OFFLU avian influenza virus characterisation meeting
29 – 30 March 2017
FAO Headquarters, Rome, Italy

Ji-Ming Chen

China Animal Health and Epidemiology Centre

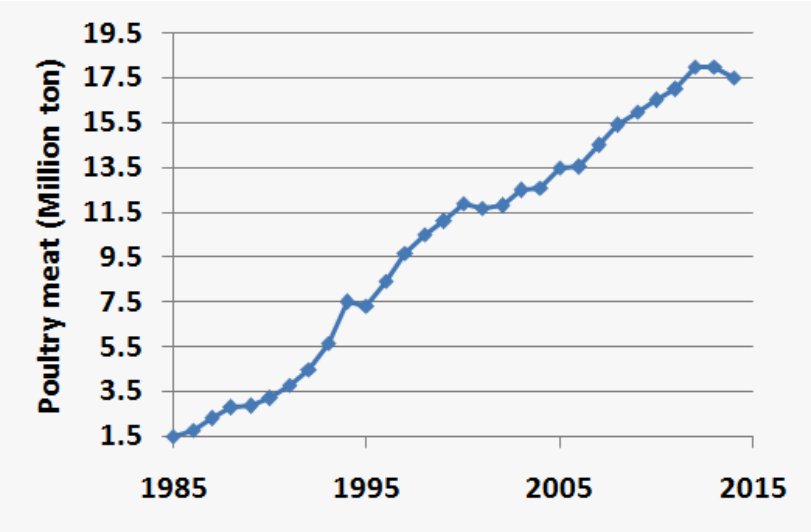
Qingdao, China

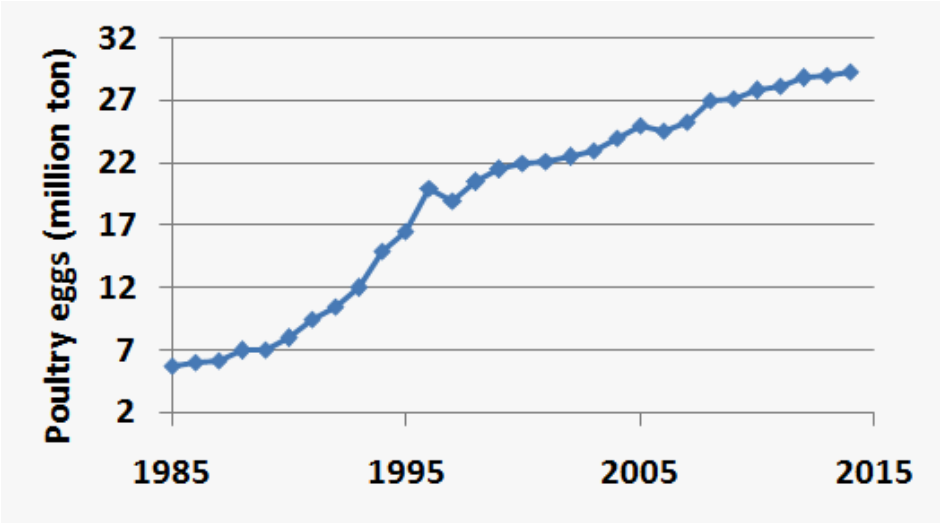
China Experience with HPAI Virus Evolution and Vaccination

- Poultry and AIVs in China
- Evolution & Vaccination of H5 HPAIV in China
- Evolution & Vaccination of H7 AIVs in China

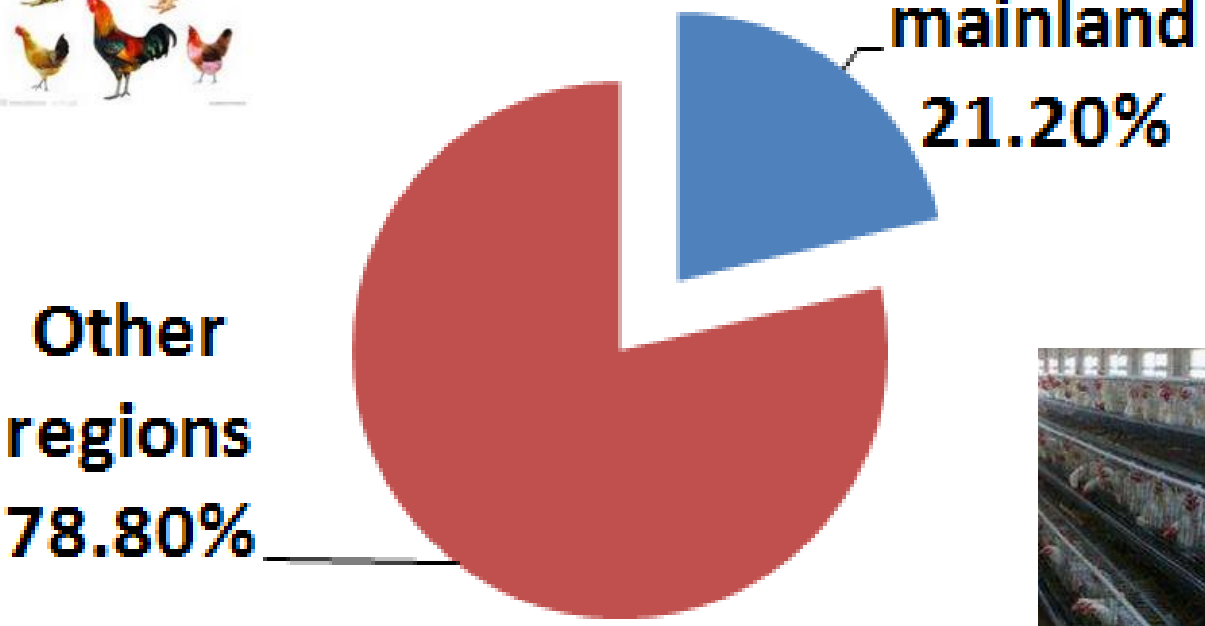
Poultry and AIVs in China

Poultry in China mainland increased by >6% annually during the past decades

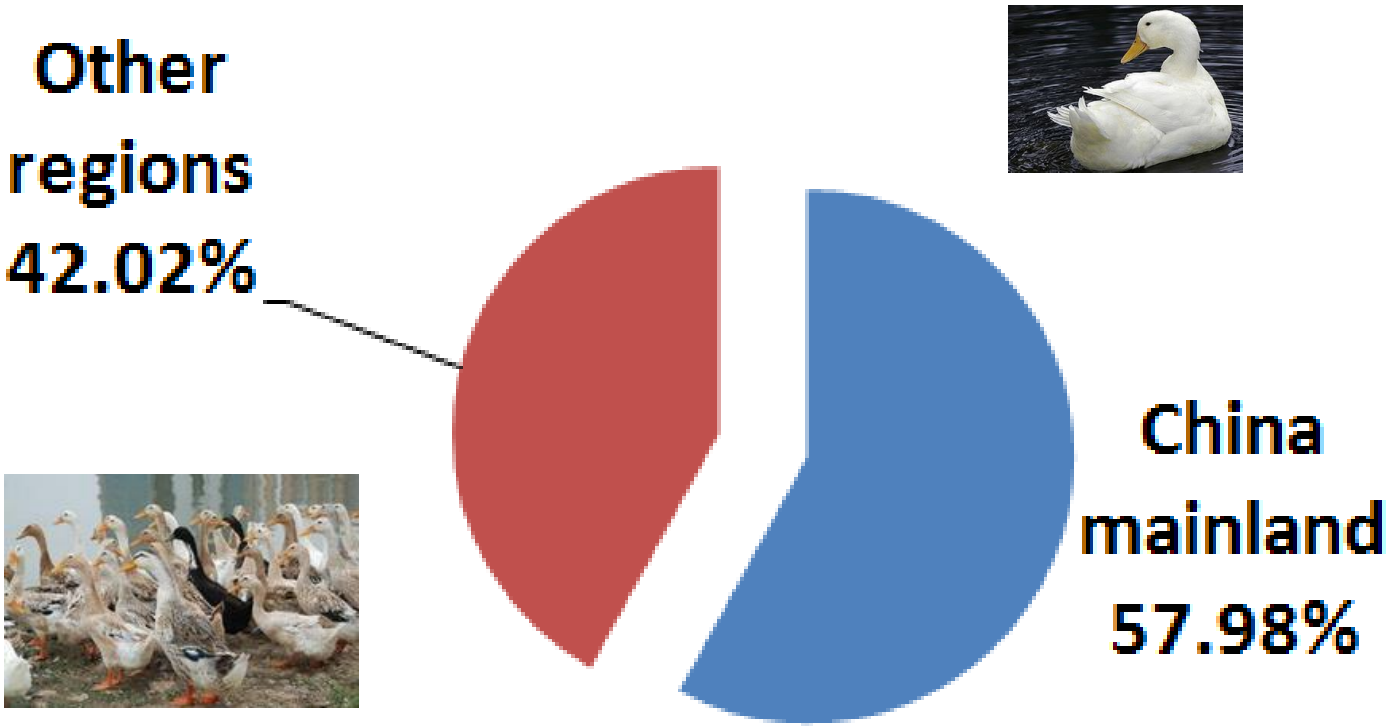




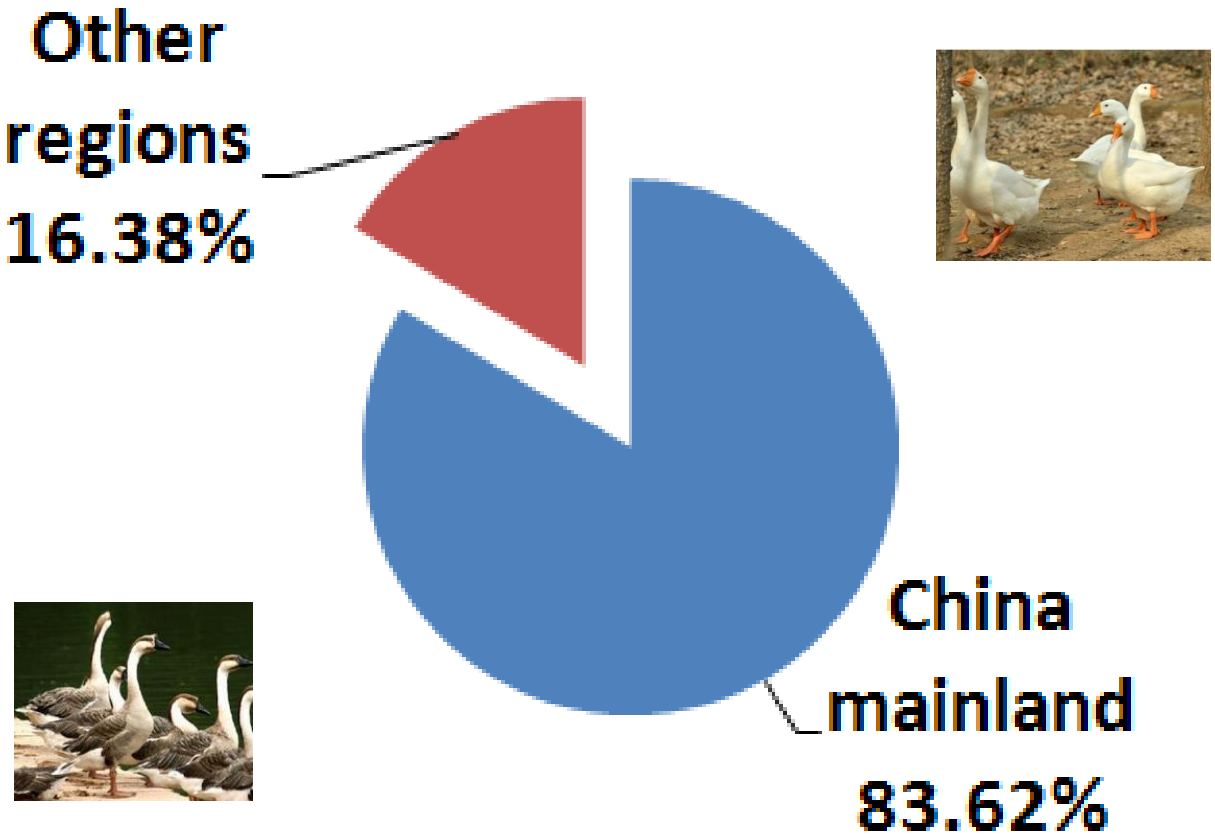
China mainland raises about one fifth chickens in the world



