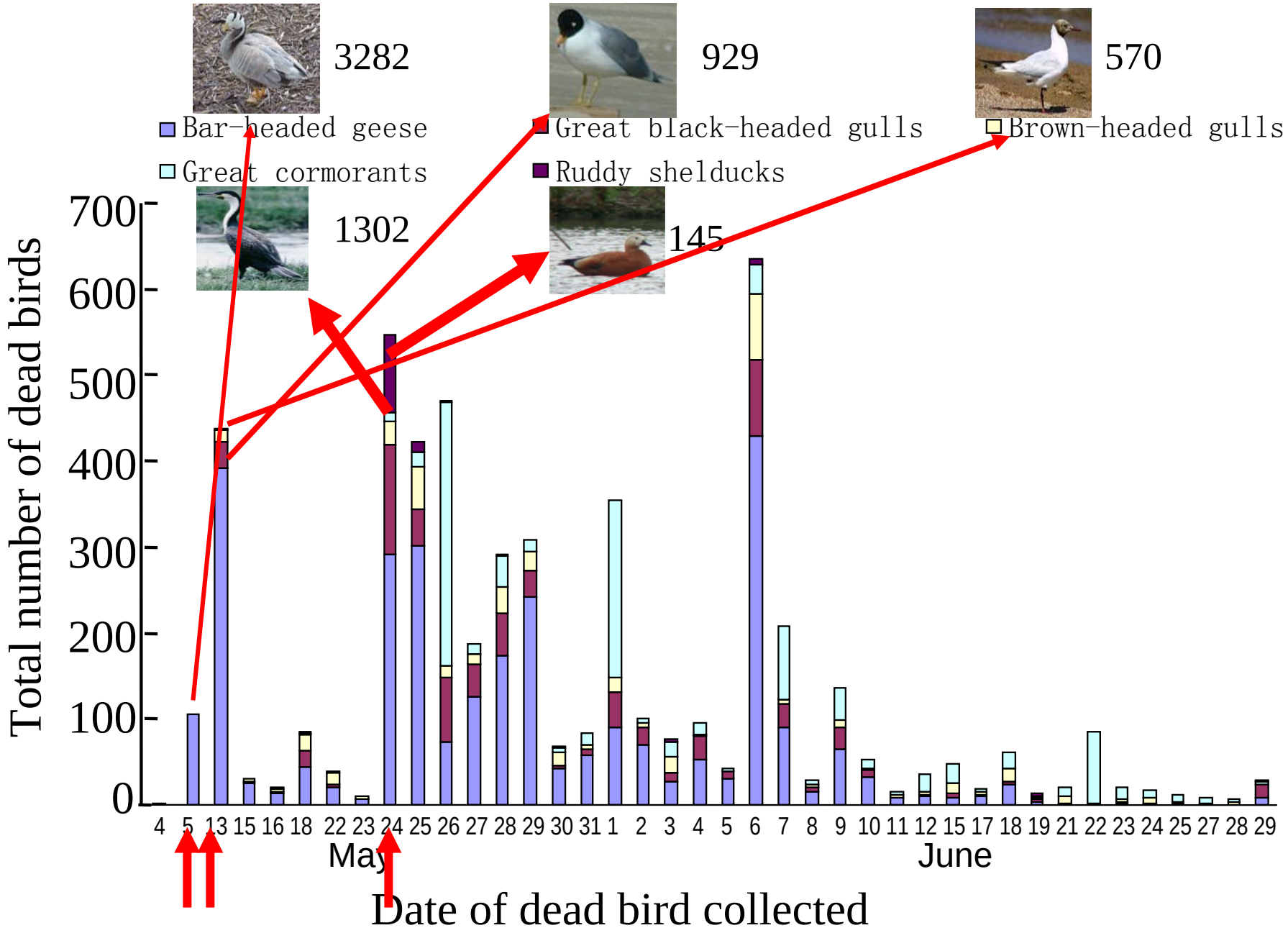
Total number of dead birds

0 100 200 300 400 500 600 700

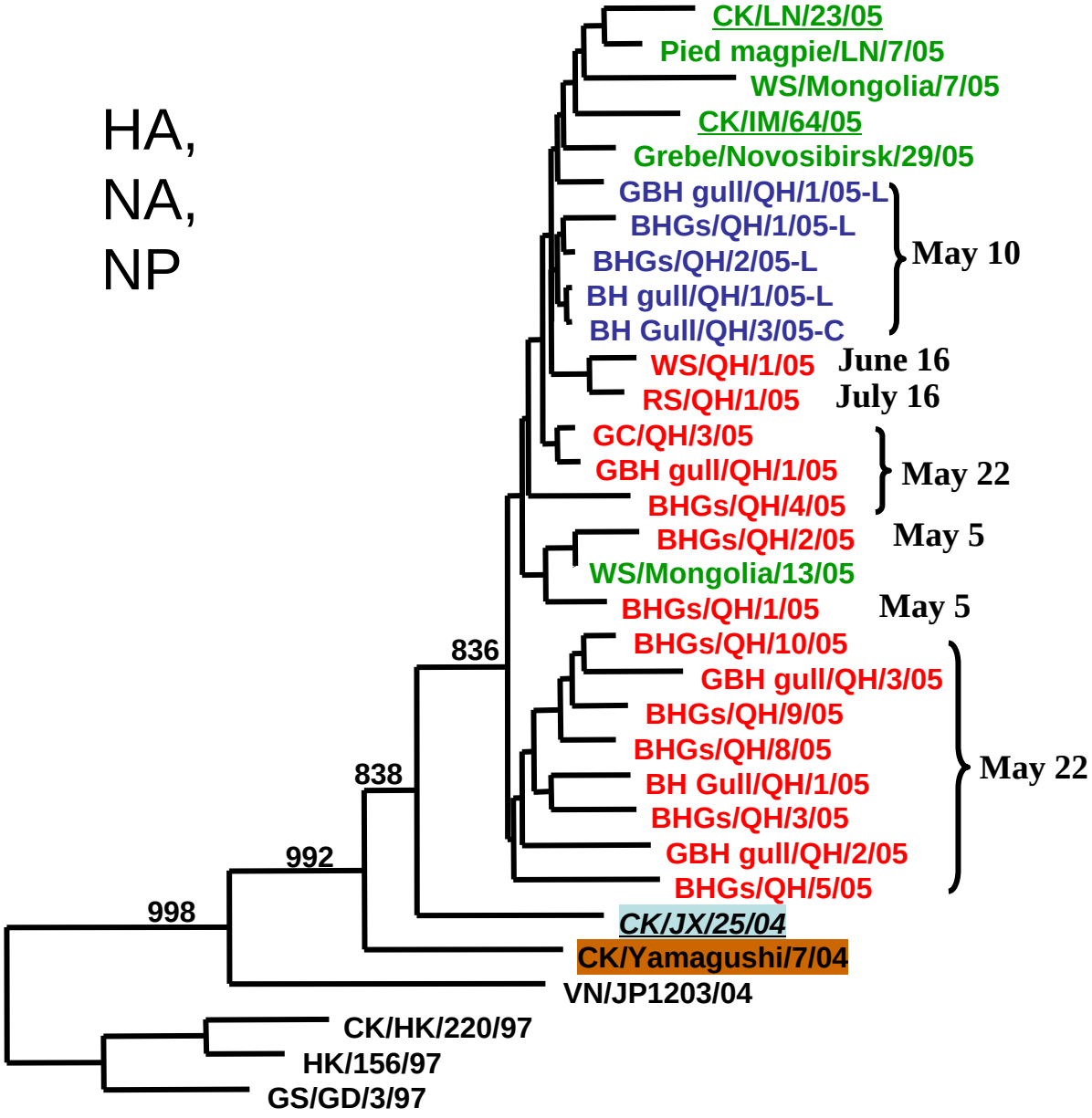
4 5 13 15 16 18 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 15 17 18 19 21 22 23 24 25 27 28 29

