Influenza AH5N1 – Human Infection

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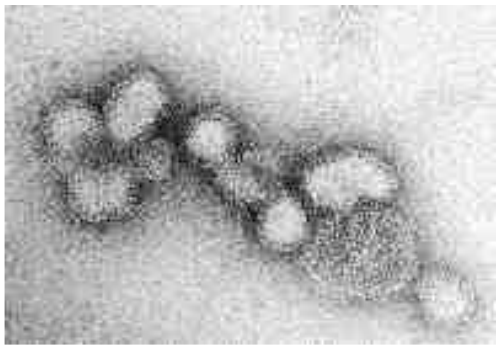


Montpellier, 15th December 2008

WHO Global Influenza Surveillance Network (GISN)

(Established in 1952)

5 WHO Collaborating Centres (Atlanta, London, Melbourne, Tokyo, Memphis)

122 National Influenza Centres (94 countries) (gaps!; IHR 2005)

Main Objectives:

- **Monitor - epidemiology of influenza and burden of disease**
  - **antigenic/genetic changes in circulating A and B viruses**
  - **geographical spread of new antigenic variants**
  - **emergence and persistence of drug resistance**

  Make biannual recommendations on human vaccine composition

- **Early detection of novel human viruses, assessment of pandemic risk**
  - **identify the virus (genetic/antigenic) – new human subtype?**
  - **identify source of infection and extent**
  - **sporadic or local clusters of infection (serological evidence)**
  - **human-human transmission?**
  - **geographical spread**
  - **develop candidate vaccine strains**

Sporadic human infections with animal viruses

Identity of the virus (genetic/antigenic) – new human subtype?

- **Swine H1N1, H1N2, H3N2 - antigenically different from human subtypes**
  (not considered likely pandemic threat)
  important to monitor changes among swine viruses
  - to readily identify source of infection (e.g. A/HK/1774/99, H3N2)
  - emergence of novel subtypes (e.g. H2N3) with increased pandemic risk

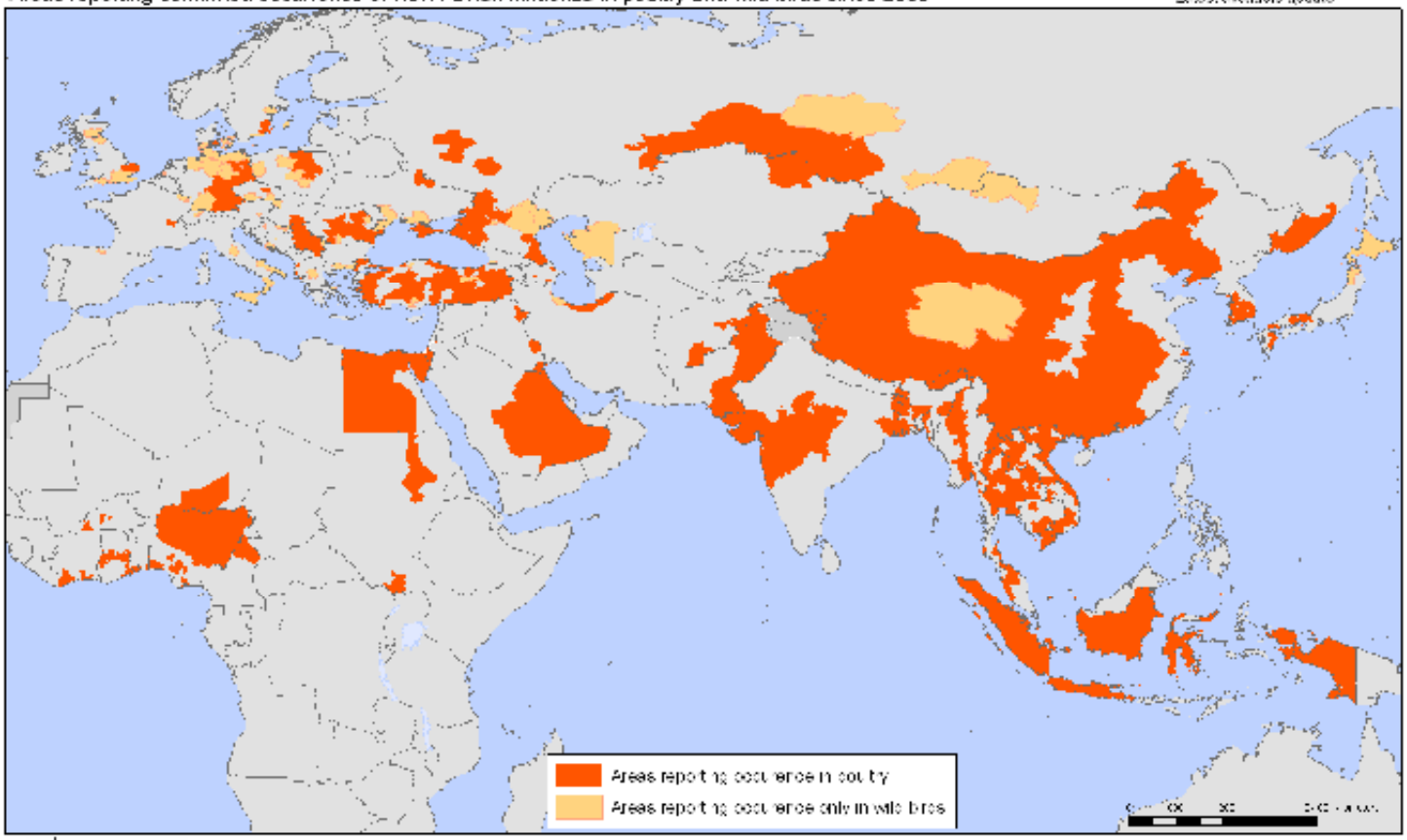
- **Avian viruses:**
  H7N7, H7N3, H7N2 - mainly mild infections, conjunctivitis
  - little change (animal/human)
  - source of infections removed
  H9N2 - mild infections; partial human receptor-binding characteristics
  - widespread
  H5N1 - highly pathogenic (~60% fatality), diverse genetically and antigenically
  - increasing spread

Influenza A (H5N1) Infection in Avain and Human Populations (1997-2008)

1997, March-May	**Outbreaks in chicken farms in Hong Kong.**
1997, May, Nov-Dec	**18 human cases, 6; all poultry slaughtered (viruses contained internal genes of H9N2 (triple reassortant) - eliminated)**
1999-2000	**Detected among geese and ducks in HK.**
2001, Apr-May	**Outbreaks among wild waterfowl in HK parks. Spread to chicken farms and live poultry markets Emergence of Z (and V) genotypes.**
2003, Feb	**2 human cases, 1 fatal (HK/Fujian, China)**
Late 2003 -	**Extensive outbreaks in E. Asia; Vietnam, Thailand, Indonesia, Malaysia, S. Korea, Japan, China, Laos, Cambodia (Z genotype dominant).**
July 2005 -	**Spread westwards to Europe; Kazakhstan, Mongolia, Russia, Romania, Turkey, Croatia and Ukraine.**
Feb 2006-	**Spread to Africa; Nigeria, Egypt, Cameroon.**
Late 2003-Sept 2008	**Identified in Africa (10 countries), Asia (25 countries) and Europe (26 countries).**
Dec 2003-Sept 2008	**387 confirmed human cases (245 fatal) in: Indonesia (137/112), Vietnam (106/52), Egypt (50/22), China (30/20), Thailand (25/17), Turkey (12/4), Azerbaijan (8/5), Cambodia (7/7), Iraq (3/2), Pakistan(3/1), Laos (2/2), Nigeria (1/1), Bangladesh (1/0), Djibouti (1/0), Myanmar (1/0).[15 countries]**

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

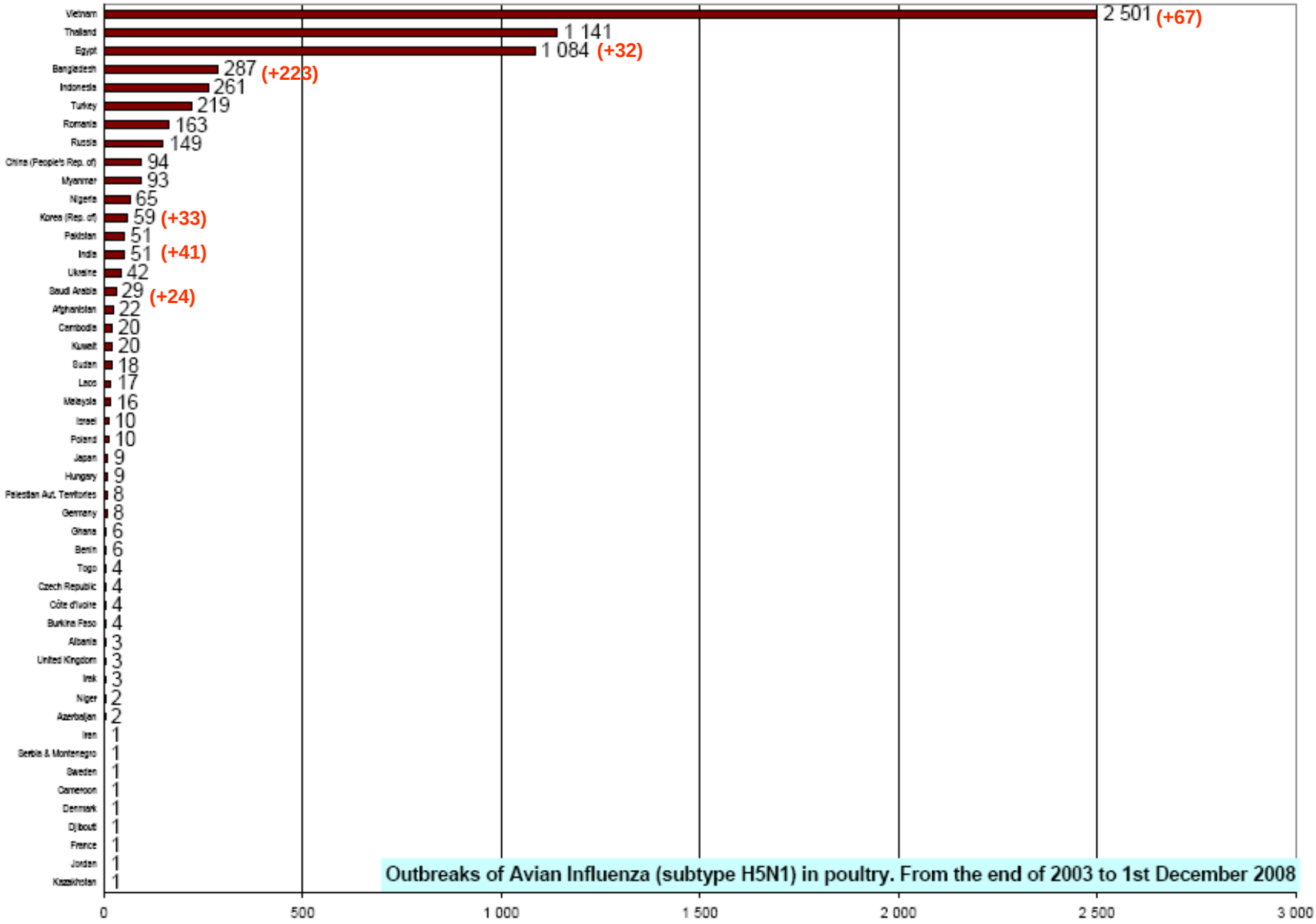
Status as of 14 November 2006
Latest available update



World Health Organization

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

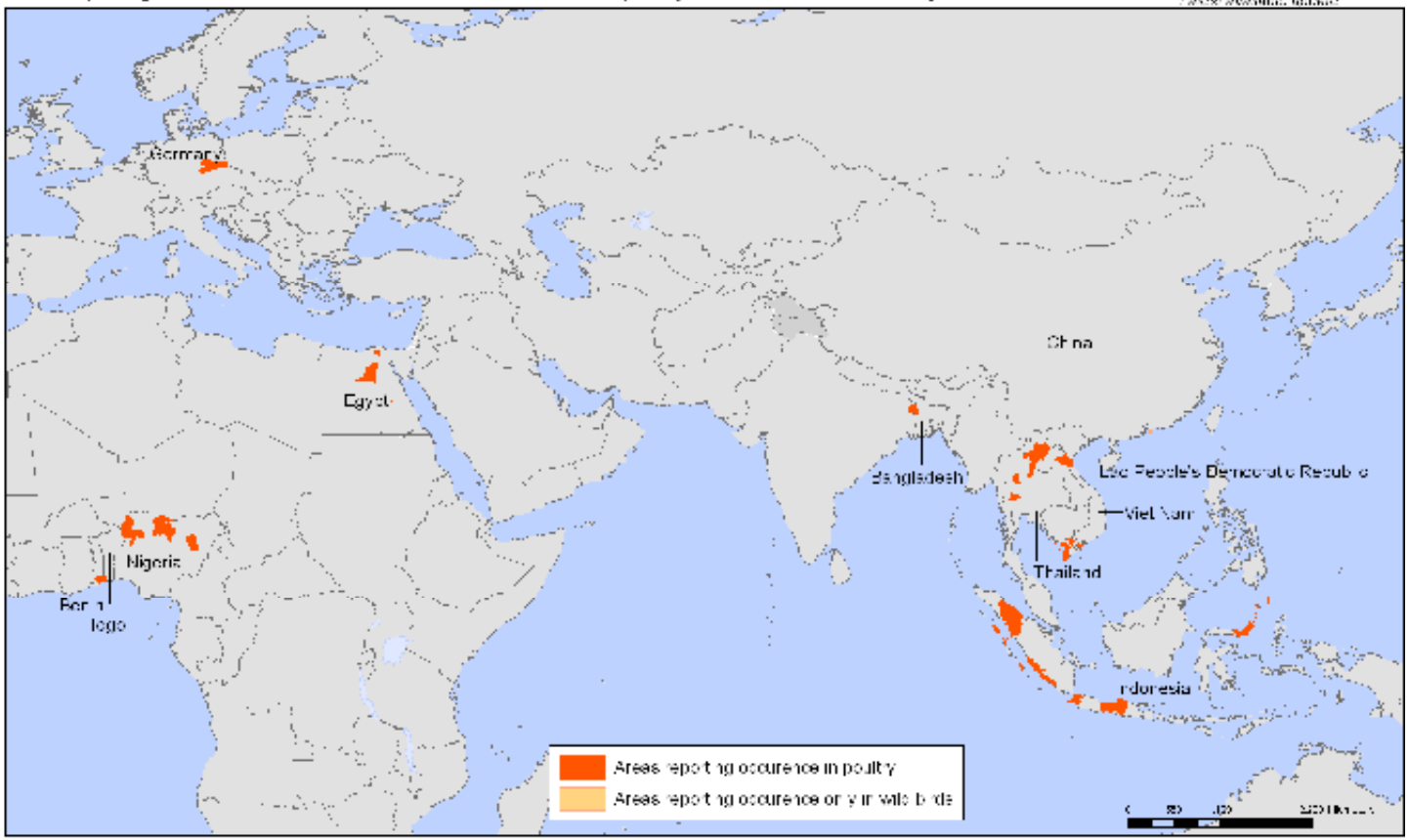
Data Source: World Organisation for Animal Health (OIE) and national governments
Map Production: Public Health Information and Geographic Information Systems (GIS) World Health Organization



