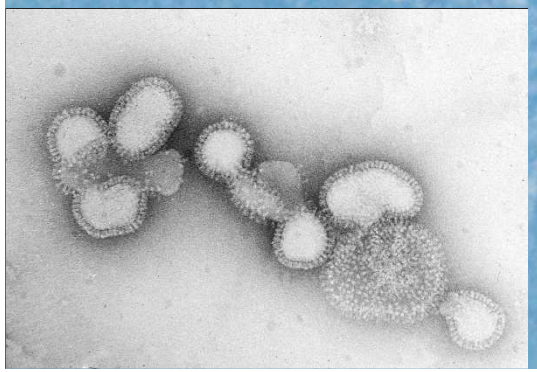
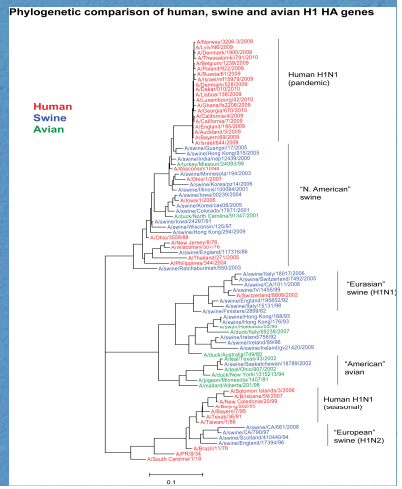
GEIG
Groupe d'Expertise et d'Information sur la Grippe

23e Rencontres du GEIG
Retours d'expériences sur la pandémie H1N1
jeudi 25 et vendredi 26 novembre 2010

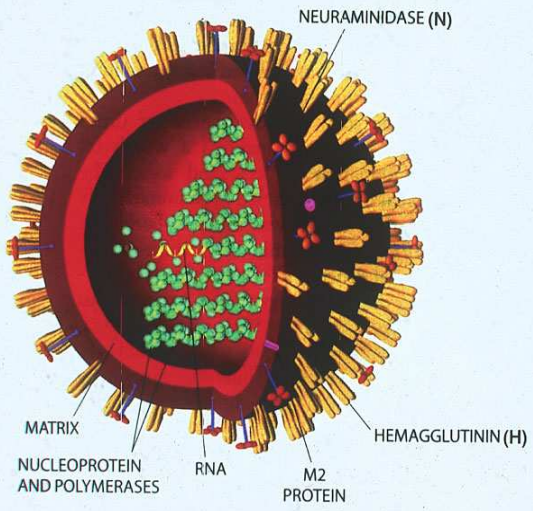
Evolution of the Viruses (pandemic A(H1N1) 2009)

Alan J. Hay
MRC National Institute for Medical Research





Influenza Virus



Birds especially aquatic birds are natural reservoirs of all influenza A viruses in other species



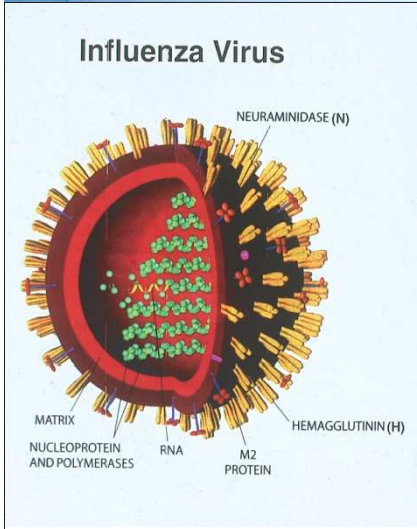

Diversity and Interspecies Transmission of Influenza A Viruses

- **Essentially avian viruses**
- **Natural host – aquatic birds**
- **Segmented genome – high frequency reassortment**
- **Large number of different subtypes (16 HA; 9 NA)**
- **Reservoir of potential pandemic/panzootic viruses**
- **Infrequent transmission to mammalian species**
- **Few subtypes endemic in:**

  Human – H1N1, H3N2, (H2N2)

  Pig – H1N1, H3N2, different reassortants

  Horse – H3N8, (H7N7)



Changes in Human Influenza A Subtypes

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1918 H1N1 (Spanish flu pandemic)

PBI HA NA

1957 H2N2 (Asian flu pandemic)

PBI HA

1968 H3N2 (Hong Kong flu pandemic)

1977 H1N1

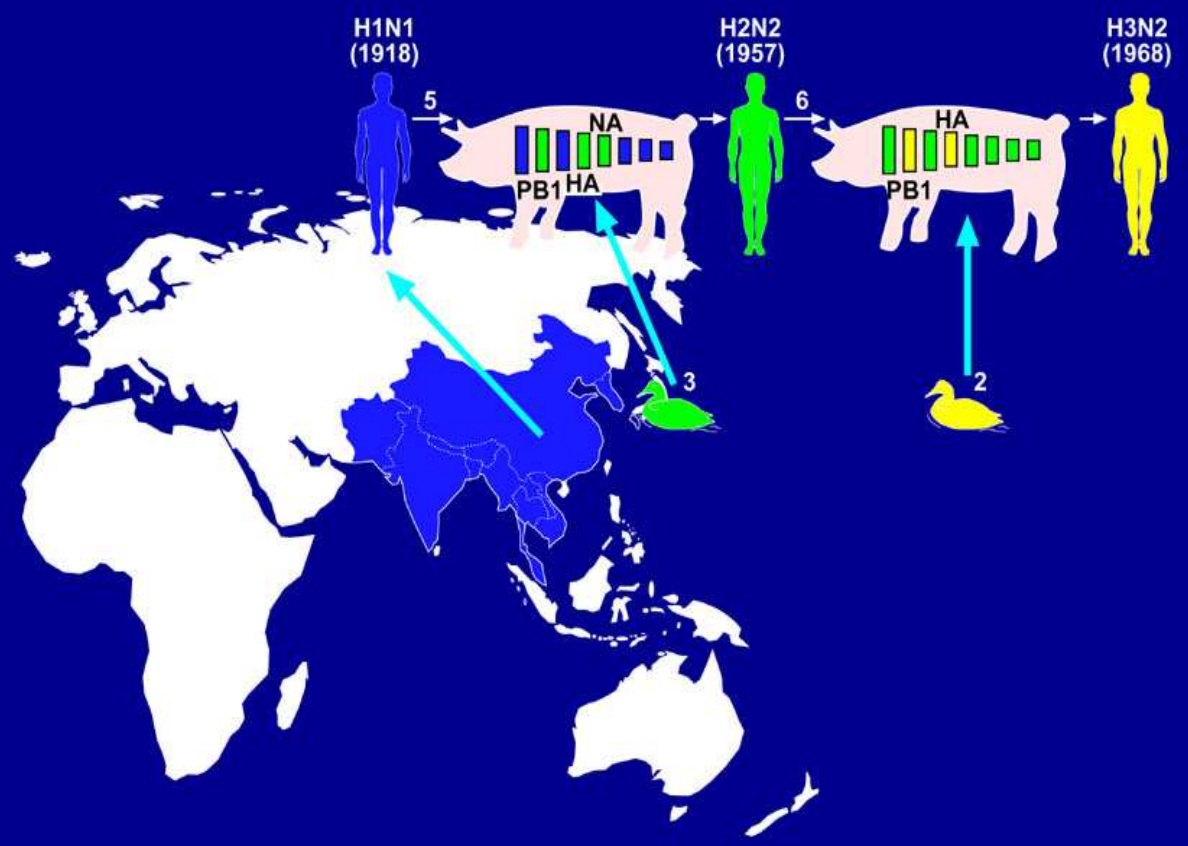
(Antigenic drift: inter-pandemic change)

2001 H1N2

("Swine" flu pandemic) 2009 H1N1

?