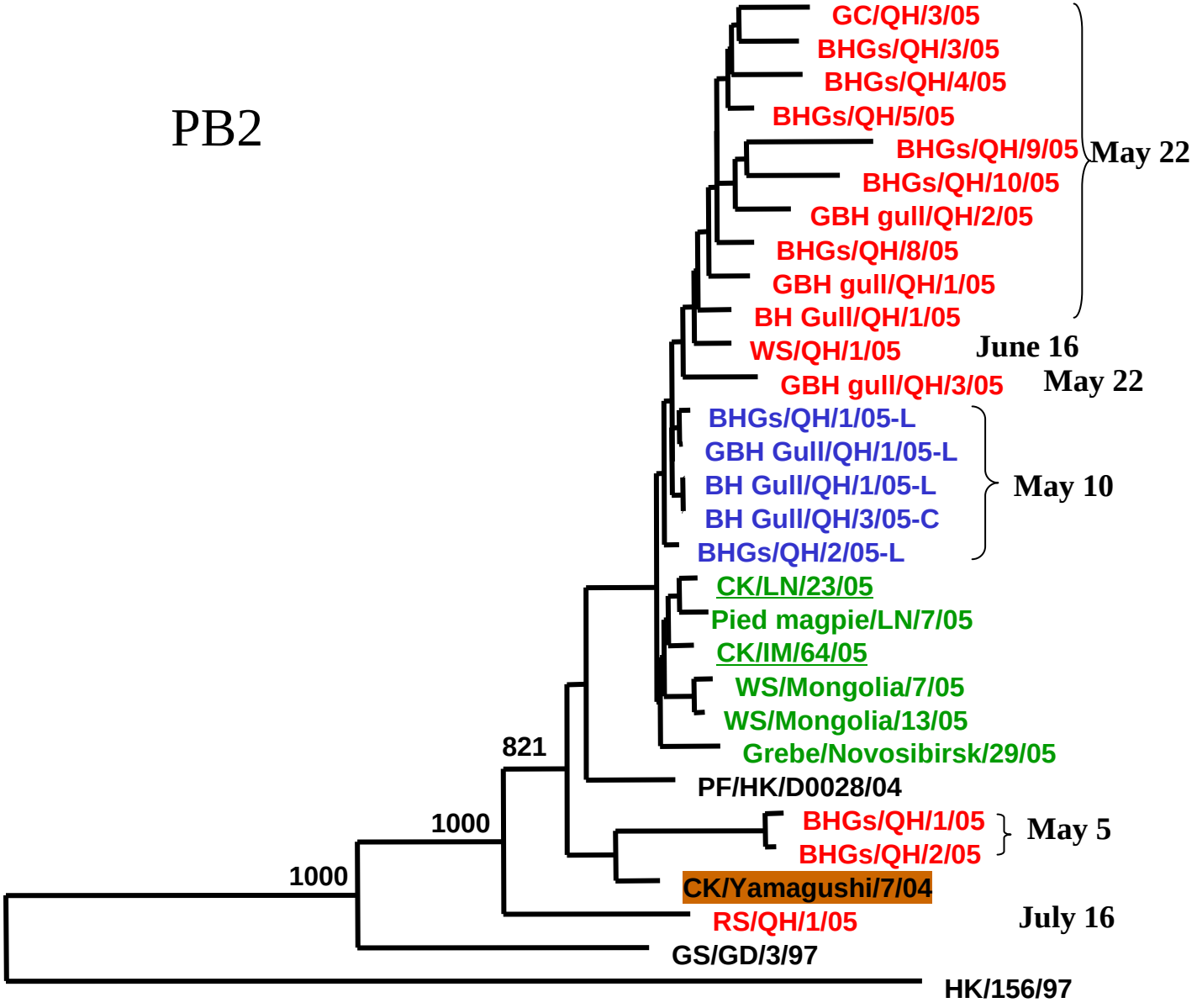
May June

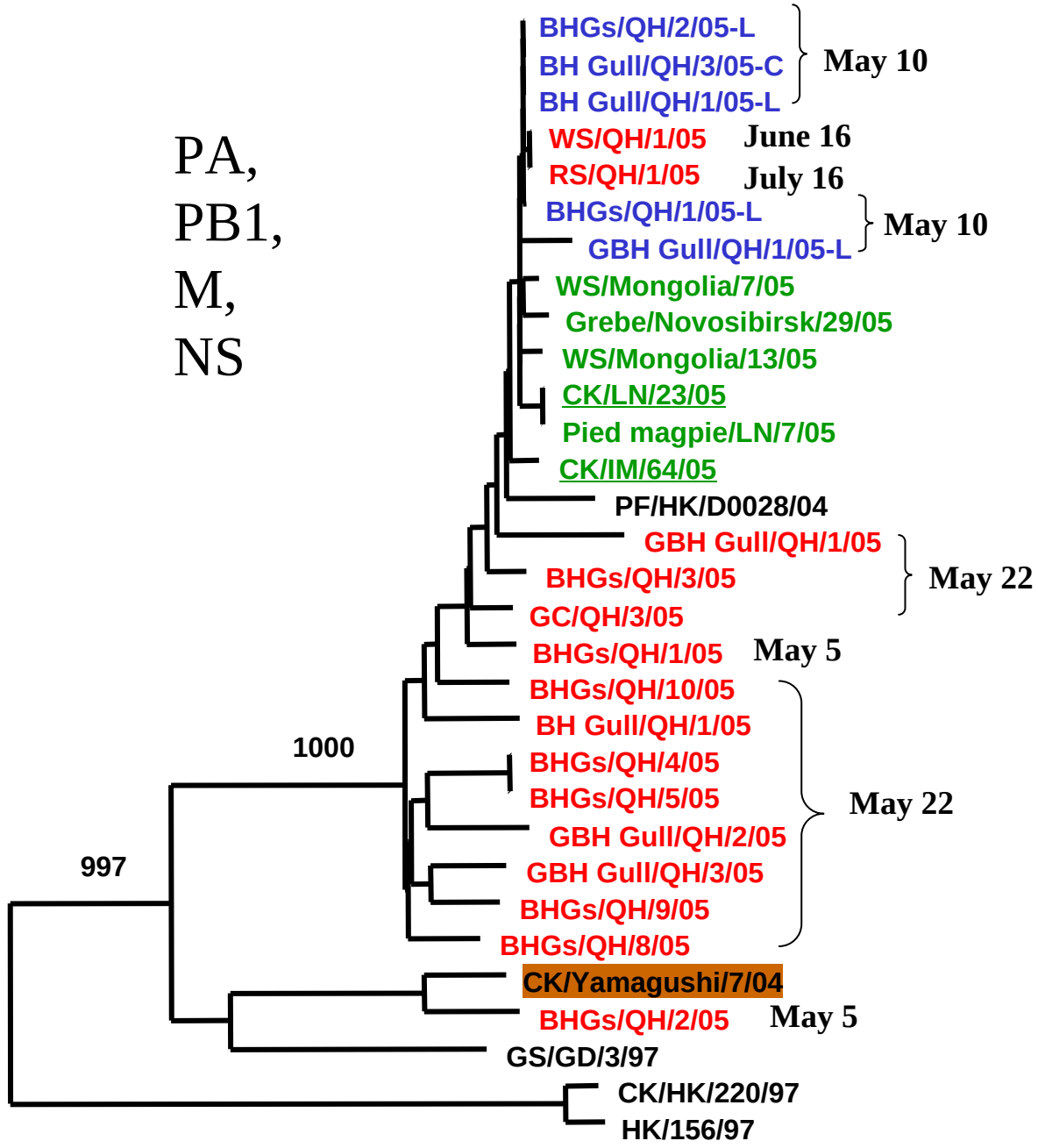
Date of dead bird collected

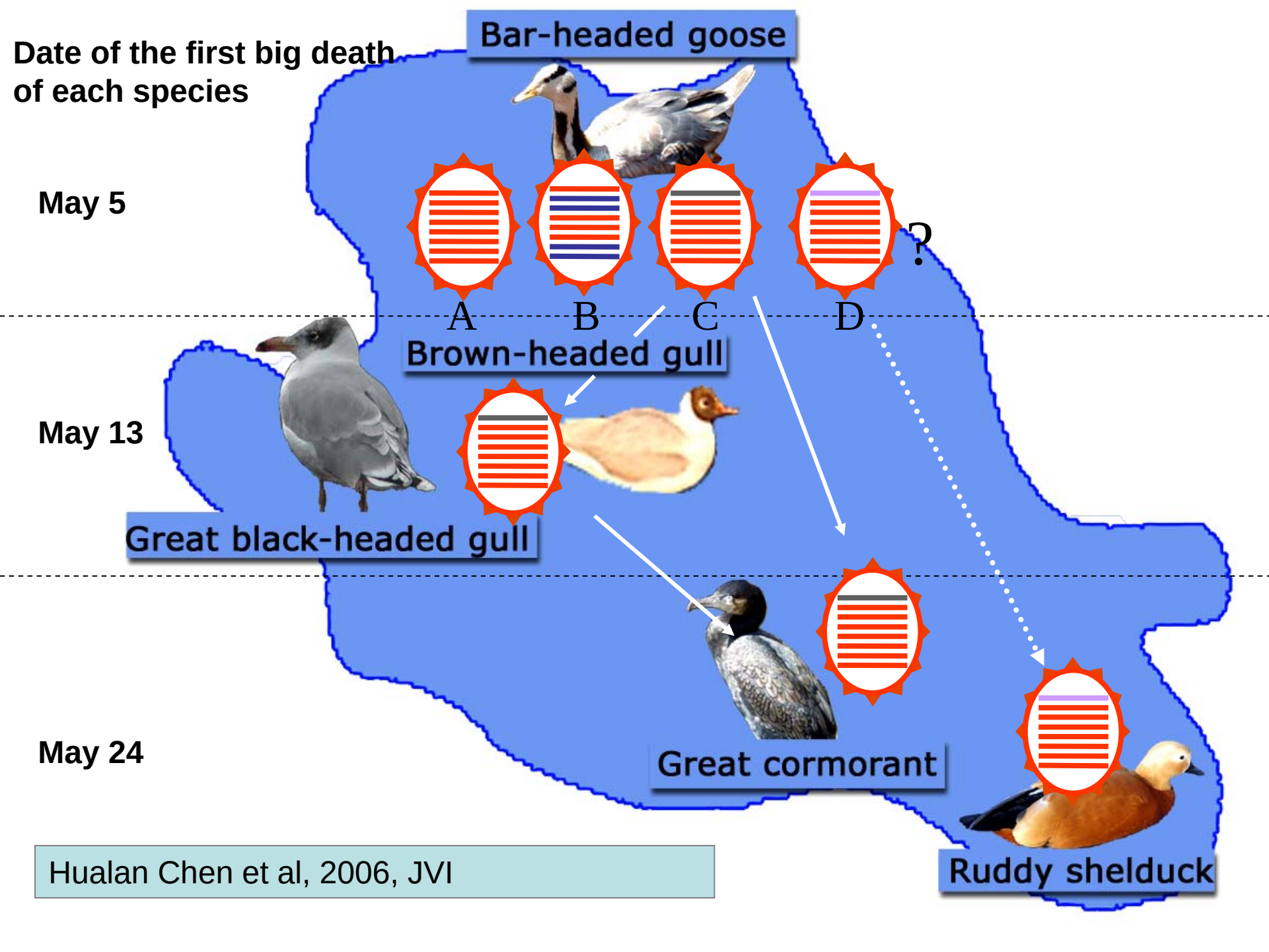

3282
929
570
Bar-headed geese
Great black-headed gulls
Brown-headed gulls
Great cormorants
Ruddy shelducks
1302
145
Total number of dead birds
700
600
500
400
300
200
100
0
4 5 13 15 16 18 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 15 17 18 19 21 22 23 24 25 27 28 29
May
June
Date of dead bird collected

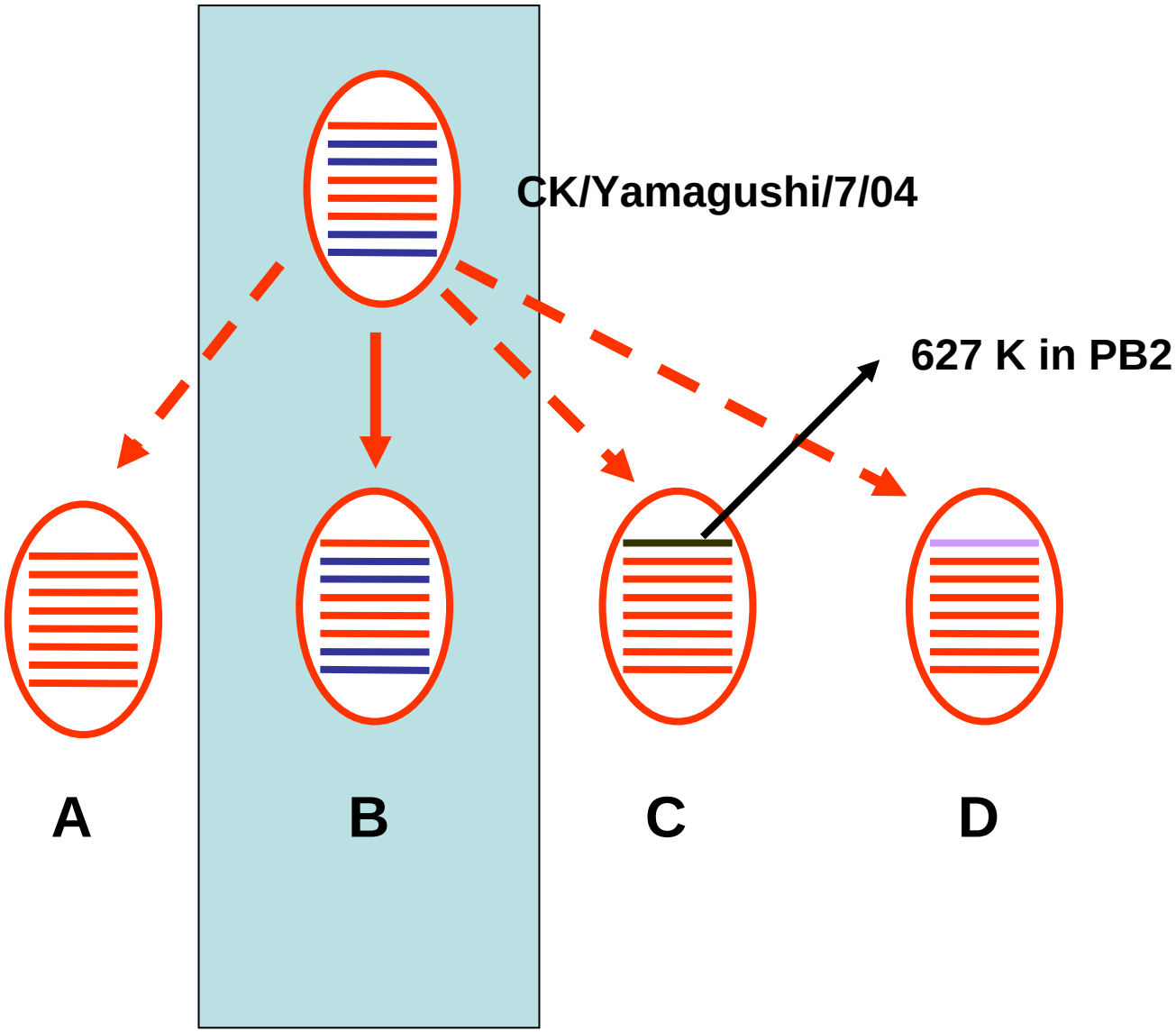
HA,
NA,
NP

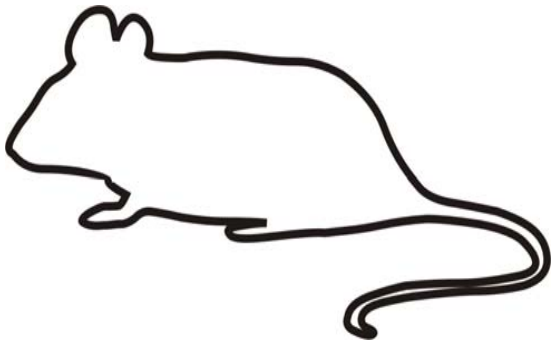

CK/LN/23/05
Pied magpie/LN/7/05
WS/Mongolia/7/05
CK/IM/64/05
Grebe/Novosibirsk/29/05
GBH gull/QH/1/05-L
BHGs/QH/1/05-L
BHGs/QH/2/05-L
BH gull/QH/1/05-L
BH Gull/QH/3/05-C
May 10
WS/QH/1/05
June 16
RS/QH/1/05
July 16
GC/QH/3/05
GBH gull/QH/1/05
BHGs/QH/4/05
May 22
BHGs/QH/2/05
May 5
WS/Mongolia/13/05
BHGs/QH/1/05
May 5
BHGs/QH/10/05
GBH gull/QH/3/05
BHGs/QH/9/05
BHGs/QH/8/05
BH Gull/QH/1/05
BHGs/QH/3/05
GBH gull/QH/2/05
BHGs/QH/5/05
May 22
836
838
992
998
CK/JX/25/04
CK/Yamagushi/7/04
VN/JP1203/04
CK/HK/220/97
HK/156/97
GS/GD/3/97


PB2
GC/QH/3/05
BHGs/QH/3/05
BHGs/QH/4/05
BHGs/QH/5/05
BHGs/QH/9/05
BHGs/QH/10/05
GBH gull/QH/2/05
BHGs/QH/8/05
GBH gull/QH/1/05
BH Gull/QH/1/05
May 22
WS/QH/1/05
June 16
GBH gull/QH/3/05
May 22
BHGs/QH/1/05-L
GBH Gull/QH/1/05-L
BH Gull/QH/1/05-L
BH Gull/QH/3/05-C
BHGs/QH/2/05-L
May 10
CK/LN/23/05
Pied magpie/LN/7/05
CK/IM/64/05
WS/Mongolia/7/05
WS/Mongolia/13/05
Grebe/Novosibirsk/29/05
821
PF/HK/D0028/04
1000
BHGs/QH/1/05
BHGs/QH/2/05
May 5
1000
CK/Yamagushi/7/04
RS/QH/1/05
July 16
GS/GD/3/97
HK/156/97

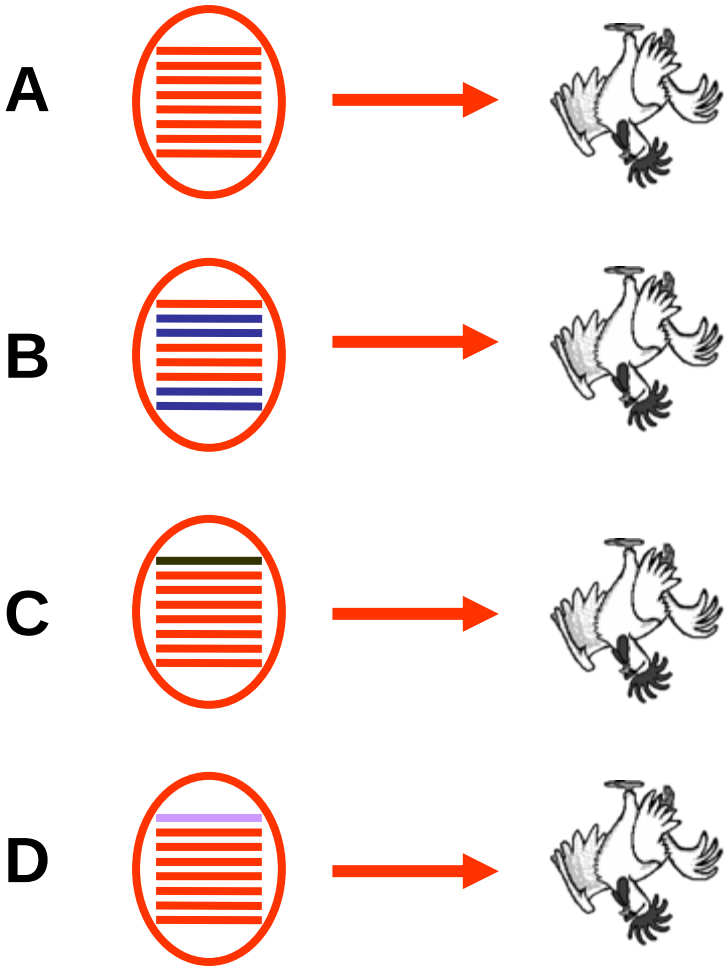

PA,
PB1,
M,
NS
BHGs/QH/2/05-L
BH Gull/QH/3/05-C
BH Gull/QH/1/05-L
May 10
WS/QH/1/05
June 16
RS/QH/1/05
July 16
BHGs/QH/1/05-L
GBH Gull/QH/1/05-L
May 10
WS/Mongolia/7/05
Grebe/Novosibirsk/29/05
WS/Mongolia/13/05
CK/LN/23/05
Pied magpie/LN/7/05
CK/IM/64/05
PF/HK/D0028/04
GBH Gull/QH/1/05
BHGs/QH/3/05
May 22
GC/QH/3/05
BHGs/QH/1/05
May 5
BHGs/QH/10/05
BH Gull/QH/1/05
1000
BHGs/QH/4/05
BHGs/QH/5/05
GBH Gull/QH/2/05
May 22
997
GBH Gull/QH/3/05
BHGs/QH/9/05
BHGs/QH/8/05
CK/Yamagushi/7/04
BHGs/QH/2/05
May 5
GS/GD/3/97
CK/HK/220/97
HK/156/97

