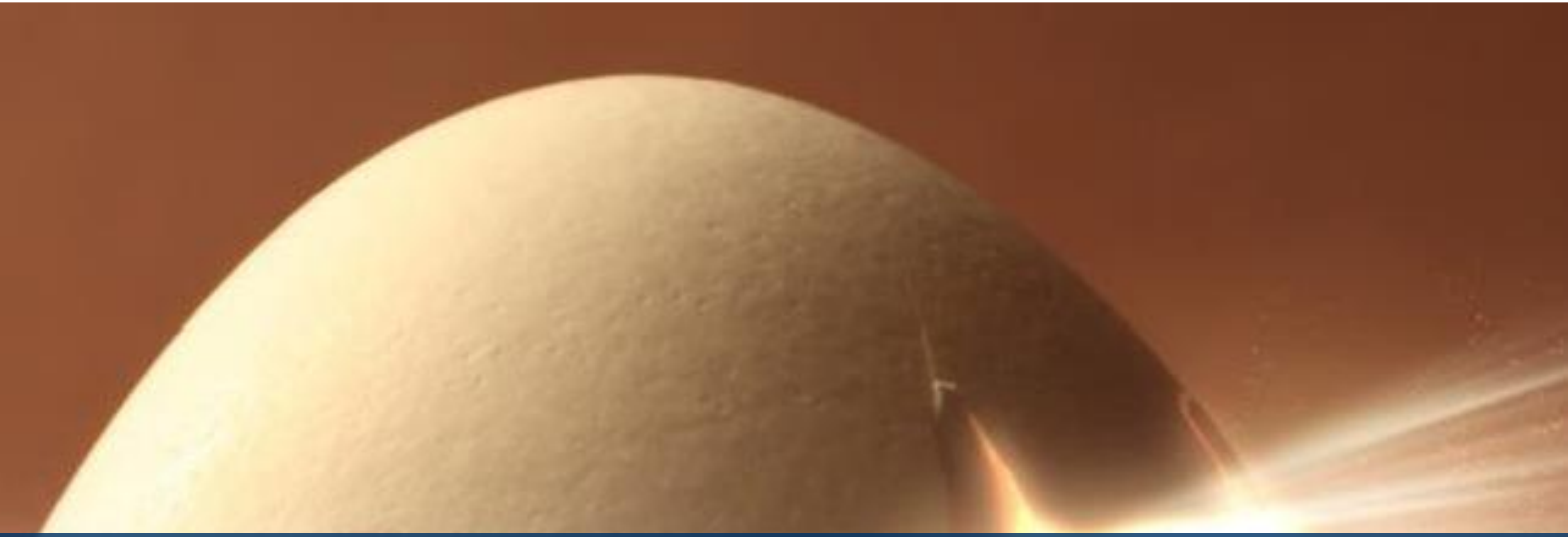


COMBAT & PREPARE FOR AVIAN INFLUENZA OUTBREAKS: (PART 2) HIGH-PATHOGEN AVIAN INFLUENZA抗击和预防禽流感爆发（二）高致病性禽流感

翻译：袁吉磊

October, 30th, 2018

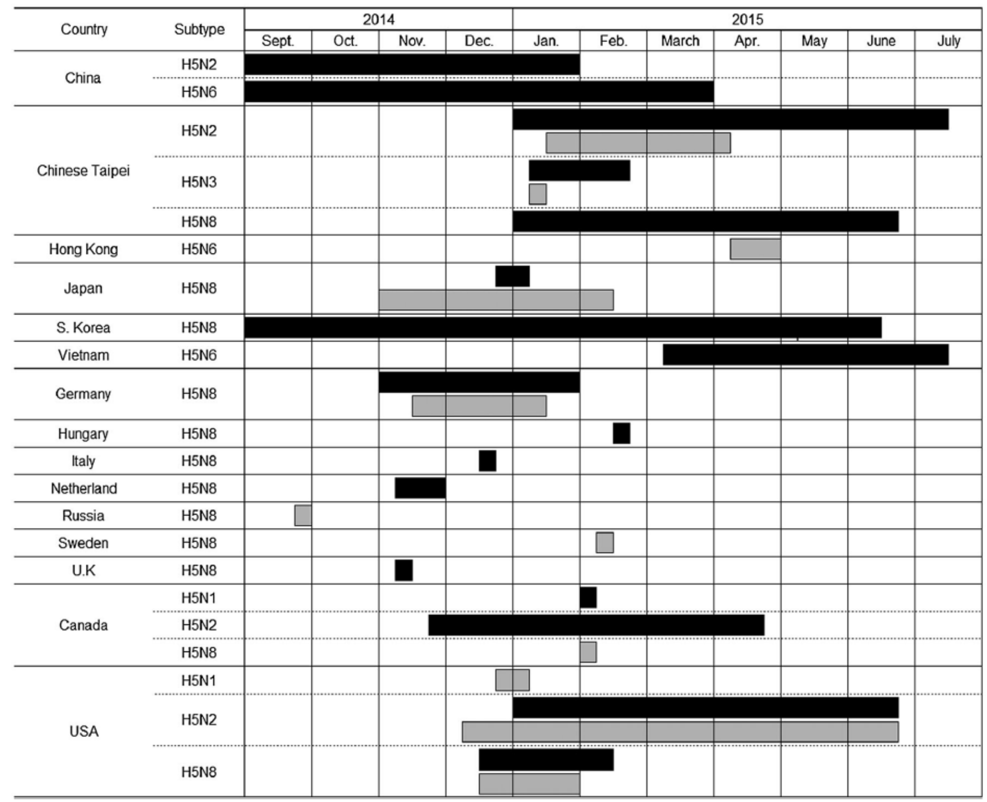
HIGHLY PATHOGENIC AVIAN INFLUENZA H5NX CLADE 2.3.4.4高致病性禽流感H5NX-2.3.4.4分支

- H5N1 HPAI is not a single virus, but a family of viruses with any clades and subclades
- H5N1 HPAI不是单一病毒，而是带有任何分支和亚分支的病毒家族。
- H5NX HPAI Clade 2.3.4.4. viruses have variable biological properties
- H5NX HPAI-2.3.4.4分支具有可变的生物学特性。

2014-2015年HPAI-2.3.4.4分支的传播

表2 2014-2015冬季H5-HPAI-2.3.4.4分支病毒毒株和爆发情况

Table 2. Clade 2.3.4.4 H5 HPAIV strains and outbreaks in the winter of 2014–2015*



*Black bars denote poultry outbreaks, and gray bars denote isolation from wild birds. Data were obtained from OIE (http://www.oie.int/animal-health-in-the-world/web-portal-on-avian-influenza/) and USDA (http://www.usda.gov/wps/portal/usda/usdahome?contentid=avian_influenza.html) web sites.