(since 1st February)

Areas reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 1 July 2008

Status as of 14 November 2008
Global avian influenza update



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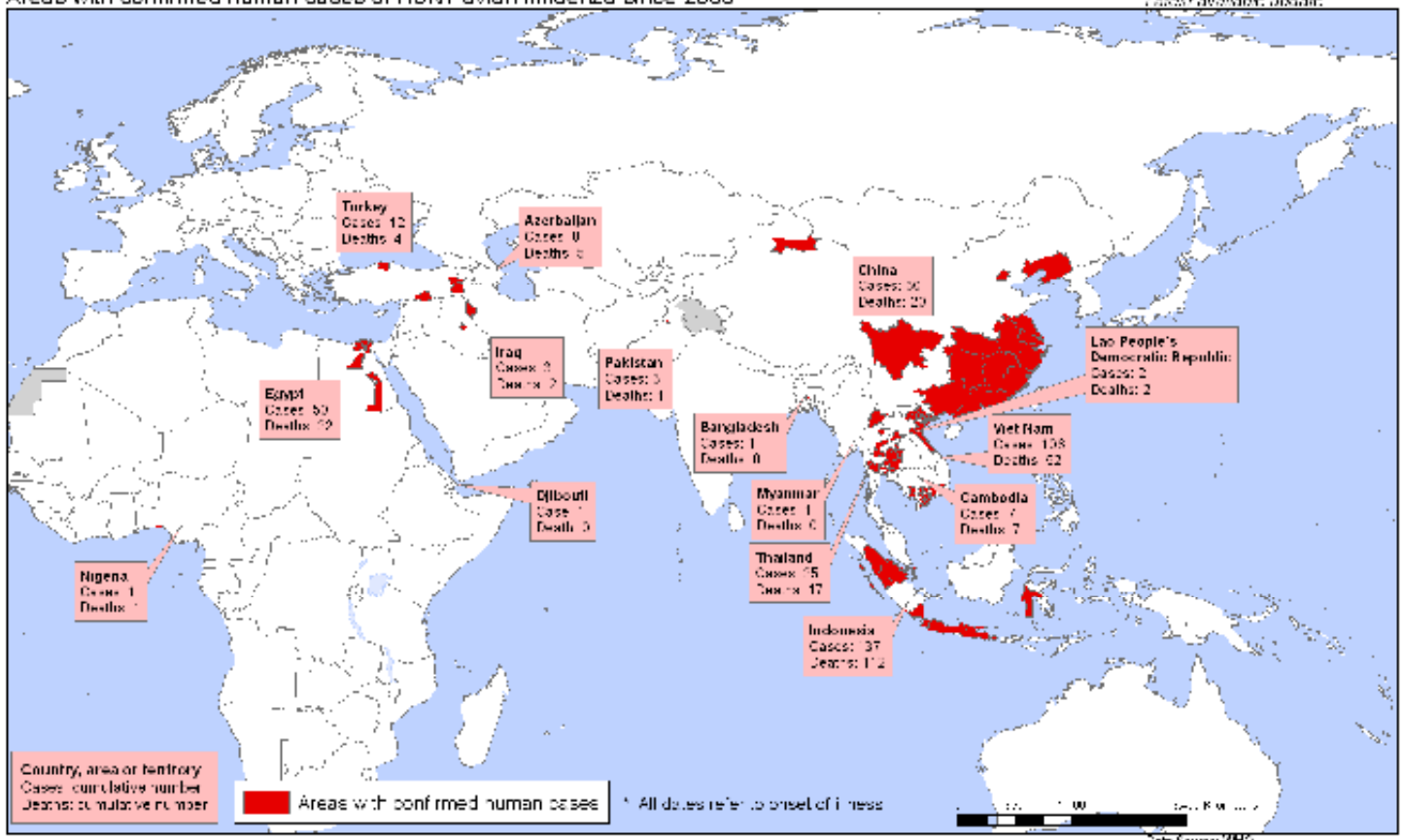
Areas with confirmed human cases of H5N1 avian influenza since 2003 *

Status as of 10 September 2008
Latest available update

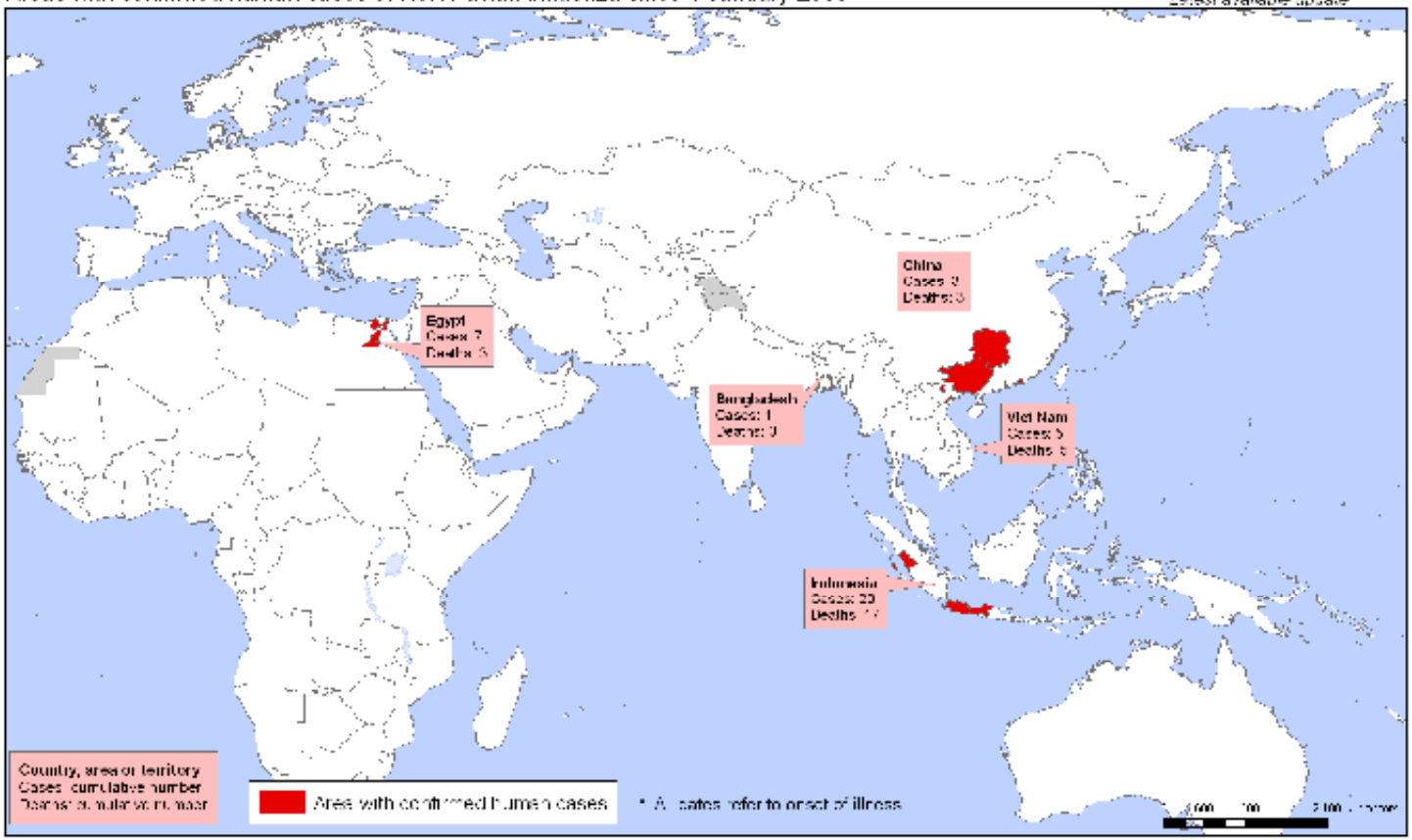


World Health Organization

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

Areas with confirmed human cases of H5N1 avian influenza since 1 January 2008 *

Status as of 10 September 2008
Latest available update



World Health Organization

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

Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

10 September 2008

Country	2003		2004		2005		2006		2007		2008		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	**8**	**5**	0	0	0	0	**8**	**5**
Bangladesh	0	0	0	0	0	0	0	0	0	0	**1**	0	**1**	0
Cambodia	0	0	0	0	**4**	**4**	**2**	**2**	**1**	**1**	0	0	**7**	**7**
China	**1**	**1**	0	0	**8**	**5**	**13**	**8**	**5**	**3**	**3**	**3**	**30**	**20**
Djibouti	0	0	0	0	0	0	**1**	0	0	0	0	0	**1**	0
Egypt	0	0	0	0	0	0	**18**	**10**	**25**	**9**	**7**	**3**	**50**	**22**
Indonesia	0	0	0	0	**20**	**13**	**55**	**45**	**42**	**37**	**20**	**17**	**137**	**112**
Iraq	0	0	0	0	0	0	**3**	**2**	0	0	0	0	**3**	**2**
Lao People's Democratic Republic	0	0	0	0	0	0	0	0	**2**	**2**	0	0	**2**	**2**
Myanmar	0	0	0	0	0	0	0	0	**1**	0	0	0	**1**	0
Nigeria	0	0	0	0	0	0	0	0	**1**	**1**	0	0	**1**	**1**
Pakistan	0	0	0	0	0	0	0	0	**3**	**1**	0	0	**3**	**1**
Thailand	0	0	**17**	**12**	**5**	**2**	**3**	**3**	0	0	0	0	**25**	**17**
Turkey	0	0	0	0	0	0	**12**	**4**	0	0	0	0	**12**	**4**
Viet Nam	**3**	**3**	**29**	**20**	**61**	**19**	0	0	**8**	**5**	**5**	**5**	**106**	**52**
Total	**4**	**4**	**46**	**32**	**98**	**43**	**115**	**79**	**88**	**59**	**36**	**28**	**387**	**245**

Total number of cases includes number of deaths.
WHO reports only laboratory-confirmed cases.
All dates refer to onset of illness.

Human H5N1 Viruses (2003-8)

- All avian genes (no reassortment with human / animal virus)

- HA (NA) - common ancestor, A/goose/Guandong/1/96

- Genotype: mostly Z; V (China – recent spread in SE Asia)
  [different from 1997 viruses (internal genes = H9N2)]

- 2004: 2 main clades 1 and 2
  2005-8: clade 2 – 3 princpal subclades (differ antigenically and geographically)
  (new nomenclature distinguishes 10 clades and multiple subclades)

- Most human infections due to contact with infected birds

- Evidence for limited human - human transmission (family clusters)

- Clinical outcome (~60 % fatality) – no apparent difference between clades/subclades: common feature is HA of gs/Gd

Genotypes of H5N1 viruses in southern China, 1996-2006

(Duan et el, 2008)

- **Rapid increase in genetic diversity (2000-)**
- **2 major reassortant events in 2001 and 2002**