Genetic Reassortment in Antigenic Shift



- Pigs proposed as intermediate host

Generation of the pandemic influenza A(H1N1) 2009 virus

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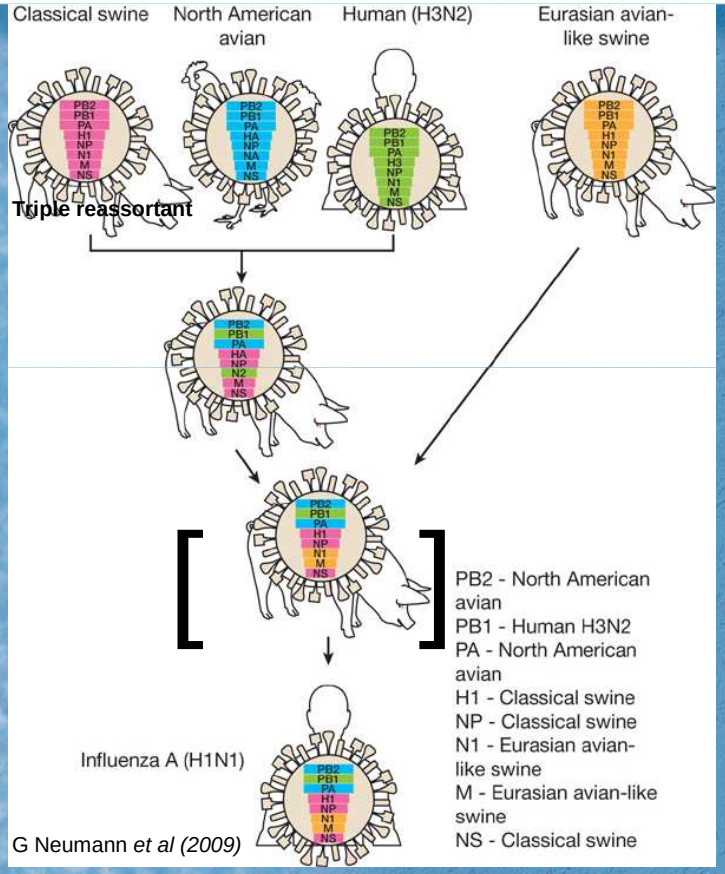
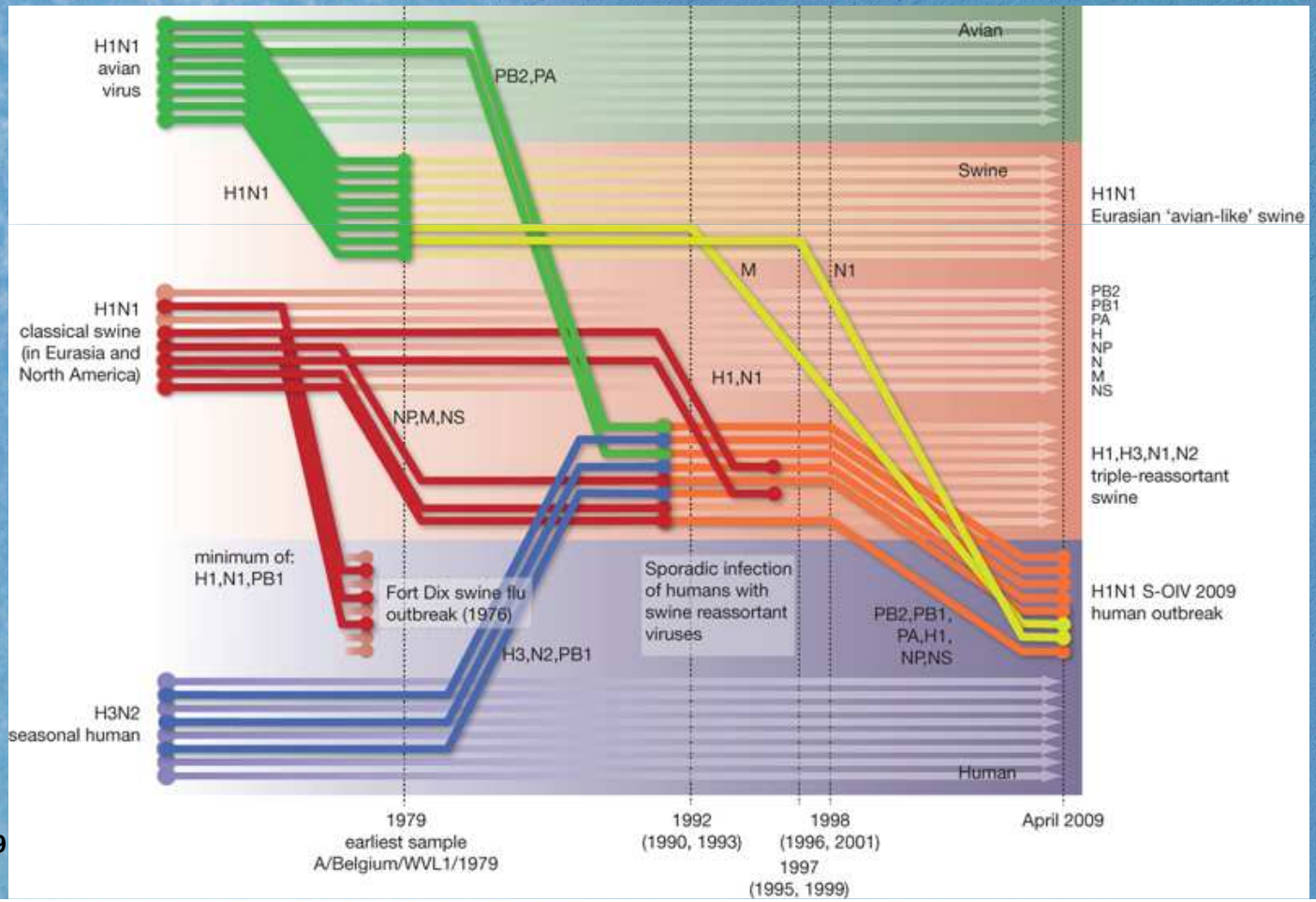


G Neumann *et al (2009)*

1998: Emergence of double(swine/human) and triple(swine/human/avian) reassortant H3N2 swine viruses
Subsequent reassortment to produce cH1N1, cH1N2, H3N1 (same 6 internal genes)
2005: Emergence of Hu-H1N1, Hu-H1N2 triple reassortants
2009: Emergence of novel human H1N1 (triple reassortant cH1 x 'avian-like' eurasian swine viruses) – **Origin?**
2009: Pandemic H1N1 virus causes outbreaks in pigs (Canada, Argentina, Australia, Germany, UK, USA) and turkeys (Chile)

Reconstruction of the sequence of reassortment events leading to the emergence of the pandemic H1N1 virus



G. Smith et al, 2009

Changes responsible for increased human-to human transmissibility of H1N1pdm?

- **Internal genes - no obvious 'human-like' changes in host range markers (e.g. PB2 E627K, D701N; but complementation by R591)**

- **HA - Receptor binding specificity (SA2,6Gal/SA2,3Gal receptors)?**
  (D186/D222 – already human-like)
  - Binding affinity?

- **NA – Improved complementarity with HA?**

Phylogenetic relationships of the HAs of pandemic H1N1 with earlier human and swine viruses

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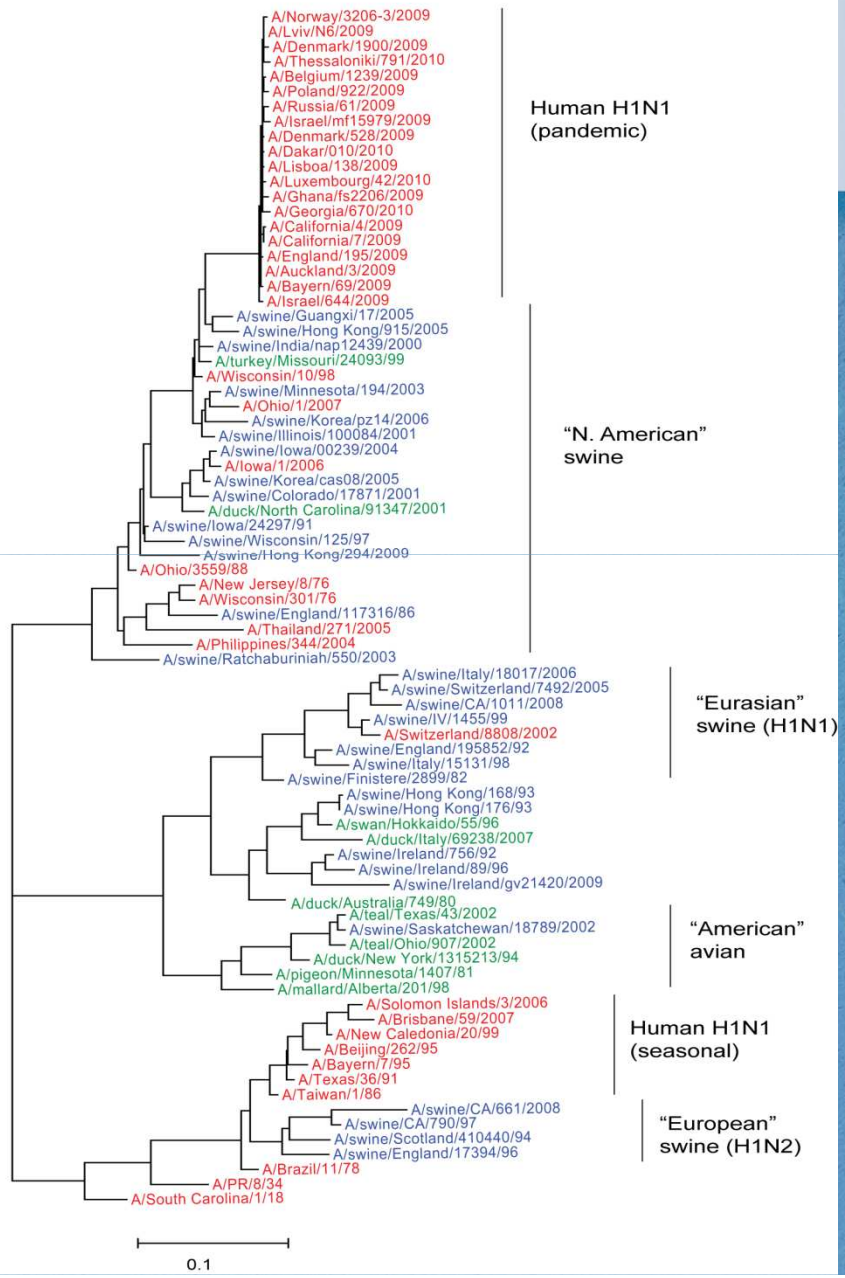
Phylogenetic comparison of human, swine and avian H1 HA genes



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Multiple introductions of avian H1N1 viruses into pigs