*黑色条形表示家禽爆发，灰色表示从野鸟分离到的。数据来源国际兽医局OIE和美国农业部。

[Saito T et Al, 2015, Rev. Med. Virol., 25, 388–405]

HIGHLY PATHOGENIC AVIAN INFLUENZA H5NX CLADE 2.3.4.4高致病性禽流感H5NX-2.3.4.4分支

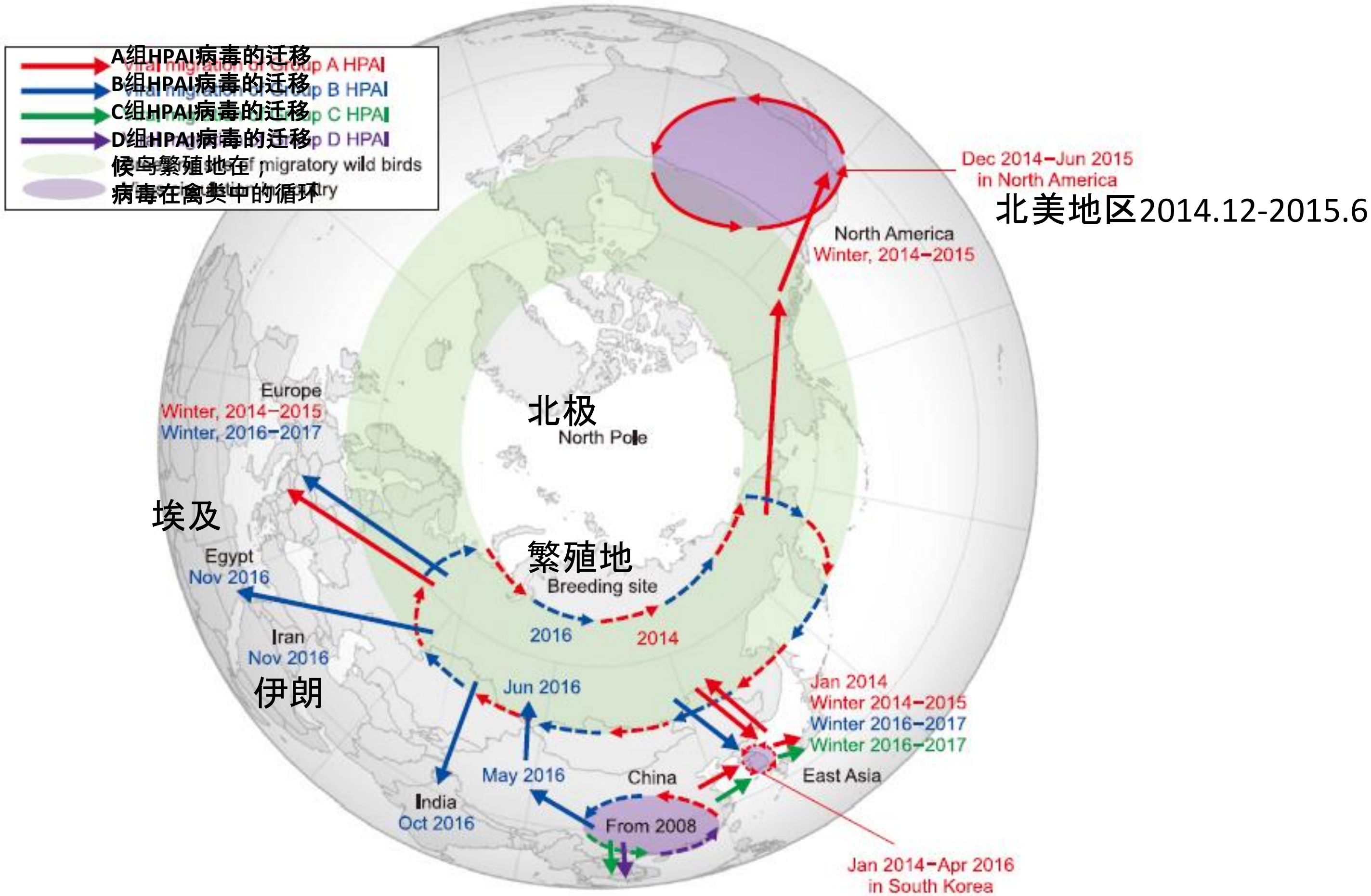


Fig. 1. Geographic map showing movement patterns of highly pathogenic avian influenza (HPAI) clade 2.3.4.4 viruses.
图1 HPAI-2.3.4.4分支病毒运动模式地图

[Lee D.H. et Al, 2017, J.Vet. Sci., 18, 269–280]

AVIAN INFLUENZA H5 ANTIGENIC DRIFTS
禽流感H5抗原漂移的研究

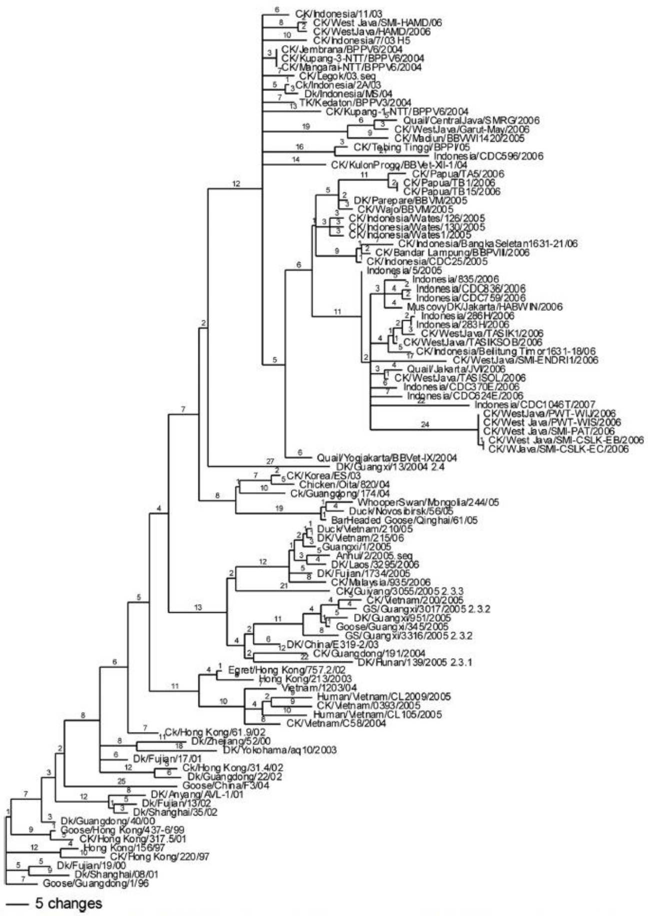


FIG 2 Phylogenetic tree of representative H5N1 isolates from Indonesia and other countries. DK and Dk, duck; TK, turkey; CK and Ck, chicken; GS, goose. The numbers on nodes are bootstrap values.