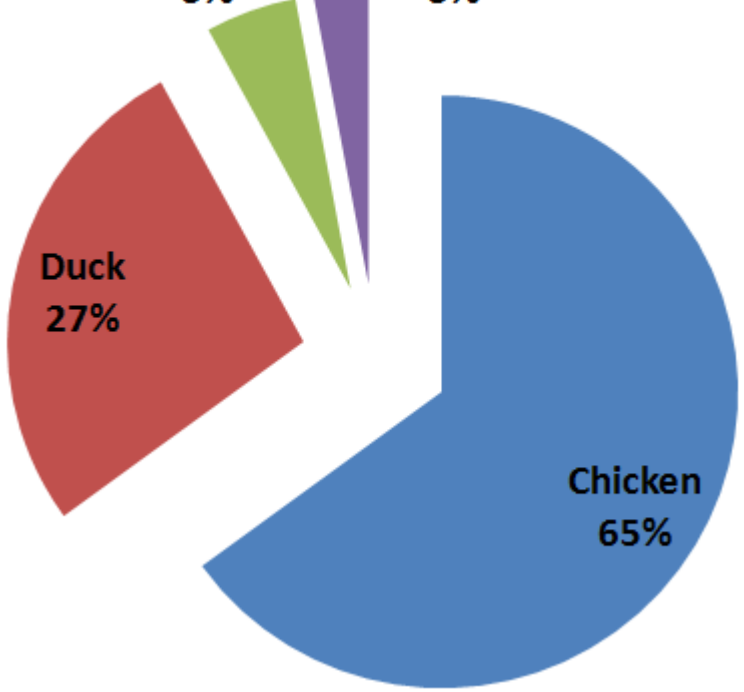
China mainland raises >50% ducks in the world



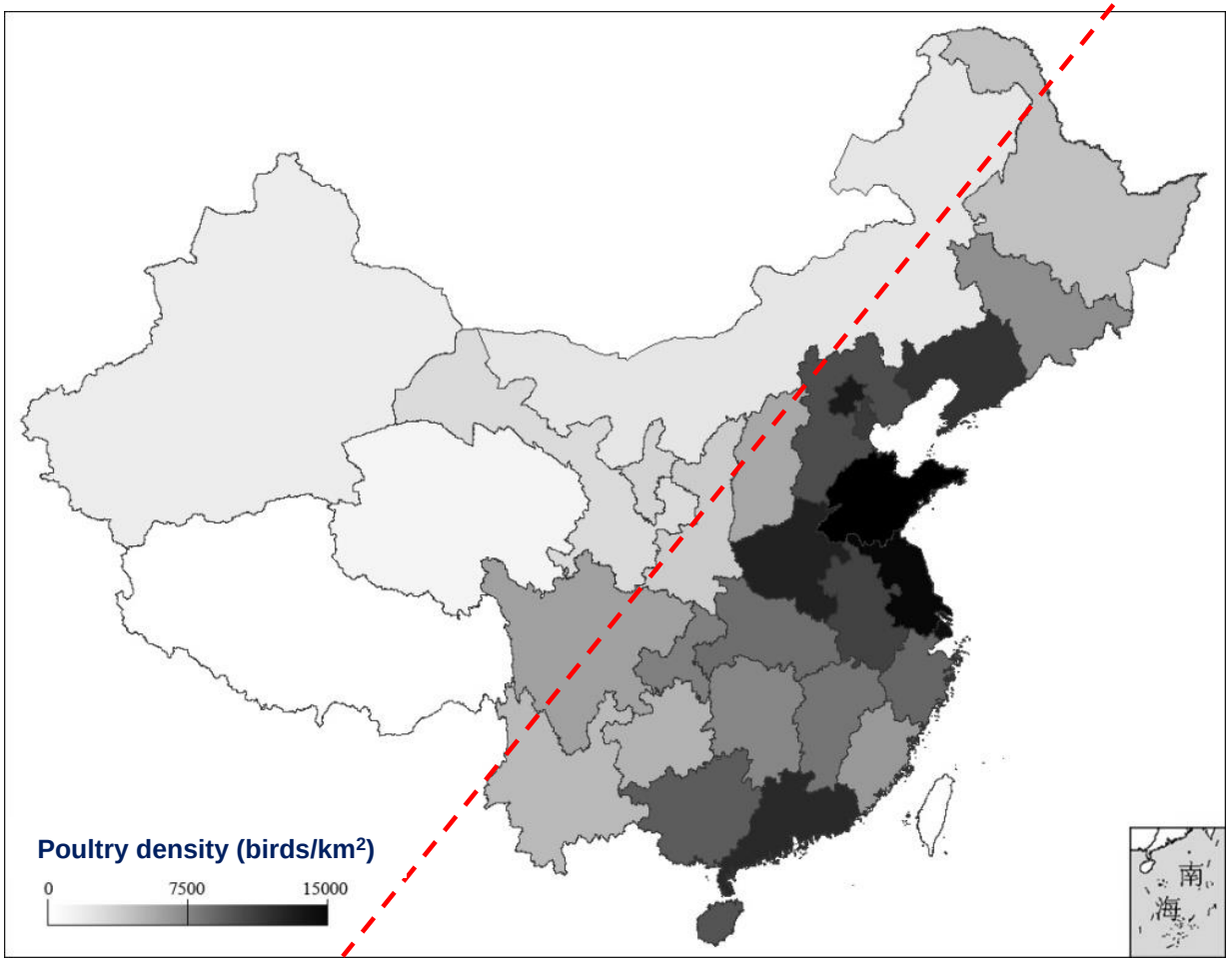
China mainland raises about four fifth geese in the world



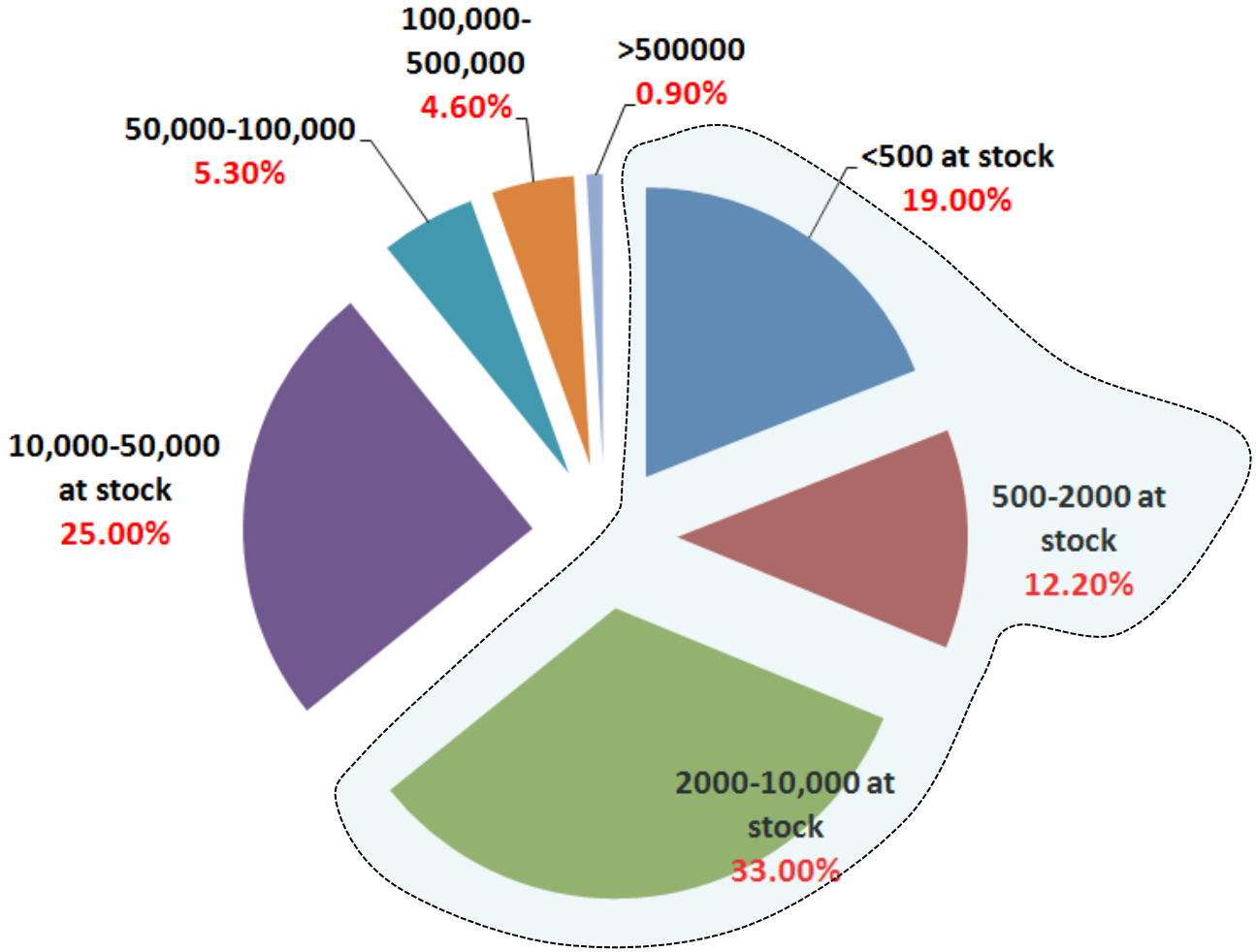
About 5.5 billion poultry birds are raised in China mainland



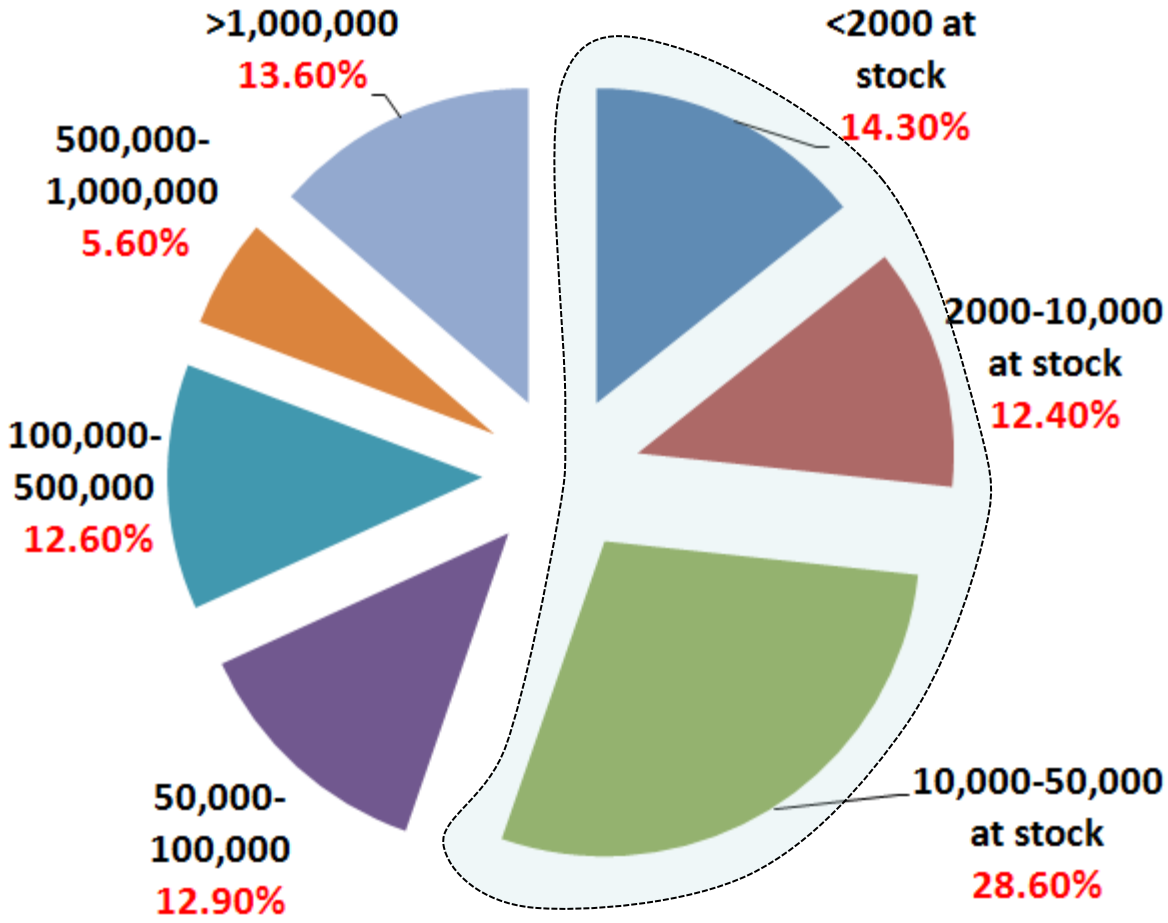
Very high poultry density in China eastern regions