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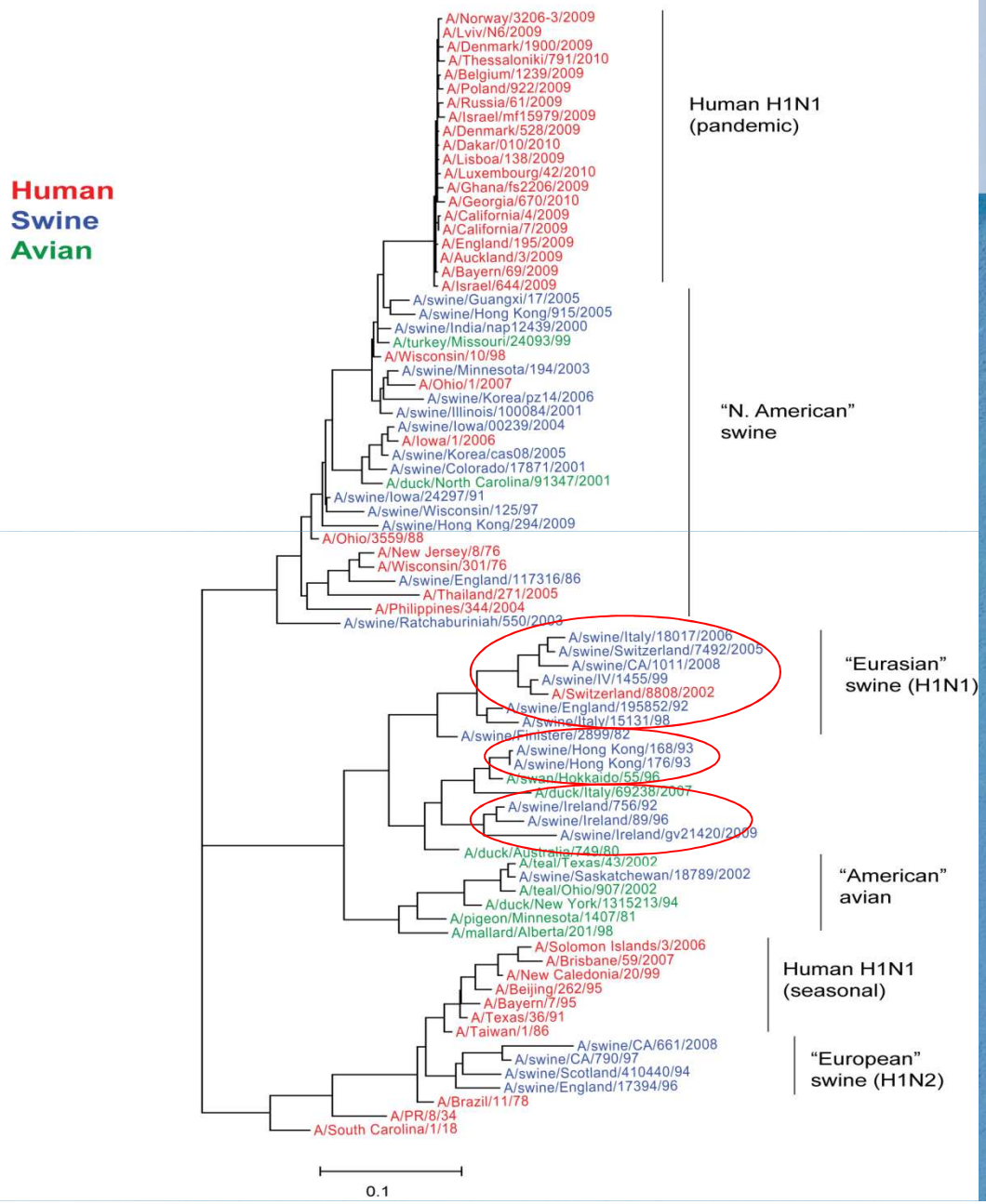
Phylogenetic comparison of human, swine and avian H1 HA genes



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Influenza virus infection

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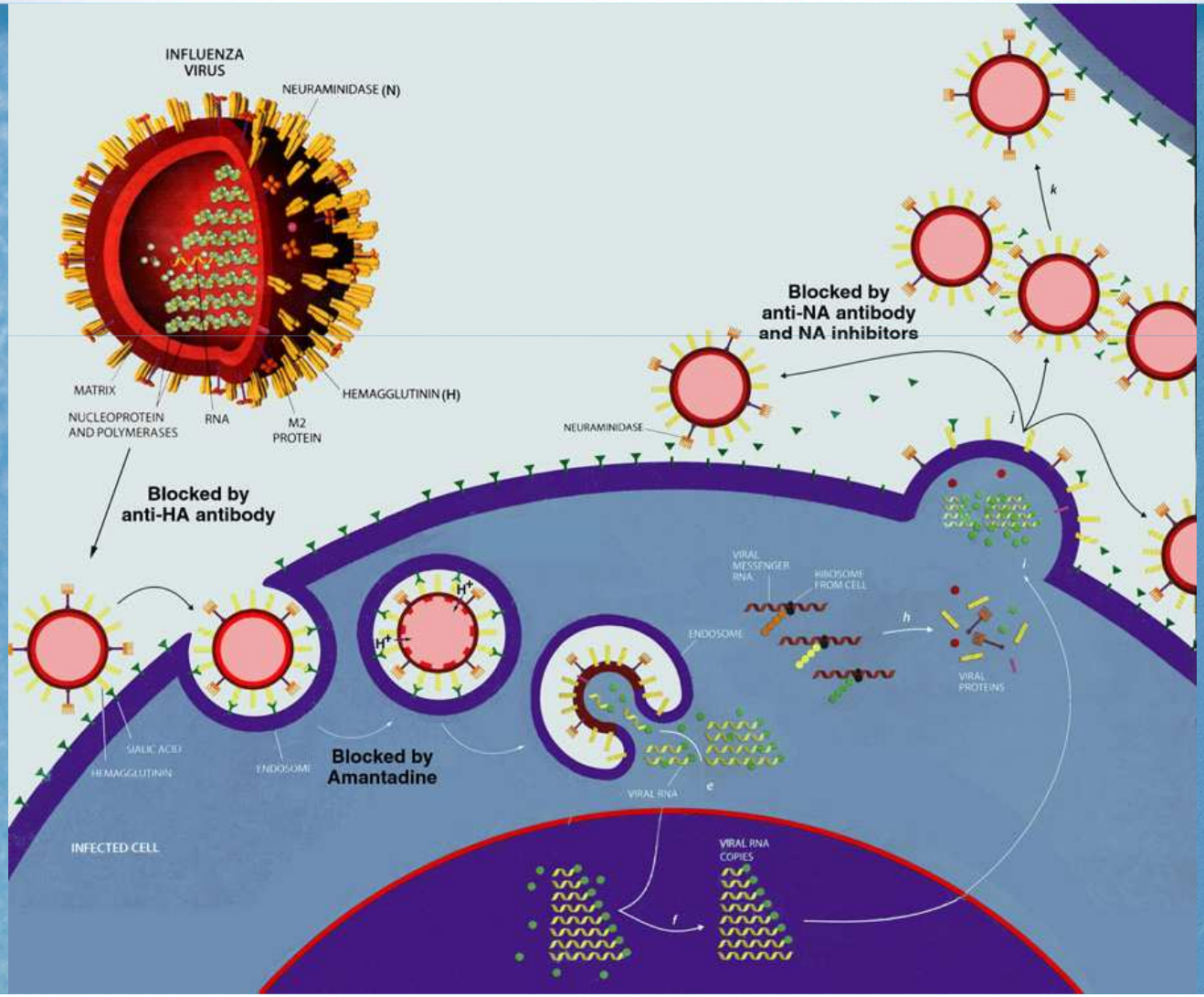
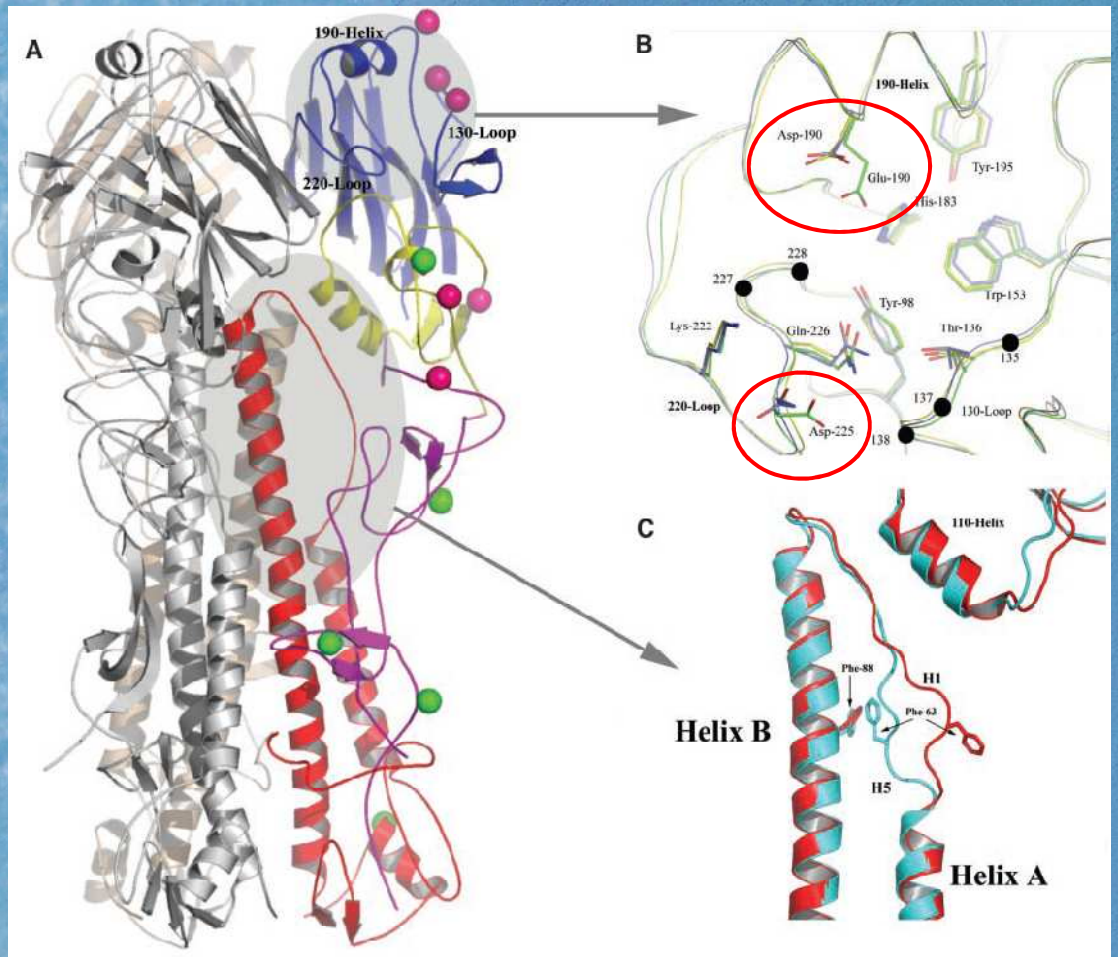
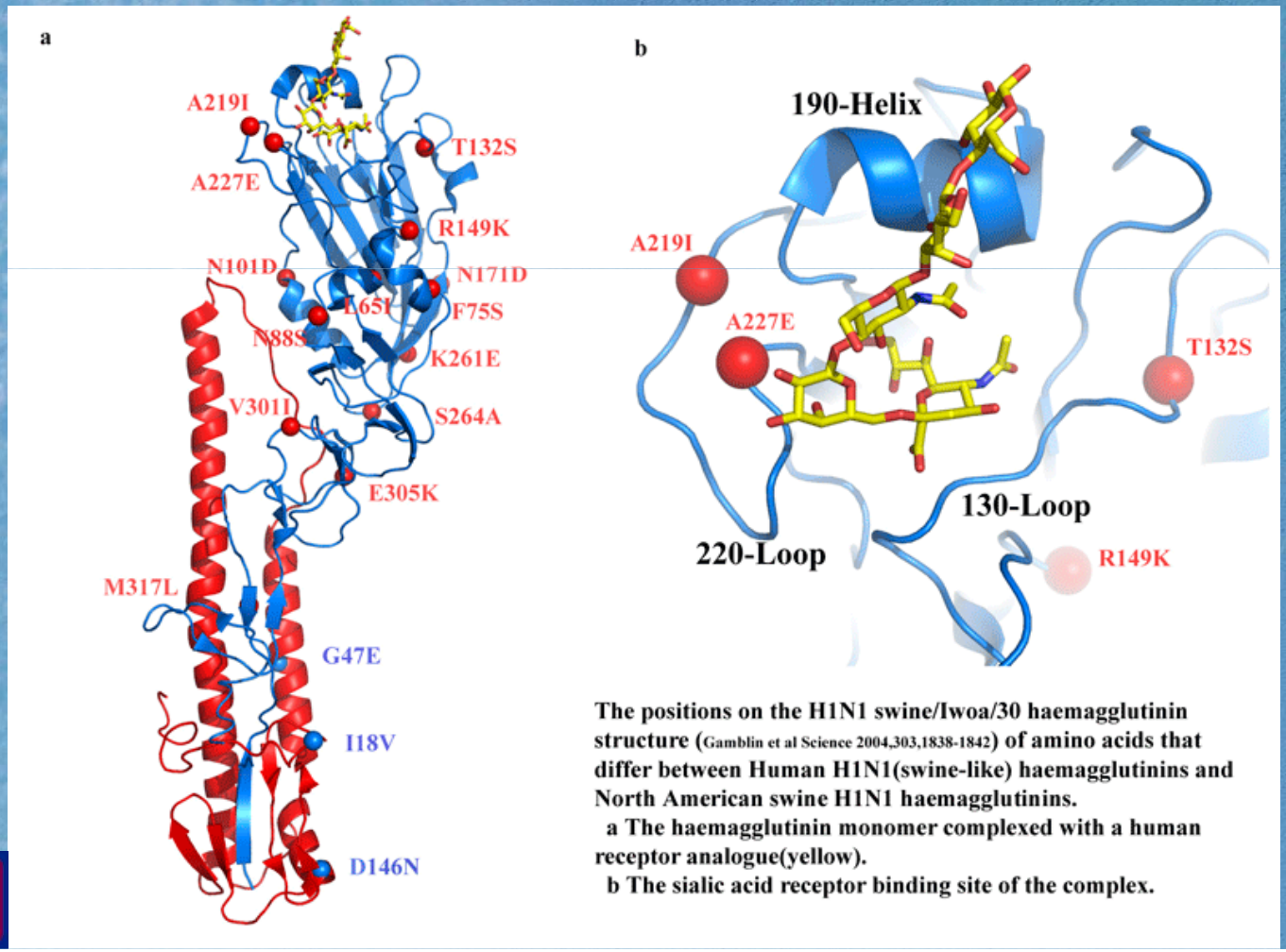