  (in domestic ducks)
- **44 genotypes identified**

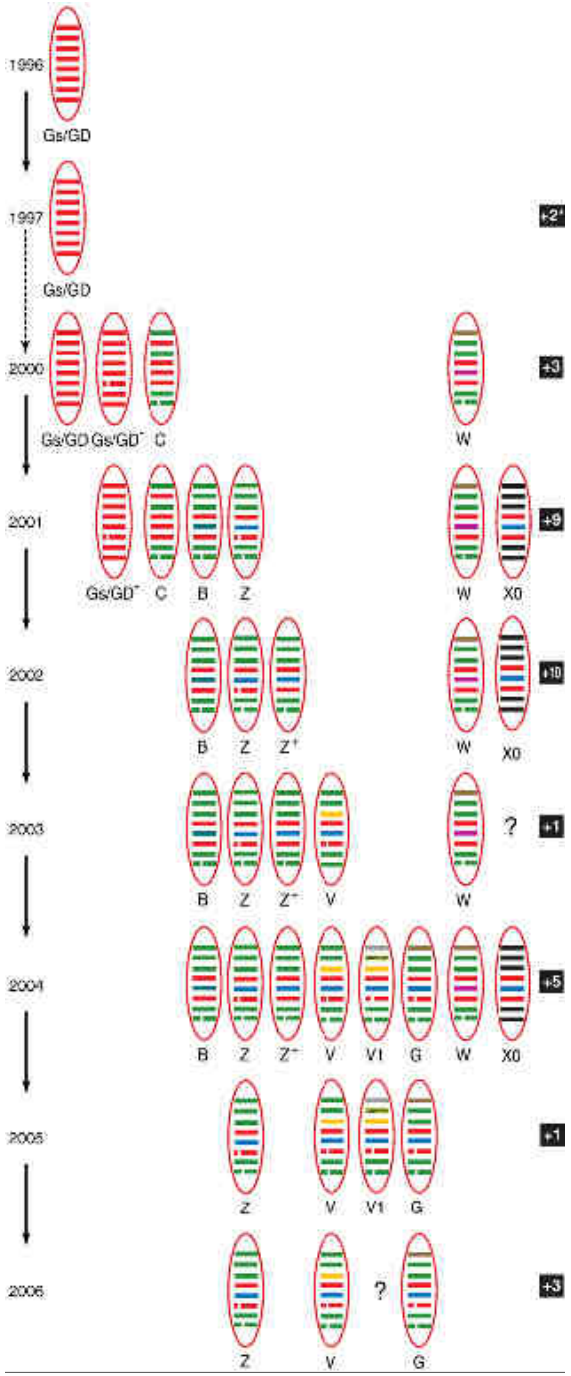
  - 10 persistent; 34 transient

  (no change in fitness)
- **2 major replacements**

  - B by Z (20 AA deletion in NA) in 2002

  - Z by V (different PA gene) in 2005

(no further reassortment with other viruses outside China)



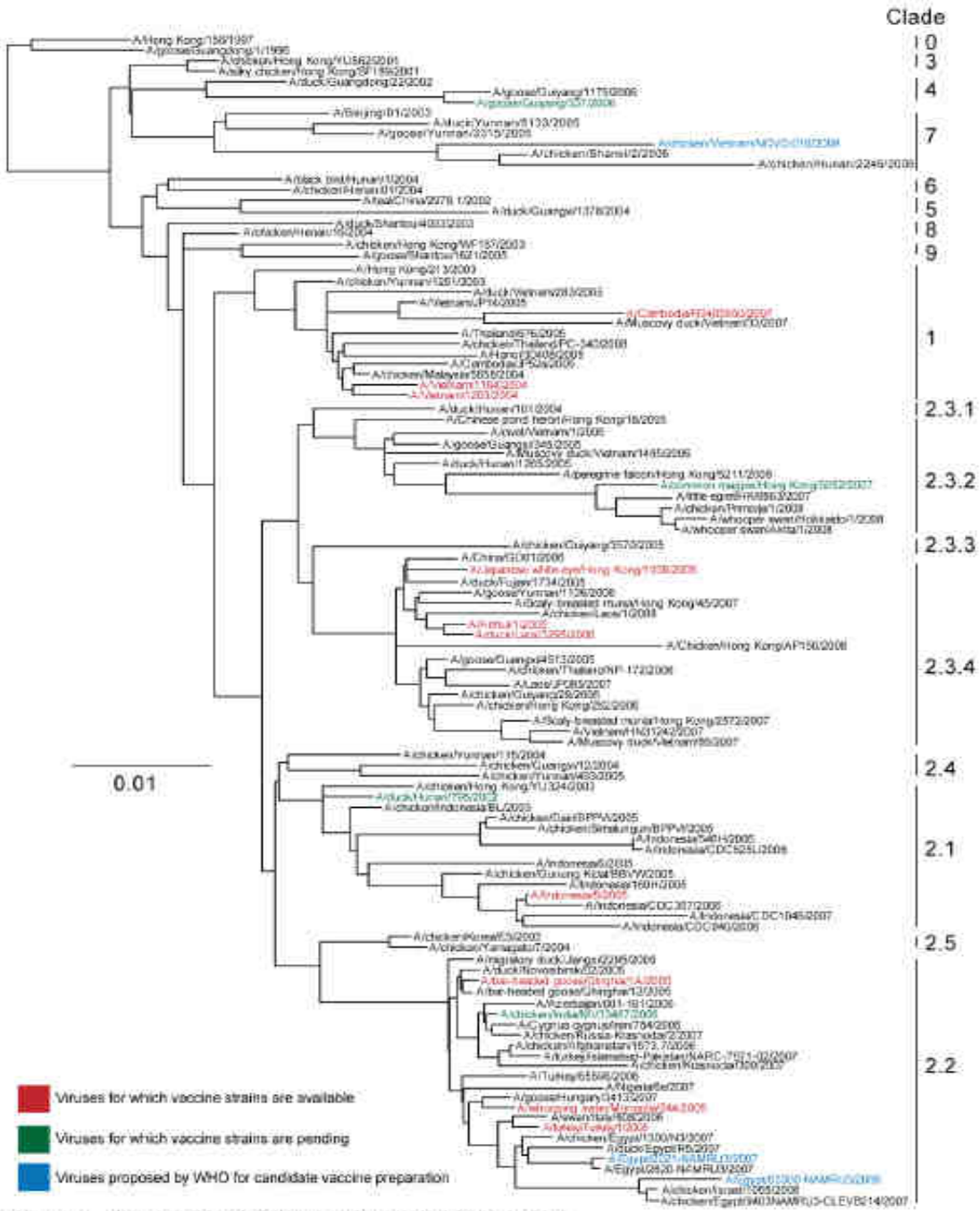


Figure 1. Phylogenetic relationships of H5N1 viruses.

Monitoring changes in novel human influenza viruses

Genetic changes/increased diversity (all genes):

- **In source viruses - genetic reassortment (avian/human)?**
  - diagnosis: update primers/probes? [H5N1)(H7N7]
  (WHO Working Group on PCR Protocols)
  - increase human infection (pandemic risk) [PB2 E627K of clade 2.2]
  - change in clinical outcome?
  - drug resistance (established mutations)?

- **Following animal to human transmission - adaptive changes?**
  (increase human transmission)
  - HA receptor binding (increased preference for 2,6 sialic acid (human) receptors)
  - Polymerase activity, e.g. PB2: E627K, D701N
  - Altered virulence?, e.g. NS1, PB1- F2

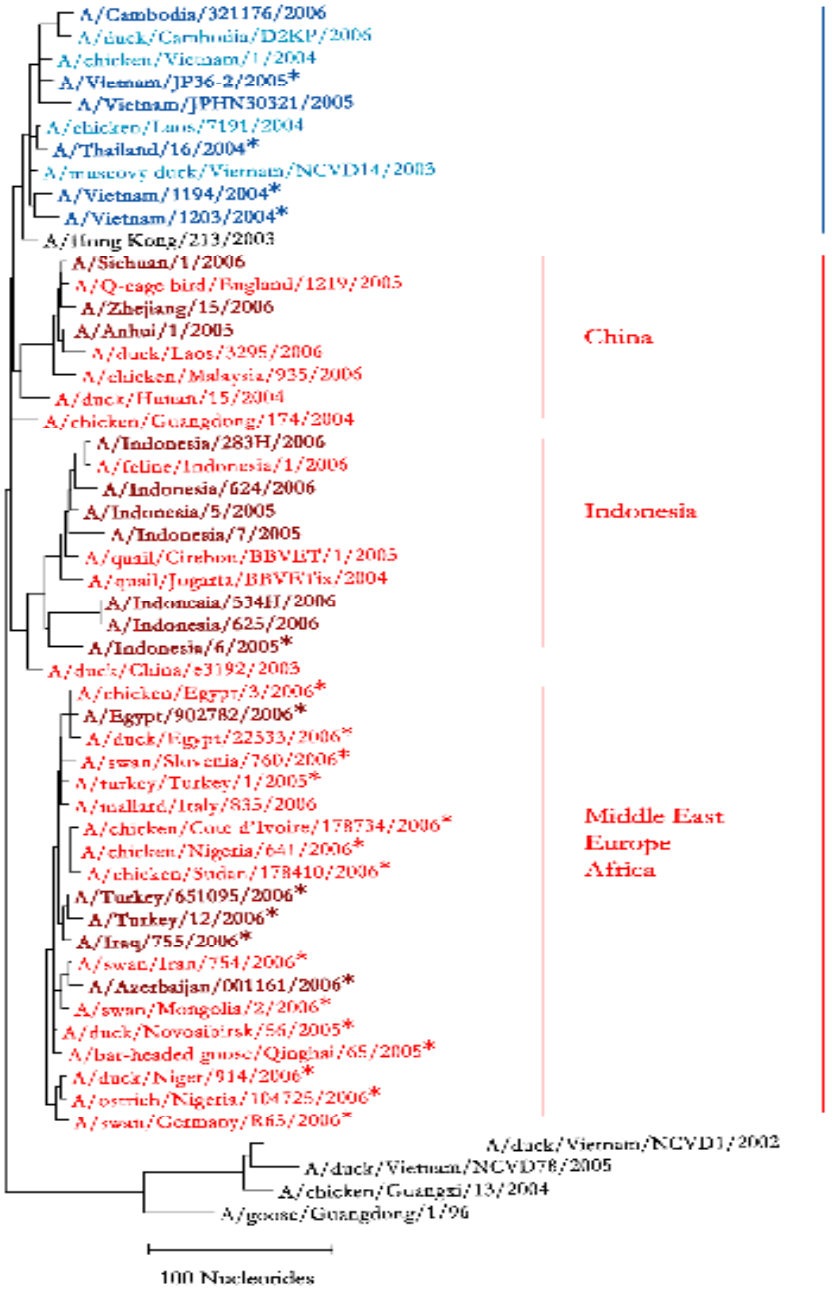
Antigenic changes/increased diversity (using ferret post-infection antisera):

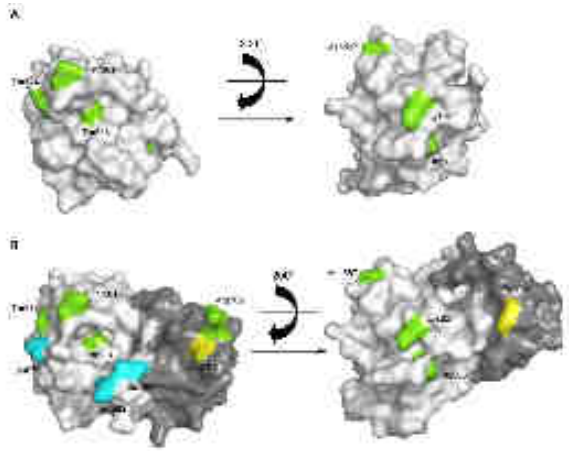
- **Diagnostics**
- **Vaccines: update of candidate strains?**
  - cross reaction/protection
- **Cross-reactivity of antibody responses to natural infection**

Resistance to anti-M2, anti-NA drugs :

- **Effectiveness of antivirals (stockpiles)**

Phylogenetic relationships between the PB2 proteins of H5N1 viruses (*K627)