>50% poultry birds are raised by small-scale farms with limited biosecurity



In 2014, 65% layers were raised by small-scale farms with <10,000 chickens at stock



In 2014, 55% broilers were raised by small-scale farms with <50,000 chickens at stock

Live bird transportation and marketing are prevalent in China mainland

Many poultry birds in China mainland are raised at open areas

Share AIVs with populous wild birds

China mainland: Eden of AIVs

Huge poultry production

High poultry density

Low poultry biosecurity

Distant live bird transportation

Prevalent live bird markets

Populous wild birds

AIVs in China we identified

Subtype	2013	2014	2015	2016
H1	√	√	√	√
H2	√	√	√	√
H3	√	√	√	√
H4	√	√	√	√
H5	√	√	√	√
H6	√	√	√	√
H7	√	√	√	√
H8	×	×	×	×
H9	√	√	√	√
H10	×	√	×	×
H11	√	√	√	√
H12-H16	×	×	×	×

H9 subtype:

most prevalent (>50%)

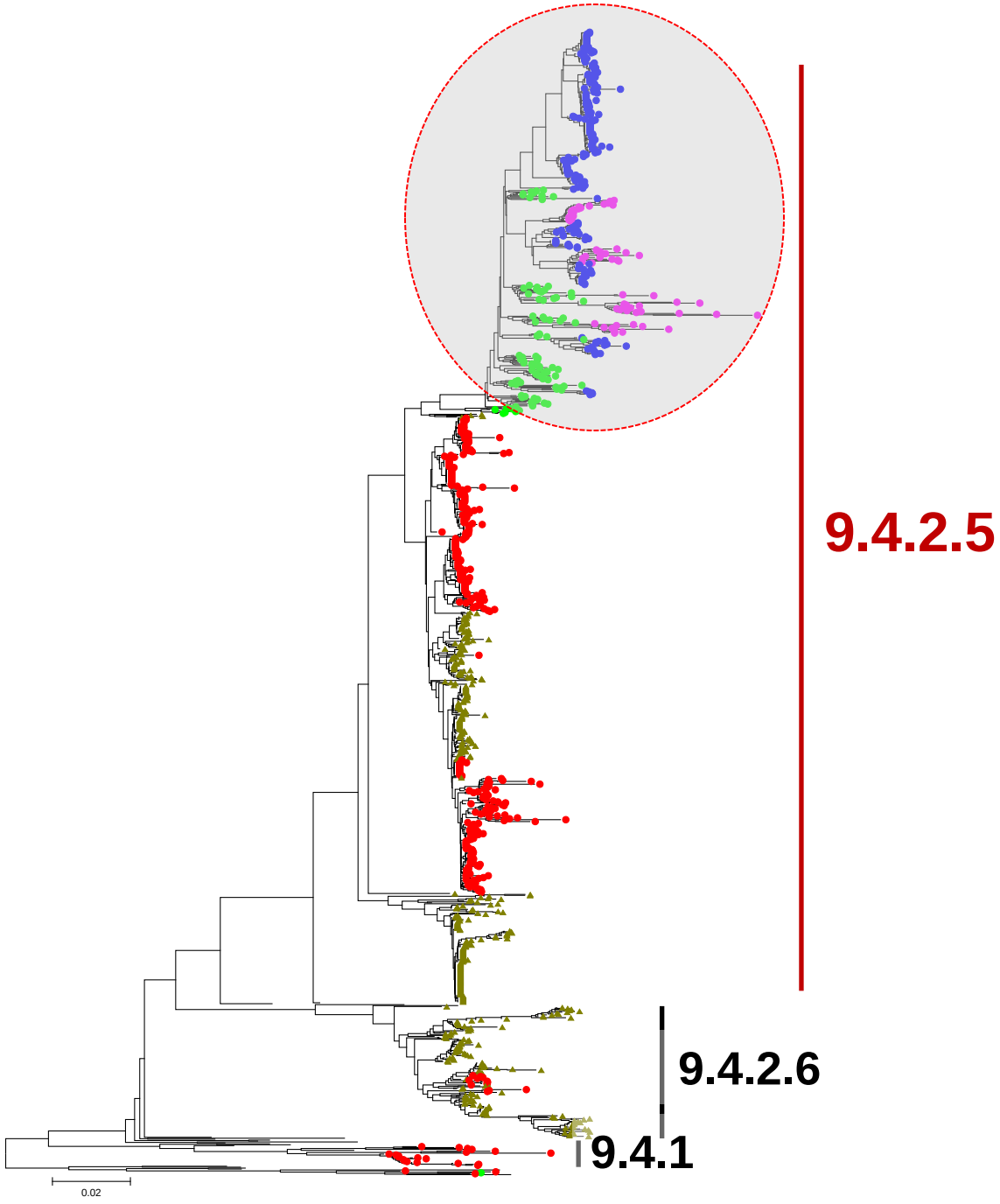
2012 ▲

2013 ●

2014 ●

2015 ●

2016 ●



Evolution & Vaccination of H5 HPAIV in China

an agony of 20 years

H5 HPAIVs included H5N1, H5N2, H5N6, H5N8 in 2016

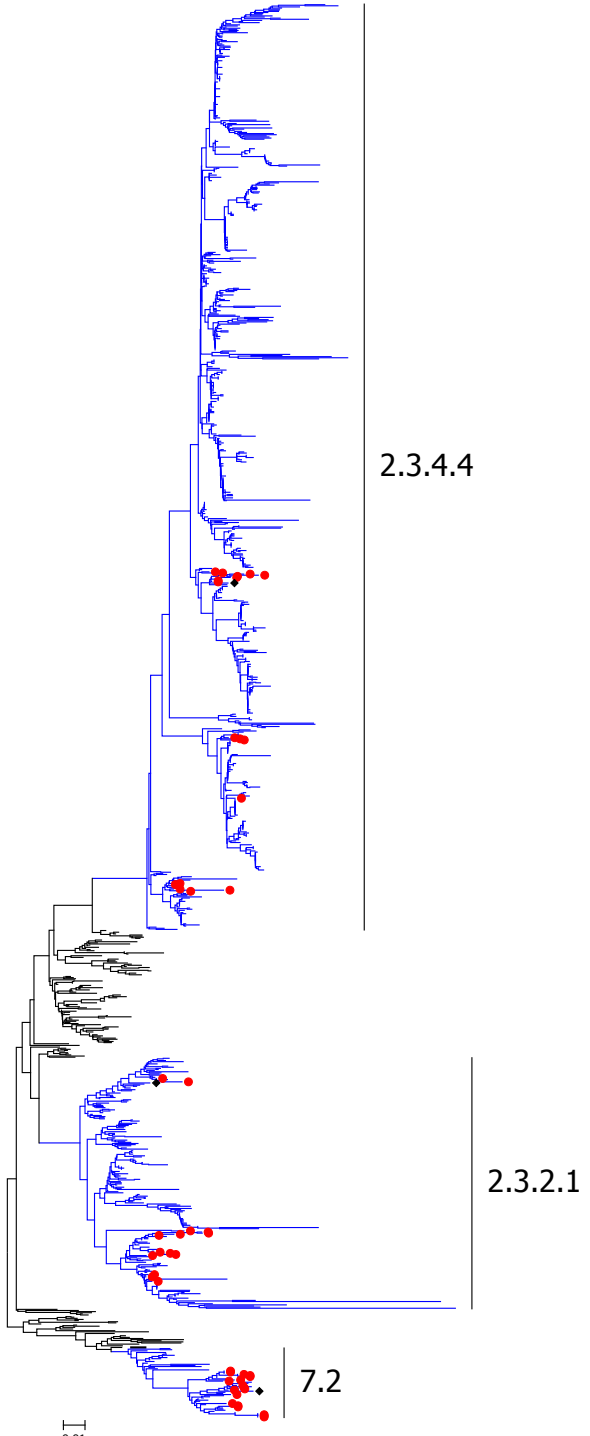
Year	Proportion (%)			
	H5N1	H5N8	H5N6	H5N2
2011	100.0	0.0	0.0	0.0
2012	92.2	3.7	0.0	4.1
2013	79.6	13.8	0.0	6.6
2014	19.3	7.1	70.2	3.3
2015	4.3	0.0	87.7	8.0
2016	6.4	0.3	63.7	31.5

H5 HPAIVs covered clades 2.3.4 and 2.3.2 in 2016

Year	Clade 2.3.2	Clade 2.3.4	Clade 7
2007	0.00%	100.0%	0.0%
2008	51.8%	25.0%	23.2%
2009	92.3%	3.4%	4.2%
2010	58.5%	28.3%	13.2%
2011	80.9%	4.6%	14.5%
2012	81.4%	9.8%	8.8%
2013	60.2%	33.0%	6.8%
2014	7.1%	92.7%	0.2%
2015	7.6%	92.4%	0.0%
2016	5.4%	94.6%	0.0%