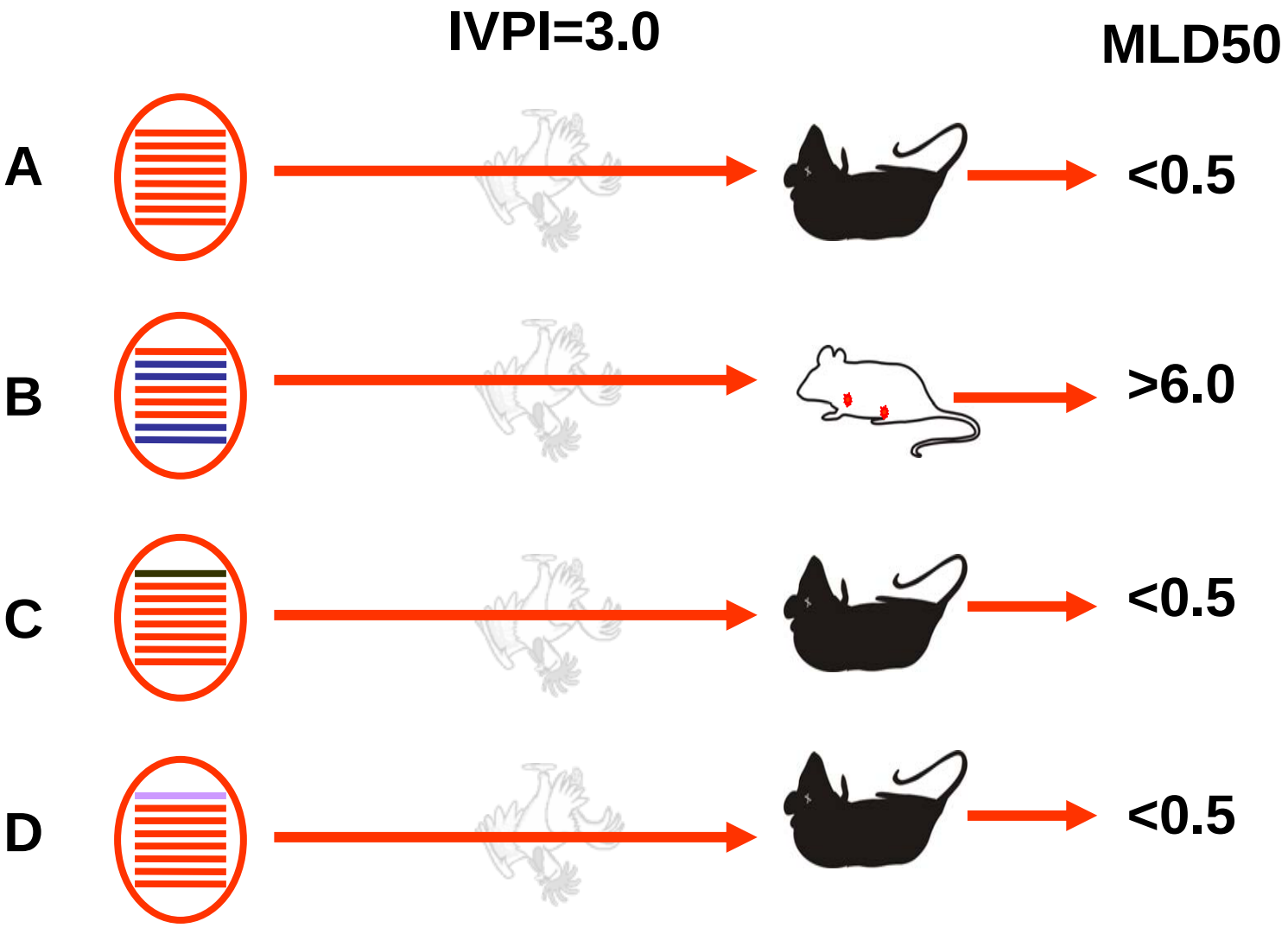

Date of the first big death of each species
Bar-headed goose
May 5
A
B
C
D
?
Brown-headed gull
May 13
Great black-headed gull
May 24
Great cormorant
Ruddy shelduck
Hualan Chen et al, 2006, JVI

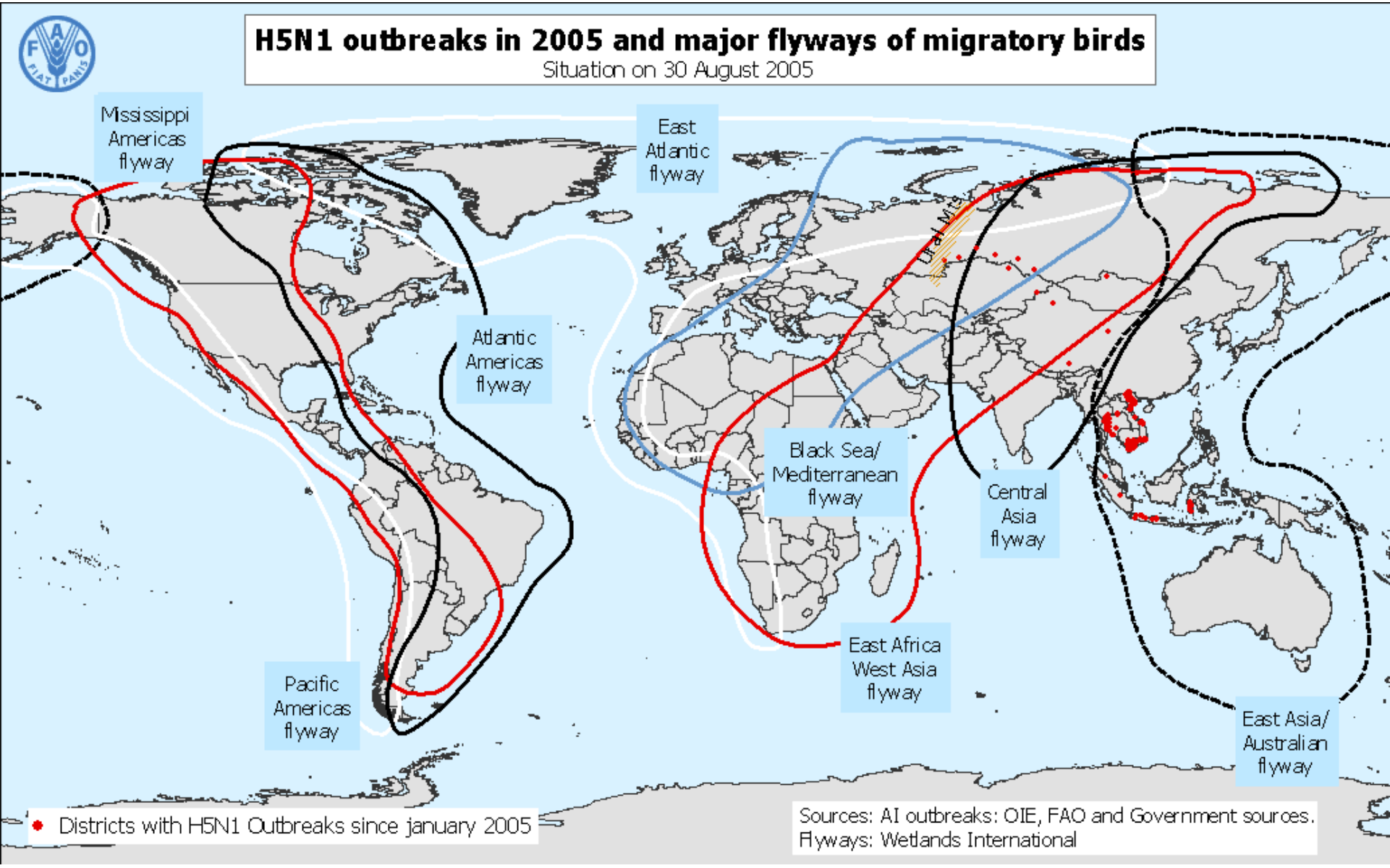

CK/Yamagushi/7/04
627 K in PB2
A
B
C
D



IVPI=3.0




IVPI=3.0
MLD50
A
<0.5
B
>6.0
C
<0.5
D
<0.5


H5N1 outbreaks in 2005 and major flyways of migratory birds
Situation on 30 August 2005
Mississippi Americas flyway
East Atlantic flyway
Ural Mts
Atlantic Americas flyway
Black Sea/ Mediterranean flyway
Central Asia flyway
East Africa West Asia flyway
Pacific Americas flyway
East Asia/ Australian flyway
Districts with H5N1 Outbreaks since january 2005
Sources: AI outbreaks: OIE, FAO and Government sources.
Flyways: Wetlands International


黑龙江
古林
辽宁
新疆维吾尔自治区
内蒙古自治区
甘肃
青海
宁夏回族自治区
陕西
山西
河北
北京
天津
山东
河南
安徽
江苏
上海
湖北
浙江
湖南
江西
福建
台湾
西藏自治区
四川
重庆
贵州
云南
广西壮族自治区
广东
澳门
海南


H5N1 outbreak in wild birds in 2006
新疆维吾尔自治区
内蒙古自治区
黑龙江
吉林
辽宁
河北
北京
天津
山西
陕西
甘肃
青海
宁夏回族自治区
西藏自治区
四川
重庆
云南
贵州
广西壮族自治区
湖北
湖南
河南
山东
安徽
江苏
上海
浙江
江西
福建
台湾
广东
澳门
海南


Antigenic variants were detected in northern China in 2006
H5N1 outbreak in wild birds in 2006
新疆维吾尔自治区
内蒙古
甘肃
青海
西藏自治区
宁夏回族自治区
陕西
山西
河北
北京
天津
辽宁
吉林
黑龙江
河南
山东
安徽
江苏
上海
湖北
浙江
四川
重庆
湖南
江西
福建
台湾
贵州
云南
广西壮族自治区
广东
澳门
海南