Receptor binding site of H1 haemagglutinin - amino acid residues important in determining specificity for human and avian viruses



Gamblin et al, 2004

Amino acid differences between HAs of human pandemic H1N1 and swine triple reassortant H1 viruses



The positions on the H1N1 swine/Iwoa/30 haemagglutinin structure (Gamblin et al Science 2004,303,1838-1842) of amino acids that differ between Human H1N1(swine-like) haemagglutinins and North American swine H1N1 haemagglutinins.
a The haemagglutinin monomer complexed with a human receptor analogue(yellow).
b The sialic acid receptor binding site of the complex.

No major change in receptor binding specificity of HA of pandemic A(H1N1)

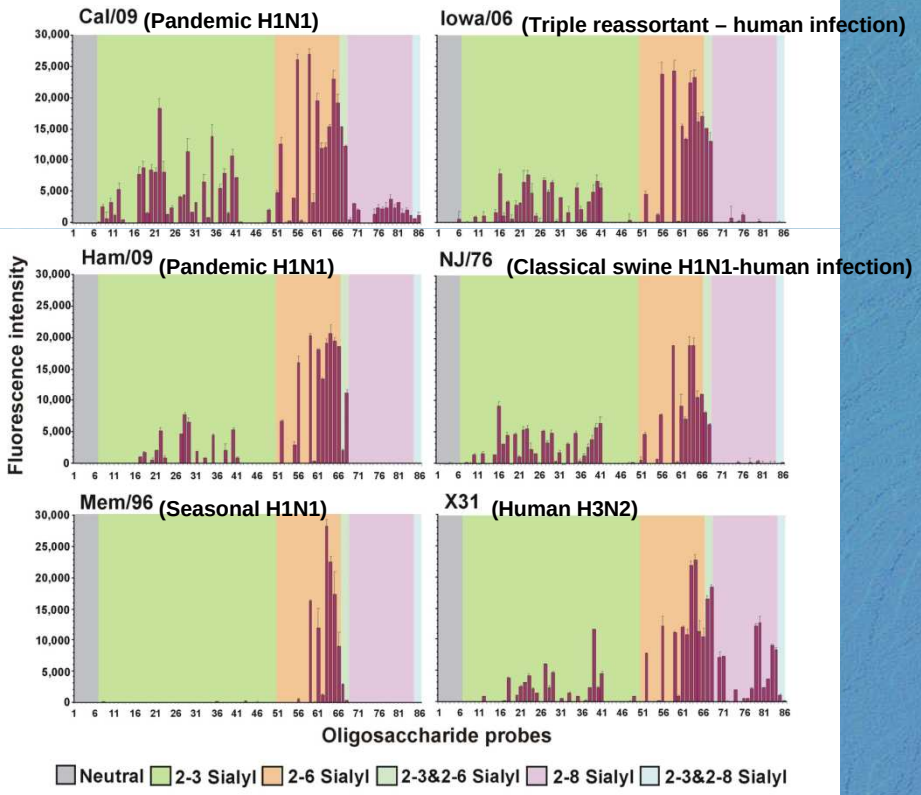


Figure 1

Childs et al, 2009

Glycan arrays – preference for binding human-like (2,6), but also avian-like (2,3), receptors

Virulence of Pandemic A(H1N1) 2009 Viruses

- Human: generally mild/moderate disease (equivalent to seasonal)
  ~ 2% severe illness
  ~ 20,000 fatal cases of confirmed H1N1 worldwide