H5 HPAIV HA

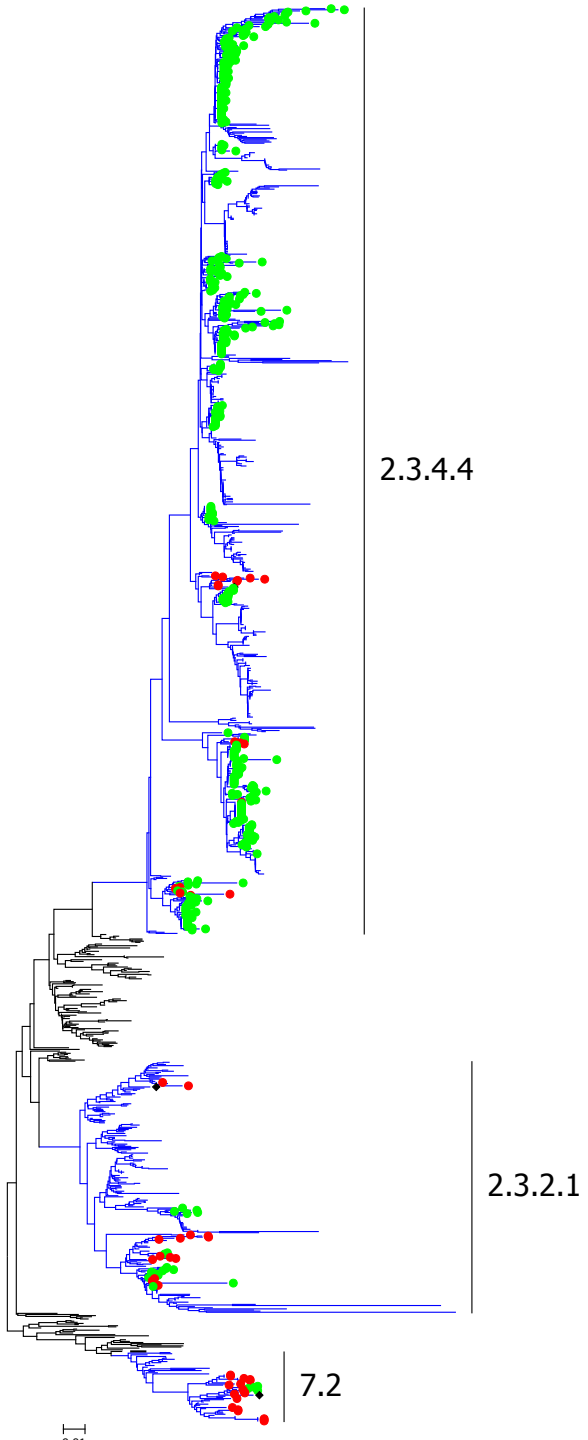
2013



H5 HPAIV HA

2013

2014

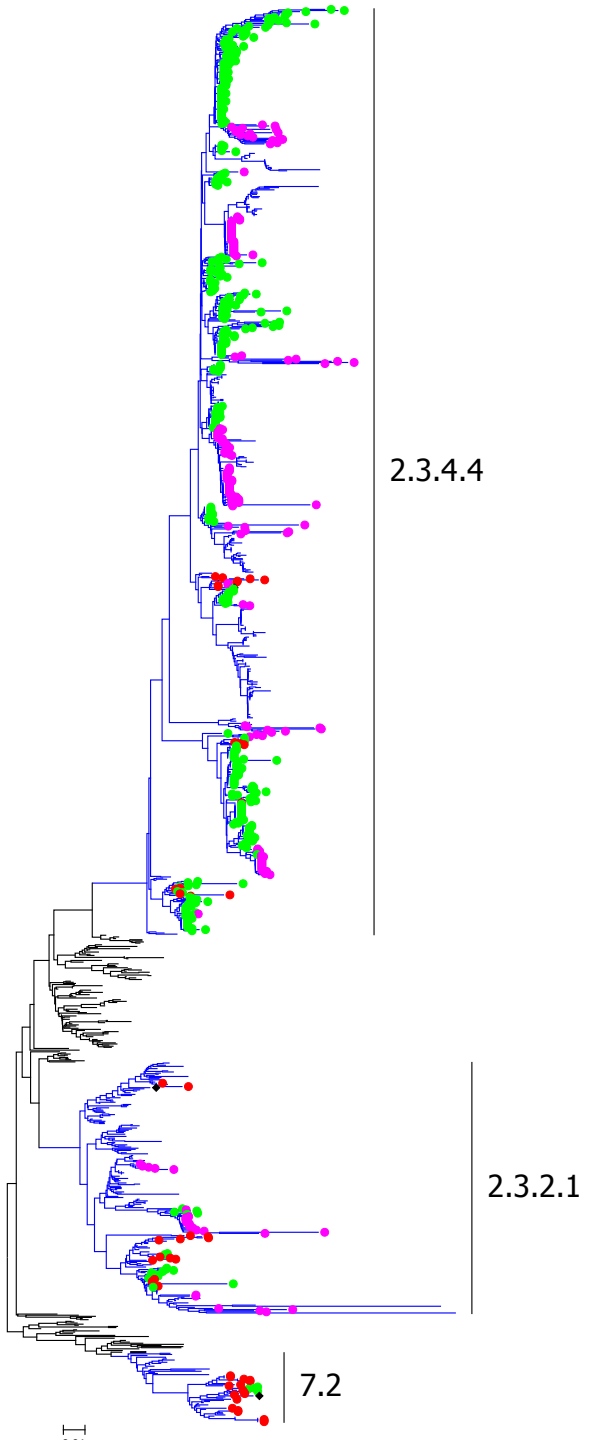


H5 HPAIV HA

2013

2014

2015



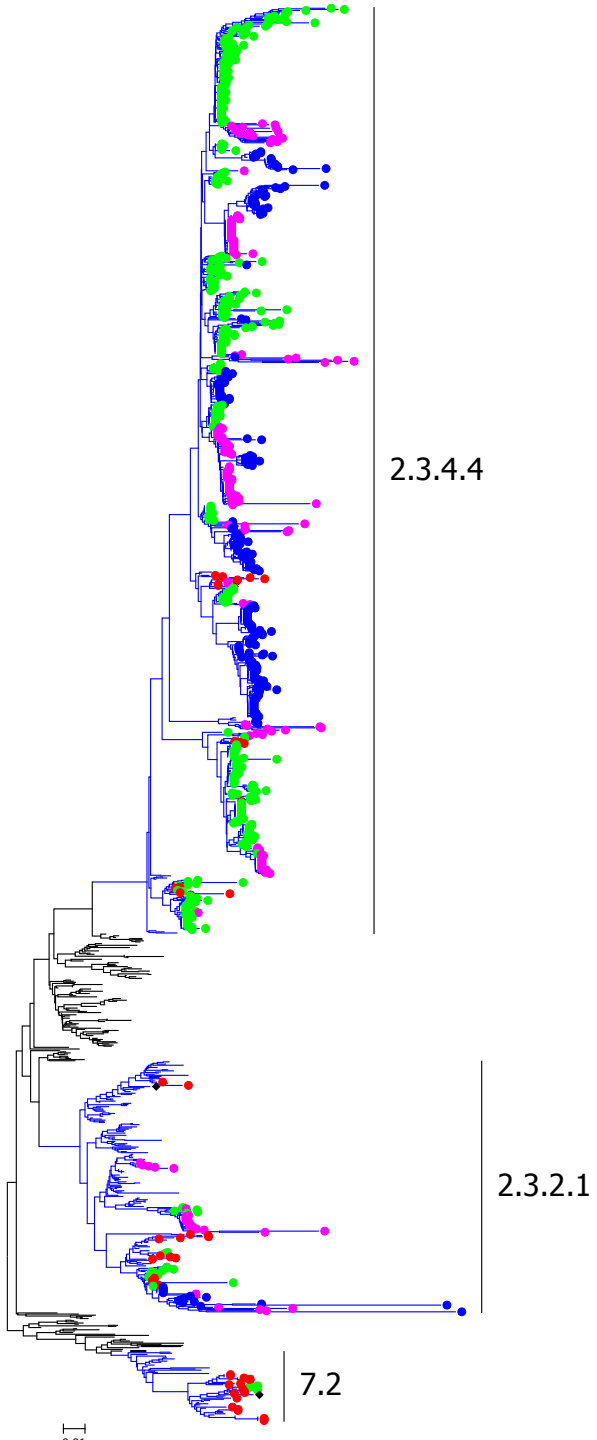
H5 HPAIV HA

2013

2014

2015

2016



China had to utilize mass vaccination against H5 HPAIVs (too high risk)

- Many birds are raised at open areas
- Many birds are raised by small-scale farms with limited biosecurity
- Frequent live birds transportation
- Prevalent live birds marketing

Producers of H5 vaccines

Multiple companies designated by China MoA including:

- **Qingdao Yebio**
- **Hohhot Jinyu**
- **Harbin Weike**
- **Luoyang QYH**
- **……**

Licensing of H5 vaccines

- **Very strict**
- **As per a series of laws and decrees**
- **Proposed by experts**
- **Inspected by China Institute of Veterinary Drug Control**
- **Approved by China MoA**

Current H5 vaccination strategy

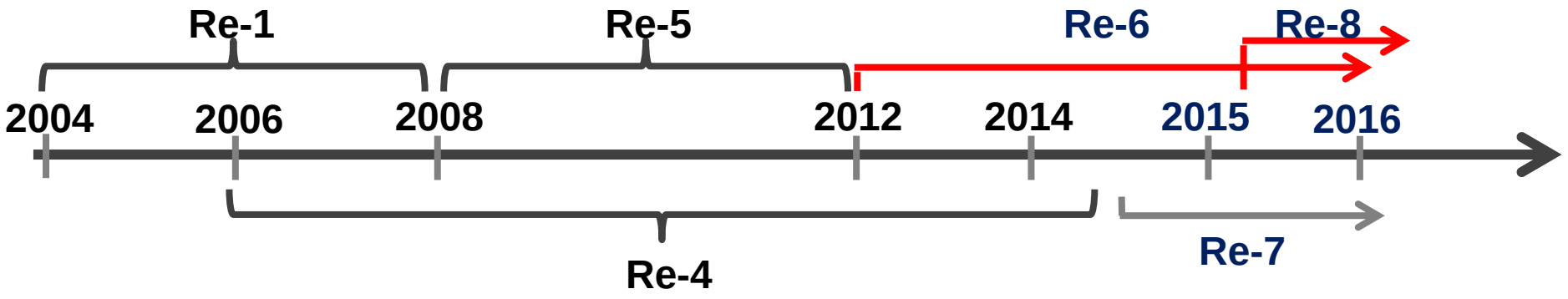
- **Maintained for a dozen of years**
- **Vaccination is funded by the government**
- **Almost all poultry birds including broilers, layers, ducks, geese, pigeons, quails in all provinces**

Actual H5 vaccination coverage

	Breeder chickens and layers	Broilers	Waterfowls (ducks)
Gross number	4-5 billions	8 billions	4 billions
Vaccination coverage rate	>80%	<20%	<30%

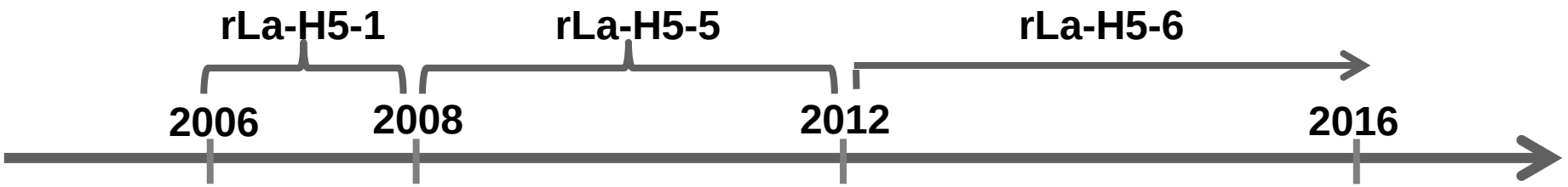
Inactivated PR8-based vaccine strains

Vaccine strains	HA clade	Applied years
Re-1	0	2004-2008
Re-4	7.2	2006-2014
Re-5	2.3.4	2008-2012
Re-6	2.3.2	2012-
Re-7	7.2	2014-
Re-8	2.3.4.4	2015-



Newcastle disease virus vectored vaccine strains

Vaccine strains	HA clade	Applied years
rLa-H5-1	0	2006-2008
rLa-H5-5	2.3.4	2008-2012
rLa-H5-6	2.3.2	2012-

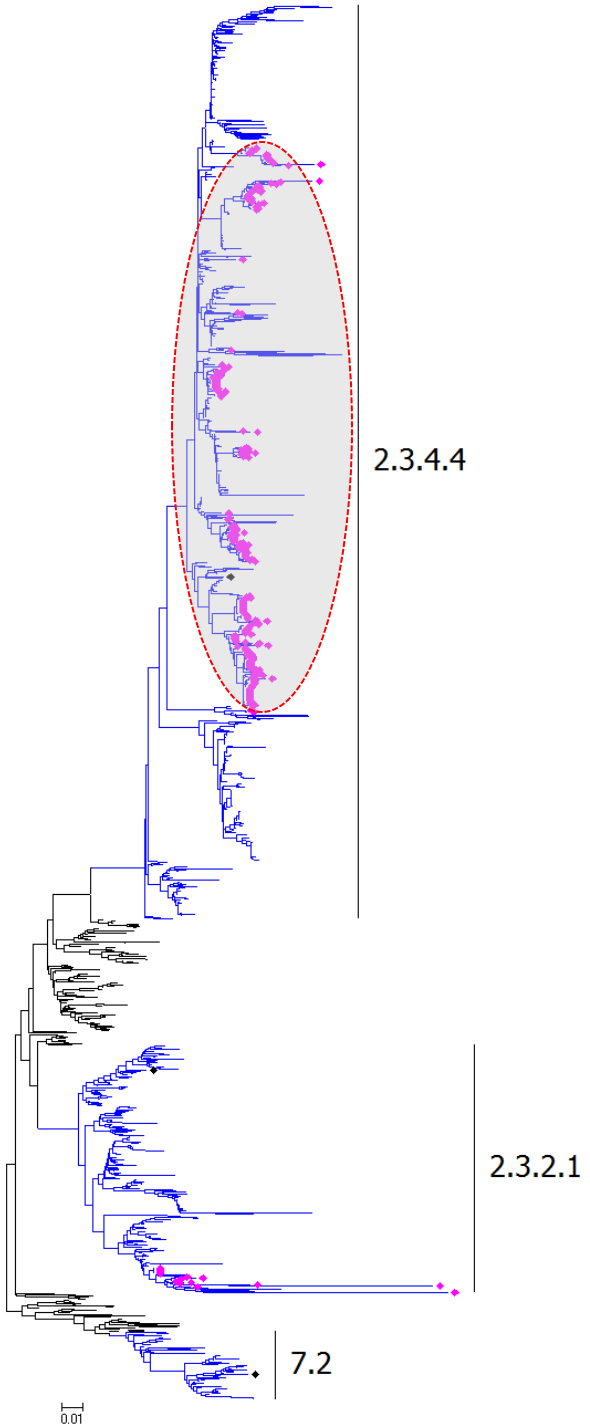


Selection/updating of vaccine strains

- **Epidemiological surveys: dominance**
- **Serological: antigenicity changes**
- **Animal challenging: protection potency**