Vaccines

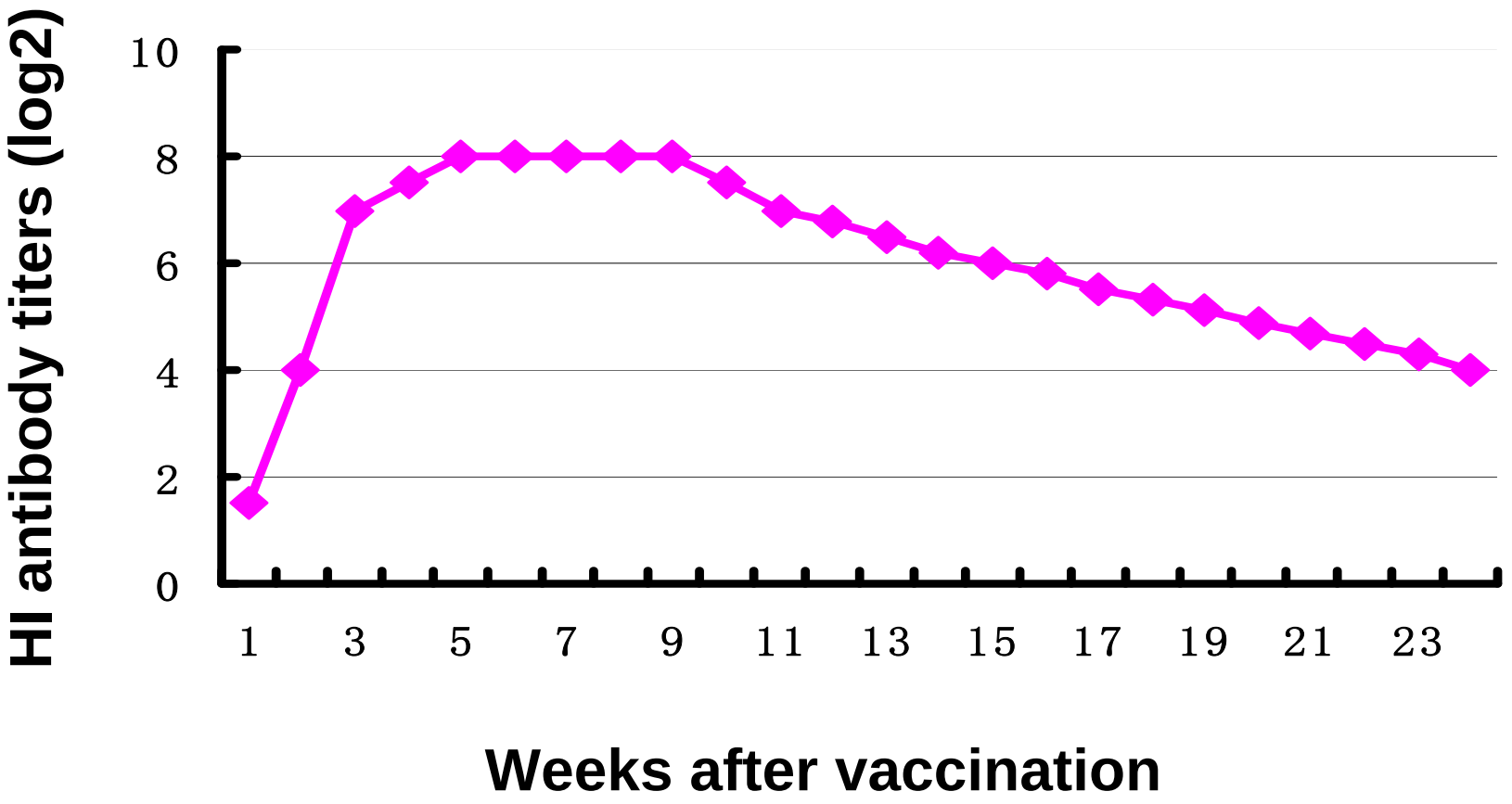
- Inactivated vaccines

  H5N2: laboratory adapted natural isolated low pathogenic H5N2 virus

  H5N1: artificial generated high growth H5N1/PR8 reassortant virus

- Live virus vectored vaccine

H5N2 vaccines is immunogenic for chickens



An ideal vaccine strain should

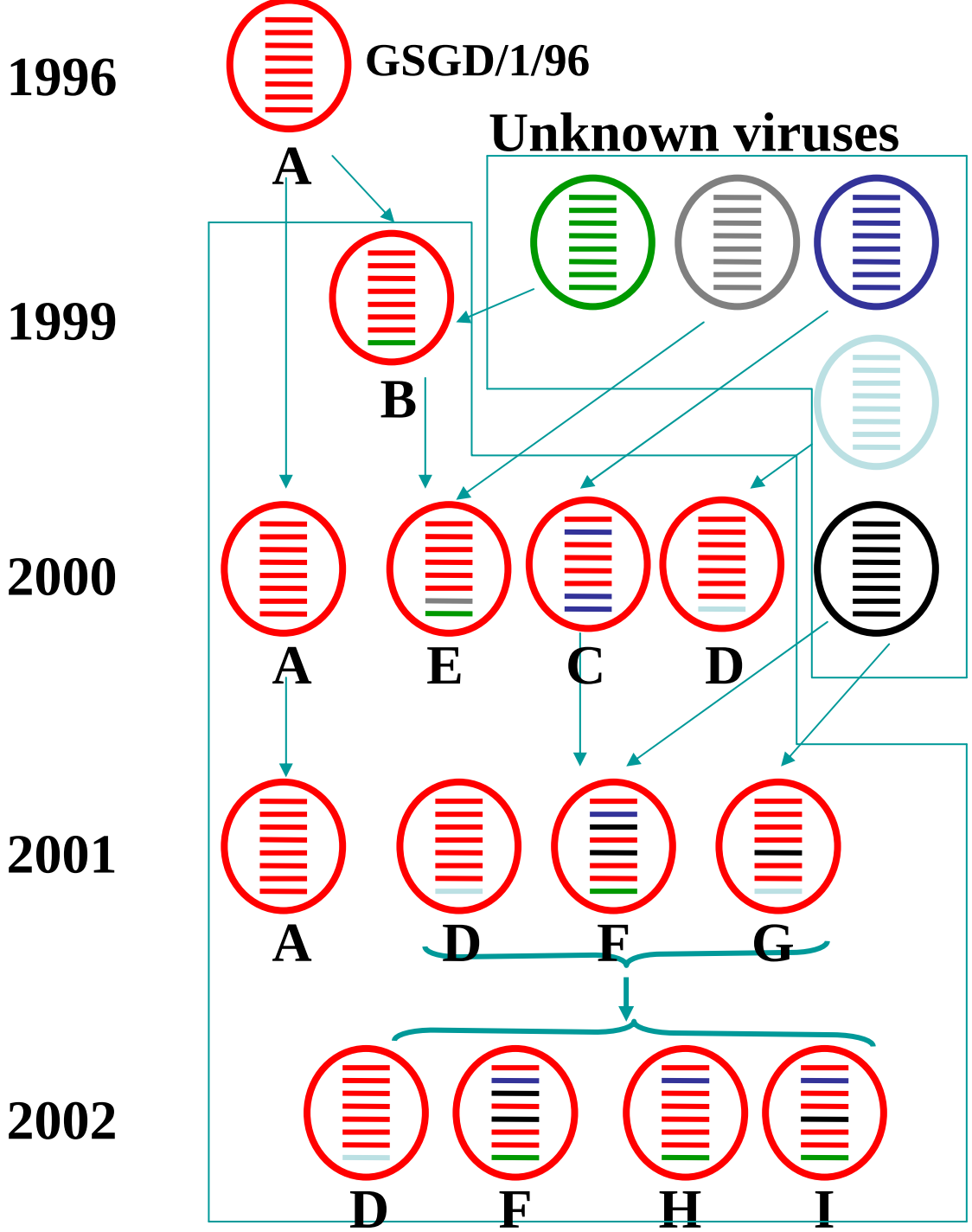
- Be a low pathogenic strain, safe to the environment

An ideal vaccine strain should

- Be a low pathogenic strain, safe to the environment
- Be able to grow well in eggs, to ensure enough antigen in the vaccine product

An ideal vaccine strain should

- Be a low pathogenic strain, safe to the environment
- Be able to grow well in eggs, to ensure enough antigen in the vaccine product
- Be well matched antigenically with the prevalence viruses



Chen H. et al, PNAS, 2004

(101): 10452-10457

Molecular modification of the HA gene of GD/96 virus

GD/96 wildtype HA:

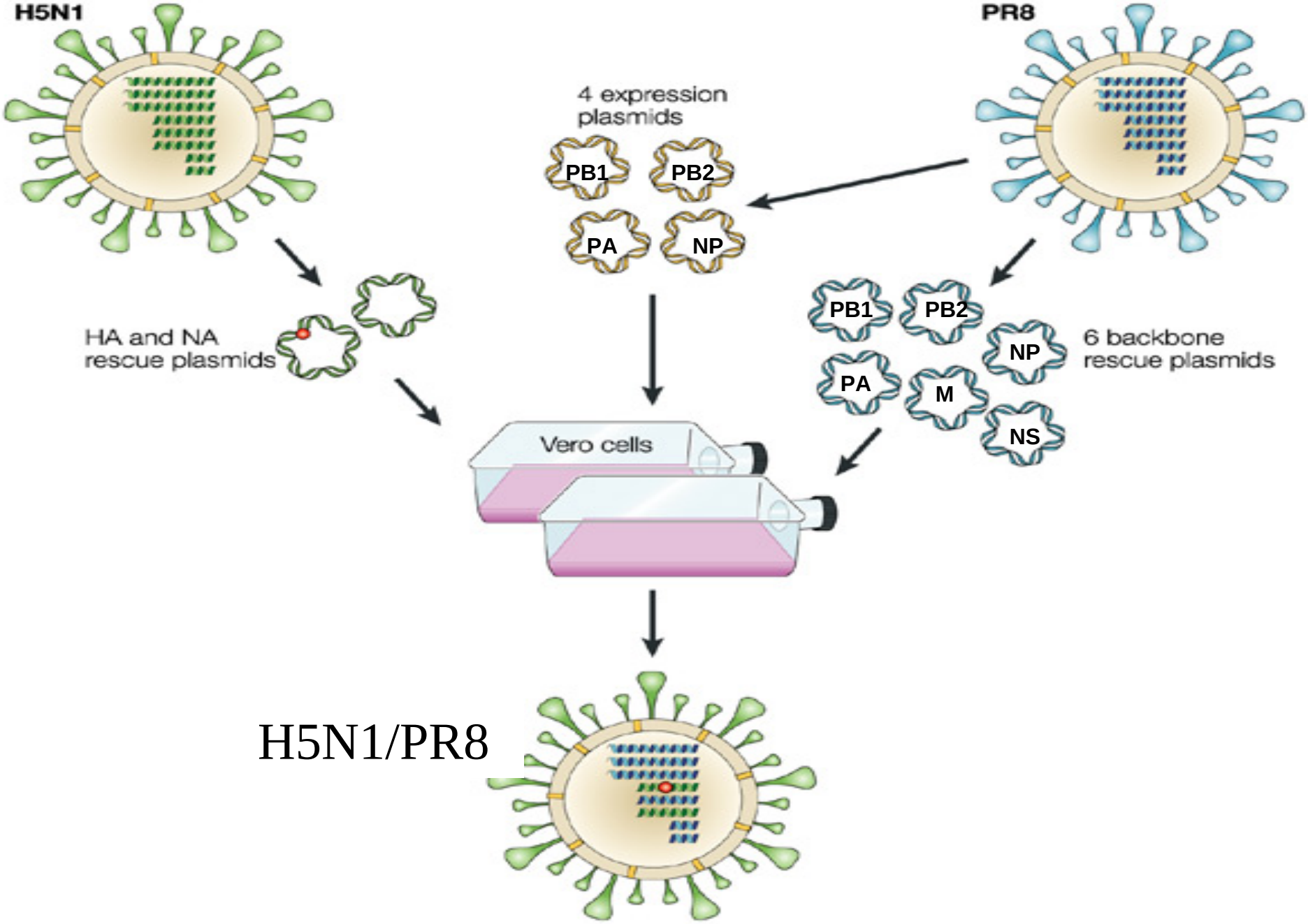
```
CCT CAA AGA GAG AGA AGA AGA AAA AAG AGA ↓ GGA CTA TTT
Pro Glu Arg Glu Arg Arg Arg Lys Lys Arg   Gly Leu Phe
        R   E   R   R   R   K   K   R
```

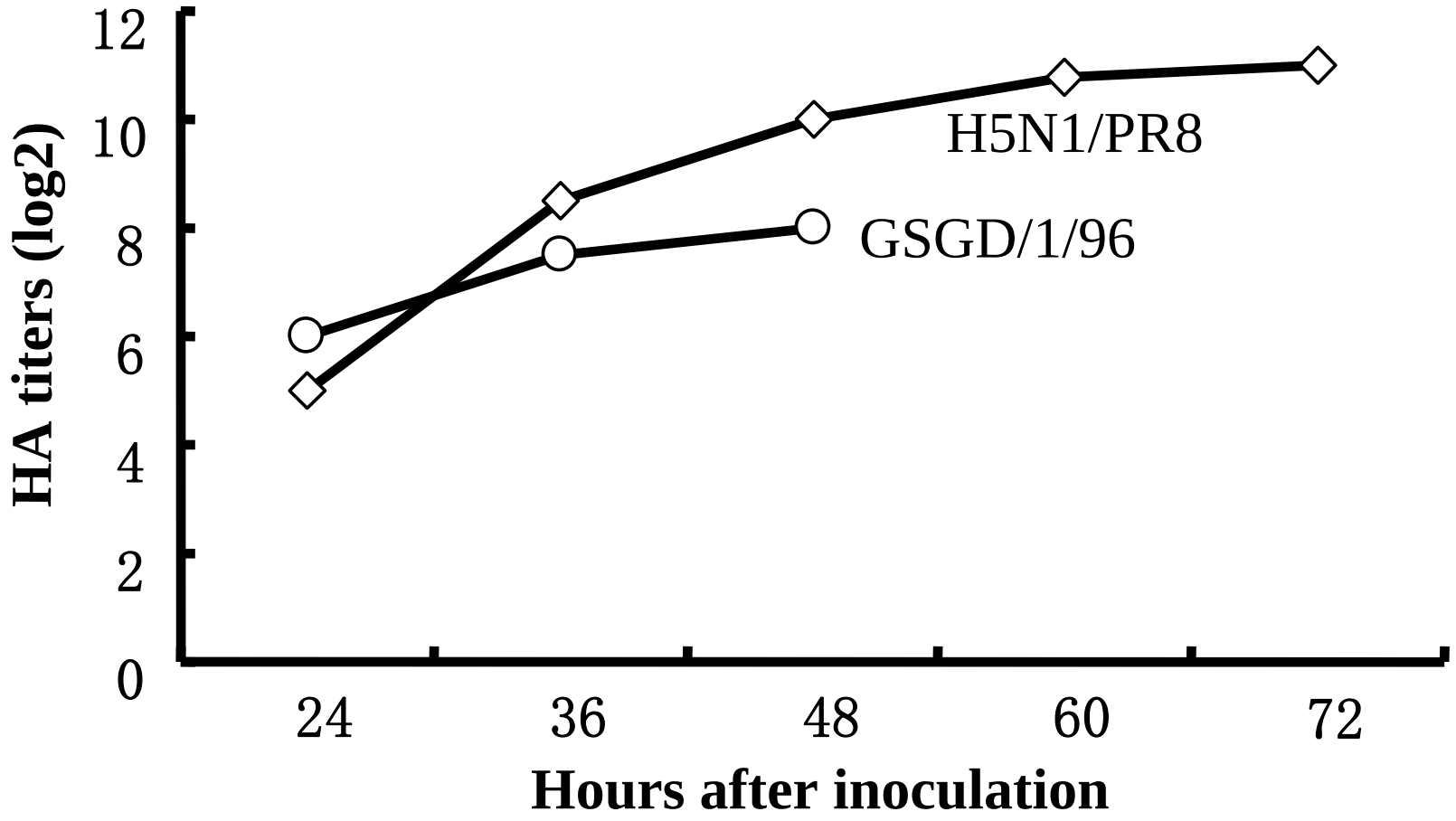
Modified HA:

```
CCT CAA CGA GAG ACG                     CGA ↓ GGA CTA TTT
Pro Glu Arg Glu Thr                     Arg   Gly Leu Phe
        R   E   T                        R
```

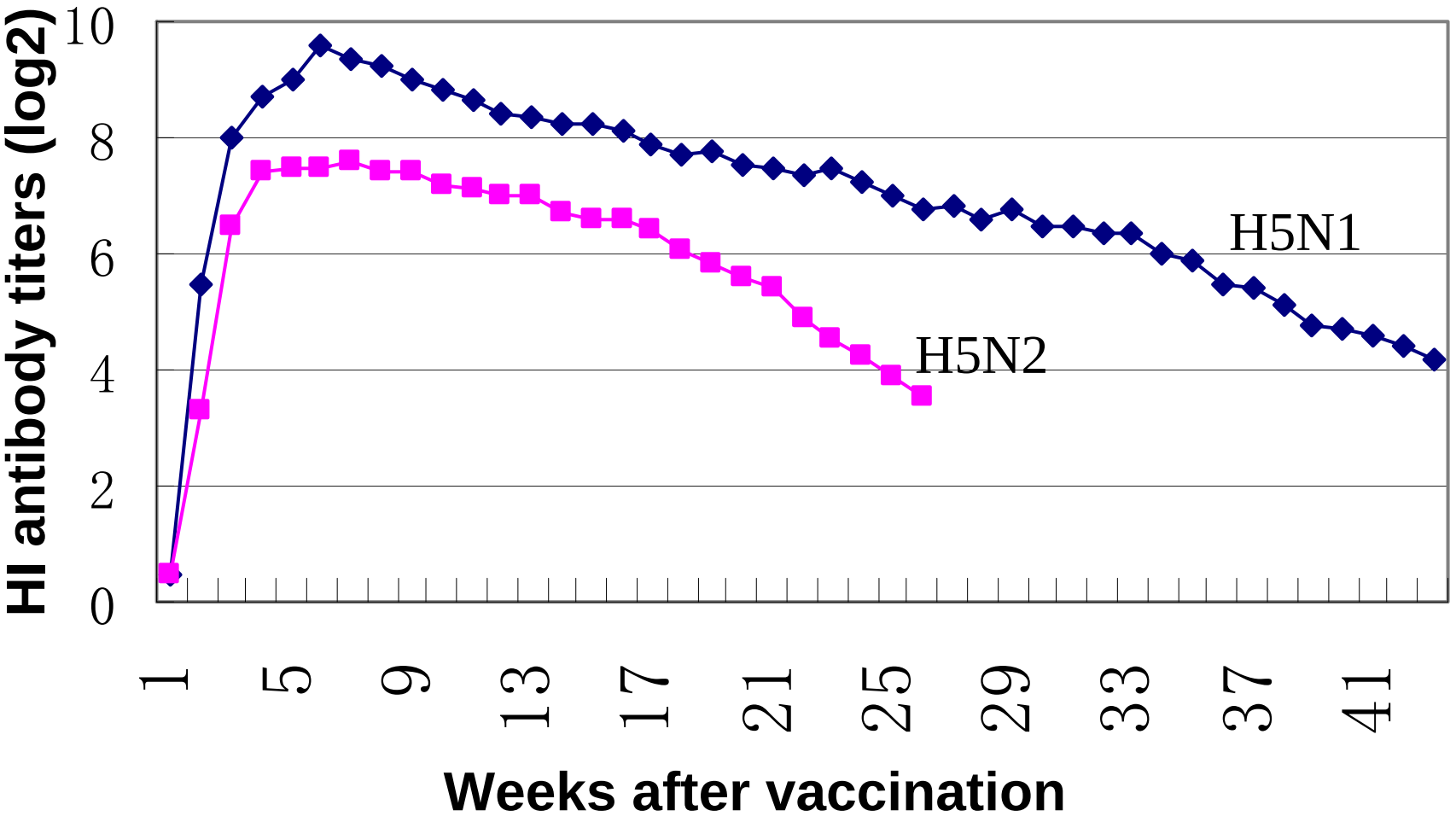
↓ Site of cleavage of into HA1 and HA2 domains.