- Human lung cells – more efficient replication than seasonal H1N1

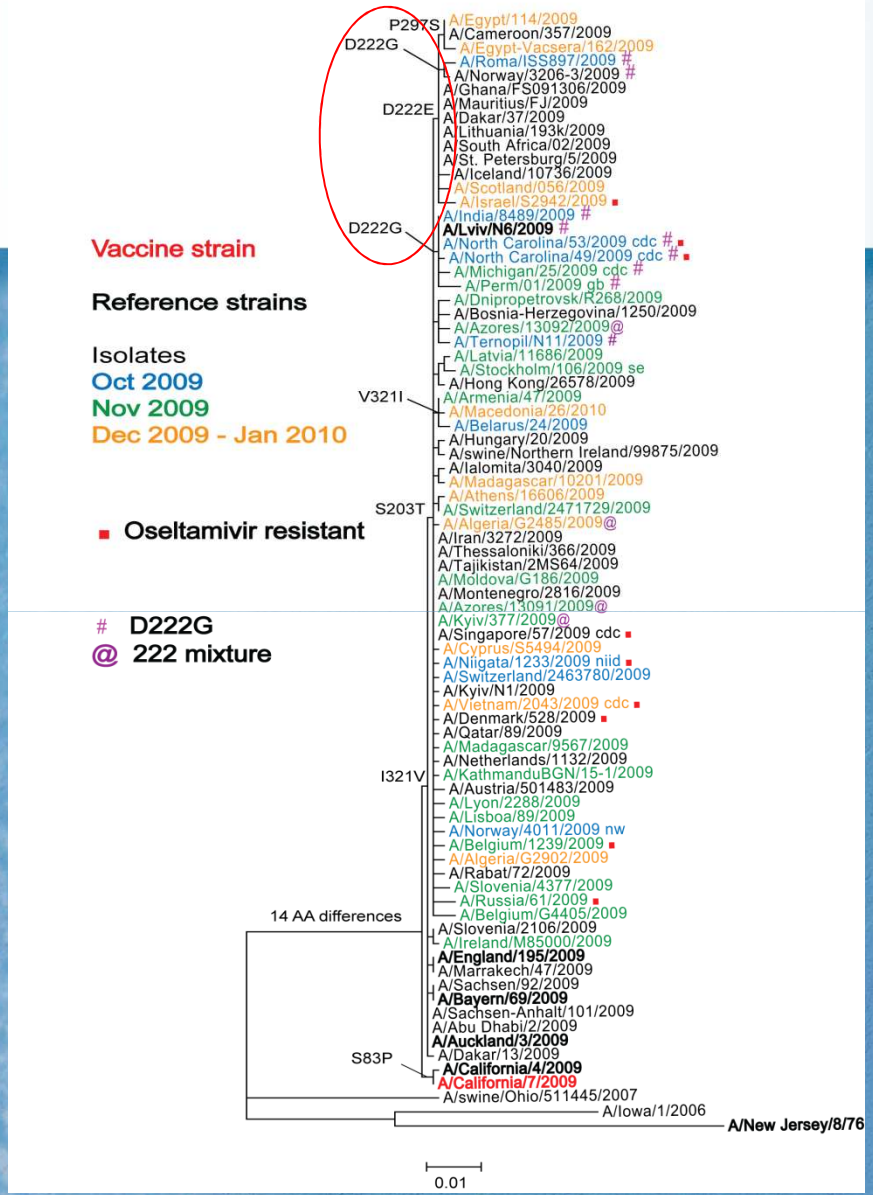
- Ferrets: - more severe disease than seasonal A(H1N1)
  - increased mortality?; increased weight loss
  - replication in LRT and URT; increased virus shedding (Munster et al, 2009)
  - PR8 hgr reassortants (vaccine viruses) less virulent

- Mice/Non-human primates - more efficient replication and more severe pathology in lungs than seasonal H1N1 (Itoh et al, 2009)

- Pigs: disease typical of earlier swine viruses
  symptoms peak at 2days, resolved by 5days; no mortality

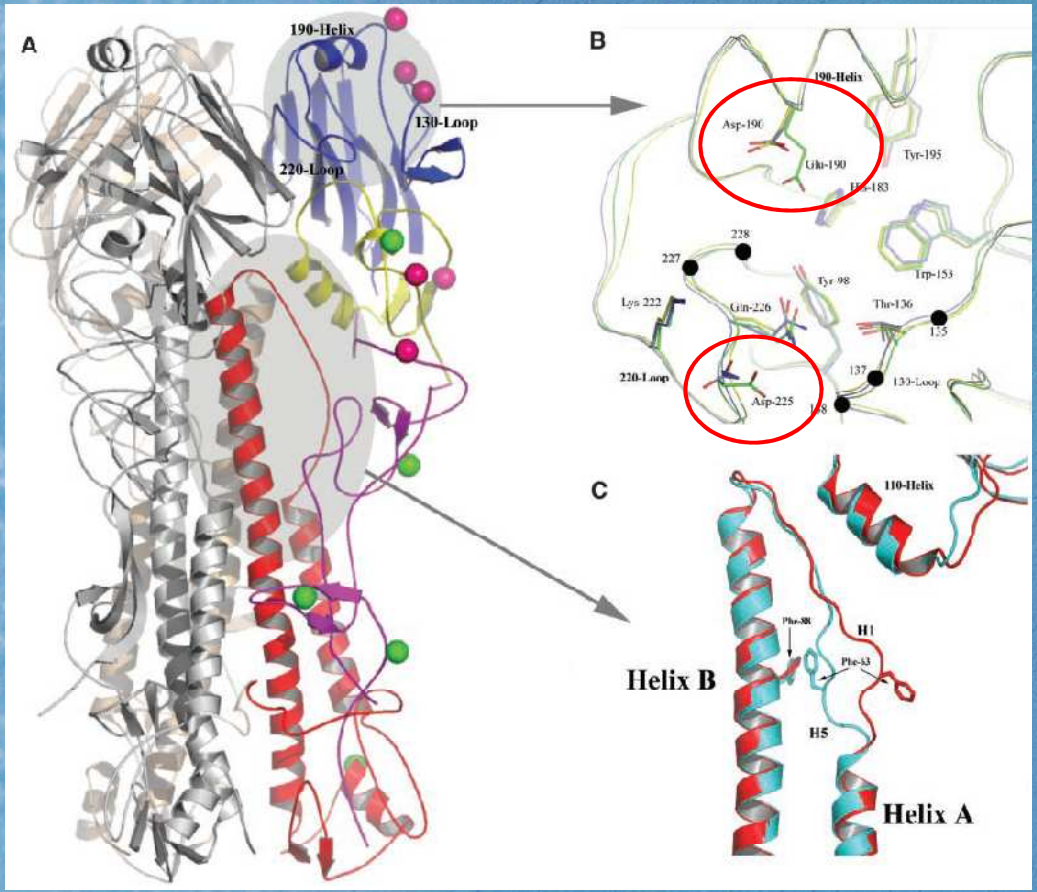
Amino acid substitutions in residue 222 of the HA receptor binding site

Figure 8. Phylogenetic comparison of pandemic A(H1N1) HA genes



- Correlation between sporadic D222G mutation and severe/fatal disease

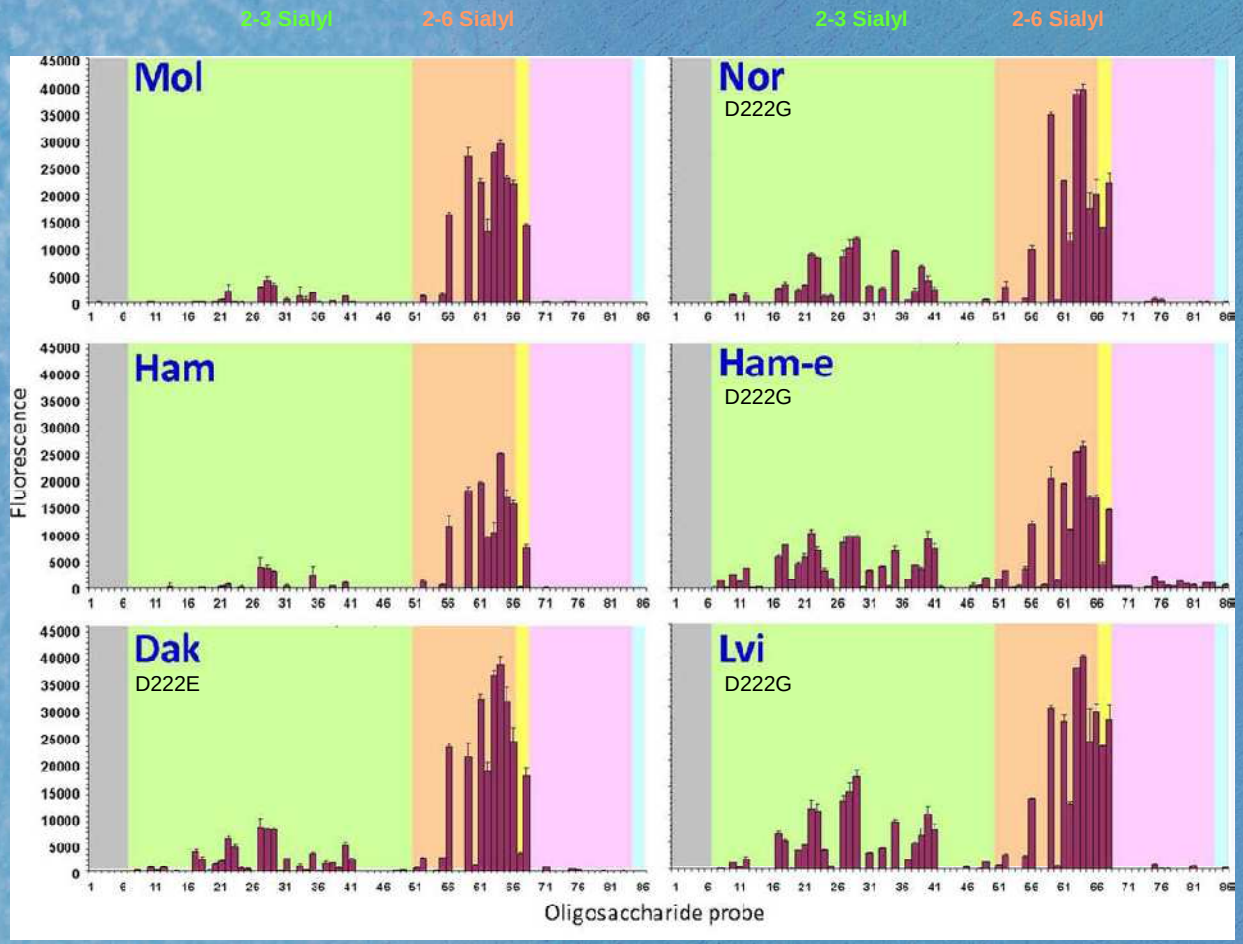
Receptor binding site of H1 haemagglutinin - amino acid residues important in determining specificity for human and avian viruses