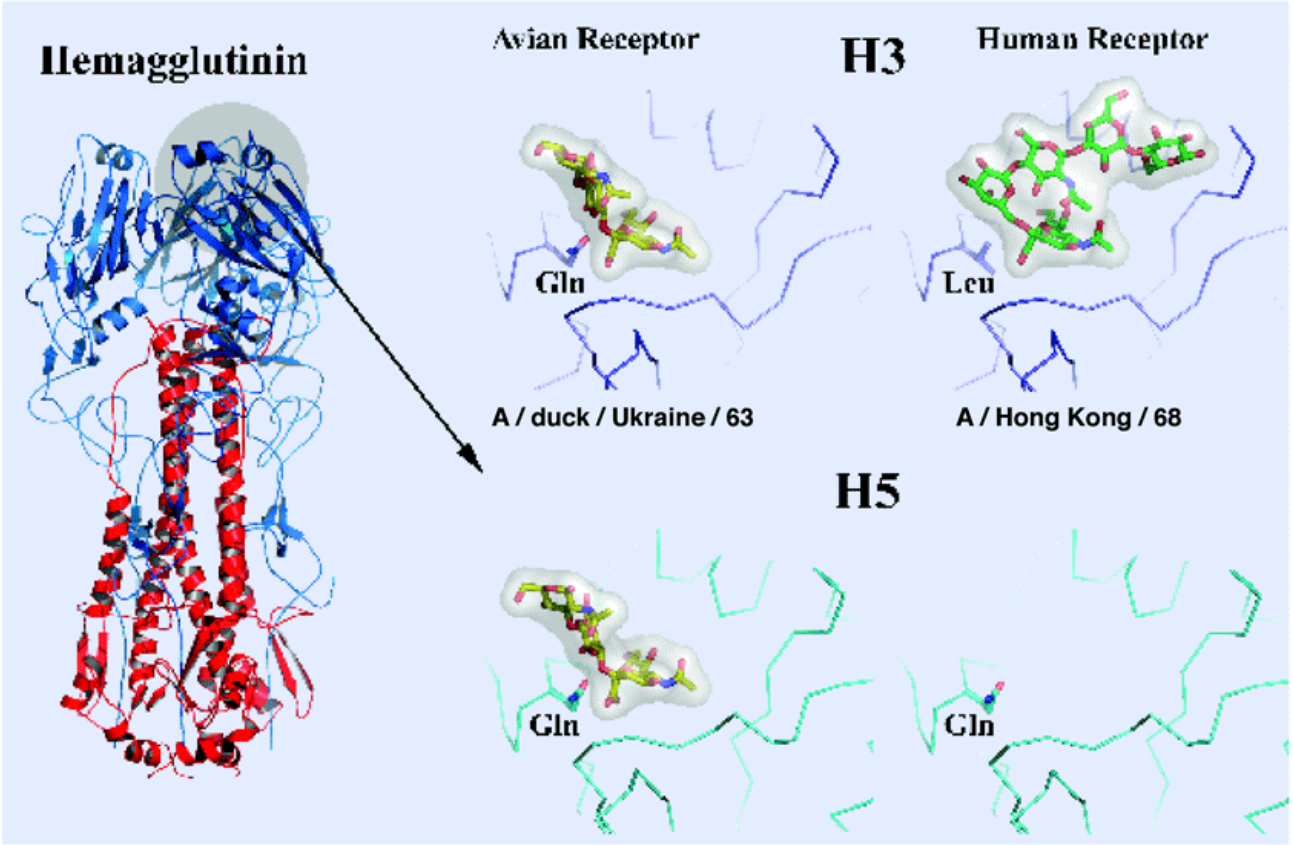




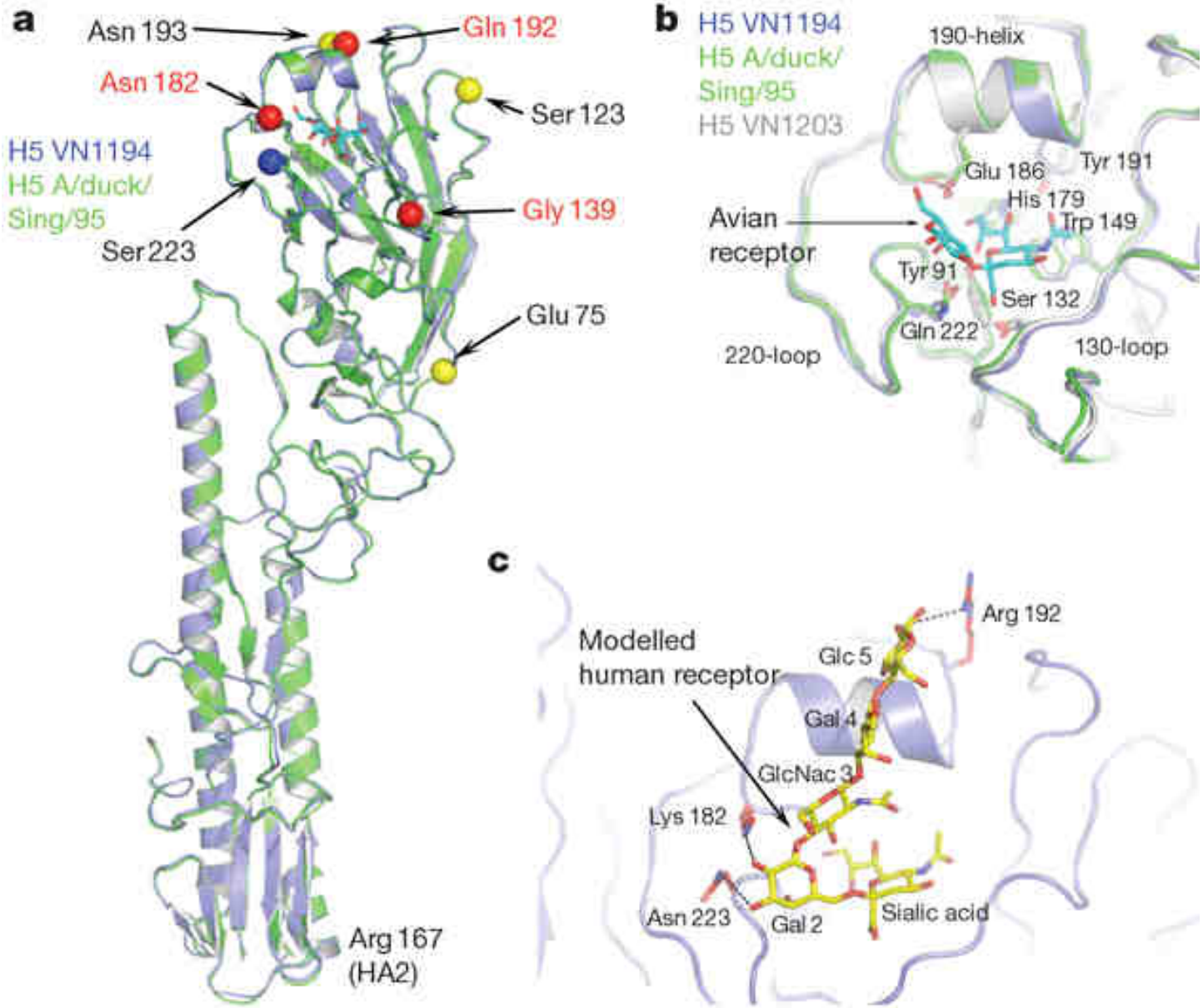
Tarendeau et al, 2008

MRC National Institute for Medical Research

Differences in Receptor Specificity of Avian and Human Influenza Viruses

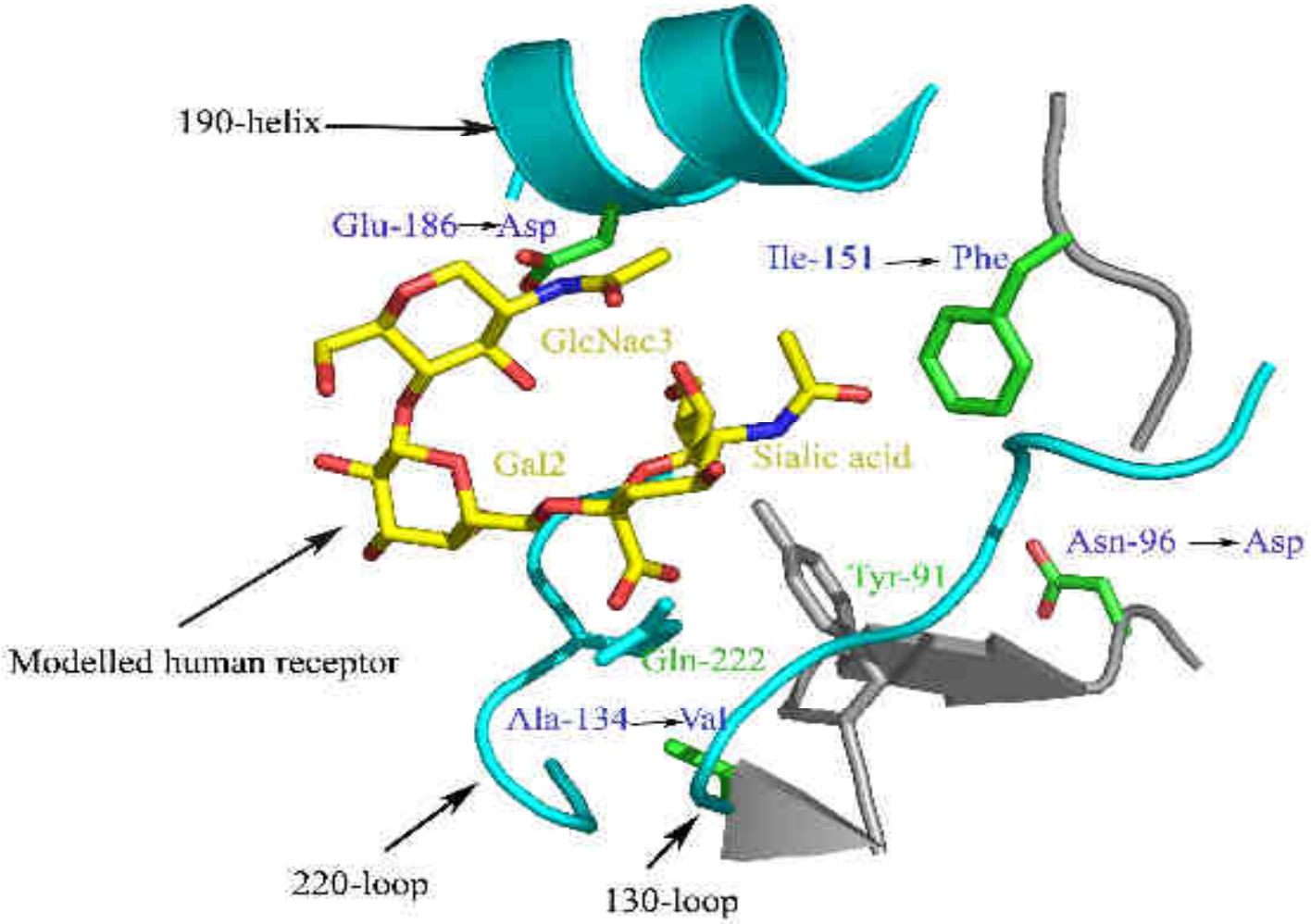


Changes in HA of H5N1 following avian to human transmission

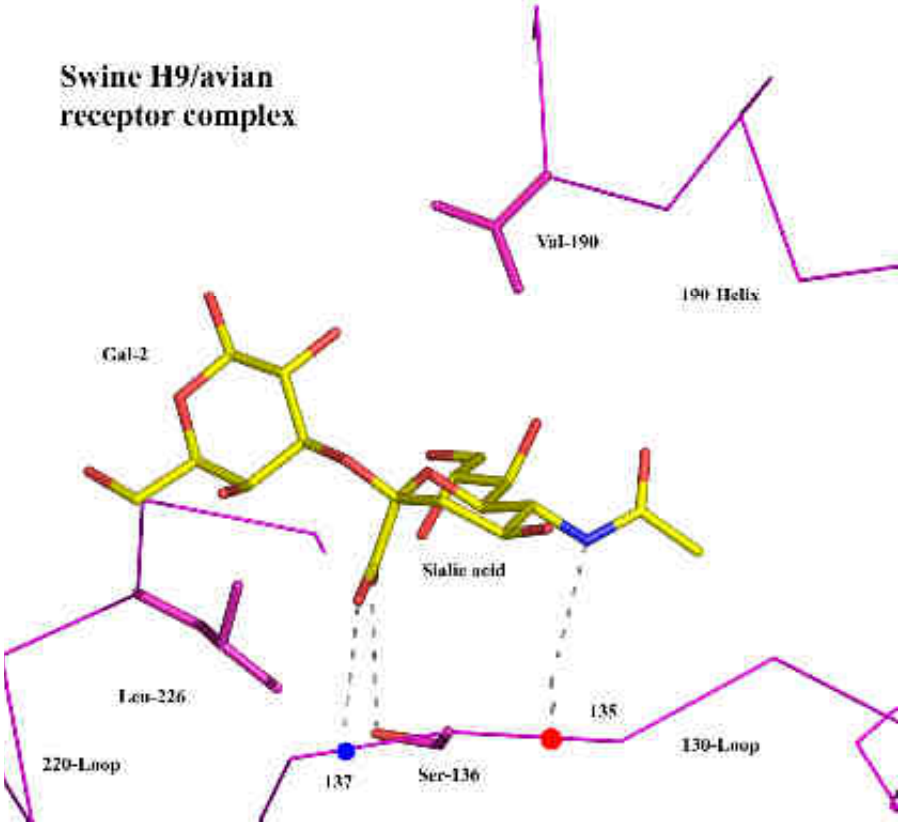


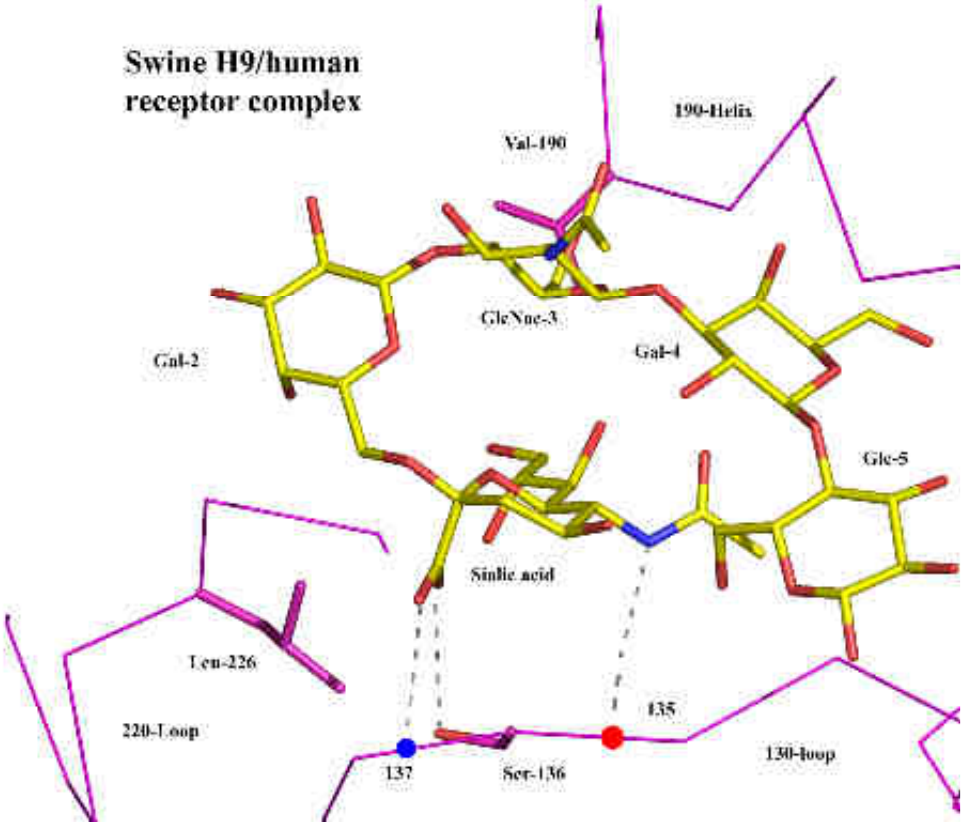
Yamada et al, 2006

Influence of mutations in HA of clade 1 H5N1 viruses on receptor binding?