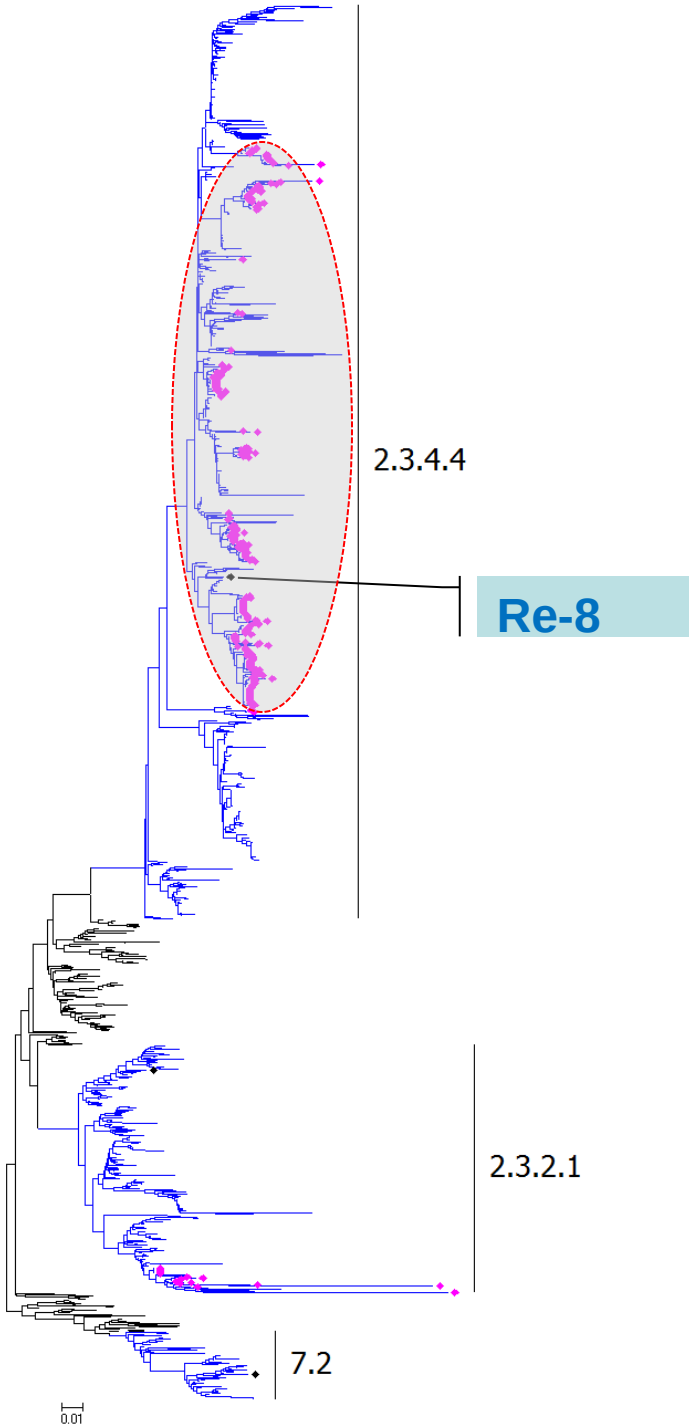
Matching of current vaccine strains

- **Field strains in 2016:** ◆



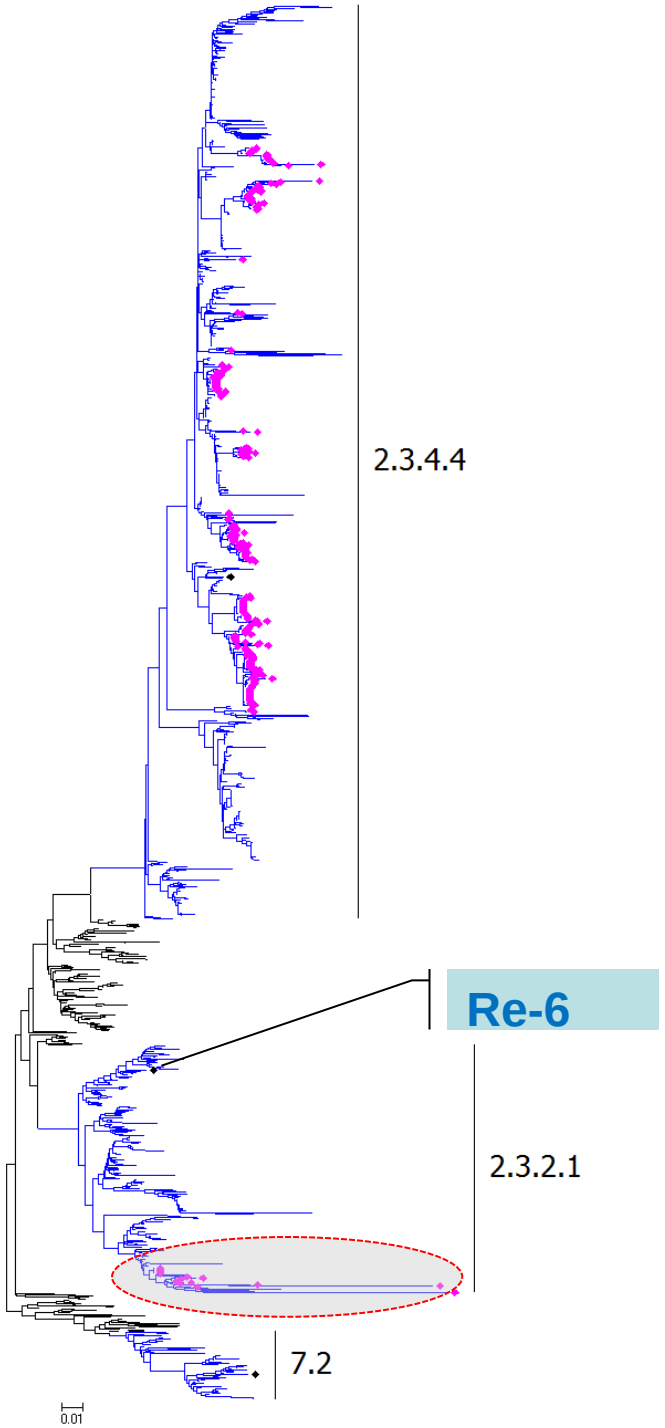
Matching of current vaccine strains

- **Field strains in 2016:** ◆
- **Re-8: match well field strains in Clade 2.3.4.4**



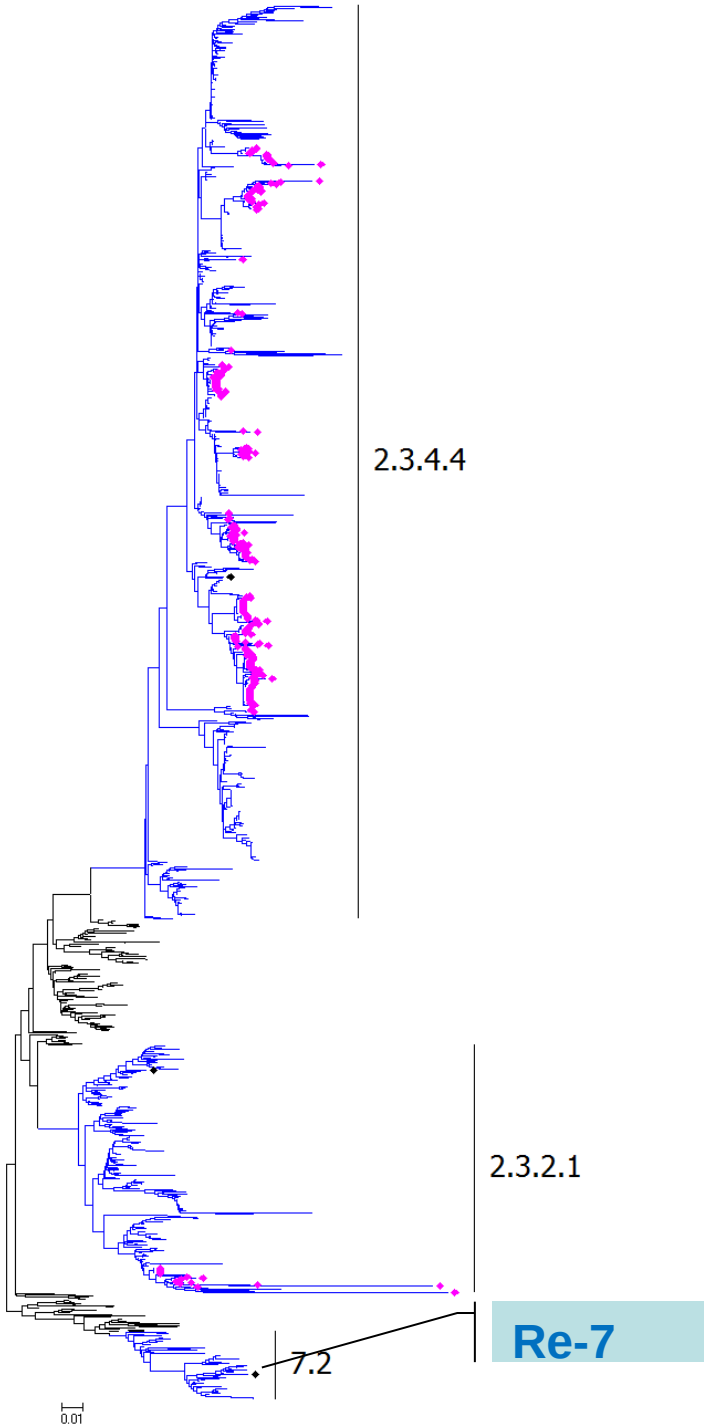
Matching of current vaccine strains

- **Field strains in 2016:** ◆
- **Re-8: match well with field strains in Clade 2.3.4.4**
- **Re-6: match not well field strains in Clade 2.3.2.1**



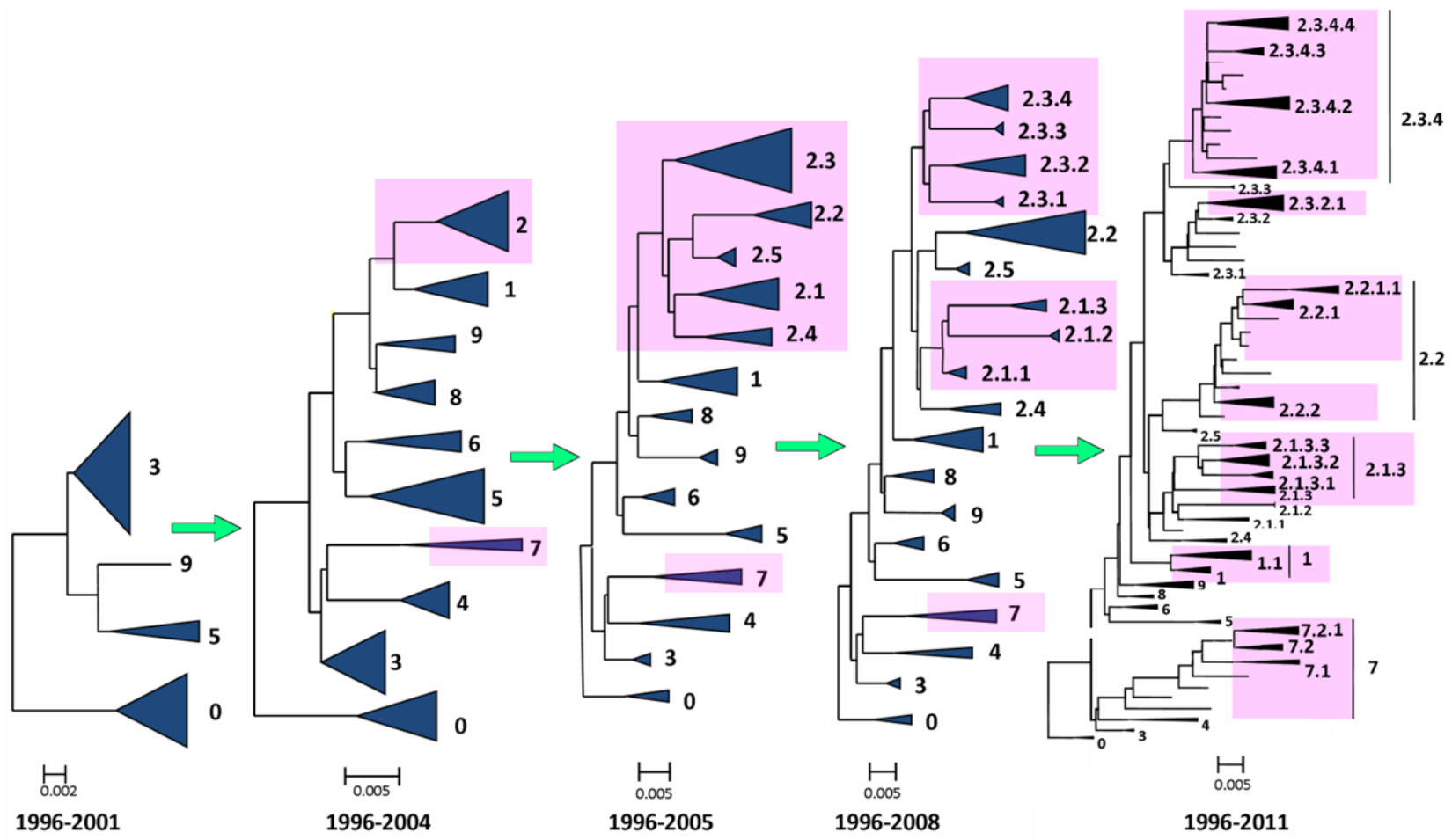
Matching of current vaccine strains

- **Field strains in 2016:** ◆
- **Re-8: match well field strains in Clade 2.3.4.4**
- **Re-6: match not well field strains in Clade 2.3.2.1**
- **Re-7: field strains in Clade 7.2 have been disappeared for 2 years (likely be excluded in 2017)**