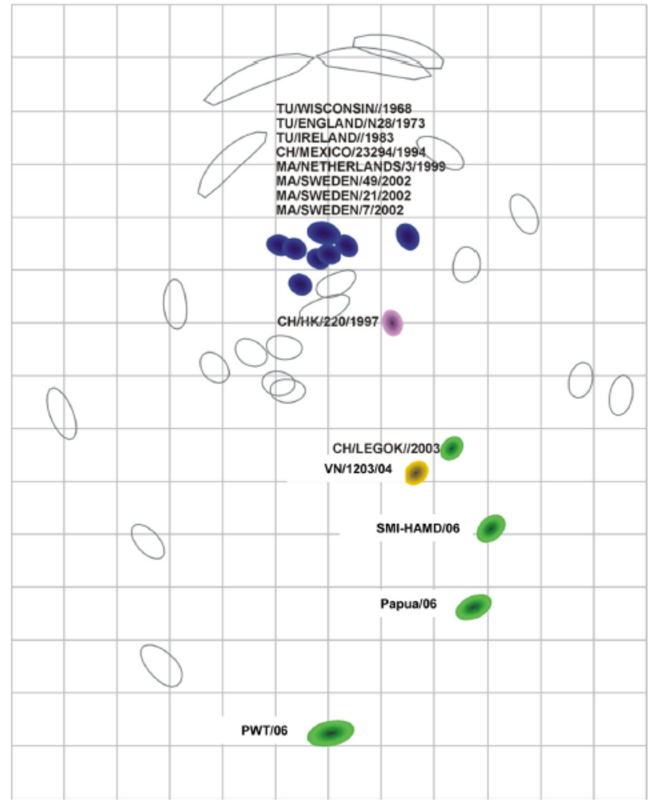
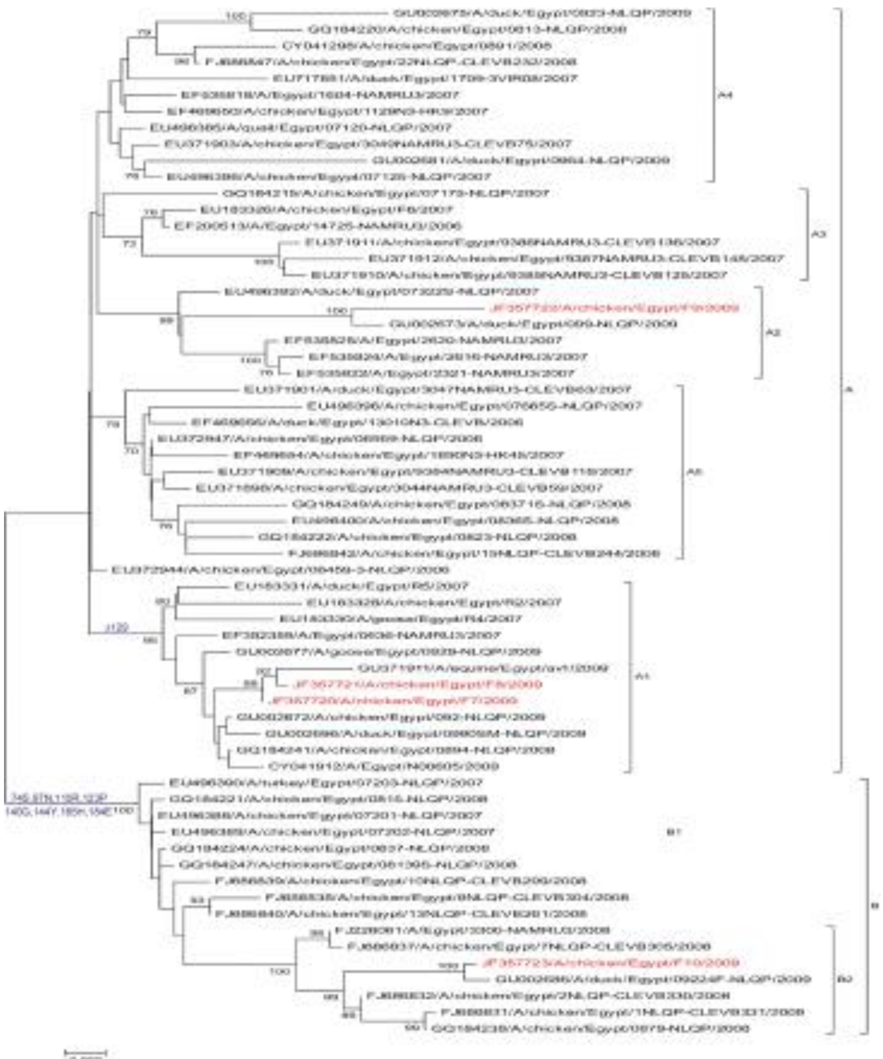


FIG 3 Cartographic map of H5 avian influenza viruses produced with chicken antisera. The colored shapes (viruses) and open shapes (antisera) are relative positions adjusted such that the distances between viruses and antisera in the map represent the corresponding HI measurements with the least error. The blue fill represents the antigenic root from classic H5 influenza viruses, including four viruses of wild bird origin (A/mallard/Netherlands/3/1999 [MA/NETHERLANDS/3/1999], A/mallard/Sweden/49/2002 [MA/SWEDEN/49/2002], A/mallard/Sweden/7/2002 [MA/SWEDEN/7/2002], and A/mallard/Sweden/21/2002 [MA/SWEDEN/21/2002]) and four viruses of poultry origin (A/turkey/Wisconsin/1968 [TU/WISCONSIN/1968], A/turkey/England/N28/1973 (TU/ENGLAND/N28/1973], A/turkey/Ireland/1983 [TU/IRELAND/1983], and A/chicken/Mexico/23294/1994 [CH/Mexico/23294/1994]). The other solid colors represent six H5N1 Guangdong lineage viruses (A/chicken/Hong Kong/220/1997 [CH/HK/220/1997], A/chicken/Legok/2003 [CH/LEGOK/2003], A/Vietnam/1203/2004 [VN/1203/04], A/chicken/West Java/SMI-HAMD/06 [SMI-HAMD/06], A/chicken/Papua/TA5/06 [Papua/06], and A/chicken/West Java/PWT-WIJ/06 [PWT/06]). Since the relative positions of antigens and antisera are determined and both the vertical and horizontal axes represent antigenic distance, the orientation of the map within these axes is free. The spacing between grid lines is 1 unit of antigenic distance, corresponding to a 2-fold dilution of antiserum in the HI assay.

图3 用鸡的抗血清生产的H5禽流感病毒的图谱。彩色形状（病毒）和开口形状（抗血清）市调整后的相对位置，在地图上病毒和抗血清的距离代表以最小的误差测得的HI值。蓝色填充代表经典H5病毒抗原根，包括4种野鸟源病毒和4种禽源病毒。另外实色代表6种广东种系病毒。由于确定了抗原和抗血清的相对位置，并且垂直轴和水平轴痘表示 抗原的距离，这些轴上地图的方向是自由的网格线间距威1格抗原的距离单位，相当于HI方法中抗血清的2倍稀释度。]

[Swayne D.E. *et Al.*, 2015, J. Virol., 89, 3746-3762]