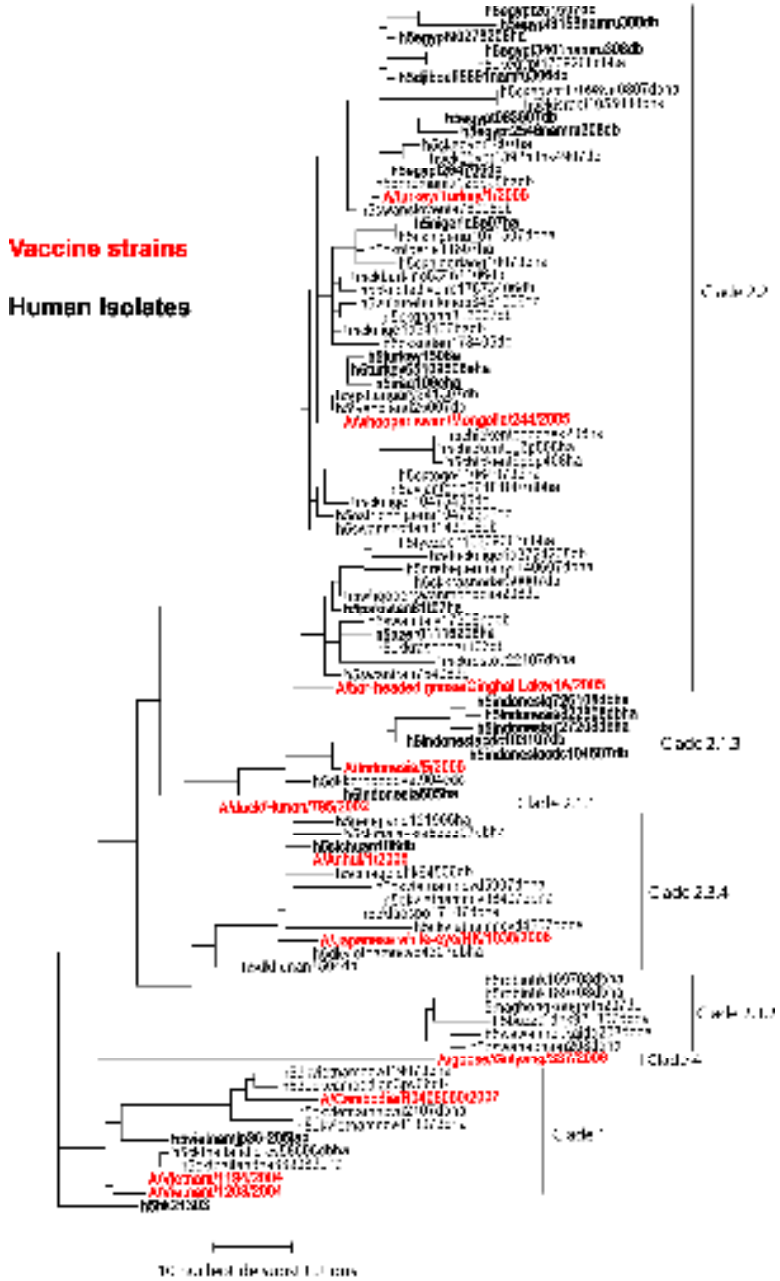


The HA of H9N2 virus, with leucine 226, has preference for the human-like (α2,6-linked sialic acid) receptor - intermediate in human adaptation? - does it pose a greater pandemic threat?





Genetic drift in HA of H5N1 viruses



Monitoring changes in novel human influenza viruses

Antigenic changes/increased diversity (using ferret post-infection antisera):

Vaccines: update of candidate strains?
- cross reaction/protection
Cross-reactivity of antibody responses to natural infection?

Table 1. Antigenic properties of H5N1 viruses

		REFERENCE FERRET ANTISERA						
REFERENCE ANTIGENS	CLADE	1 VN/1203	1.1 IND/5	1.1 DK/HU	2.1 MS/294	2.2.1 VD/VN	2.3.4 AH/1	7 CK/VN
A/Viet Nam/1203/2004 (VN/1203)	1	320	20	<40	<10	40	-	<10
A/Indonesia/5/2005 (IND/5)	1.1	10	640	80	80	160	-	80
A/duck/Hunan/795/2002 (DK/HU)	1.1	80	1280	160	-	-	40	-
A/whooper swan/Mongolia/244/2005 (MG/244)	2.2	20	160	160	320	80	-	-
A/mallard duck/Viet Nam/1855/2006 (MD/VN)	2.2.1	40	160	-	160	320	<10	-
A/Anhui/1/2005 (ANH/1)	2.3.4	40	320	-	<10	-	640	<10
A/chicken/Viet Nam/NCVD 016/2008 (CK/VN)	7	<10	<10	-	-	-	<10	640
TEST ANTIGENS								
A/Indonesia/05/2005	1	160	20	-	<10		40	-
A/duck/Viet Nam/NCVD16/2007	1	40	<10	-	<10		<10	-
A/Indonesia/CDC1043/2006	2.1	<10	640	-	160		160	-
A/Indonesia/CDC670/2006	2.1	40	80	40	20		<10	-
A/Turkey/65-596/2006	2.2	160	1280	-	5120		320	-
A/Egypt/3300-NAMRU3/2008	2.2	<10	320	-	320		<10	-
A/Egypt/3439-NAMRU3/2008	2.2	<10	160		80		20	
A/Bangladesh/20/2005/2008	2.2	<10	320	-	320		<10	-
A/common magpie/Hong Kong/5052/2007	2.2.1	80	320	40	-	640	<10	-
A/duck/Viet Nam/NCVD16/2007	2.3.4	<10	80	-	<10		320	-
A/chicken/Viet Nam/NCVD247/2007	2.3.4	20	<10	-	<10		40	-
A/duck/Viet Nam/NCVD87/2007	2.3.4	<10	<10	-	<10		80	-
A/chicken/Hong Kong/AP156/2008	2.3.4	<10	<10	40	<10	40	<10	-
A/chicken/Viet Nam/NCVD-04/2008	7	<10	<10	-	-	-	<10	40

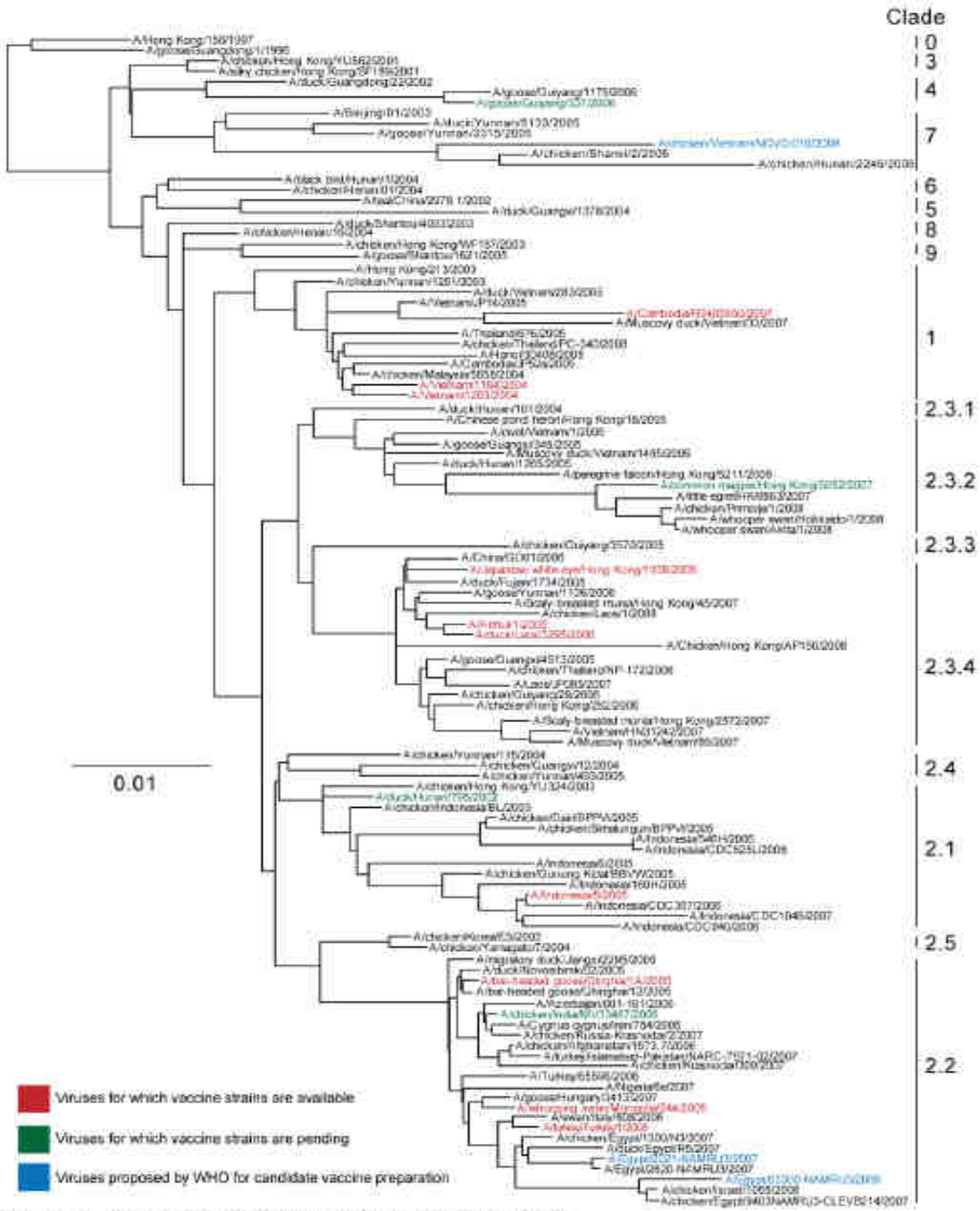
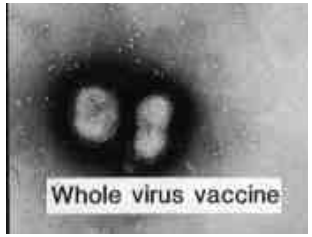
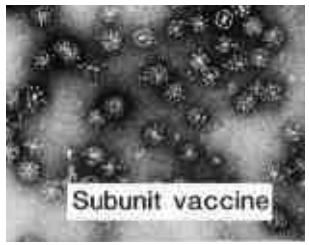


Figure 1. Phylogenetic relationships of H5N1 viruses.

Pandemic vaccines under clinical evaluation
(WHO, July 2008: >70 trials)

- Whole virus without adjuvant – Baxter (Vero), Austria
- Whole virus with Al – 4 Japanese companies; Omninvest, Hungary; GSK, Germany; Sinovac, China
- Split without adjuvant – Sanofi P, USA
- Split with Al - Sanofi P, France; Sanofi P, USA ;CSL, Australia
- Split with oil/water emulsion - Sanofi P, France; GSK, Germany
- Subunit with Al – Microgen, Russia
- Subunit with oil/water emulsion – Novartis, Italy; Microgen, Russia
- Rec HA/M2– Protein Science, Acambis, Merck, Vaxinnate, Novavax, USA; Cytos, Switzerland
- DNA – NIH, USA; Vical, USA
- Live attenuated virus – Medimmune, USA, Inst Exp Med, Russia



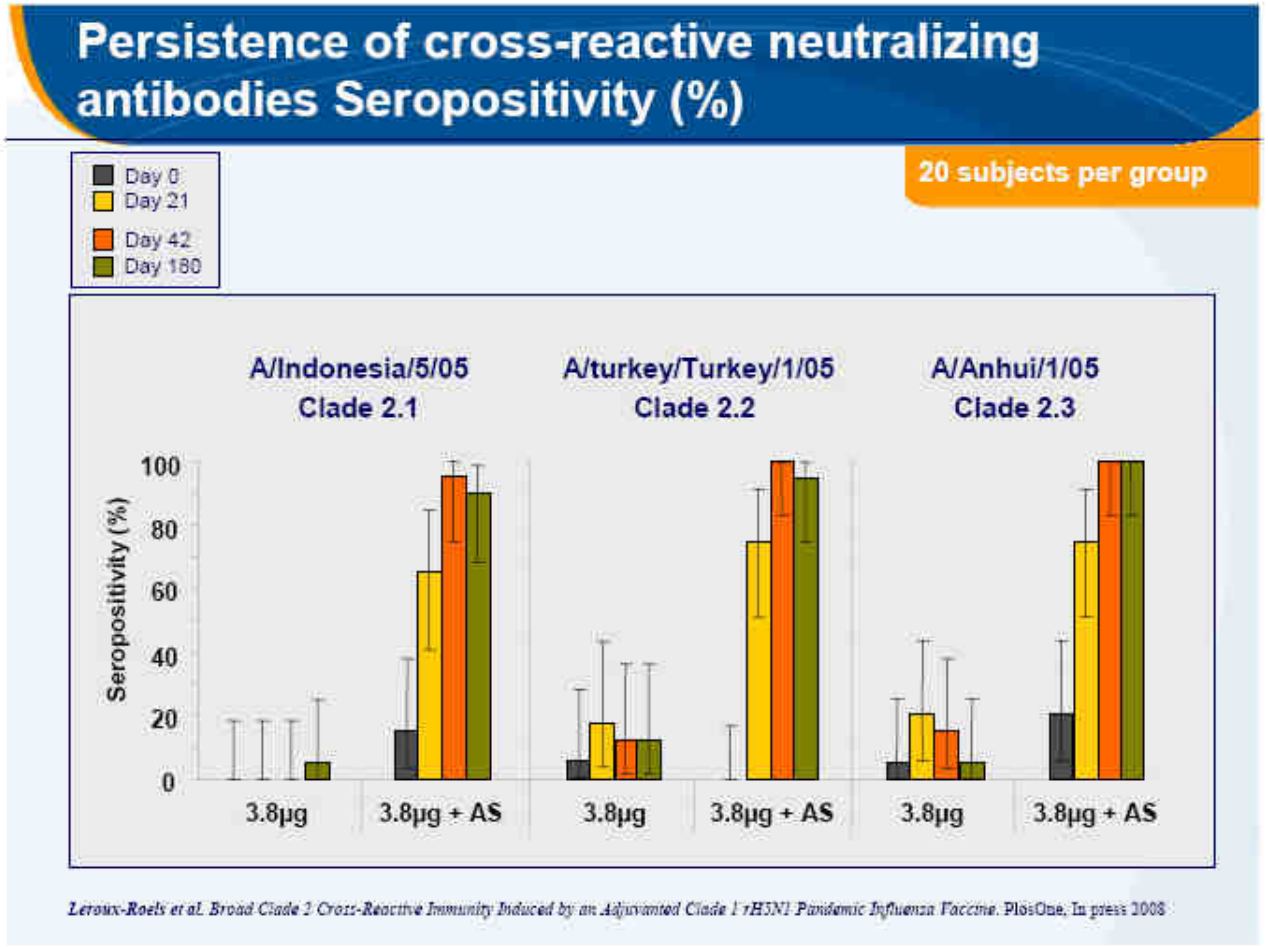


Results from H5N1 vaccine trials

Type of vaccine	'Compliance' with EU licensing criteria
Split vaccine no adjuvant (Sanofi P)	Need two doses of 90 µg
Split/subunit vaccine with Al (Various)	Need two doses of 15-45 µg
Whole virus (egg) with Al (Various)	Need two doses of 5-15 µg
Subunit with MF59 adjuvant (Novartis)	Need two doses of 7.5 µg
Whole virus Vero cell culture, no adjuvant (Baxter)	Need two doses of 7.5 µg
Split vaccine with AS adjuvant (GSK)	Need two doses of 3.8 µg
Split vaccine with AF03 adjuvant (Sanofi P)	Need two doses of 1.9 µg
Whole virus vaccine with Al (Omninvest)	Need one dose of 6 µg