H5 vaccine strains should not be updated too frequently

- **The strain updating is expensive**
- **A new mutant may not become dominant since many mutants (e.g. Clades 3, 2.3.1, 2.3.4.2) shall disappear naturally**



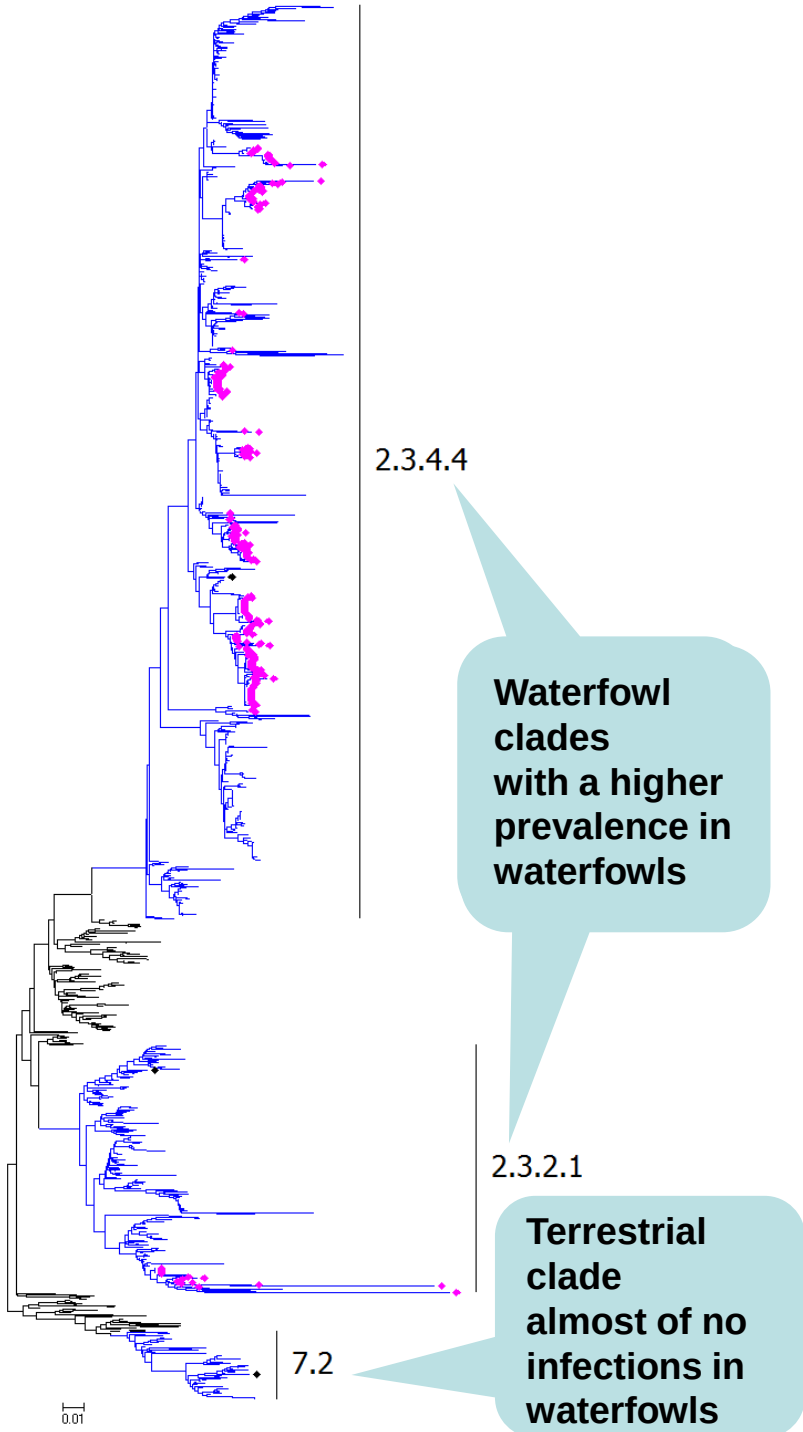
Multiple clades have disappeared naturally

Vaccine updating criteria (personally assumed)

- **The proportion of a mutant in all H5 HPAIVs should be >30%**
- **The HI titer should be of ≥16-fold differences between the mutant and original strains**
- **The protection efficacy of a new vaccine strain targeting the mutant should higher by >30% than the original one**

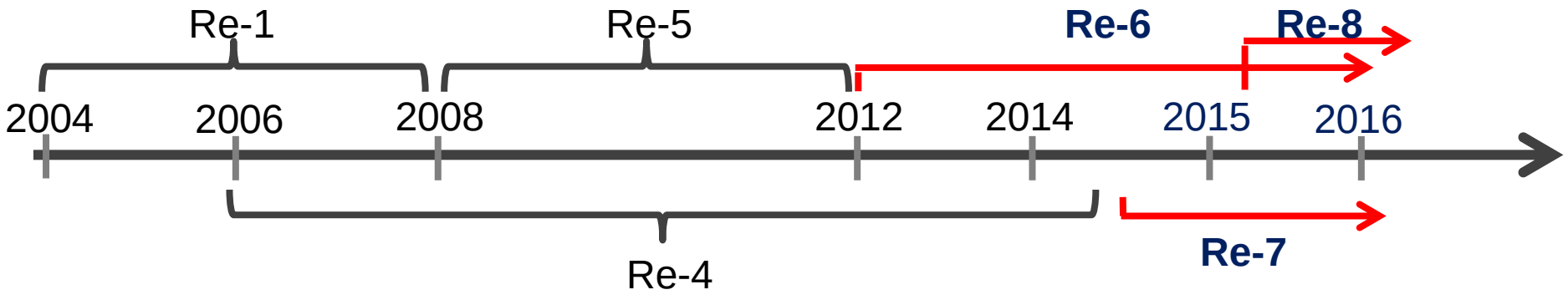
H5 vaccination:

very effective for inhibiting the terrestrial clade 7.2 which caused few infections in waterfowls

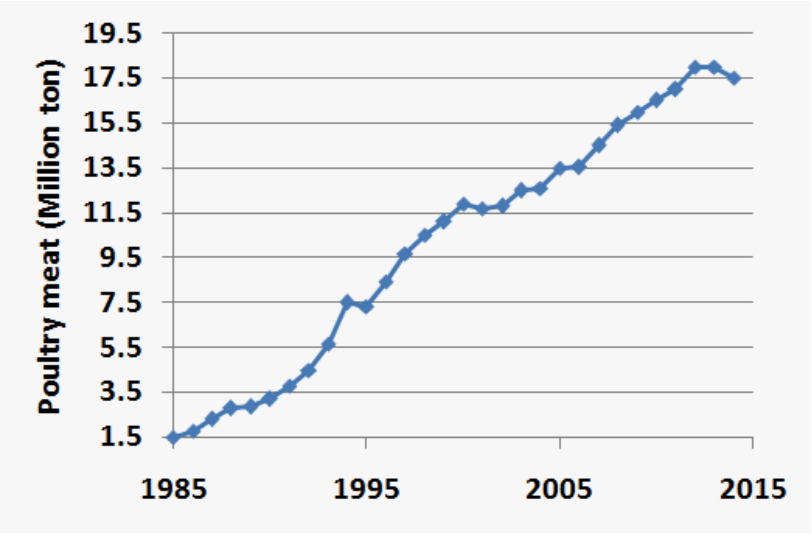


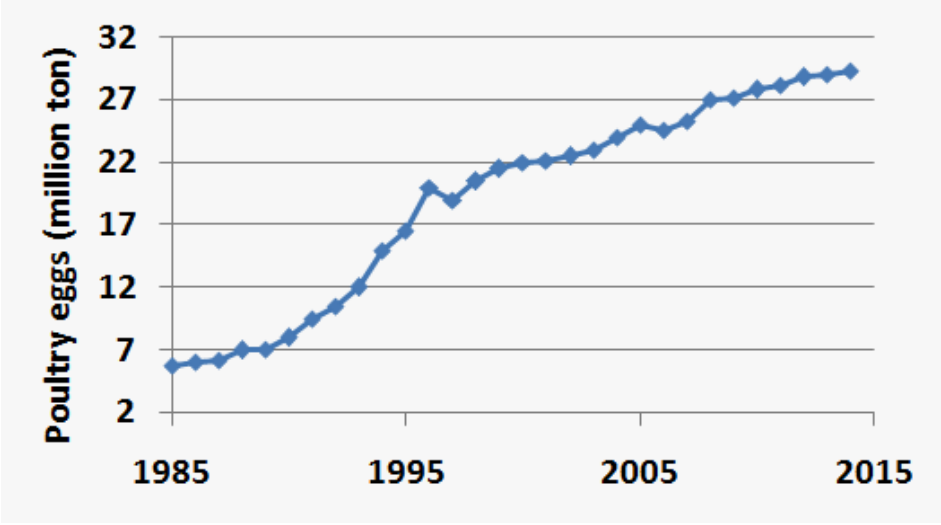
H5 vaccination against waterfowl clades

- **Quite effective for inhibiting waterfowl clades 2.3.2 and 2.3.4 within 1-3 years**
- **Thereafter, more and more vaccine-escaping mutants emerged**
- **Usually vaccine strains should be updated within 4 years**



H5 vaccination:
stabilizing and developing the poultry industry in China





H5 vaccination:

saving many small-scale poultry farms which are of great social significance (advantage)

Small-scale poultry farms are of great HPAI transmission risk (disadvantage)

H5 vaccination:
too expensive and tough in management

H5 vaccination:
led to silent circulation in vaccinated fowls

Subclinical Highly Pathogenic Avian Influenza Virus Infection in Vaccinated Chickens, China

Qing-Xia Ma, Wen-Ming Jiang, Shuo Liu, Su-Chun Wang, Qing-Ye Zhuang, Guang-Yu Hou, Xiang-Ming Liu, Zheng-Hong Sui, and Ji-Ming Chen

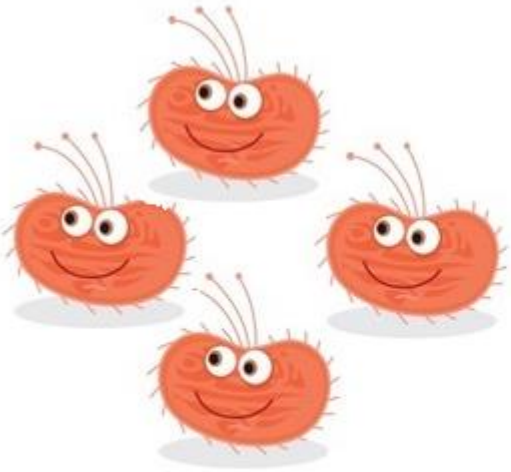
Subclinical infection of vaccinated chickens with a highly pathogenic avian influenza A(H5N2) virus was identified through routine surveillance in China. Investigation suggested that the virus has evolved into multiple genotypes. To better control transmission of the virus, we recommend a strengthened program of education, biosecurity, rapid diagnostics, surveillance, and elimination of infected poultry.