AVIAN INFLUENZA H5 ANTIGENIC DRIFTS
禽流感H5抗原漂移的研究

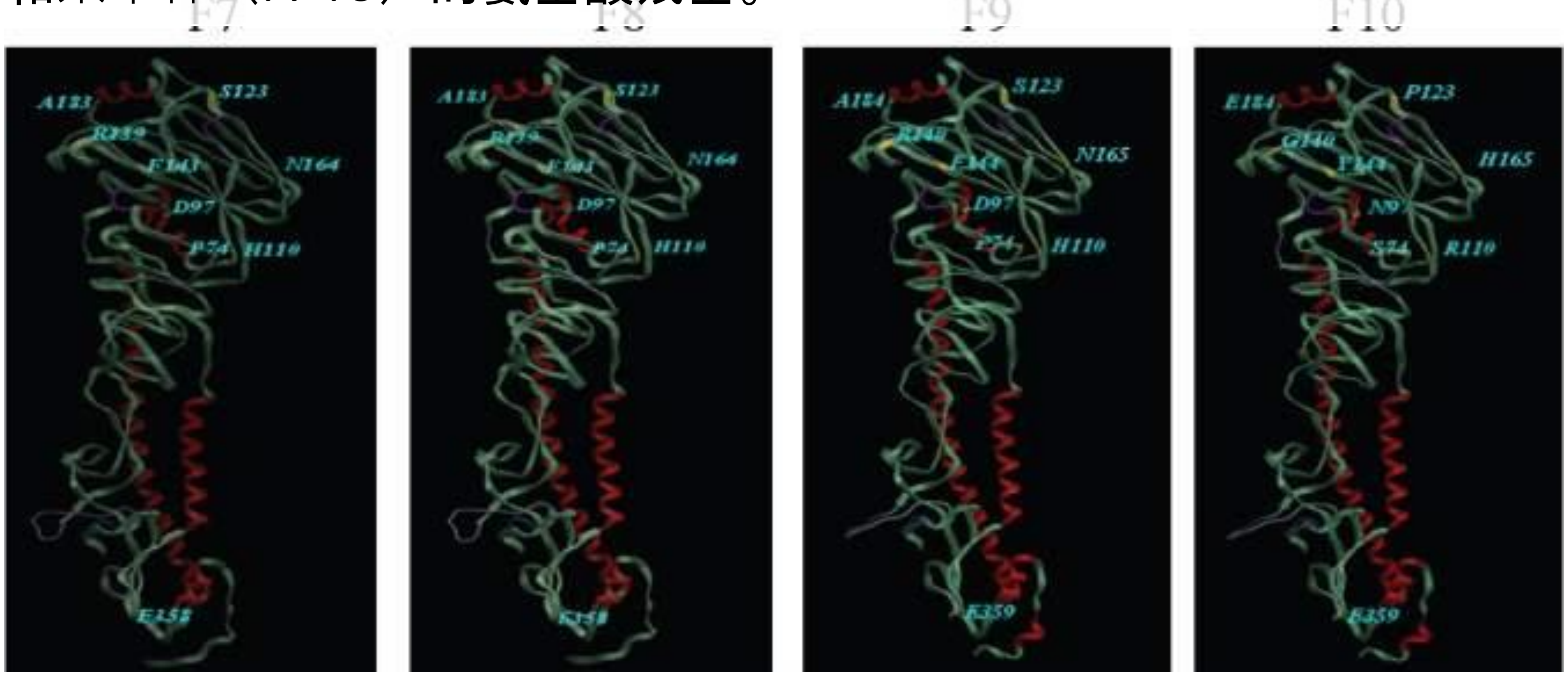


Phylogenetic tree of viral HA sequences of the Egyptian H5N1 viruses generated by neighbor-joining analysis
邻接法分析得出的埃及**H5N1病毒的HA基因序列的**进化树。

[Abdel-Moneim A.S. et Al., 2011, Virol. J., 8, 283-209]

AVIAN INFLUENZA H5 ANTIGENIC DRIFTS
禽流感H5抗原漂移的研究

Ribbon diagram of the trimeric HA molecule with depicts the amino acid residues differentiating variant strain (F10) from parent ones (F7-F9) shown in yellow三聚体HA分子的带状图，描述了区分变异株（F10）和亲本株（F7-F9）的氨基酸残基。



[Abdel-Moneim A.S. *et Al.*, 2011, Virol. J, 8, 283-209]

HP AVIAN INFLUENZA VACCINATION STRATEGIES
高致病性禽流感免疫接种策略

Available technologies – vector vaccines
可用技术-载体疫苗
Fowl Pox (H5, H7)
Newcastle disease virus (H5)
HVT (H5)
鸡痘（ H5,H7)
新城疫病毒（H5)
HVT(H5)

Available technologies – inactivated vaccines可用技术-灭活疫苗
Full virus – ovoculture (H5, H7, H9)
Reverse genetics (Re series China H5)
Expression systems – baculovirus (H5, H7)Etc. 全病毒-胚内培养（H5,H7,H9)
反向遗传学（ Re系列中国H5)
表达系统-杆状病毒（H5,H7)等等。

Vaccination programs免疫接种程序

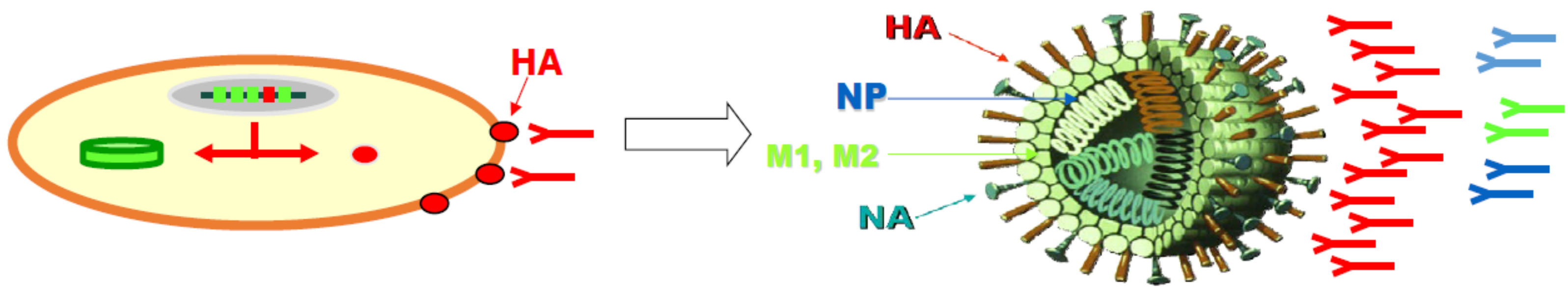
Day-old application of vector vaccines (vFP + vHVT)， Combo inactivated vaccines with other components like ND， Compatibilities with other vaccines of inac vaccines used， starting from less susceptibility to maternal antibodies载体疫苗(vFP + vHVT)的应用，结合含有像ND成分的灭活苗，考虑到灭活疫苗和其他疫苗的兼容性， 先从对母抗不敏感的开始使用。

HP AVIAN INFLUENZA VACCINATION STRATEGIES高致病性禽流感免疫策略

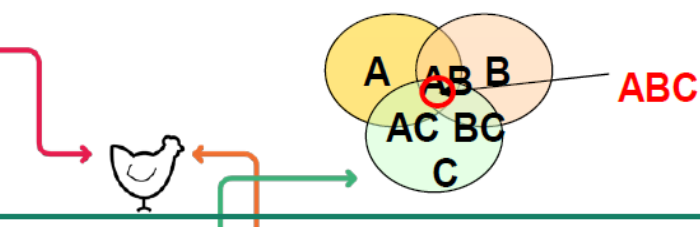
Enlargement of cross-protection spectrum by combining vaccines – PRIME-BOOST联合疫苗来扩大交叉保护谱-加强免疫

Currently H5& H7 to come

1. **Different antigen presentation** 1、与众不同的抗原递呈
   - **Fowlpox:** Cell-Mediated Immunity -鸡痘：细胞介导免疫
   - **Inactivated:** Humoral Immunity -灭活疫苗：体液免疫
2. **Boost directed to protective antigen** (HA)
   2、针对保护性抗原HA增强作用



3. **Boost with a different HA** to increase response against conserved epitopes (broader response) 3、用不同的HA来增强针对保守抗原表位的免疫反应（来获得更广泛的反应）