Residues that were mutagenized are underlined.

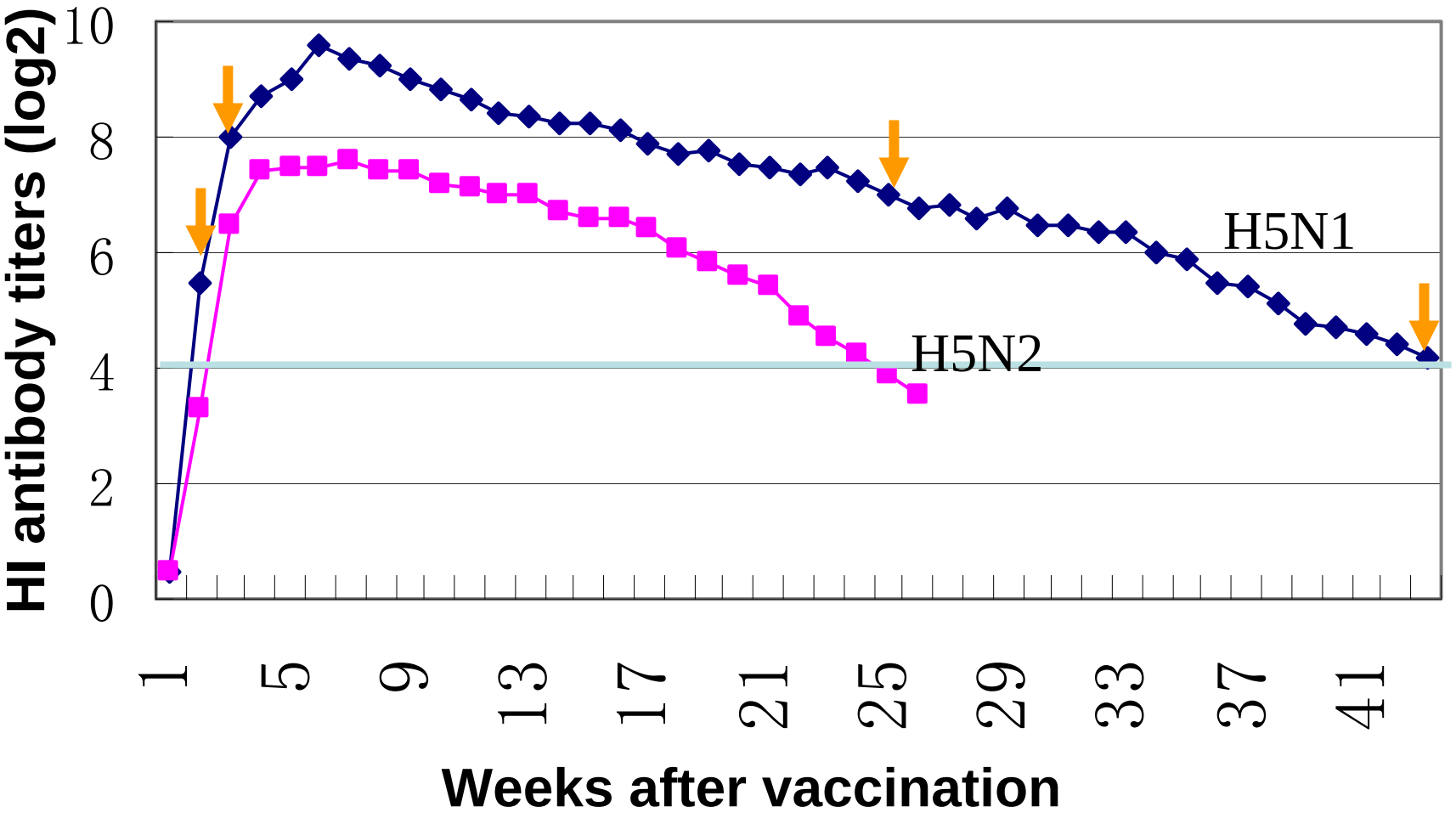

H5N1
PR8
4 expression plasmids
PB1
PB2
PA
NP
HA and NA rescue plasmids
PB1
PB2
NP
PA
M
NS
6 backbone rescue plasmids
Vero cells
H5N1/PR8


12
10
8
6
4
2
0
HA titers (log2)
H5N1/PR8
GSGD/1/96
24
36
48
60
72
Hours after inoculation

HI antibody duration induced by H5 inactivated vaccines



One dose inoculation in SPF chickens could provide 43 weeks protection



Does this vaccine work for waterfowls?

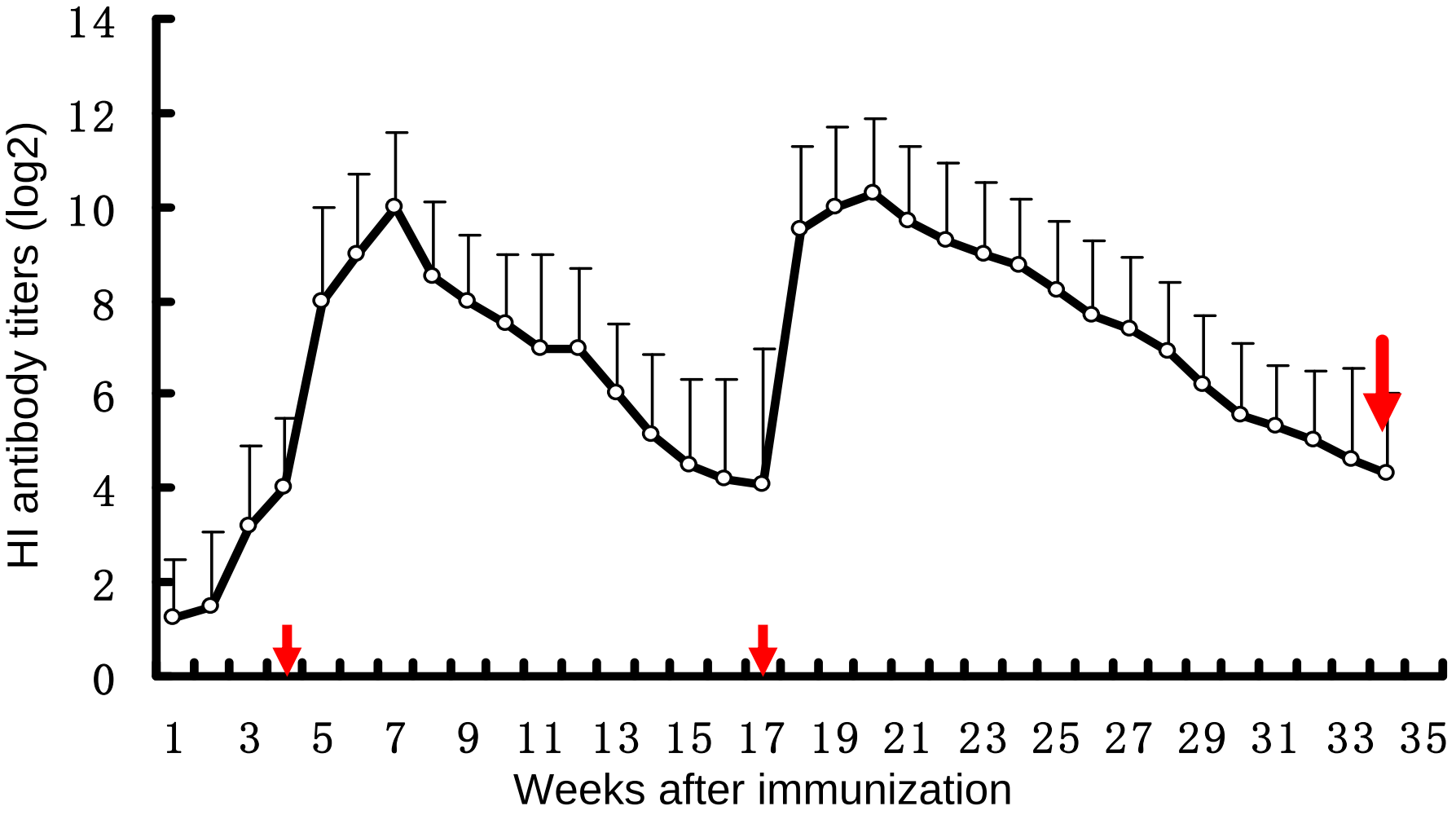
Vaccine efficacy in geese

Groups	Challenged time	Virus isolation at different days after challenge						Survival /total
		Day 3		Day 5		Day 7		
		Oroph.	Cloacal	Oroph.	Cloacal	Oroph.	Cloacal	
Vaccinated	2	1/5	0/5	4/5	3/5	1/3	2/3	3/5
Control	2	3/5	0/5	5/5	3/5	/	/	0/5
Vaccinated	3	0/5	0/5	0/5	0/5	0/5	0/5	5/5
Control	3	0/5	1/5	5/5	3/5	2/2	2/2	0/5

Vaccine efficacy in Ducks

Groups	Challenge time (wpi)	Virus isolation at different days after challenge						Survival/ total
		Day 3		Day 5		Day 7		
		Oroph.	Cloacal	Oroph.l	Cloacal	Oroph.	Cloacal	
Vaccinated	3	0/30	0/30	0/30	0/30	0/30	0/30	30/30
Control	3	13/15	8/15	7/15	5/15	0/2	0/2	2/15

HI antibody duration in geese-field test

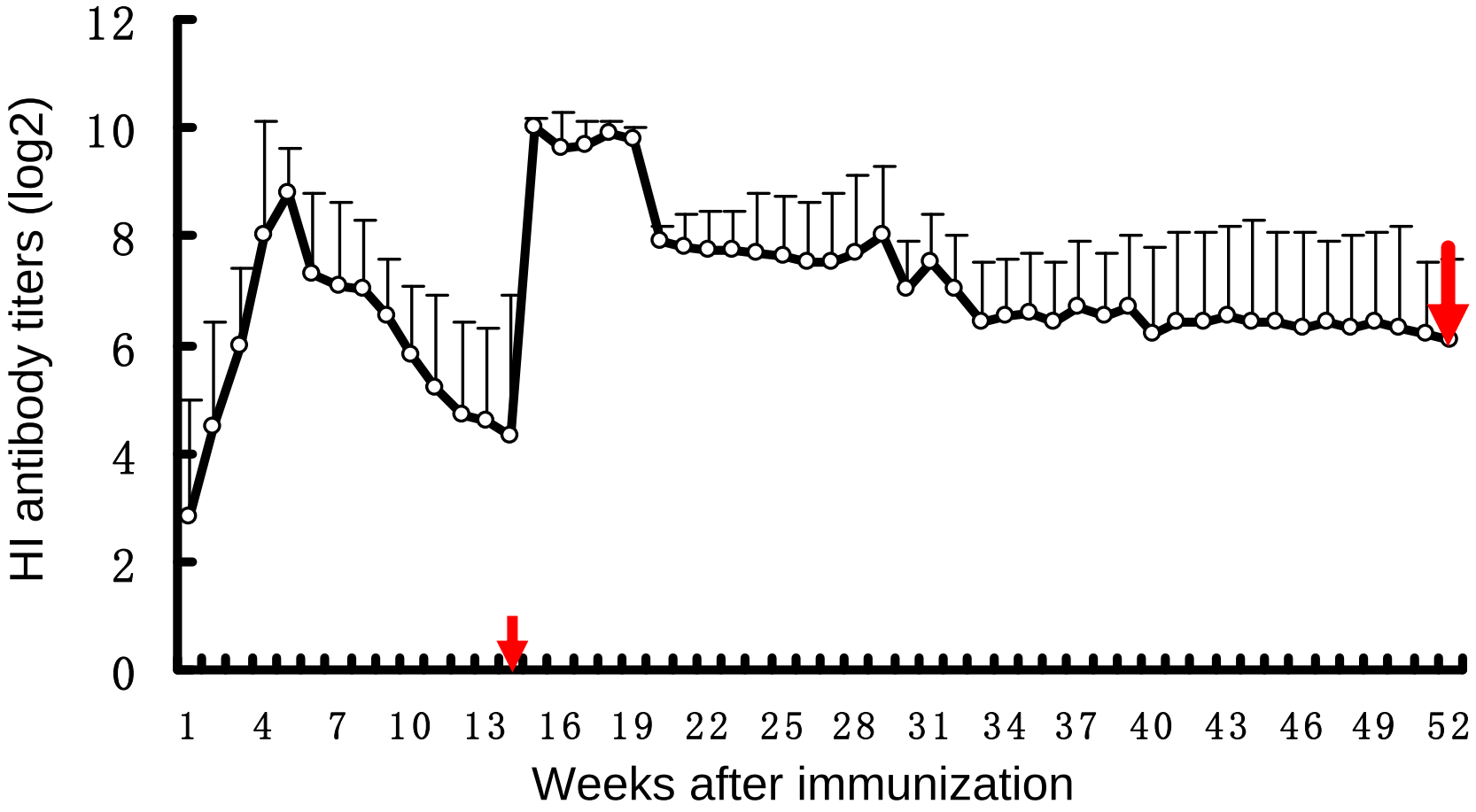


Guobin Tian, et al. Virology, 2005, 341: 153 – 162

Vaccinated field geese could be completely protected

Groups	Virus isolation at different days after challenge						Survival/ total
	Day 3		Day 5		Day 7		
	Oroph.	Cloacal	Oroph.l	Cloacal	Oroph.	Cloacal	
Vaccinated[a]	0/10	0/10	0/10	0/10	0/10	0/10	10/10
Control[b]	9/10	4/10	9/9	3/9	/	/	0/10