The Study

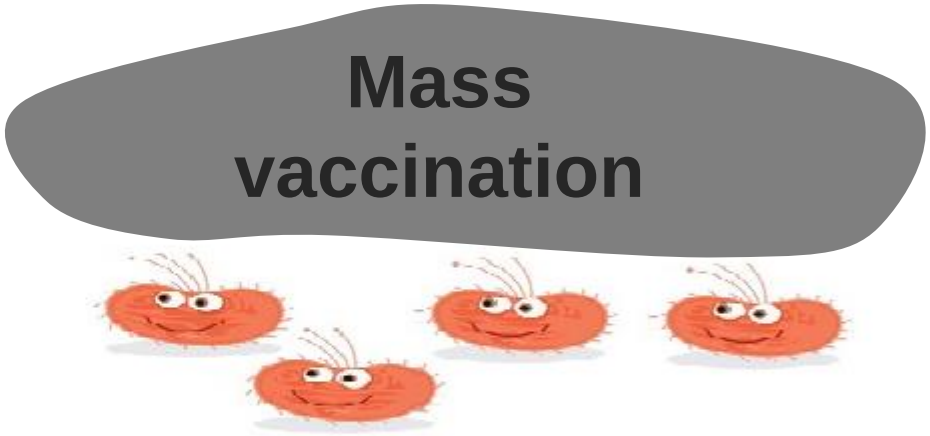
During routine surveillance in January 2014, we collected 30 swab samples from chickens on an egg-laying chicken farm populated by 12,000 320-day-old and 6,000 150-day-old chickens without any clinical signs, in Qingdao, Shandong Province, China. Of those, 5 were positive by real-time reverse transcription PCR for detection of the hemagglutinin (HA) gene of H5 HPAI viruses. The flock was culled immediately after the diagnosis, and an H5N2 subtype HPAI virus was isolated by inoculating specific-pathogen-free embryonated eggs with the collected samples. The entire viral genome of the virus, A/chicken/Qingdao/1/2014(H5N2), abbreviated as H5N2qd14, was sequenced and analyzed as described (*4*). The sequences were deposited in GenBank under accession nos. KJ683877–KJ683884. We used the full-length sequences for each gene in phylogenetic analyses.

The HA protein of the H5N2qd14 virus has multiple basic amino acid residues (PQIEGRRRKR↓GLF) at the cleavage site, categorizing it as an HPAI virus. Its intravenous pathogenicity index is 2.84, determined by intravenously inoculating 10 chickens, which were 6 weeks old and specific pathogen–free, with 0.1 mL of a 1:10 dilution of

H5 vaccination:
accelerated the viral mutation



No vaccination, no pressure, we need not mutate that fast



We have to mutate faster to escape from the vaccination

Country	Period	Mass Vaccination	HA1substitution rate (10^{-3} substitution/site/yr)	
			HA1 gene	HA2 gene
China	2005–2010	Yes	7.28	3.97
Indonesia	2003–2009	Yes	7.75	4.37
China	1996–2004	No	3.37	2.34
Thailand	2004–2008	No	2.69	2.44

Article

Chinese Science Bulletin

Molecular Virology

July 2012 Vol.57 No.19: 2419–2424
doi: 10.1007/s11434-012-5215-y

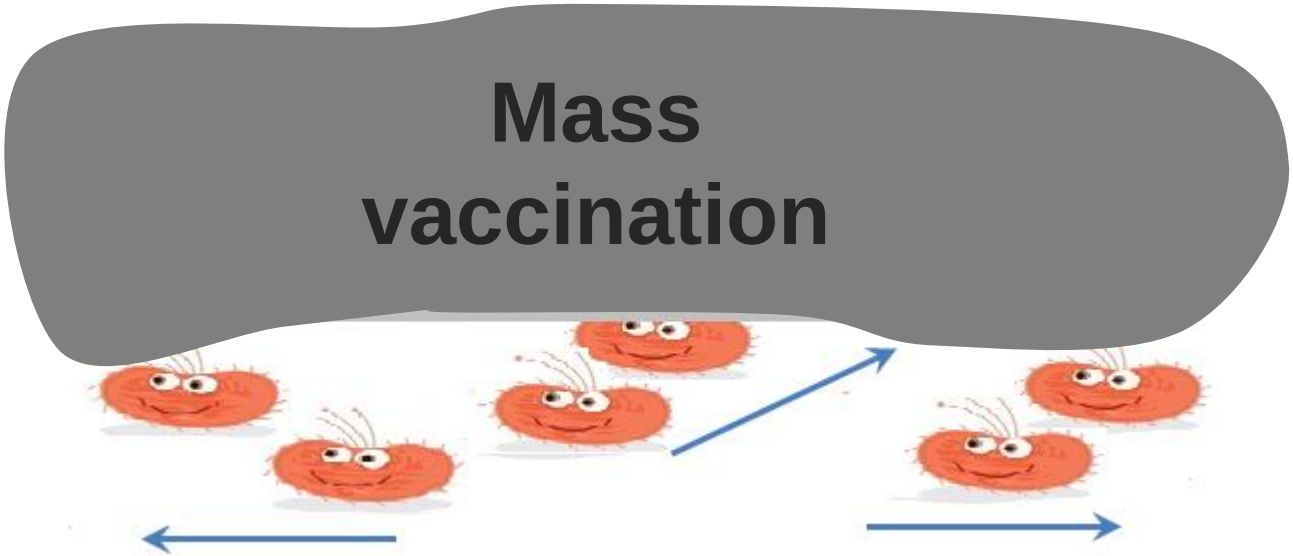
Increased substitution rate in H5N1 avian influenza viruses during mass vaccination of poultry

WANG ZhaoGuo[1], JIANG WenMing[2], LIU Shuo[2], HOU GuangYu[2], LI JinPing[2], WANG ZhiYu[1*] & CHEN JiMing[2*]

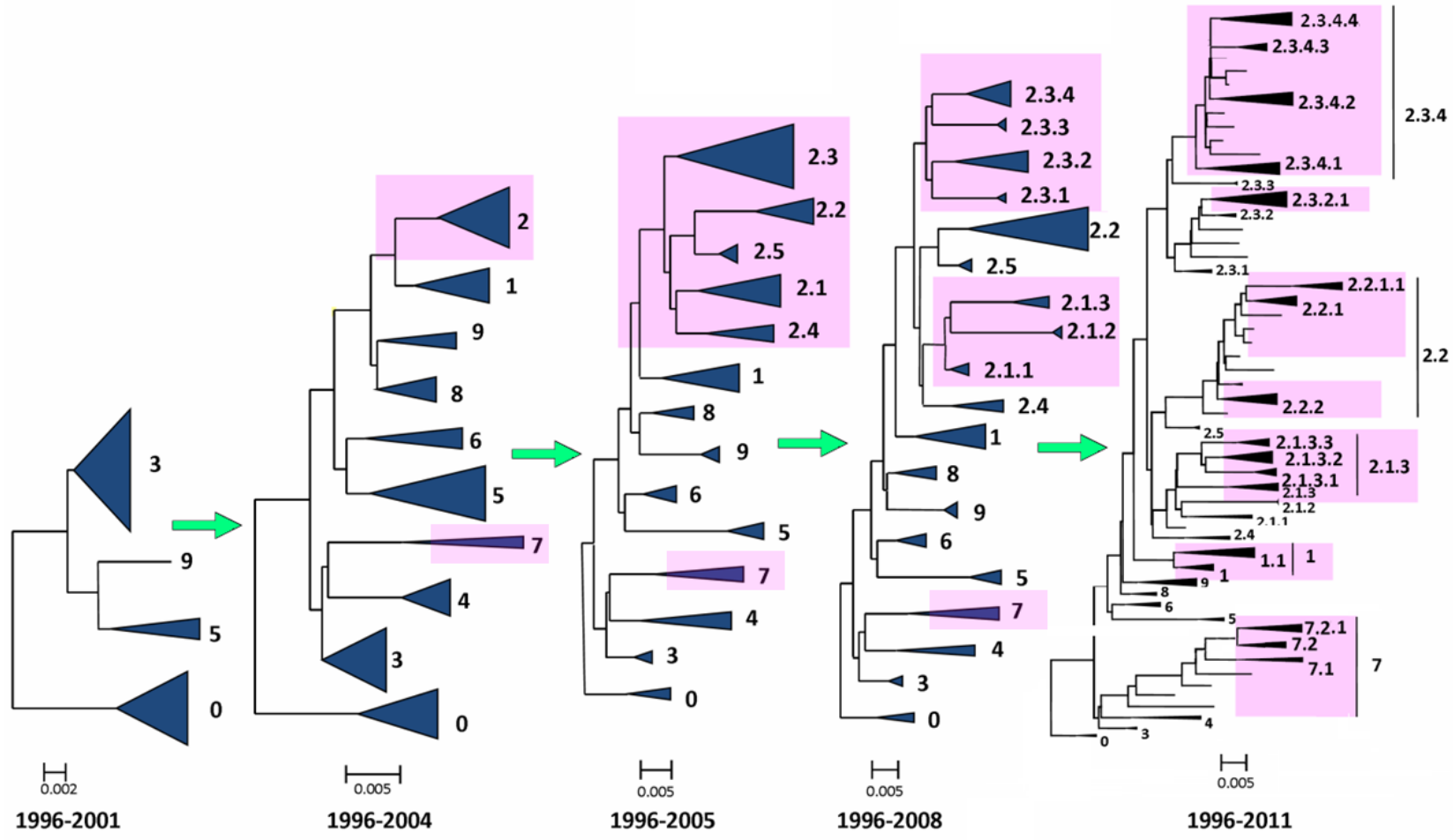
[1] *College of Public Health, Shandong University, Jinan 250012, China;*
[2] *China Animal Health and Epidemiology Center, Qingdao 266032, China*

Received November 29, 2011; accepted March 8, 2012

H5 vaccination:
accelerating the viral diversification



The virus can escape from vaccination via various venues: diversification


3
9
5
0
0.002
1996-2001
2
1
9
8
6
5
7
4
3
0
0.005
1996-2004
2.3
2.2
2.5
2.1
2.4
1
8
9
6
5
7
4
3
0
0.005
1996-2005
2.3.4
2.3.3
2.3.2
2.3.1
2.2
2.5
2.1.3
2.1.2
2.1.1
2.4
1
8
9
6
5
7
4
3
0
0.005
1996-2008
2.3.4.4
2.3.4.3
2.3.4.2
2.3.4.1
2.3.4
2.3.3
2.3.2.1
2.3.2
2.3.1
2.2.1.1
2.2.1
2.2
2.2.2
2.5
2.1.3.3
2.1.3.2
2.1.3.1
2.1.3
2.1.3
2.1.2
2.1.1
2.4
1.1
1
1
9
8
6
5
7.2.1
7.2
7.1
7
4
3
0
0.005
1996-2011

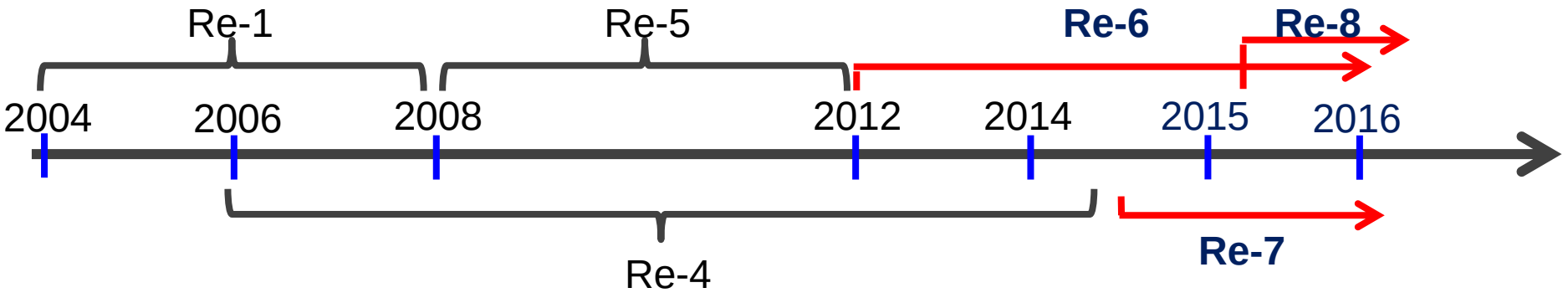
Genetic distances between the 3 clades have increased

	2005-2006	2007-2008	2009-2010	2011-2012
2.3.2 vs 2.3.4	3.31%	6.60%	9.21%	10.05%
2.3.2 vs 7.2	7.07%	11.31%	12.77%	13.19%
2.3.4 vs 7.2	7.05%	9.63%	11.59%	12.52%