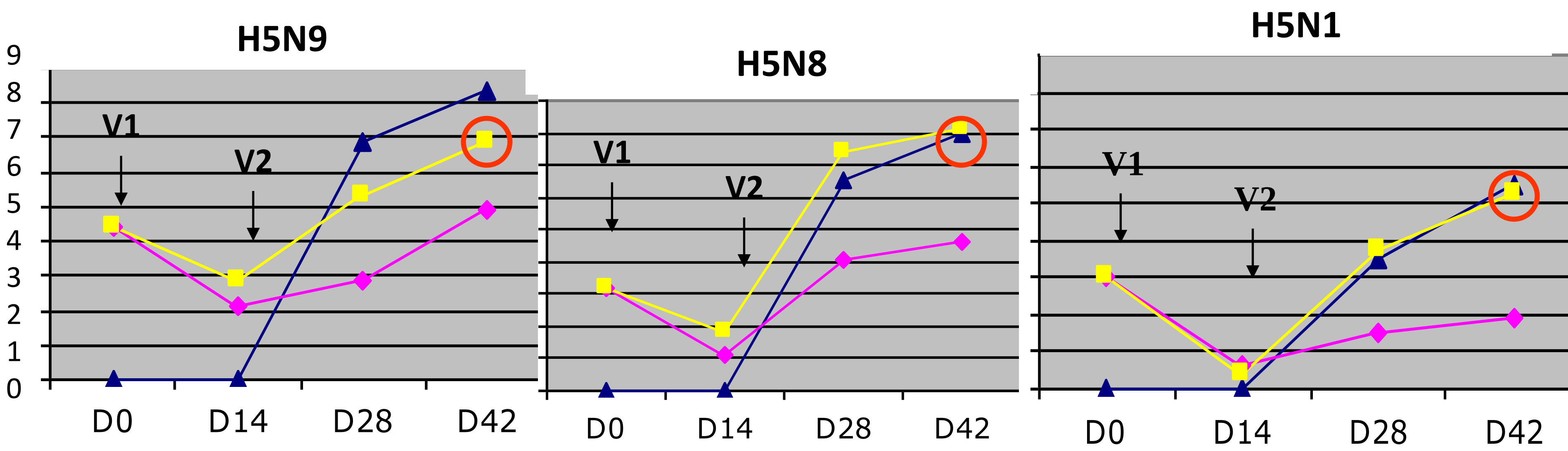
2针灭活疫苗

B + B ⇒ B mainly	(2 shots of inactivated vaccine)
A + B ⇒ AB + ABC	(prime-boost) 加强免疫

HP AVIAN INFLUENZA VACCINATION STRATEGIES

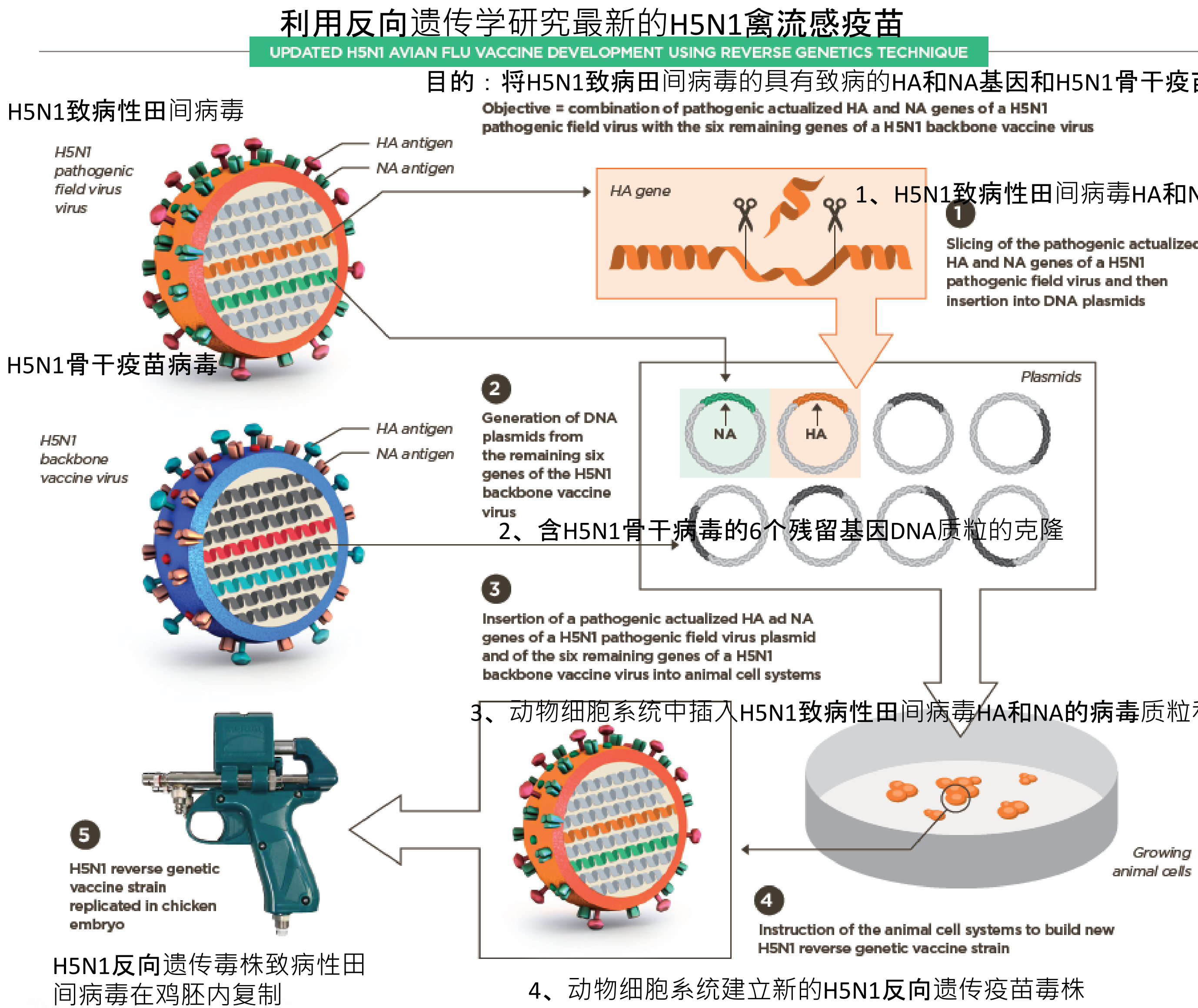
VACCINATION AGAINST AVIAN INFLUENZA H5N1 HP Prime-boost protocol H5N1高致病性禽流感加强免疫计划

	Breeder 种鸡	D0 （1日龄）	D14 （14日龄）
▲	SPF	-	H5N9 It
◆	AI 3x	-	H5N9 It
■	AI 3x	vFP89	H5N9 It



FP AIV-H5 priming overcomes MDA interference on inactivated vaccine鸡痘载体疫苗克服了母抗对灭活疫苗干扰的缺陷

AVIAN INFLUENZA H&N PROTEIN ENGINEERING
禽流感H&N蛋白工程



Re series:

H5N1 Re-5

H5N1 Re-6

H5N1 Re-8

H7N9 Re-1

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

- Engineering of the insert to match currently present H5N1 HPAI viruses
  与目前流行的H5N1型HPAI病毒匹配的插入物的工程
- Expression of antigen in insect cells after infection with a recombinant baculovirus encoding for inserted sequence编码插入序列的重组杆状病毒感染后在昆虫细胞内的抗原表达

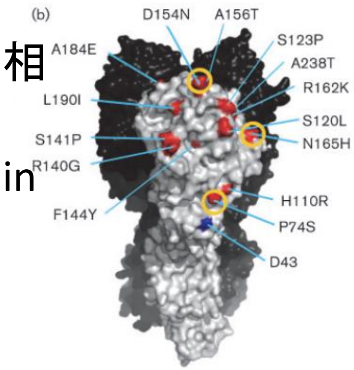
A/Dk/China/E319-2/03 (H5N1) **Clade 2.3.2**