HI antibody duration in ducks-field test



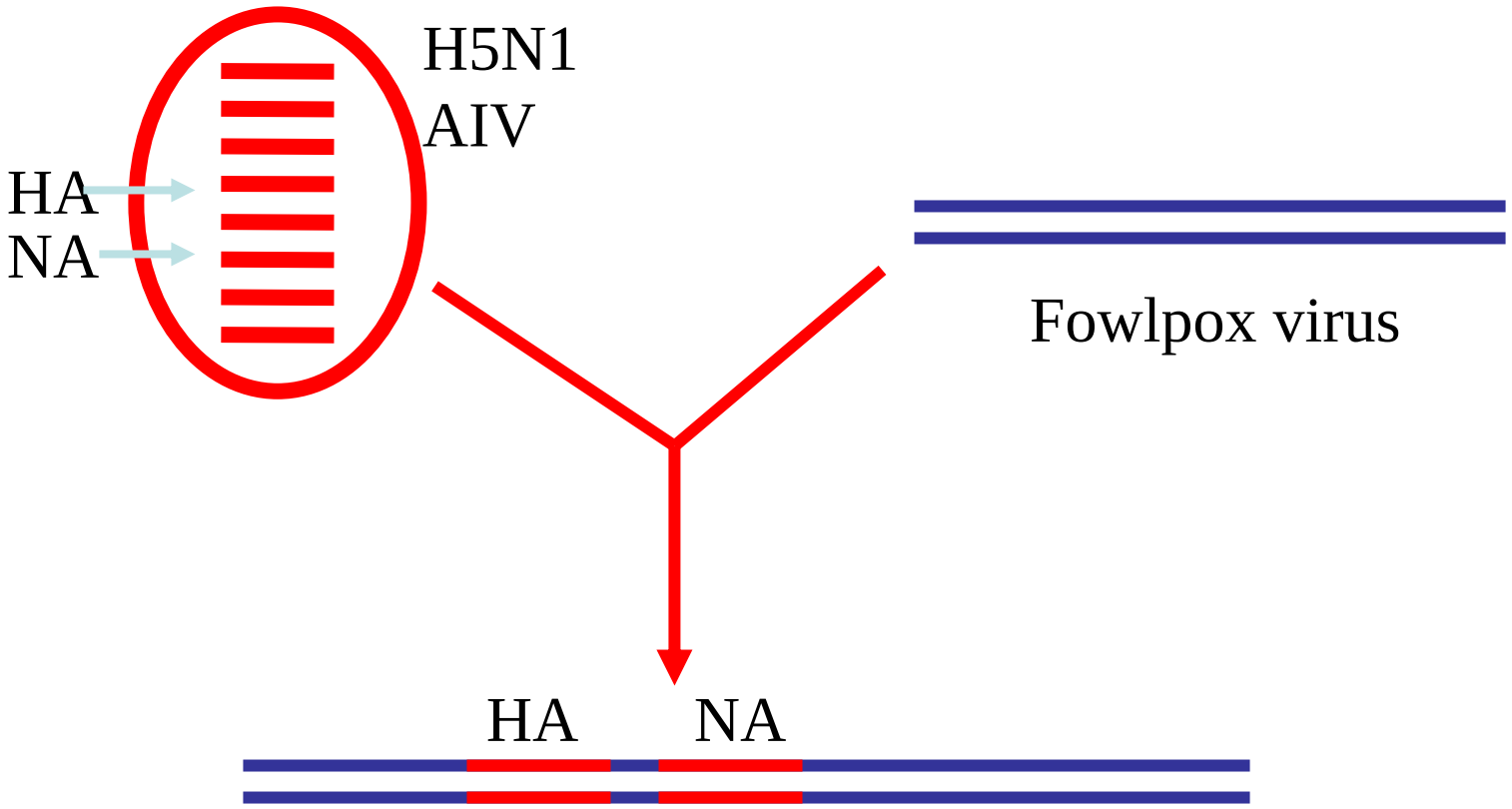
Guobin Tian, et al. Virology, 2005, 341: 153 – 162

Vaccinated field ducks could be completely protected

Groups	Virus isolation at different days after challenge						Survival/ total
	Day 3		Day 5		Day 7		
	Oroph.	Cloacal	Oroph.l	Cloacal	Oroph.	Cloacal	
Vaccinated[a]	2/10[c]	0/10	0/10	0/10	0/10	0/10	10/10
Control[b]	10/10	3/10	5/9	1/9	0/9	0/9	9/10

Live virus vectored vaccines

- Recombinant fowlpox vaccine
- Recombinant Newcastle disease vaccine



Recombinant H5N1 fowlpox virus

A recombinant fowlpox virus vectored vaccine is very well immunogenic in chickens

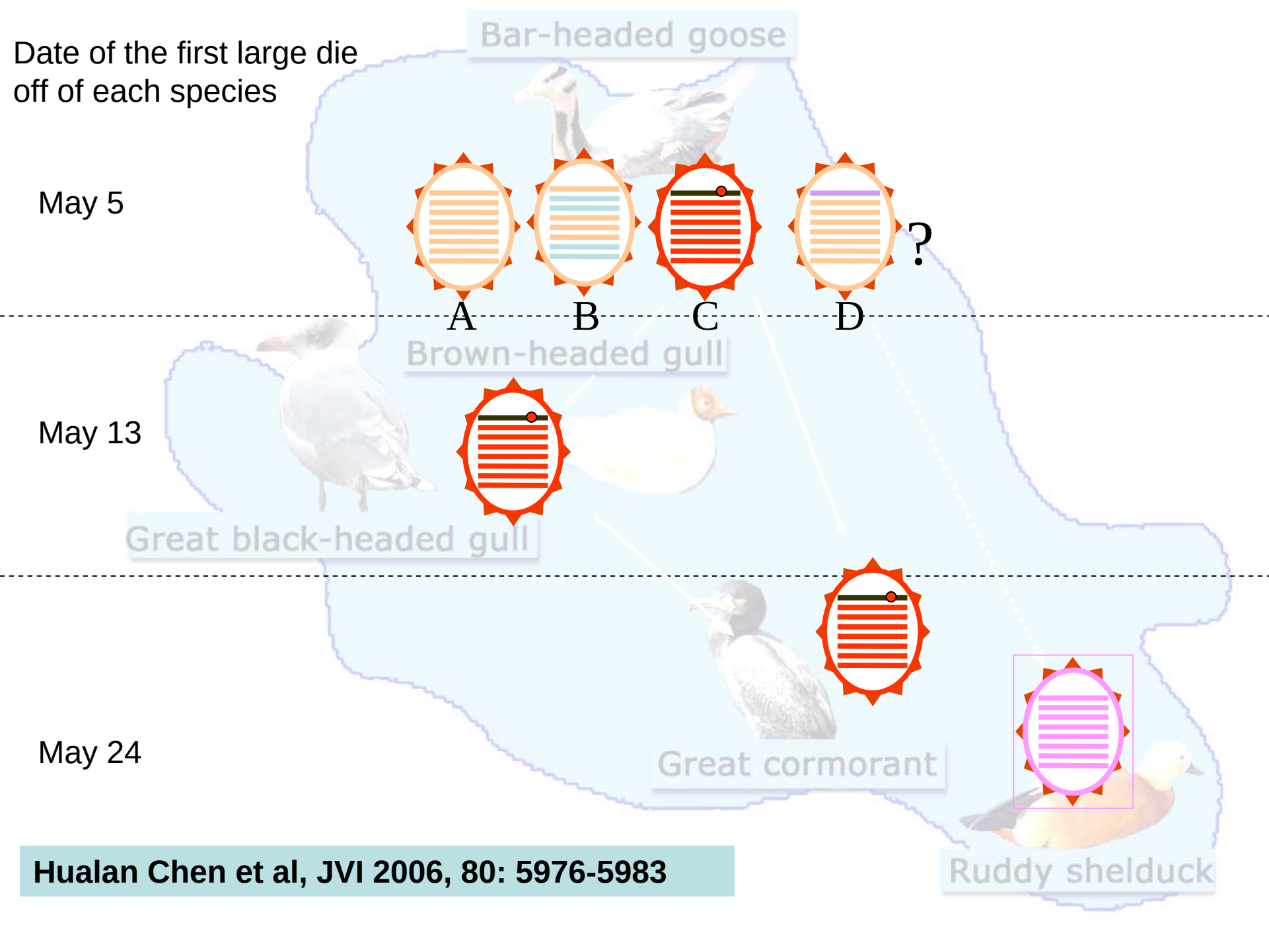
Weeks post vaccination	HI antibody (log2)	AGP antibody	Protection	
			Disease	Death
1	3.58	—	0/10	0/10
2	6.82	—	0/10	0/10
3	7.02	—	0/10	0/10
4	6.56	—	0/10	0/10
5-10	6.52	—	0/10	0/10
11-20	5.89	—	0/10	0/10
21-30	4.80	—	0/10	0/10
31-40	3.68	—	0/10	0/10
41-45	2.96	—	0/10	0/10

Newcastle disease virus

- Holding great promise as a vector for human emerging infectious disease vaccine development
- Very suitable to be a vector for H5 avian influenza vaccine development

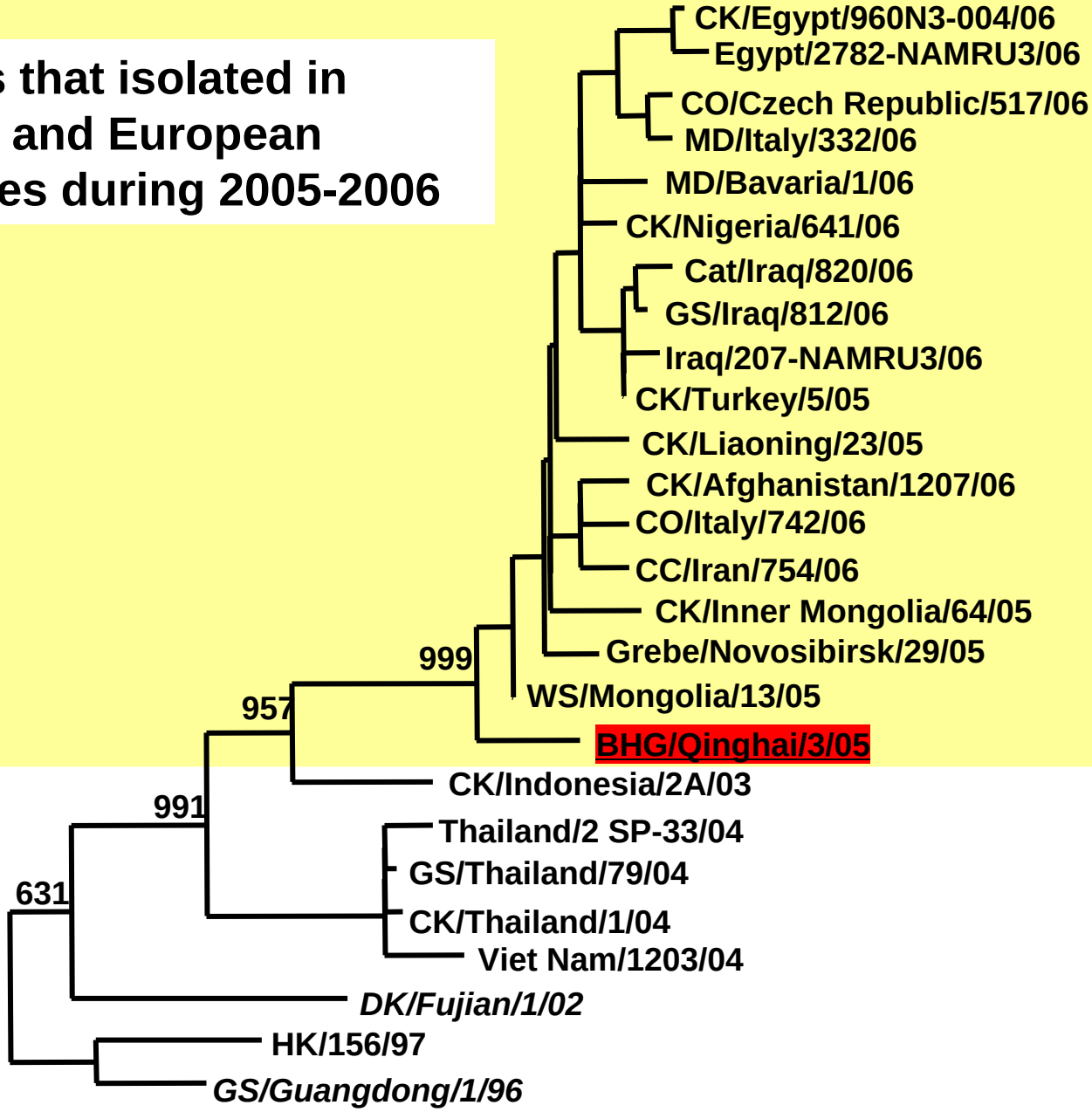
➢ **Will prevent two list A poultry diseases**