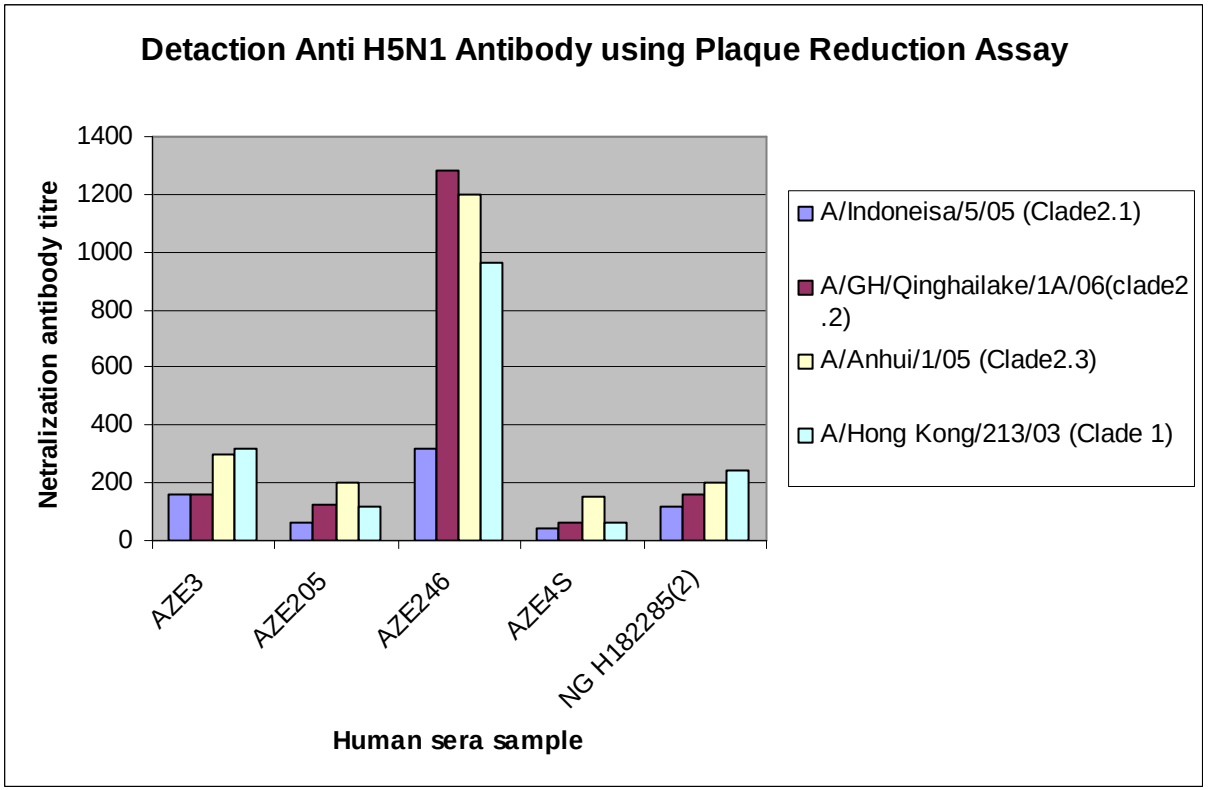
Data published or presented at WHO meetings, 2007, 2008

Cross reactivity of antibody stimulated by GSK AS adjuvanted split vaccine



E Hanon, WHO 2008; I Leroux-Roels et al, PLoS ONE, 2008, Feb 27; 3 (2): e1665

Cross-Clade Reactivity of Anti-H5N1 Antibodies Detected by the Plaque Reduction Assay (1)



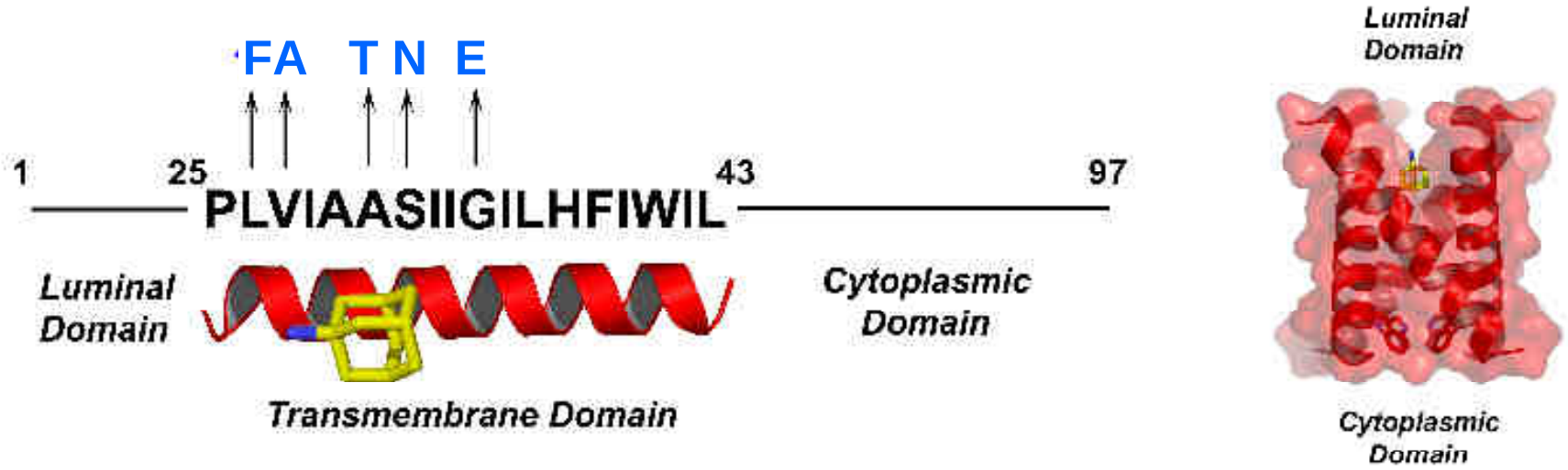
Sera from four confirmed cases of H5N1 clade 2.2 infection of humans and a confirmed case from Nigeria were assayed against the panel of viruses indicated. All sera showed cross-reactivity to the four viruses clade/subclade representatives used.

MRC National Institute for Medical Research

Antiviral Agents for Influenza

Class/agent	Brand name	Route
M2 inhibitors		
Amantadine	*Symmetrel*	Oral
Rimantadine	*Flumadine*	Oral
NA inhibitors		
Zanamivir (GG167)	*Relenza*	Inhaled
Oseltamivir (GS4104)	*Tamiflu*	Oral

Amantadine resistance mutations in the M2 channel protein

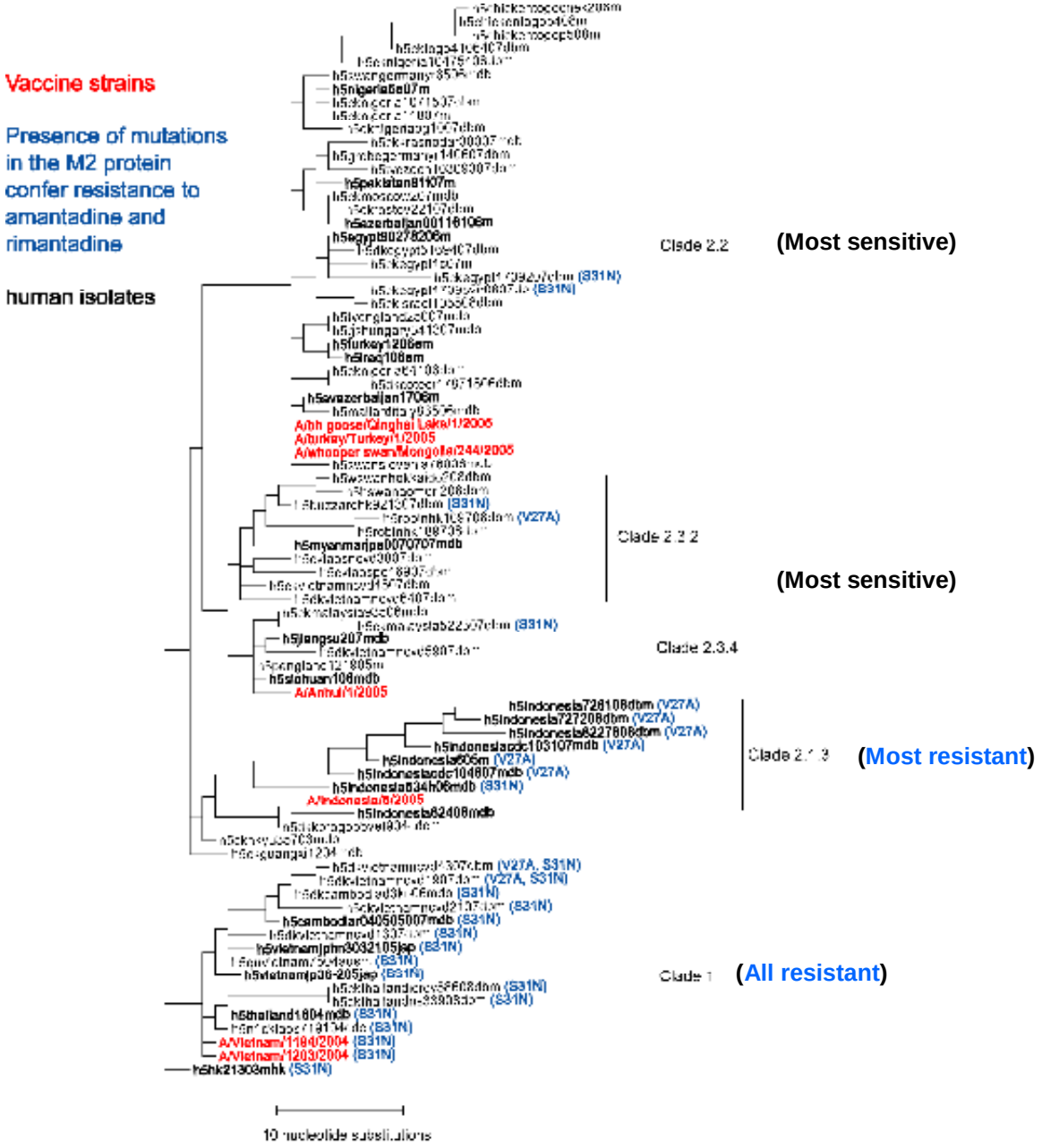


- Well-defined mutations correlate with resistance *in vitro* and *in vivo*
- Screen for resistance by sequencing M gene (pyrosequencing)

Emergence of amantadine resistance in human and animal viruses

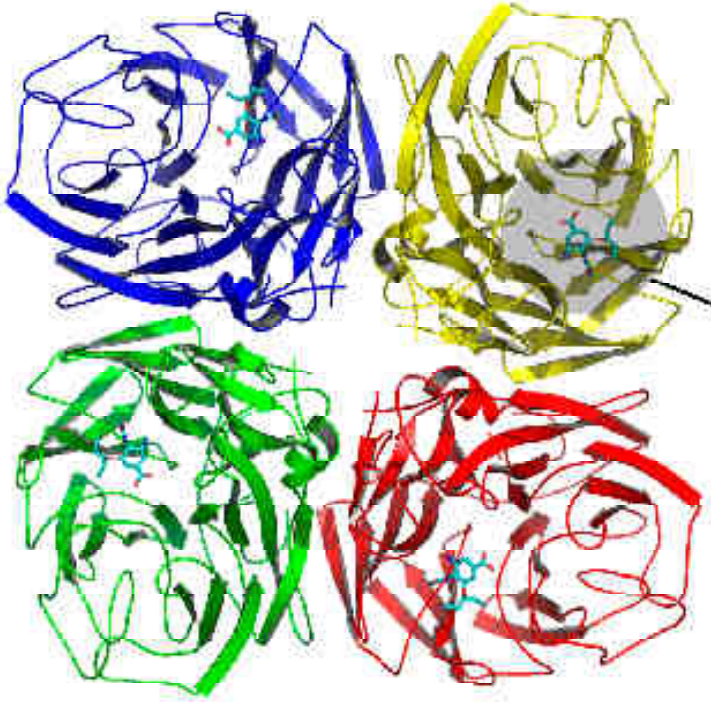
- Pre 1980's – low incidence; approx. 1%
- Mid 1980's – European swine H1N1 viruses
  (sporadic human cases)
- 2000 - avian H5N1,H9N2 (S.E. Asia)
- 2003 - avian H5N1 clade 1 (and human cases)
  H5N3(S.E. Asia)
  H7N2(N. America)
- 2003 - human H3N2 (China/Hong Kong; worldwide)
- 2006 - human H1N1 emergent variant