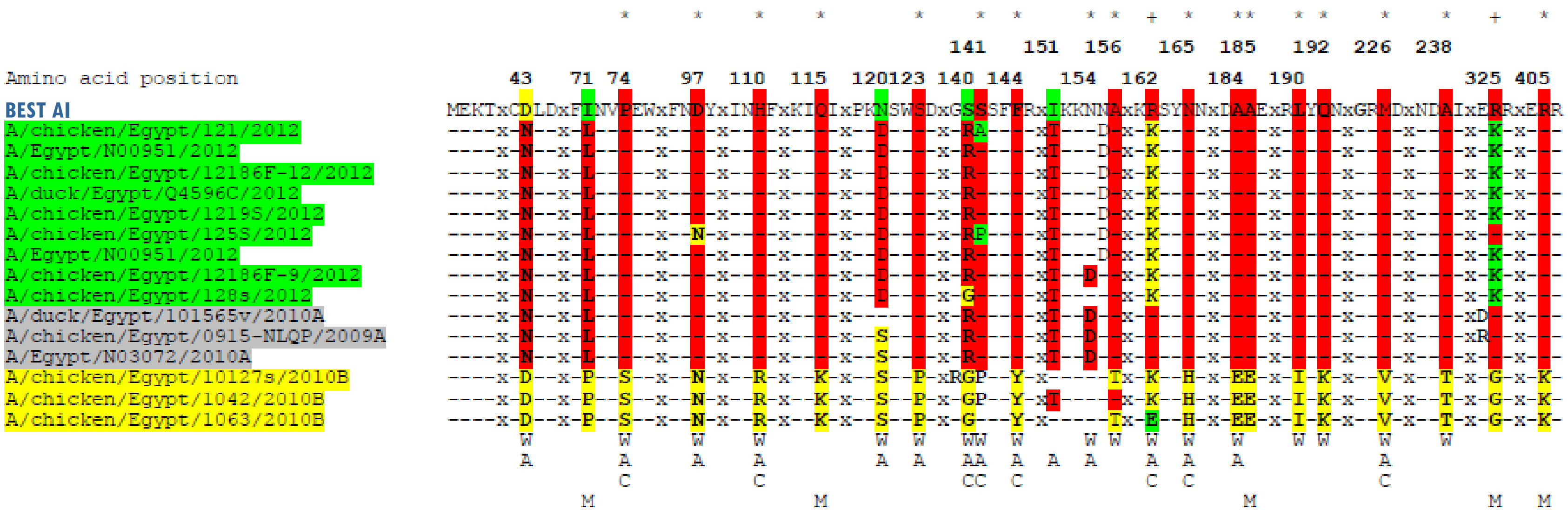
↓

AI rH5

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

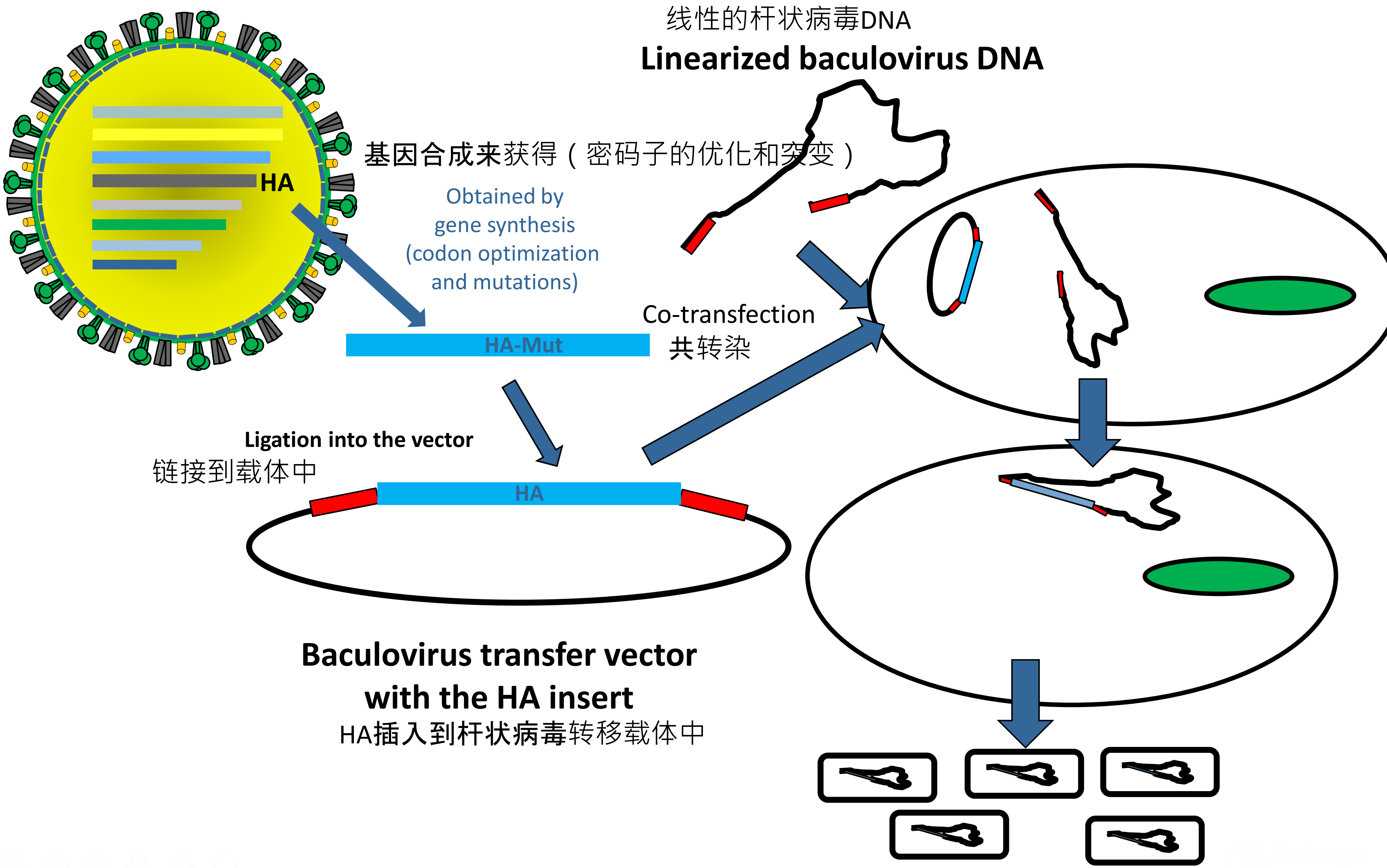
- The AI component resembles closely the currently prevalent Clade A H5N1 HPAI viruses禽流感重组的成分和当前流行的分支A H5N1 HPAI病毒非常相似。
- 18 out of 24 amino acids specific for the Clade A H5N1 viruses are present in the HA antigen
- 分支A H5N1病毒中的特异的24个氨基酸中由18个是存在于HA抗原中





[Abdelwhab et Al., 2012, Virus Genes, 45, 14–23]
[Cattoli G. et Al., 2011, J Virol, 85, 8718–8724]
[Watanabe et Al, 2012, J Gen Virol, 93, 2215-2226]