Gamblin et al, 2004

(Numbering: 190 and 225 in H3 correspond to 186 and 222 in H1)

The D222G substitution causes an increase in binding to 2-3 receptors



Liu et al, 2010

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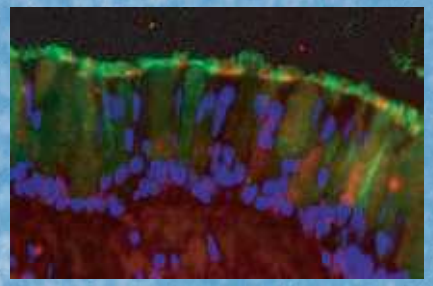
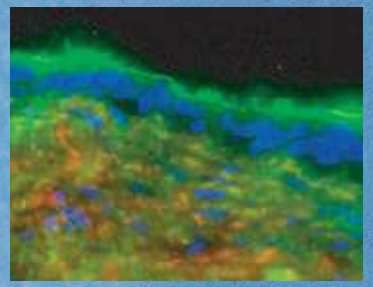
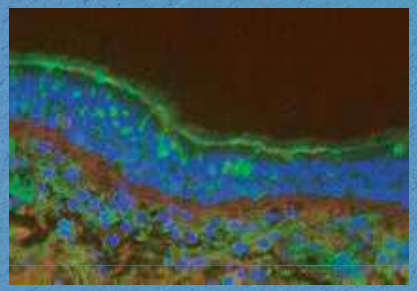
SA2,3Gal receptors more prevalent in lower respiratory tract



Nasal

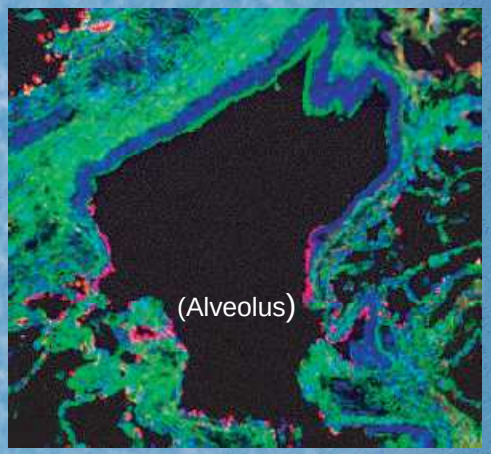


Sinus

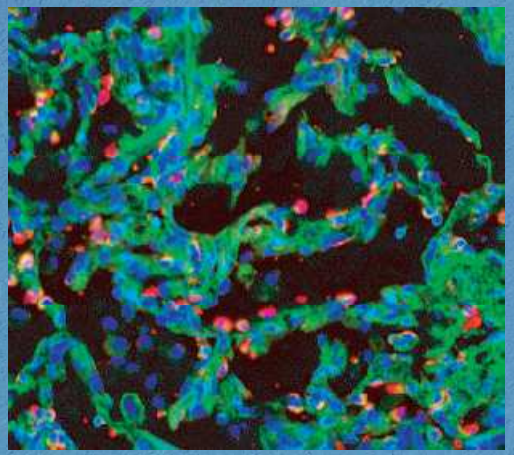


Bronchus

Green: Sambucus nigra lectin staining of SA2,6Gal receptors



Bronchiole



Alveolus

Red: Maackia amurensis lectin staining of SA2,3Gal receptors

Shinya et al, 2006

Viruses with D222G substitution

- Detected sporadically; correlation with severe disease
- Increased binding to 2-3 sialyl receptors
- Increased infection of ciliated human airway epithelial cells
- More frequent identification in LRT specimens
- Tropism in pigs: 222D nasal; 222G in lung
- Cause or consequence of severe LRT infection?
- Infrequent transmission (in contrast to D222E)

Changes responsible for increased human-to human transmissibility of H1N1pdm?

- **Internal genes - no obvious 'human-like' changes in host range markers (e.g. PB2 E627K, D701N; but complementation by R591)**

- **HA - Receptor binding specificity (SA2,6Gal/SA2,3Gal receptors)?**
  (D186/D222 – already human-like)
  - Binding affinity?

- **NA – Improved complementarity with HA?**

- **M1/M2(amantadine resistance)?**

Emergence of amantadine resistance in human and animal viruses

- Pre 1980's - low incidence; approx. 1%
- Mid 1980's - European swine viruses
  (sporadic human cases)
- 2000 - avian H5N1, H9N2 (SE Asia)
- 2003 - avian H5N1 clade 1; human cases
  H5N3(SE Asia)
  H7N2(N America)
- 2003 - - human H3N2 (China/Hong Kong; worldwide)
- 2006 - 09 - human H1N1 emergent variant (clade 2C)
- 2009 - pandemic H1N1

Origin of amantadine resistance (M2 S31N) of pandemic H1N1 viruses

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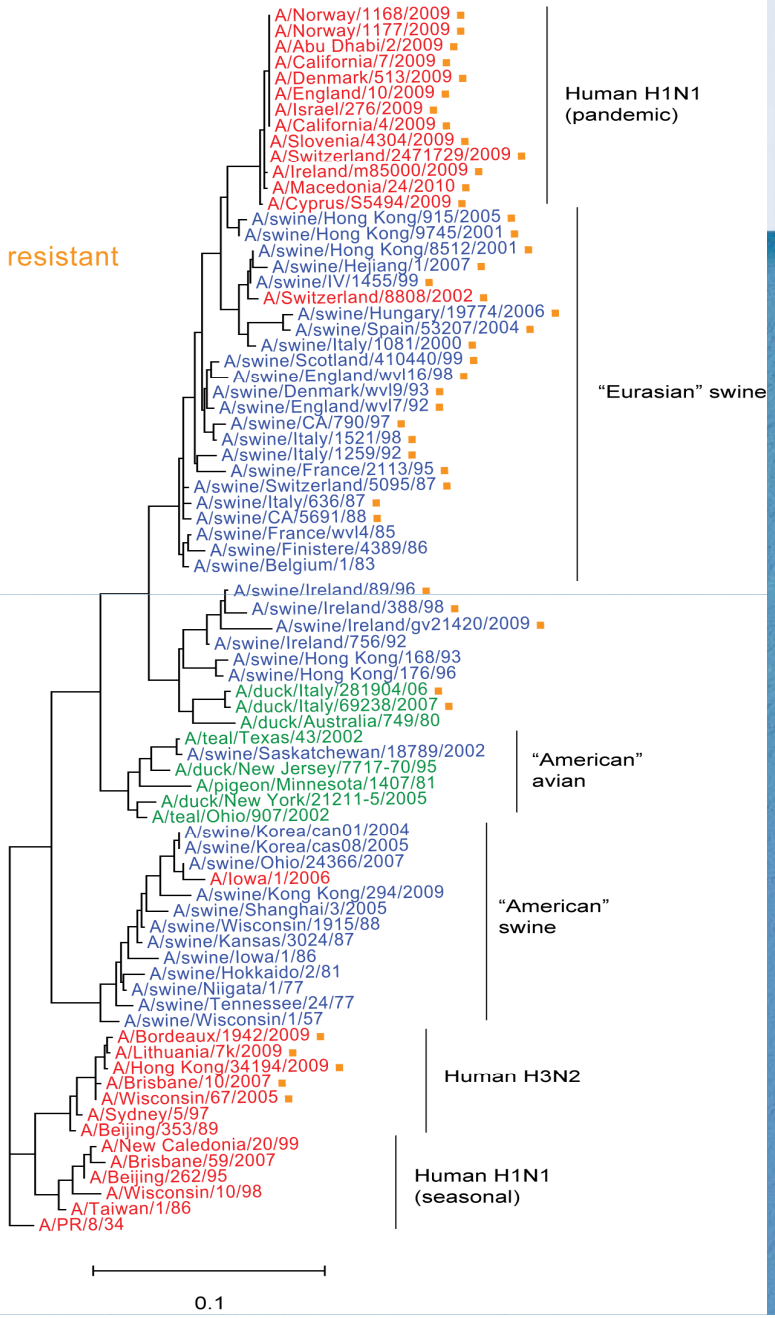
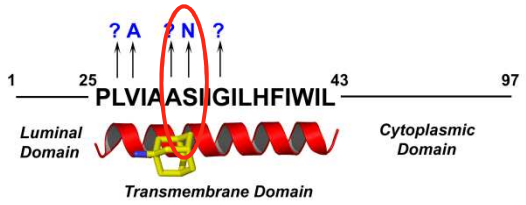
Phylogenetic comparison of human, swine and avian M genes