M genes of H5N1 viruses – amantadine resistance

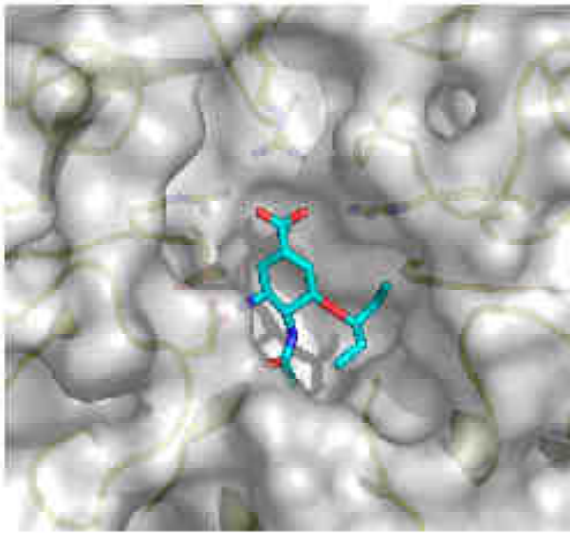


Structure Based Drug Design

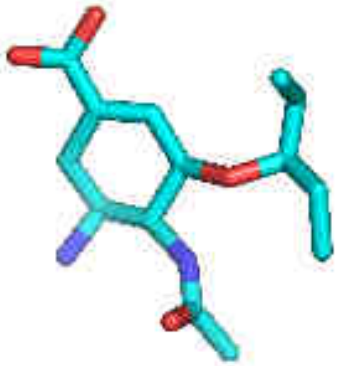
Neuraminidase



In complex with Oseltamivir

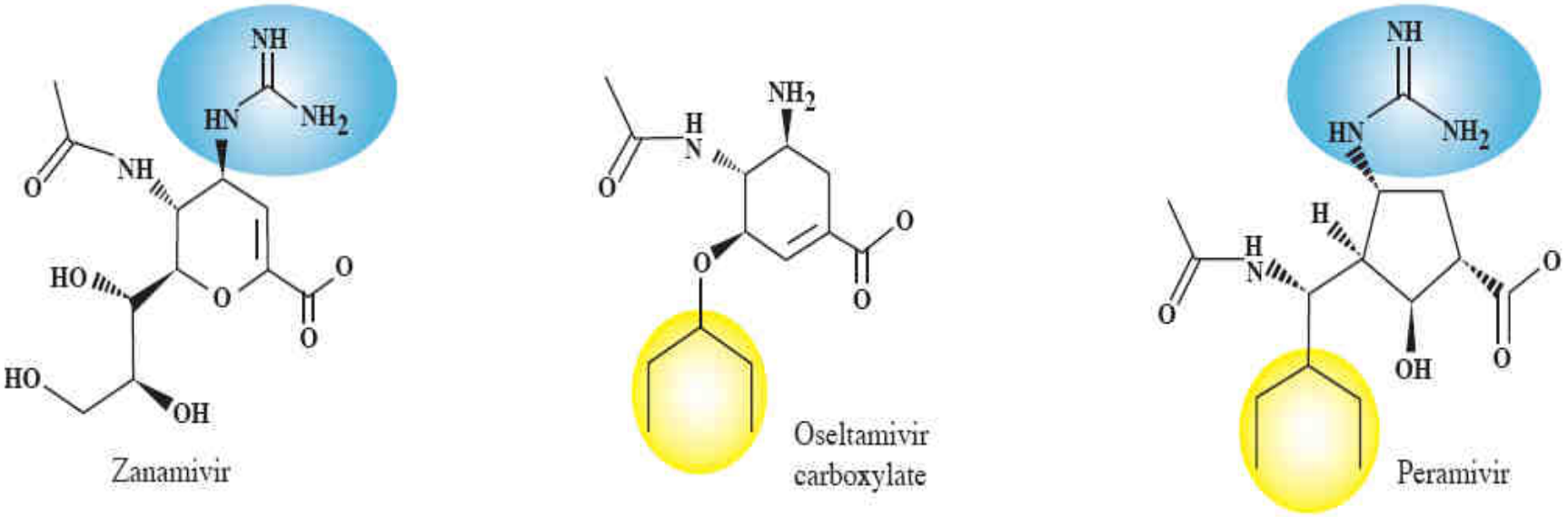


DANA
low μM



Tamiflu

low nM



Structures of the inhibitors of the neuraminidases of influenza A and B viruses. The guanidino and ethylpropoxy substituents of zanamivir and oseltamivir, respectively, both present in peramivir, are highlighted.

Emergence of resistance to zanamivir and oseltamivir

Clinical trials

- Zanamivir – low (?)
- Oseltamivir – adults: 0.3%(4/1228)
  children: 4%(17/421)

Oseltamivir treatment

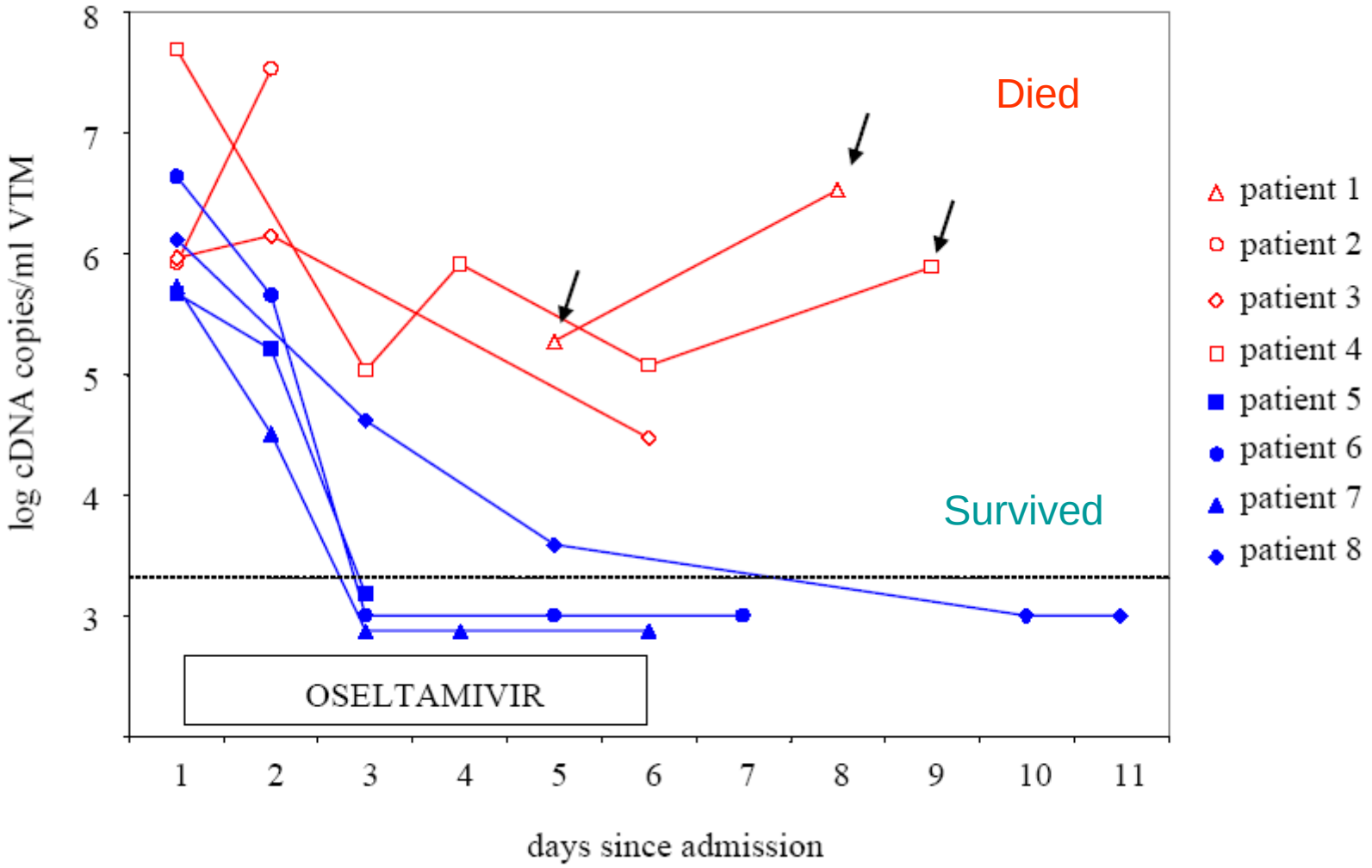
- Japanese children – H1N1: 16%(7/43) (Ward et al,2005)
  - H3N2: 18%(9/50) (Kiso et al, 2004)
  - B : 1.4%(1/74) (Hatakeyama et al, 2007)
- Japanese(untreated) – B: 1.7%(7/422) (Hatakeyama et al)
- H5N1-infected patients: 25%(2/8) (de Jong et al, 2005)

Current A H1N1 viruses (Late 2007 – 2008)

- Emergence and worldwide spread of oseltamivir resistance
  80 - 100% in e.g. South Africa, Senegal, Australia, Philippines, Uruguay

Effect of Oseltamivir Treatment on Virus Load in H5N1 Patients

[arrows indicate resistant virus (H274Y)]



M. de Jong et al. N. Eng. J. Med. 2005

Table 2. Drug susceptibility of influenza A and B viruses recovered from oseltamivir- or zanamivir-treated patients

Virus type/ subtype	Drug treatment	NA[1] substitution	Drug susceptibility[2]		
			oseltamivir	zanamivir	peramivir
AH3N2	Oseltamivir	R292K	R	'R'	R
		E119V	R	S	S
		E119V+I222V	R	S	'R'
		N294S	'R'	S	S
		Δ244-247	R	S	
AH1N1	Oseltamivir	H274Y	R	S	R
AH5N1	Oseltamivir	H274Y	R	S	
		N294S	'R'	S	
B	Oseltamivir	D198N	'R'	'R'	S
	Zanamivir	R152K	R	R	R

[1]Amino acids numbered according to N2 sequence.
[2]Determined by NA assay. R = High level resistance; 'R' = intermediate reduction in drug susceptibility; S = relatively little change in drug susceptibility.

Effects of oseltamivir resistance mutations in ferrets (and mice)

- **H3N2: R292K** – reduced infectivity/virulence, not transmitted
  E119V – similar infectivity/virulence (?), transmitted

- **H1N1: H274Y** – reduced infectivity/virulence, reduced transmission
  (currently circulating H1N1 viruses!)
  N294S - lower replication/virulence (in mice)

- **H5N1: H274Y** - reduced replication, (similar virulence in mice)
  N294S – similar virulence (in mice)

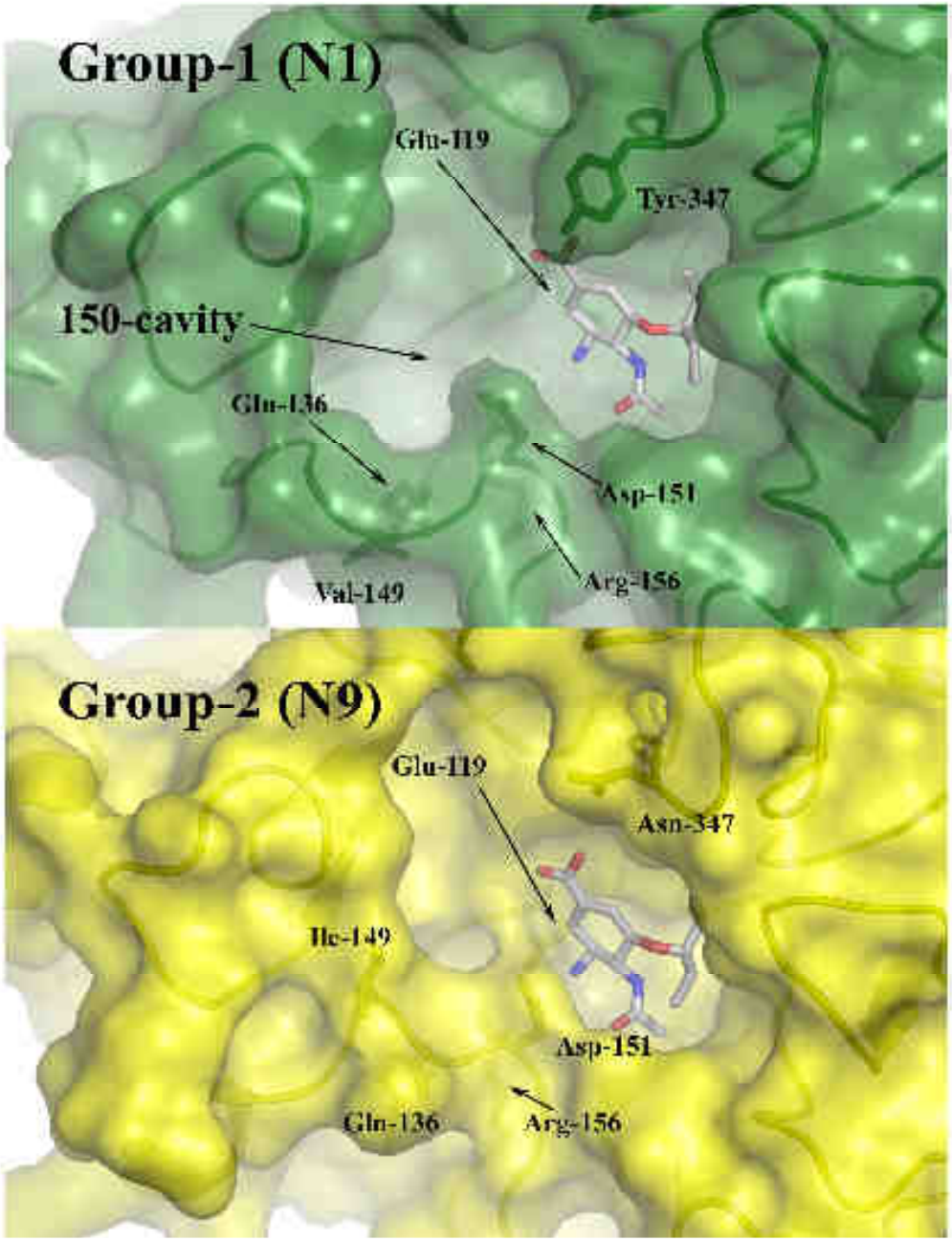
- **B: R152K(Z) -** reduced infectivity/virulence
  D198N - similar infectivity/virulence