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

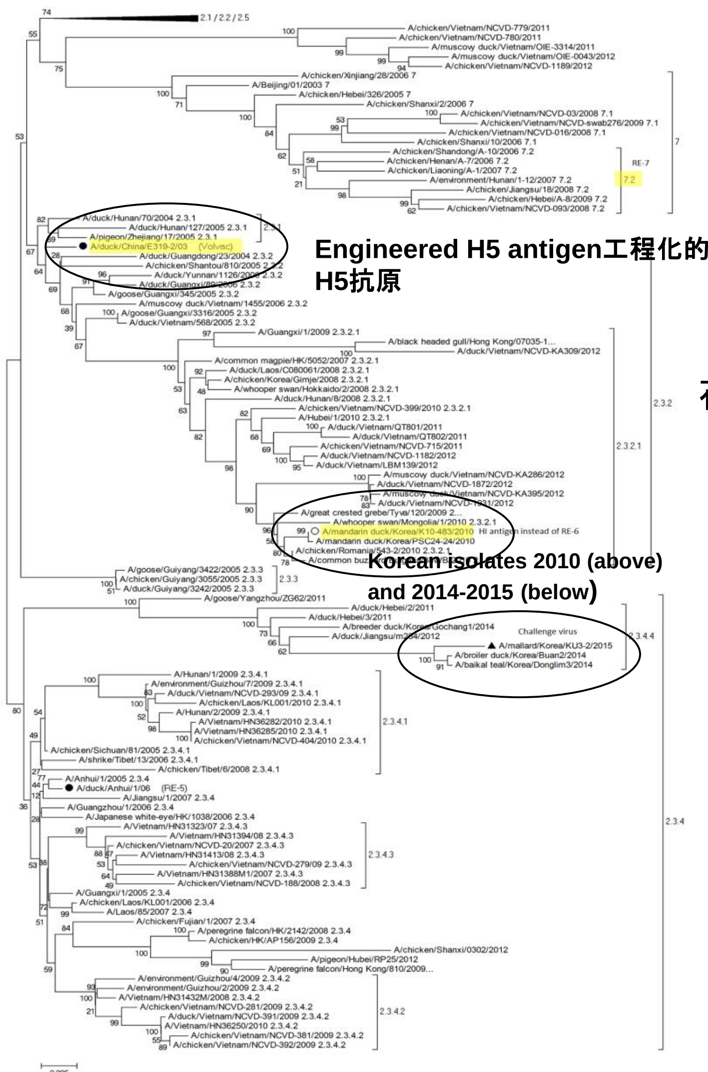


AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

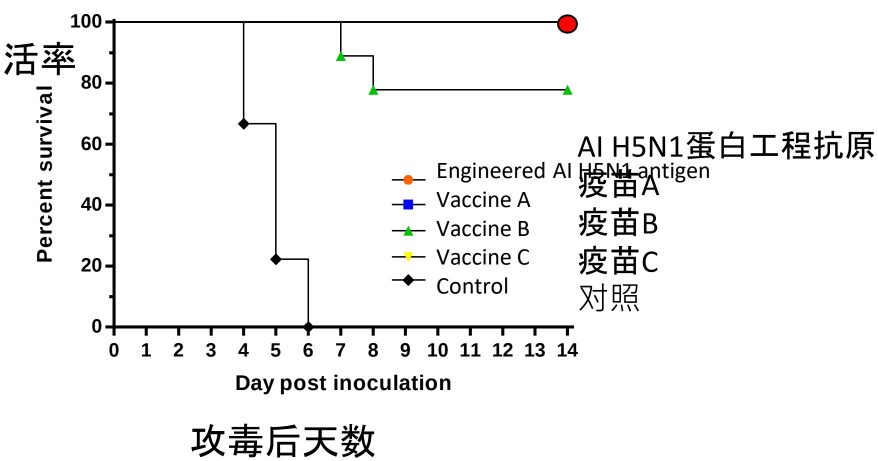
组别 Group	挑战病毒的起源国家 Country of Origin of challenge virus	挑战病毒的起源种类 Species of Origin of challenge virus	分离年份 Year of isolation	病毒分支 Virus Clade	死亡率和临床症状的保护力 Protection against mortality and clinical signs	最大排毒天数（PCR+） Maximum shedding in days (Positives by PCR)
1	墨西哥 Mexico (H5N2)	Chicken 鸡	2004	0	100%	Not done 未做
2	Vietnam 越南	Duck 鸭	2005	2.3.2	100%	3
3	Spain 西班牙	Chicken 鸡	2006	2.2	100%	4
4	Egypt 埃及	Chicken 鸡	2008	2.2.1.1**	90%	3
5	Egypt 埃及	Chicken 鸡	2010	2.2.1*	100%	Absent 缺乏）
6	Egypt 埃及	Chicken 鸡	2010	2.2.1.1**	80%	7
7	Egypt 埃及	Chicken 鸡	2012	2.2.1*	100%	7

* Also known as Clade A
** Also known as Clade B

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程



Phylogenetic tree representing different isolates of Korean AI H5NX virus 韩国AI H5NX病毒不同分离株呈现的进化树



AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

组别	死亡数/总数	口腔排毒数/总数	泄殖腔排毒数/总数	排毒天数：口腔/泄殖腔
Group	**Mortality/total**	**Oral Shedding/ total**	**Cloacal shedding/ total**	**Days shedding Oral/Cloacal**
Engineered H5	**0/9**	**6/9**	**0/9**	**3.5 / 0**
Controls	**9/9**	**9/9**	**9/9**	**3.9 / 3.7**

- Antigenic drift is the challenge抗原漂移是挑战
- Cross-protection induced by vaccination programs
- 免疫接种引起的交叉保护
- Engineered proteins to optimize immune response
- 工程蛋白来优化免疫反应

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

Volvac® B.E.S.T. AI+ND KV RSA H5N8 challenge study

Volvac B.E.S.T AI+ND KV RSA **疫苗**H5N8 **攻毒保**护研究

Zero mortalities were recorded in vaccinated birds, according to the Sponsor's definition, the vaccine attained 100% efficacy in this study. (Section 7.2, pg18-19).

根据发起者的定义，试验表明：免疫接种的鸡死亡率为零，本研究中该疫苗获得**100%的有效性**

Volvac B.E.S.T AI+ND KV induced a drastic reduction of shedding in vaccinated chickens. At the respective peak shedding points, non-vaccinated birds in Group B shed an estimated mean of 14.84 million viral particles per tracheal swab compared to 115,507 viral particles per tracheal swab in Group A. Group B shed an average 822,603 viral copies per cloacal swab compared to 217 in Group A (Section 7.4, pg26-29).

Volvac B.E.S.T AI+ND KV **可以极大的降低免疫**鸡的排毒时间。同时在各自的排毒高点，未免疫的B组，**气管棉拭子排毒量**为1484**万**，**而免疫**组A对应的为115507；**泄殖腔拭子排毒量分**别为，B组为822603，**而**A组为217**个病毒拷**贝数。