Date of the first large die off of each species

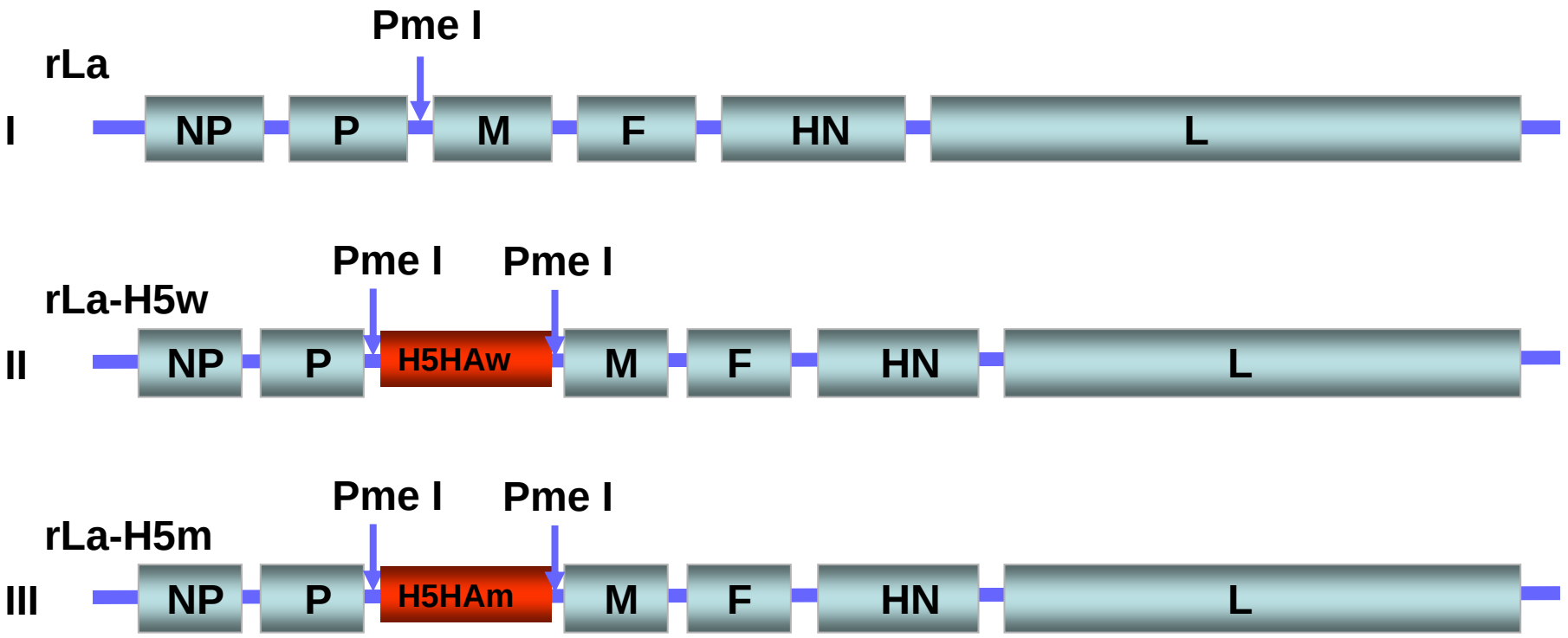


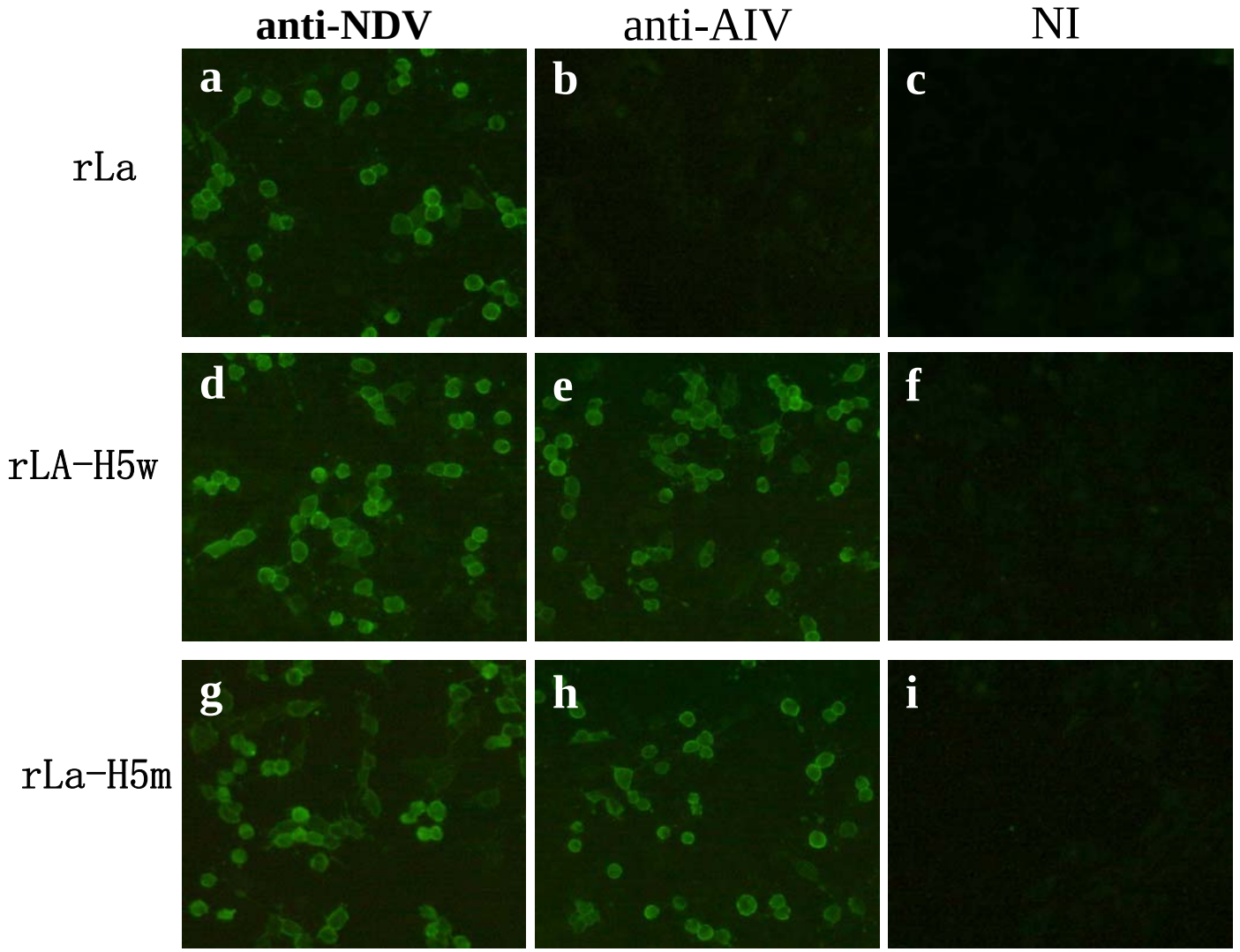
Hualan Chen et al, JVI 2006, 80: 5976-5983

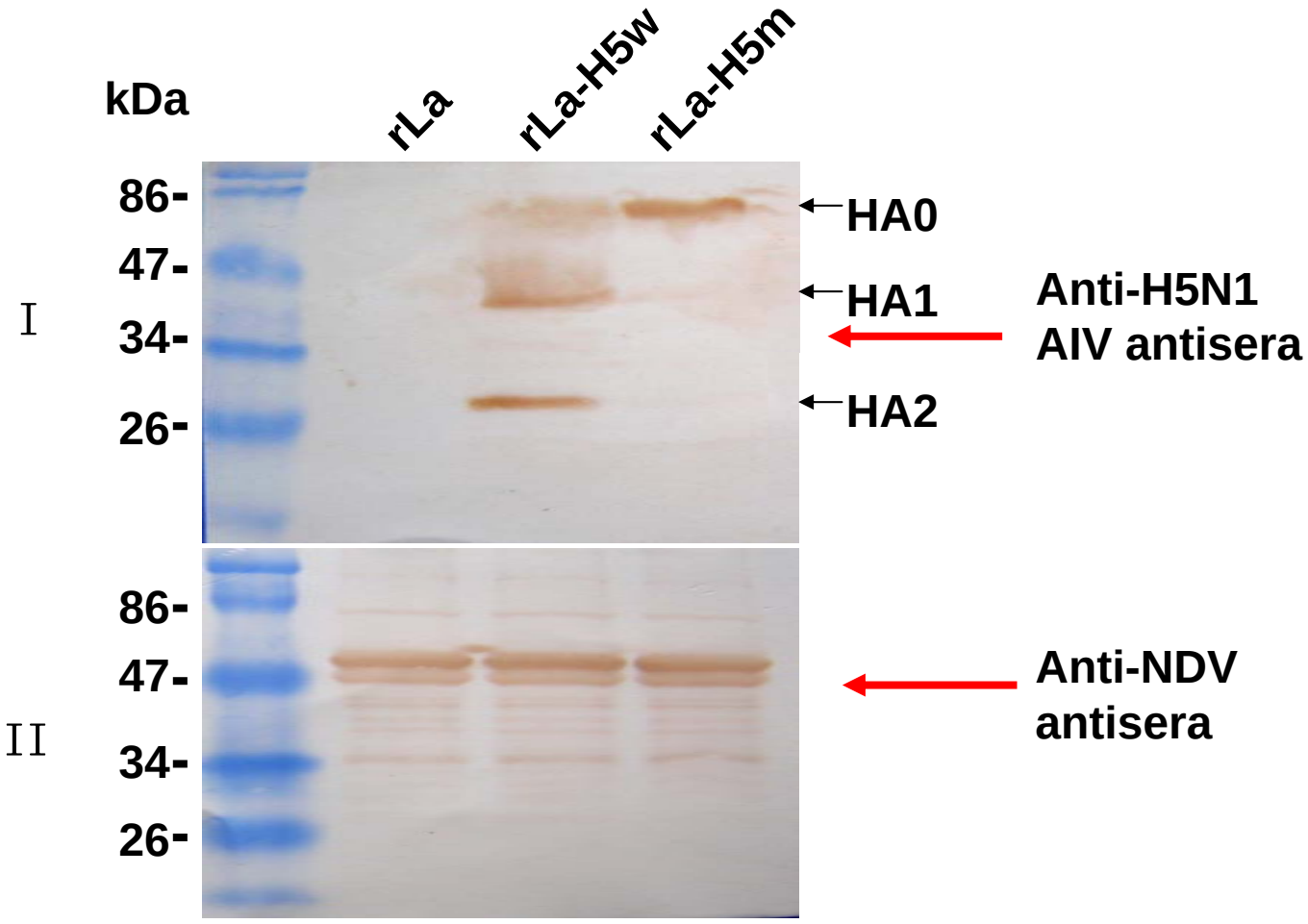
Viruses that isolated in African and European countries during 2005-2006



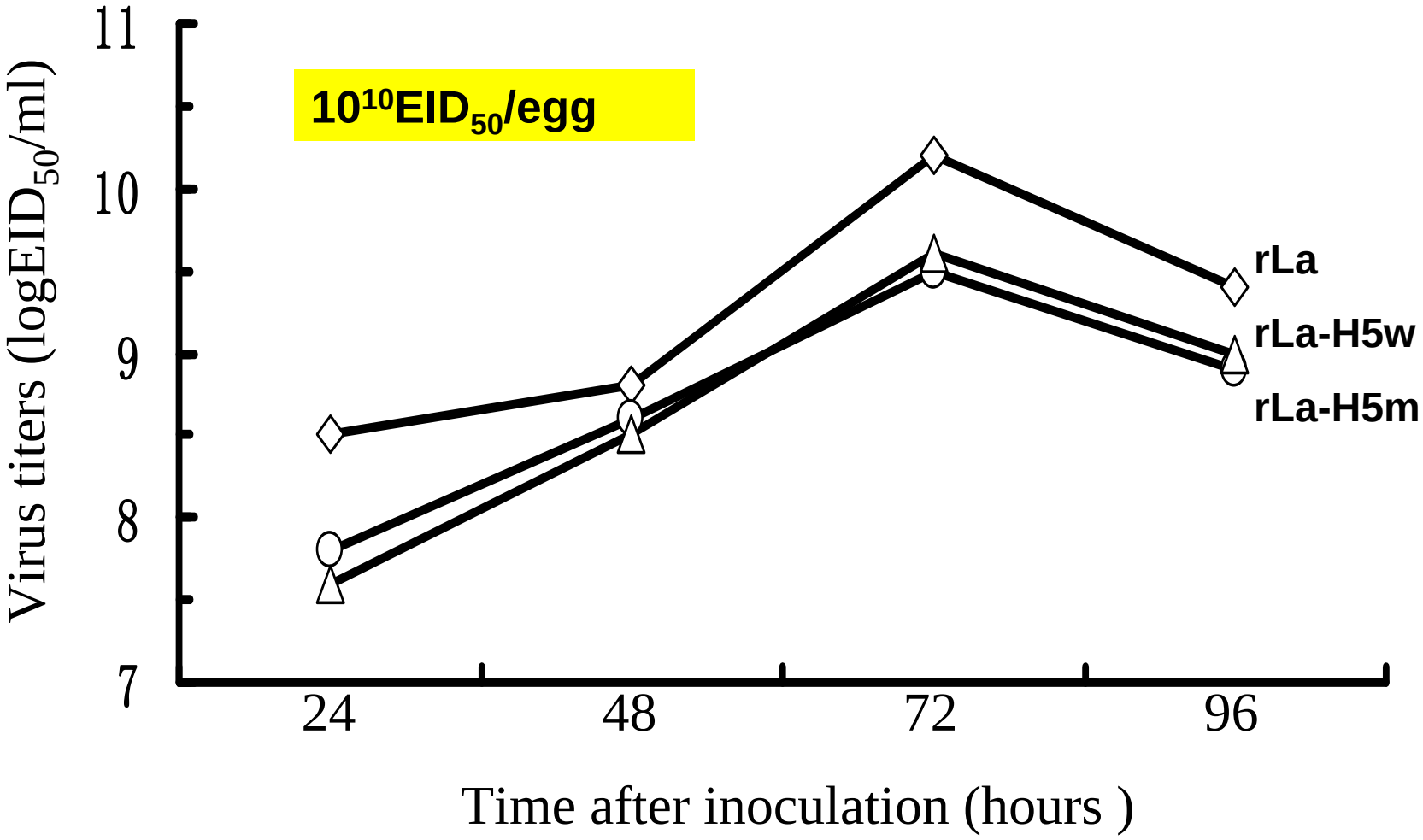
Generation of a recombinant Newcastle disease virus with the H5HA gene




anti-NDV
anti-AIV
NI
rLa
rLA-H5w
rLa-H5m
a
b
c
d
e
f
g
h
i


kDa
rLa
rLa-H5w
rLa-H5m
86-
47-
34-
26-
I
HA0
HA1
HA2
Anti-H5N1
AIV antisera
86-
47-
34-
26-
II
Anti-NDV
antisera

The recombinant virus grows as well as the wild type NDV virus



Biological properties of the rescued NDV and recombinant viruses

Virus	MDT (hrs)	Pathogenicity in chickens	
		ICPI	IVPI
rLa	>120	0.4	0
rLa-H5w	>120	0	0
rLa-H5m	>120	0	0