Cross-protection of vaccines declined

More vaccine strains needed

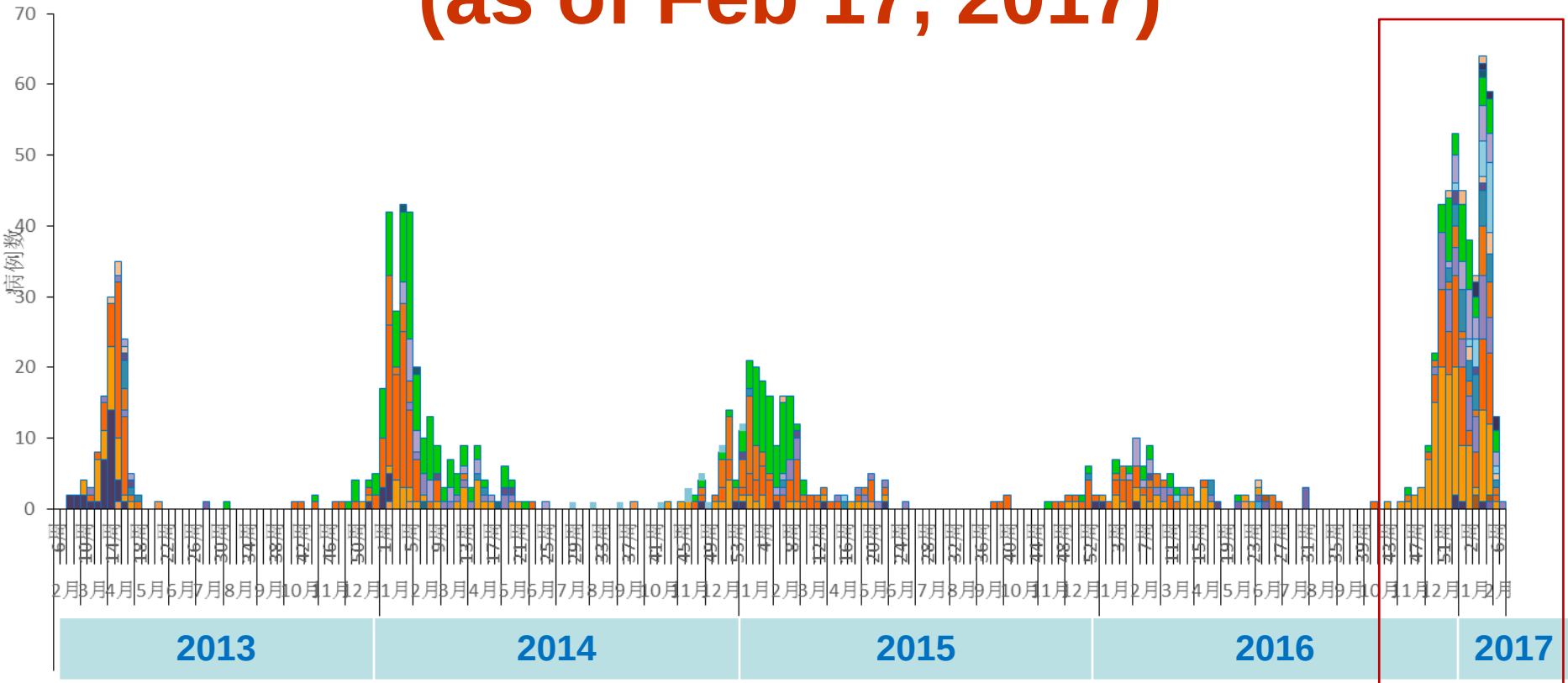
- **Before 2006, one vaccine strain was enough**
- **2006-2009, two vaccine strains (bivalent vaccine) were needed**
- **In 2010s, sometimes three vaccine strains (trivalent vaccine) were needed**



Evolution & Vaccination of H7 HPAIV in China

Five waves of H7N9: human cases

(as of Feb 17, 2017)



Province	12	13	14	15	16
Case	134	304	219	118	436
Death	44	127	100	48	141
CFR	33%	42%	46%	41%	32

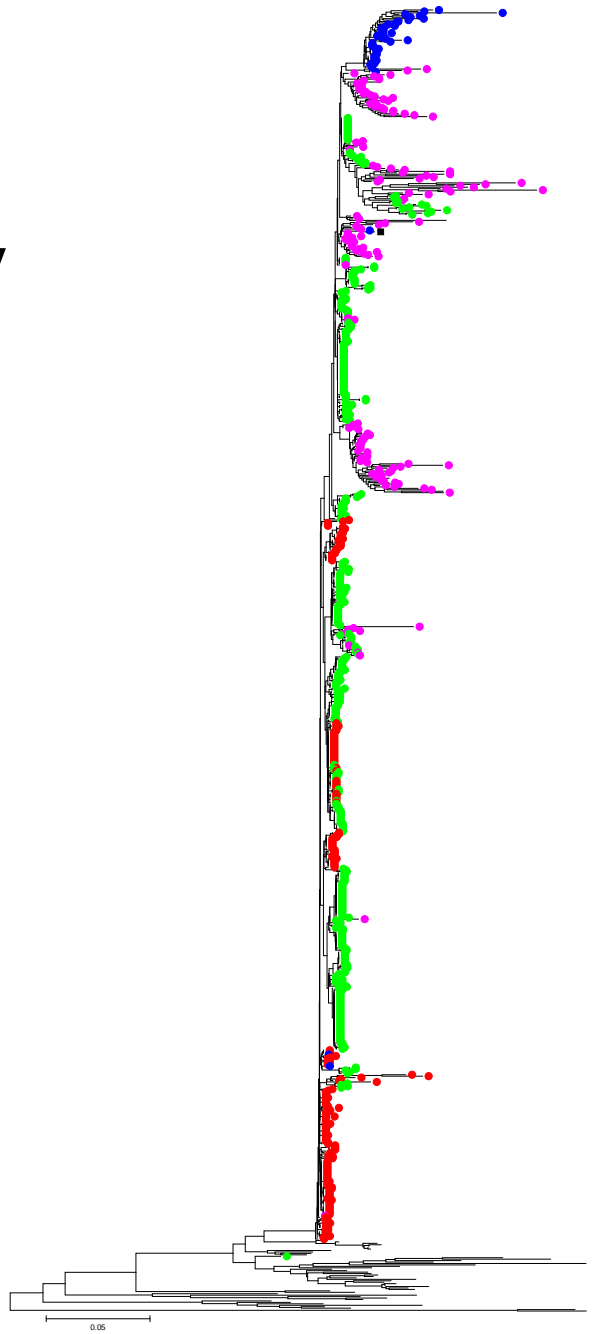
H7 AIV HA gene Changed gradually

2013 ●

2014 ●

2015 ●

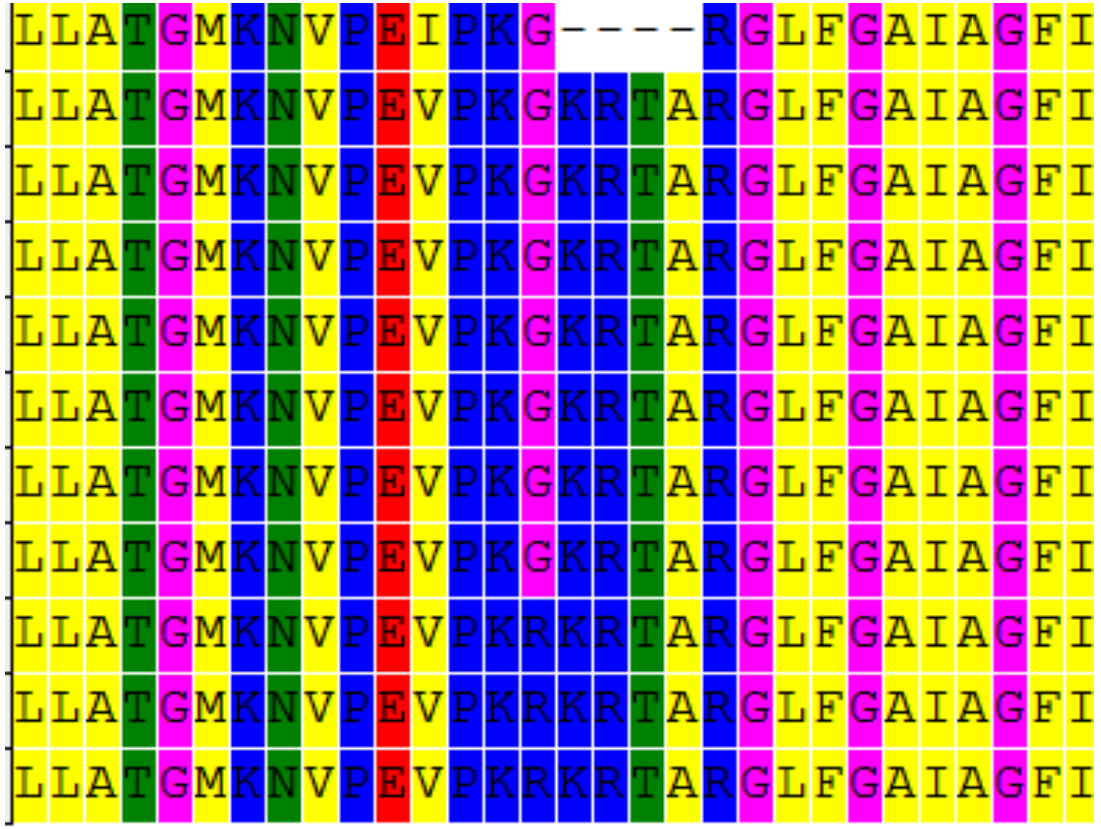
2016 ●



Frequent reassortment with H9N2

J Virol, 2016 90(12):5561-5573

H7N9 LPAIV mutated into HPAIV in Guangdong province in 2016 and in Hunan province in 2017



Intensive risk analysis: ECDC

http://ecdc.europa.eu/en/publications/Publications/09-mar-2017-RRA-update-6-influenza-A-(H7N9)-China-Taiwan.pdf

Risks in general

- **Of great danger threatening the poultry in China**
- **No evidence of increased risk threatening public health has been identified**

Vaccination against H7 AIVs?

Heatedly debated

Disadvantages of vaccination

- **Expensive**
- **Laborious**
- **Accelerate viral mutation**
- **Lead to subclinical infections**
- **Lead to unfailing fails**

Advantages

- **Not that expensive: replace Re-7 in the H5 vaccine by a H7N9 strain as H5_clade 7.2 has disappeared for 2 years**

Advantages

- **Not that expensive: replace Re-7 in the H5 vaccine by a H7N9 strain as H5_clade 7.2 has disappeared for 2 years**
- **Not that laborious: combine the H5 and H7 vaccines / only one shot**

Advantages

- **Not that expensive: replace Re-7 in the H5 vaccine by a H7N9 strain as H5_clade 7.2 has disappeared for 2 years**
- **Not that laborious: combine the H5 and H7 vaccines / only one shot**
- **May not accelerate viral mutation: massively vaccinate against H7 only for one to three times**

H5 mass vaccination: for years

H7 mass vaccination: for emergence control

Advantages

- **Not that expensive: replace Re-7 in the H5 vaccine by a H7N9 strain as H5_clade 7.2 has disappeared for 2 years**
- **Not that laborious: combine the H5 and H7 vaccines / only one shot**
- **May not accelerate viral mutation: massively vaccinate against H7 only for one to three times**
- **Subclinical infections are not new: subclinical H7N9 LPAIV infections has been prevalent before vaccination**

Advantages

- **Not that expensive: replace Re-7 in the H5 vaccine by a H7N9 strain as H5_clade 7.2 has disappeared for 2 years**
- **Not that laborious: combine H5 and H7 vaccines / only one shot**
- **May not accelerate viral mutation: massively vaccinate against H7 only for one to three times**
- **Subclinical infections are not new: subclinical H7N9 LPAIV infections has been prevalent before vaccination**
- **May highly effective: H7N9 is also terrestrial AIVs, and vaccination against the terrestrial H5 clade 7.2 was highly effective**

Dual aspects of H5 & H7 crises

- Caused great economic loses
- Caused thousands of severs human cases

- Spur modernization of the poultry
- Spur development of veterinary technologies
- Spur optimization of animal disease control

Thank you!

Contact: jmchen678@qq.com