OSELTAMIVIR BINDS TO BOTH OPEN AND CLOSED CONFORMATIONS

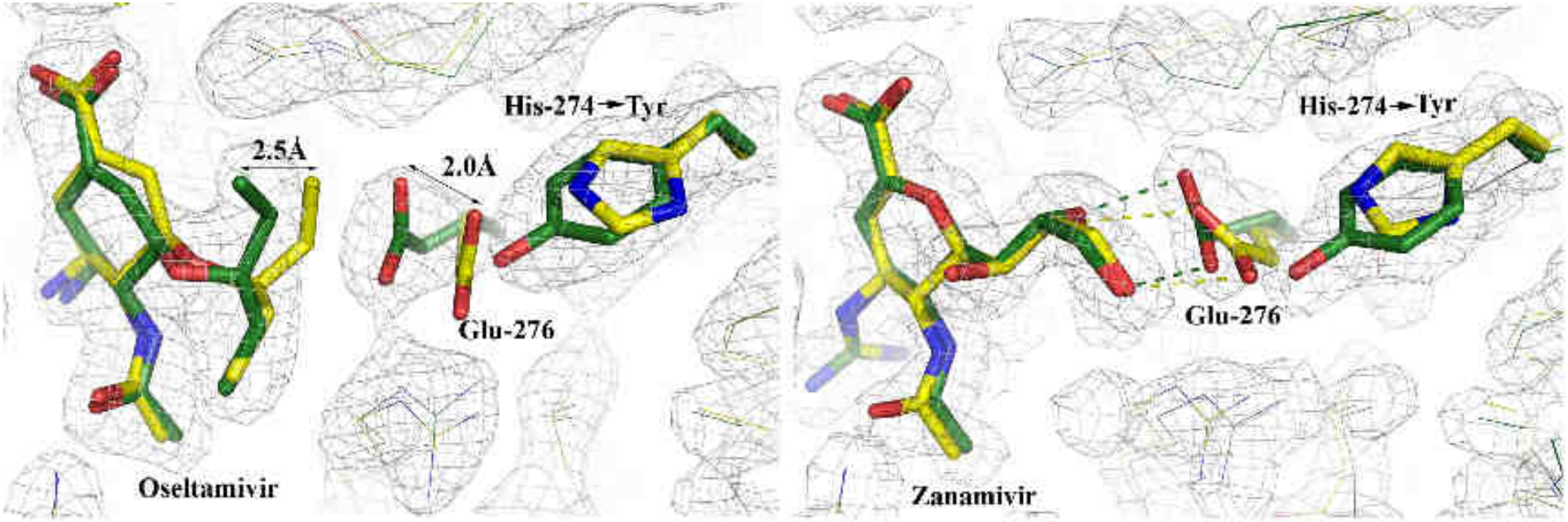
Group 1 NAs:

Additional 150 cavity adjacent to catalytic site (not seen in group 2 NAs)

-target for additional drug design



Effects of the H274Y mutation on the location of Glu 276 of N1 of A/Vietnam/1203/04(H5N1) in complex with with oseltamivir or zanamivir



Wild type (yellow); H274Y mutant (green)

Table 1 | Activity, binding and kinetic parameters for N1 neuraminidases

NA type	V_m relative to wild type	K_m (μM)	Oseltamivir relative K_i*	Zanamivir relative K_i†	k_{on} ($\mu M^{-1} s^{-1}$) oseltamivir	k_{off} (s^{-1}) oseltamivir ($\times 10^4$)	k_{on} ($\mu M^{-1} s^{-1}$) zanamivir	k_{off} (s^{-1}) zanamivir ($\times 10^4$)
Wild type	1.0	6.3	1.0	1.0	2.52 (0.21)	8.1 (1.2)	0.95 (0.08)	0.95 (0.13)
His274Tyr	0.8	27.0	265	1.9	0.24 (0.06)	180 (30)‡	0.35 (0.02)	0.67 (0.08)
Asn294Ser	1.15	53.0	81	7.2	1.1 (0.18)	235 (40)‡	0.52 (0.04)	3.7 (0.6)
Tyr252His	0.94	7.5	0.1	1.2	3.9 (0.15)	1.25 (0.13)	1.38 (0.15)	1.66 (0.33)

K_m values are from three determinations; K_i values from at least six measurements. Values in parentheses represent the standard deviations obtained from linear least squares fits to k_{obs} values as a function of substrate and inhibitor concentrations, as shown in Supplementary Information. k_{on} and k_{off} are the association and dissociation rate constants, respectively.

* Oseltamivir relative K_i is K_i(mutant)/K_i(wild type), where wild type = 0.32 nM.

† Zanamivir relative K_i is K_i(mutant)/K_i(wild-type), where wild type = 0.1nM.

‡ Directly determined.

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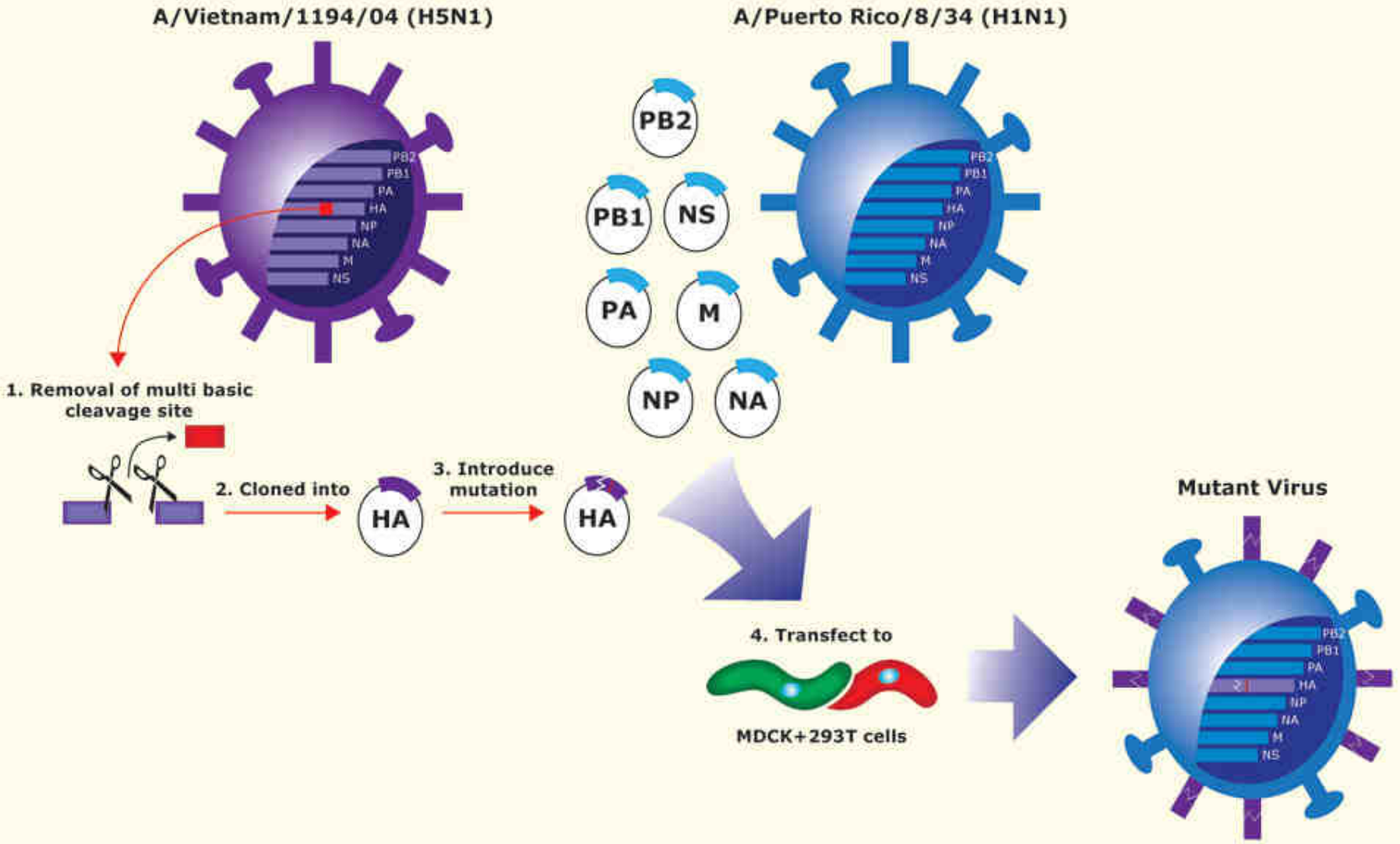
VLA, Weybridge
Ian Brown

Mutant Viruses Produced Via Reverse Genetic System

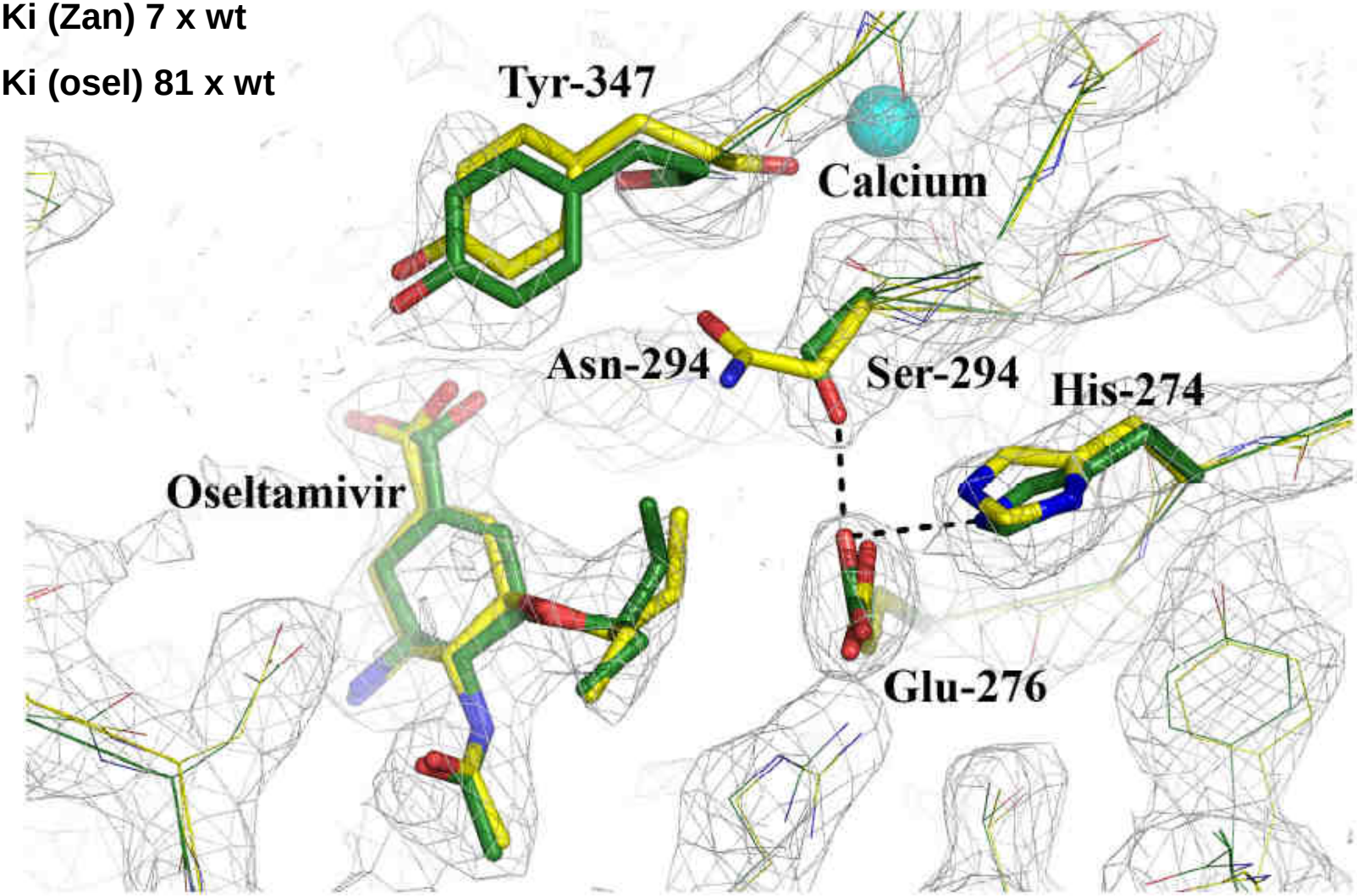


Effect of the Asn294Ser mutation on oseltamivir binding to N1 of H5N1

Km 8 x wt

Ki (Zan) 7 x wt

Ki (osel) 81 x wt



Phylogenetic comparison of **H5N1** neuraminidase genes