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

Volvac® B.E.S.T. AI+ND KV RSA H5N8 challenge study

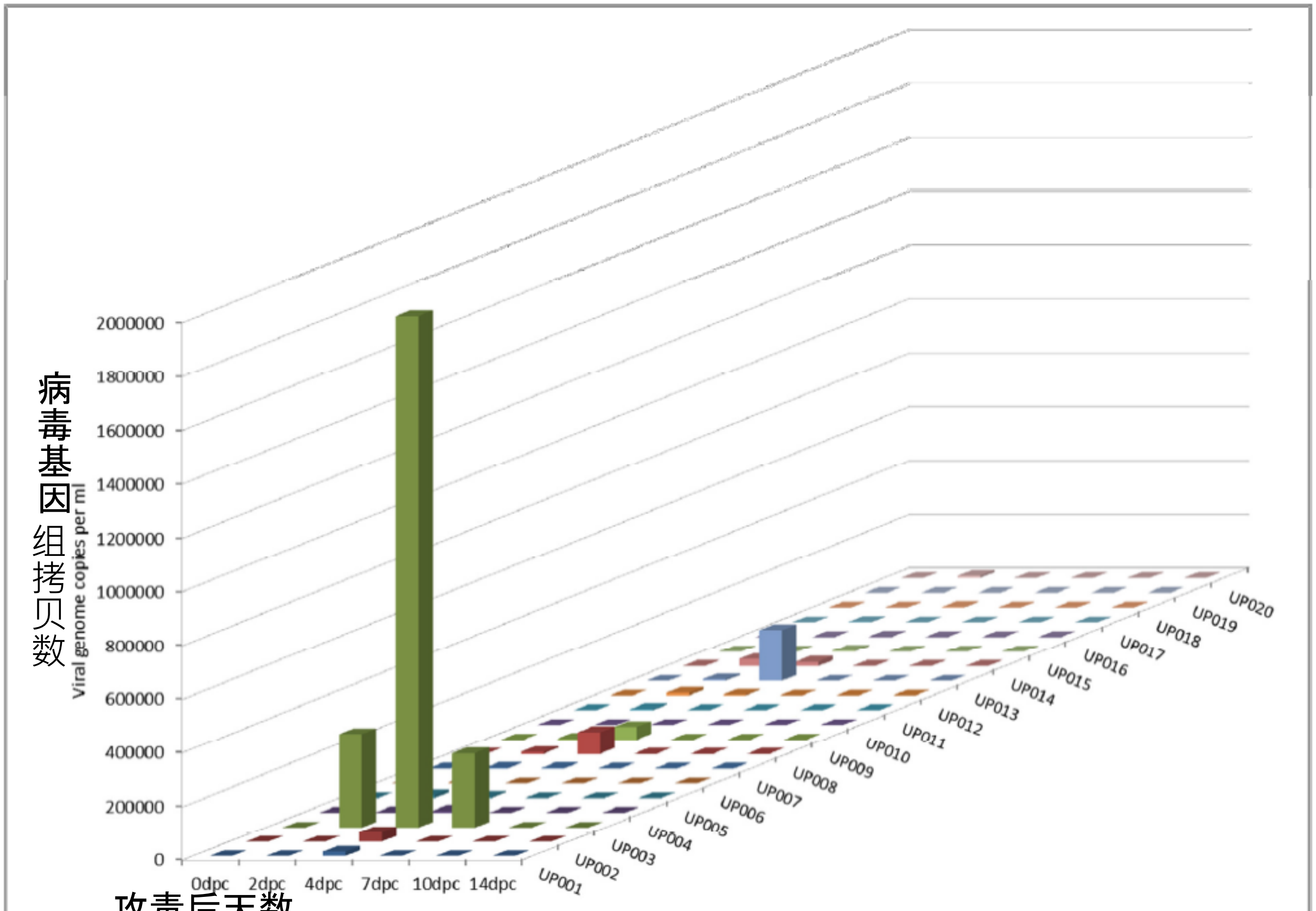


Fig 1: Oropharyngeal shedding of HPAI H5N8 virus in Group A (vaccinated) chickens.

表1 A组（接种疫苗）HPAI H5N8咽部排毒情况

AVIAN INFLUENZA H5 PROTEIN ENGINEERING
禽流感H&N蛋白工程

Volvac® B.E.S.T. AI+ND KV RSA H5N8 challenge study

表14：DIVA血清型检测结果分析

Table 14: Interpreting serological test results for DIVA

		SEROLOGY TEST RESULT 血清学结果	
	Volvac B.E.S.T AI+ND KV鸡群中产生疫苗反应	IDEXX AI ELISA	IDvet H5 ELISA
INTERPRETATION	VOLVAC® B.E.S.T AI+ND KV VACCINE REACTION IN THE FLOCK	-	+
	H5 INFLUENZA FIELD CHALLENGE IN THE FLOCK 田间鸡群存在H5挑战风险	+	+

VACCINATION AGAINST HP AVIAN INFLUENZA WITH A STRATEGY高致病性禽流感免疫策略

Prevention and control:预防和控制

- Biosecurity生物安全
- Continuous monitoring持续监测
- Eradication programs根除计划
- Training at all production levels所有生产水平的培训
- **Vaccination** (prevents clinical signs and mortality and significantly reduce virus shedding) 免疫接种（防止临床症状和死亡率，极大降低病毒排毒）

VACCINATION AGAINST HP AVIAN INFLUENZA WITH A STRATEGY高致病性禽流感免疫策略

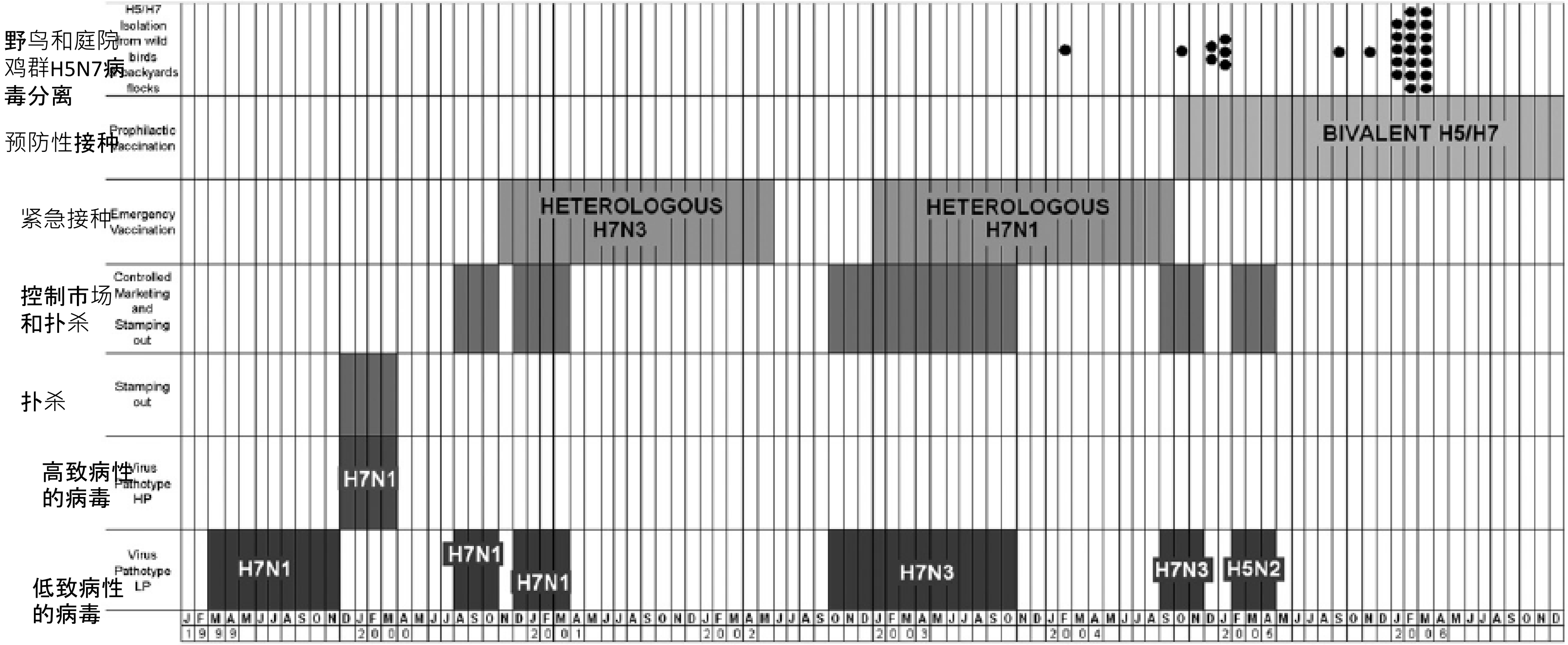


Fig. 1. Occurrence and duration of outbreaks, strains involved and control measures applied in Italy to combat Avian Influenza introductions between 1999 and 2006.

1999-2006年间意大利抗击禽流感疫情的发生和持续时间，所涉及的毒株和采取的措施

[Capua I. et Al., 2007, Vaccine, 25, 4987-4995]

Thank You!谢谢