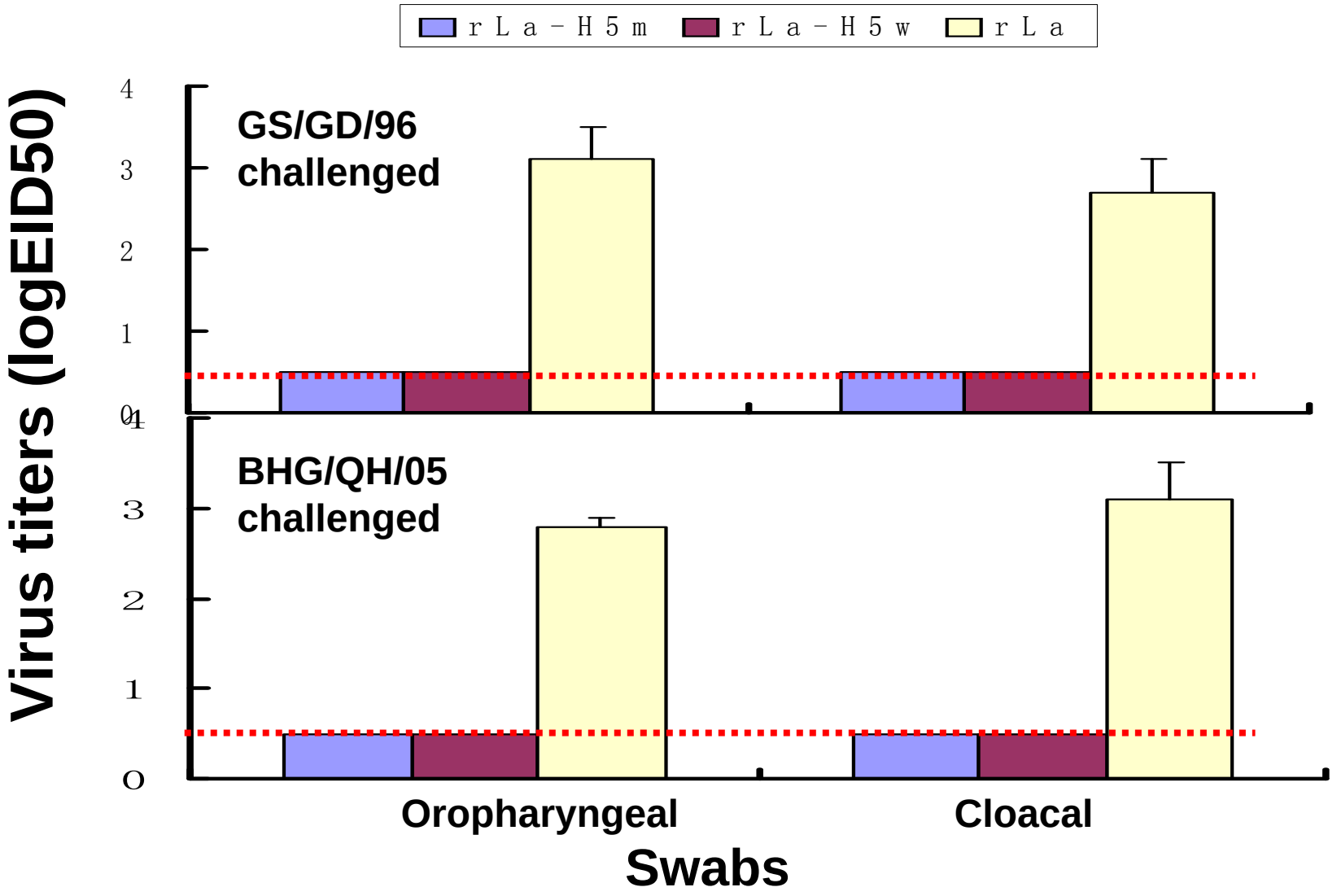
Protective efficacy against the highly pathogenic NDV challenge

Vaccines	HI antibody titers to NDV (log2)	Manifestation of chickens	
		No. sick/total	No. dead/total
rLa	7.3±0.4	0/10	0/10
rLa-H5w	6.6±0.3	0/10	0/10
rLa-H5m	6.8±0.4	0/10	0/10
PBS	<1	10/10	10/10

Protective efficacy against highly pathogenic H5N1 virus challenge

Challenge virus	Vaccines	HI antibody titers (log2)	Survival /total
GS/GD/96	rLa-H5m	4.2	10/10
	rLa-H5w	4.3	10/10
	rLa	<1	0/10
BHG/QH/05	rLa-H5m	8.8	10/10
	rLa-H5w	8.9	10/10
	rLa	<1	0/10

Virus shedding of chickens on day 3 p.c.

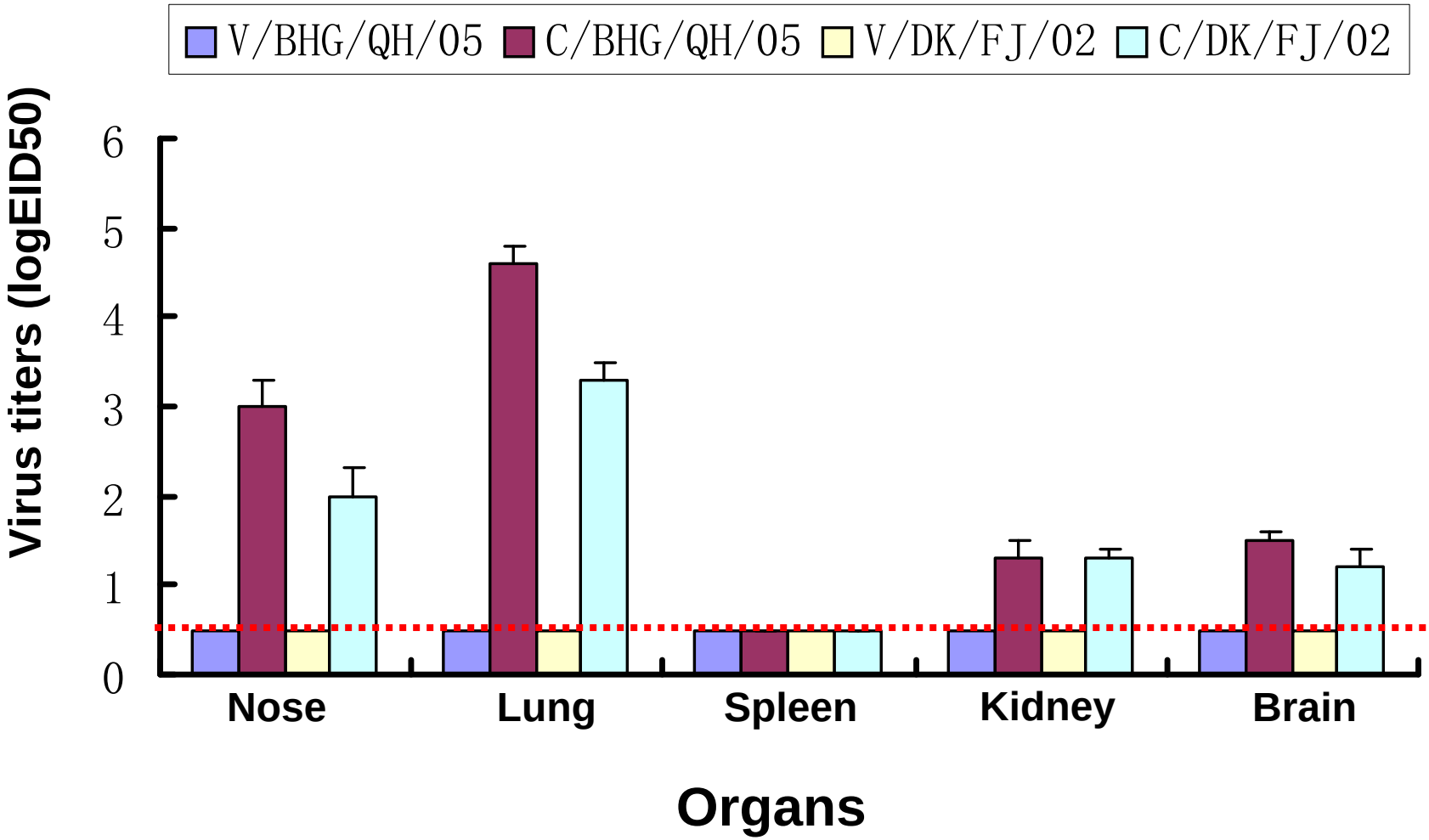


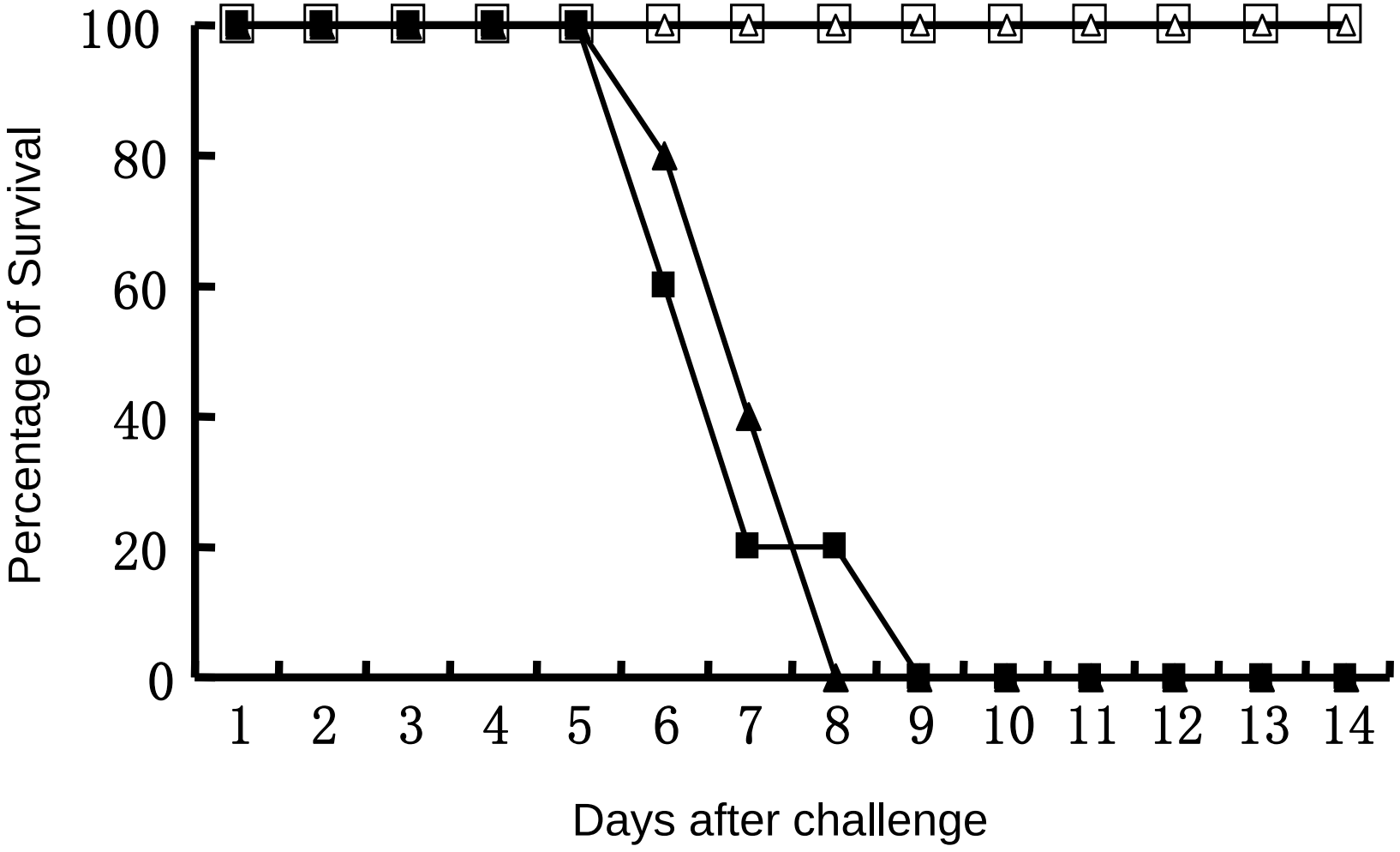
Does this vaccine work for mammalian animals?

- Balb/C mice were vaccinated by intraperitoneal injection with two doses of $10^8 EID_{50}$ of rLa-H5w in a 3 week interval
- Challenged with two HPAIV:

  BHG/QH/05 and **DK/FJ/02**

Virus replication in organs of mice on day 3 post challenge

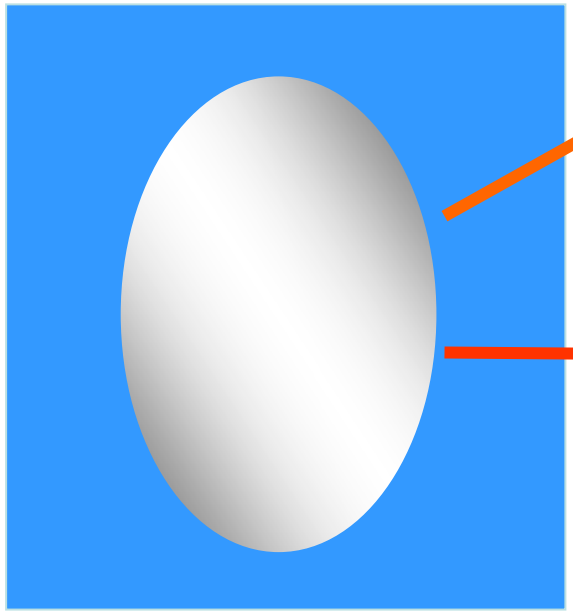




Vaccinated with rLa (▲, ■) or rLa-H5w (△, □) , and challenged with BHG/QH/05 (△, ▲) or DK/FJ/02 (□, ■)

Vaccine seed virus generated

Surface gene donor virus	H5N1/PR8	Recombinant NDV virus	Recombinant fowlpox virus
GS/GD/96	Yes, widely used	Yes, widely used	Yes, widely used
BHG/QH/05	yes	yes	No
AH/1/05	yes	yes	No
CK/SX/06	Yes, used in limited area	yes	pending



50 doses

AI of ND inactivated vaccines

4000 doses

NDV-based recombinant vaccine

Summary

- Four major genotype of H5N1 influenza viruses were identified in China during 2005-2006. Though the viruses showed some degree of antigenic variation, the widely used GSGD/96 virus based vaccine are still effective to three groups of the four groups viruses. A Shanxi virus based vaccine has been developed and applied in the field for the control real antigenic drifted viruses. Therefore, the vaccines used in the poultry in China have been very well evaluated and targeted, and proved to be effective.

Reference

- Jinying Ge, et al, Hualan Chen. Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. **Journal of Virology,** 2006, 80, epub., Oct. 18th .
- Zejun Li, et al, Hualan Chen. The NS1 Gene Contributes to the Virulence of H5N1 Avian Influenza Viruses. **Journal of Virology,** 2006, 80：11115-11123, epub., Sept. 13th .
- Hualan Chen, et al. Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. **Journal of Virology,** 2006, 80(12):5976-83.
- Zejun Li, Hualan Chen, et al. Molecular basis associated with replication of duck H5N1 influenza viruses in a mammalian mouse model. **Journal of Virology,** 2005, **79:** 12058–12064
- Guobin Tian, Hualan Chen. Protective efficacy in chickens, geese and ducks of an H5N1 inactivated vaccine developed by reverse genetics. **Virology**, 2005, 341: 153 – 162
- H. Chen. et al. The evolution of H5N1 influenza viruses in ducks in Southern China. **Proc. Natl. Acad. Sci. USA,** 2004, 101(28): 10452-10457

Acknowledgements

- Dr. R. Webster
- Dr. Y. Kawaoka
- Dr. N. Cox
- Dr. P. Palese
- Dr. G. Brownlee
- Dr. Kangzhen Yu
- Dr. Zejun Li
- Dr. Guohua Deng
- Dr. Guobin Tian
- Dr. Yanbing Li
- Dr. Yongping Jiang
- Dr. Zhigao Bu