■ Amantadine resistant

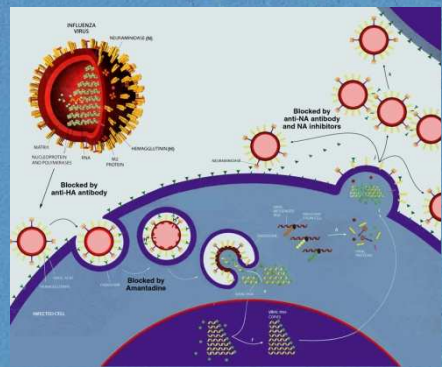
Human
Swine
Avian





Adaptive changes in NA (in relation to HA)

- **Alteration in enzyme (receptor destroying) activity – to complement receptor binding changes in HA**
- **Emergence of oseltamivir-resistant (H275Y) seasonal H1N1 viruses - alteration in enzyme activity?**
- **[Contribution of NA to receptor binding of recent H3N2 virus isolates - role in attachment?]**



Emergence of oseltamivir-resistant seasonal H1N1 viruses during 2007-2008

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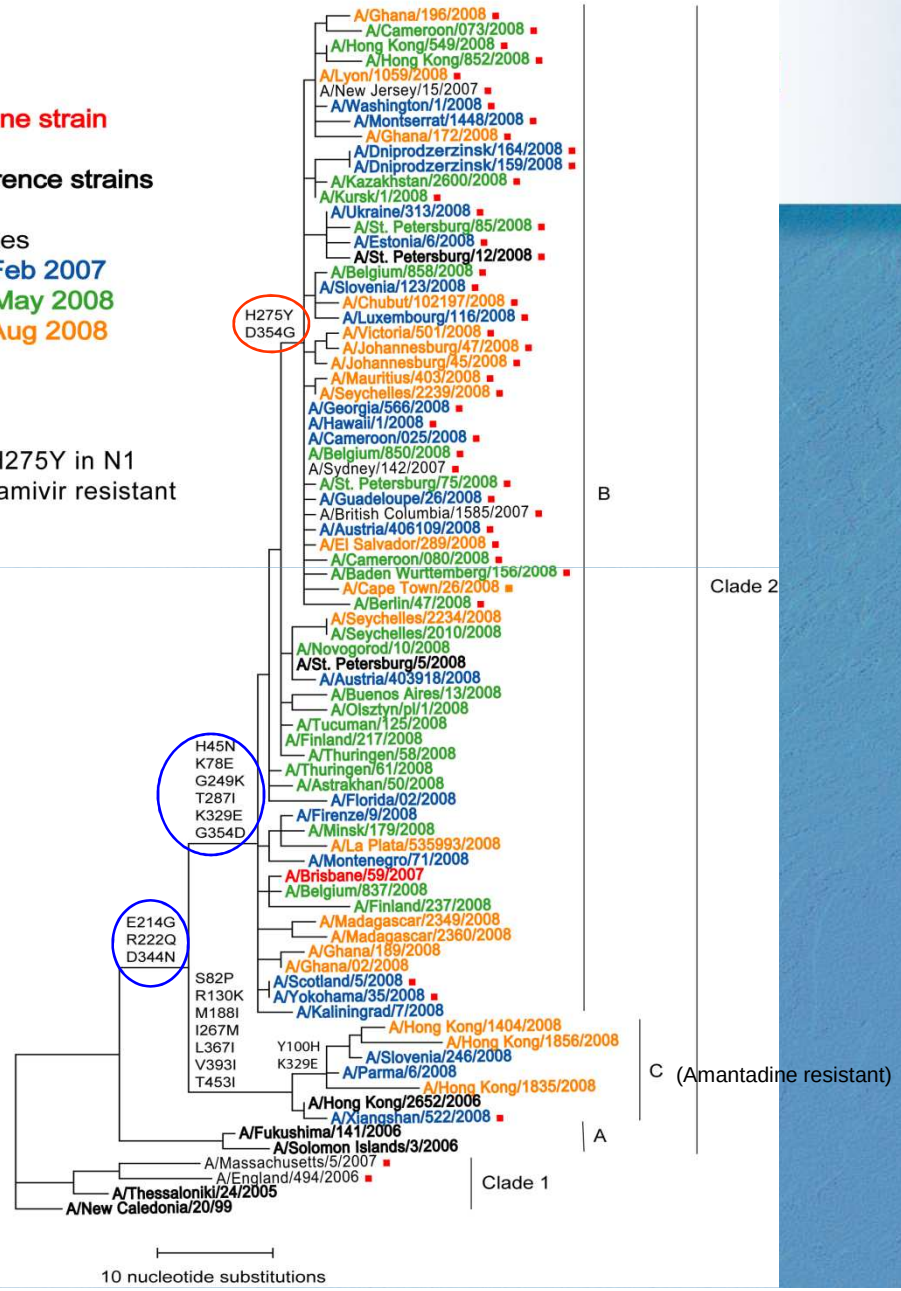
Figure 2. Phylogenetic comparison of N1 neurmindase genes

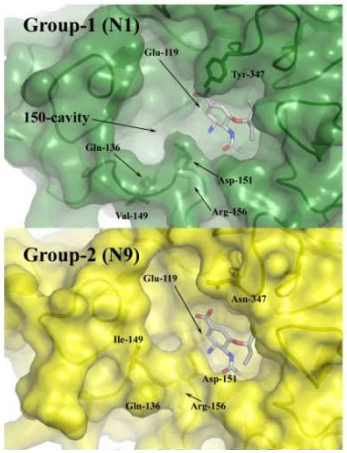
Vaccine strain

Reference strains

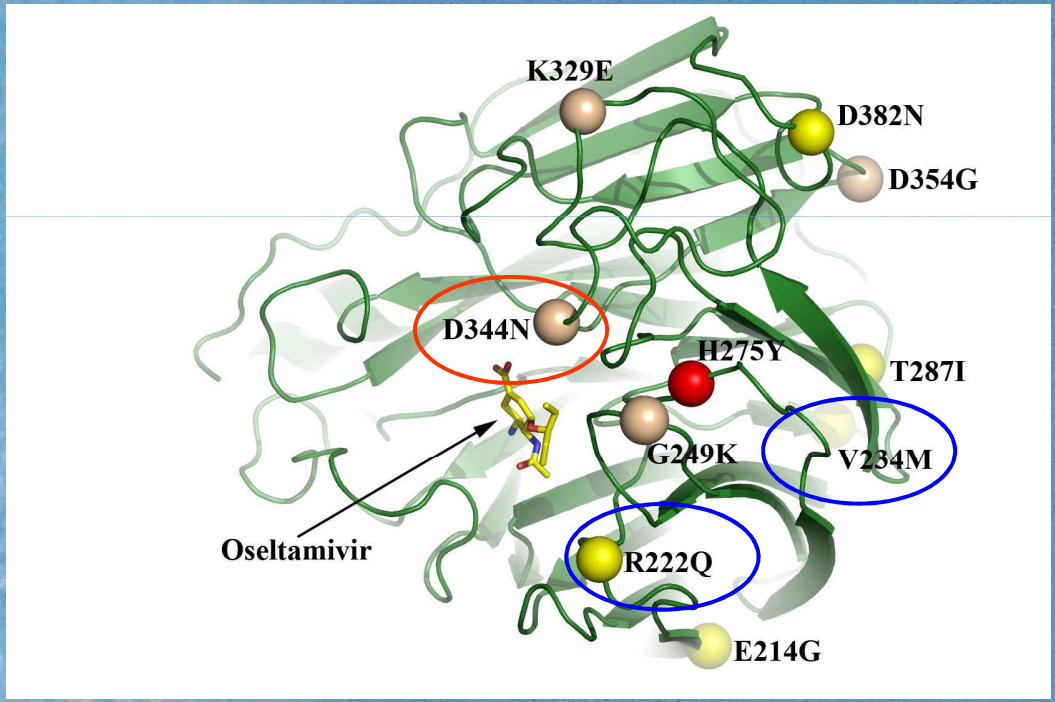
Isolates
Jan-Feb 2007
Mar-May 2008
Jun-Aug 2008

■ H275Y in N1
Oseltamivir resistant





Locations of recent amino acid changes in the NAs of seasonal H1N1 viruses relative to the catalytic site and H275Y oseltamivir-resistant mutation



Compensatory mutations:

- **D344N - increase in NA activity**
- **R222Q + V234M – increase in cell surface expression of NA (Bloom et al, 2010)**

H275Y – adaptive change?; resistance to oseltamivir simply coincidental

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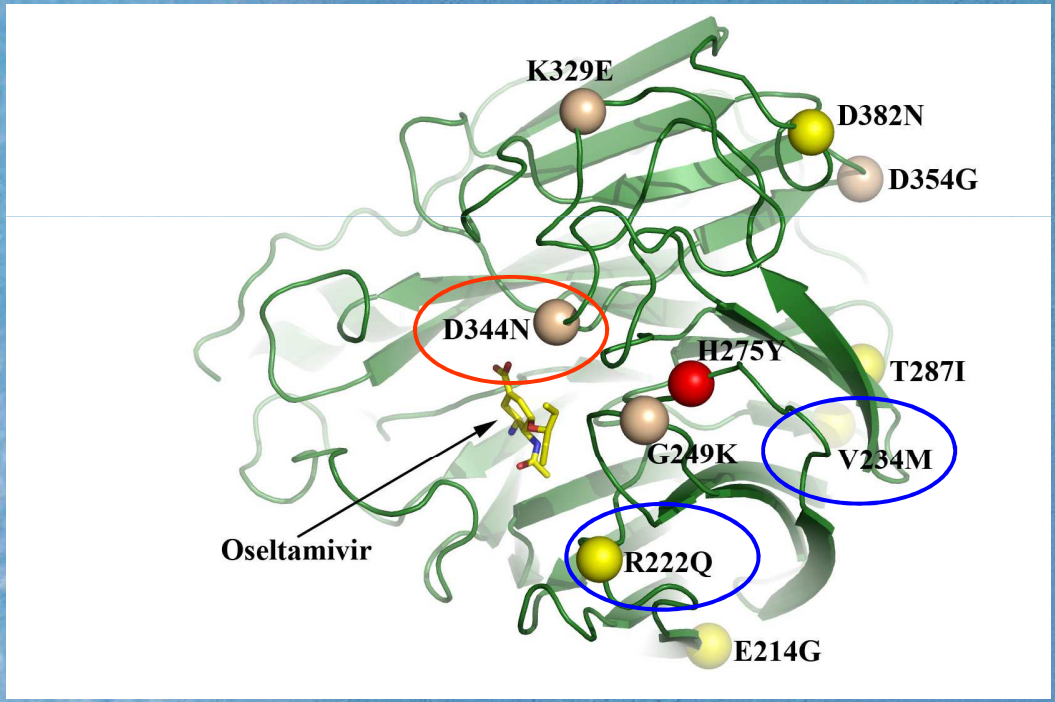

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H275Y – adaptive change?; resistance to oseltamivir simply coincidental

Oseltamivir-resistant pandemic H1N1 viruses: sporadic (~300 cases to August 2010)

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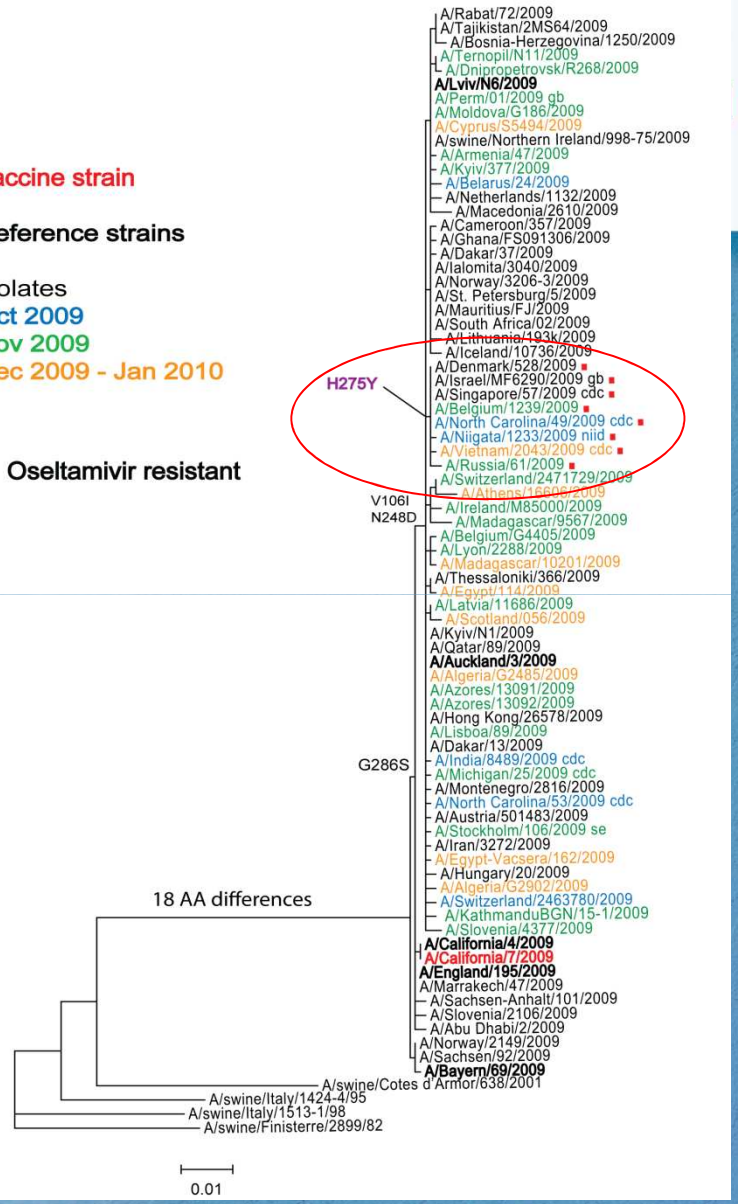
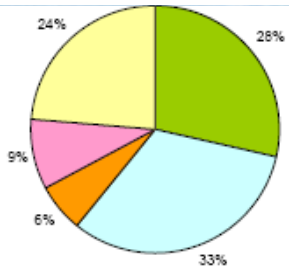
Figure 11. Phylogenetic comparison of pandemic A(H1N1) NA genes





(Effect of H275Y on transmission in animals – contradictory data)

Pandemic H1N1 virus antigenically similar to triple reassortant swine viruses

Table 1 Results of haemagglutination inhibition tests of influenza A(H1N1) viruses with post-infection ferret sera[A]

	Ferret antisera to reference viruses (titres)				
	A/swine/Iowa/30	A/Illinois/9/2007[B]	A/California/4/2009[C]	A/California/7/2009[D]	A/Brisbane/59/2007[E]
Antigens					
A/swine/Iowa/30	320	5	5	5	<10
A/Illinois/09/2007	160	5120	2560	5120	<10
A/California/04/2009	20	1280	1280	1280	<10
A/California/07/2009	80	1280	1280	1280	<10
A/Brisbane/59/2007	20	ND[5]	<40	<40	160
Novel A (H1N1) isolates					
A/Texas/05/2009	160	2560	1280	1280	<10
A/Mexico/4108/2009	160	1280	1280	1280	<10
A/Mexico/4596/2009	160	2560	1280	1280	<10
A/Mexico/4646/2009	160	1280	1280	1280	<10
A/New York/18/2009	160	2560	2560	2560	<10
A/Washington/11/2009	20	2560	1280	1280	<10
A/New Mexico/04/2009	80	2560	2560	2560	<10
A/El Salvador/211/2009	40	2560	1280	2560	<10
A/El Salvador/213/2009	20	1280	640	1280	<10
A/Hawaii/09/2009	80	2560	2560	1280	<10
A/Costa Rica/4314/2009	80	2560	2560	2560	<10
A/Costa Rica/4857/2009	20	1280	640	1280	<10
A/England/195/2009	20	ND	1280	1280	<10
A/Israel/644/2009	20	ND	1280	1280	<10
A/Netherlands/602/2009	20	ND	1280	1280	<10
A/Auckland/1/2009	20	ND	1280	2560	<10
A/Auckland/3/2009	20	ND	1280	2560	<10

[A] Data provided by WHO Collaborating Centres for influenza
[B] Antisera raised against earlier human influenza A (H1N1) virus associated with swine infection
[C] Antisera raised against the emergent novel human influenza A (H1N1) virus
[D] Antisera raised against human seasonal influenza A (H1N1) virus
[E] ND = not determined

Increased heterogeneity in HA sequence of recent pandemic H1N1 viruses

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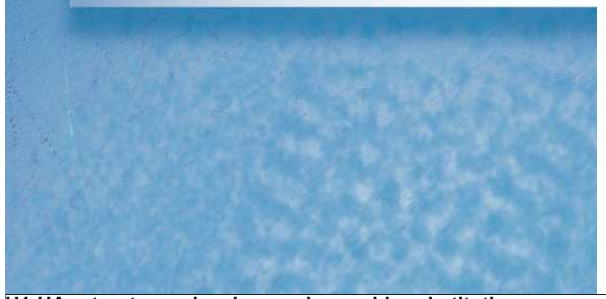
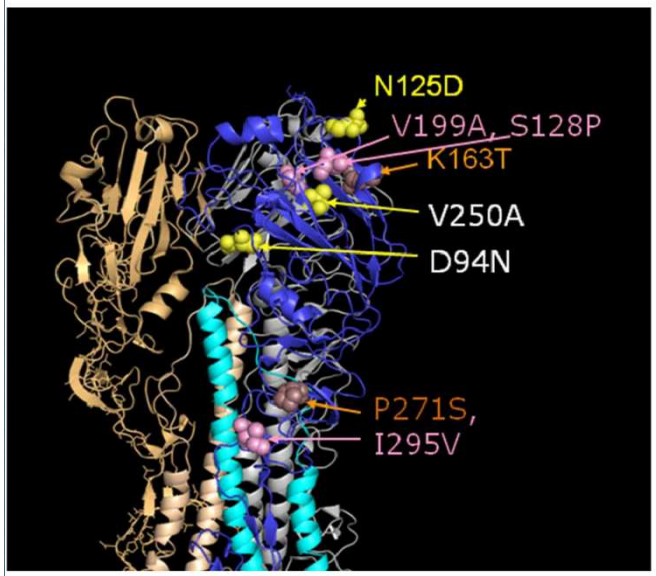


H1-HA structure showing amino acid substitutions associated with emerging genetic groups of H1 pandemic virus



Two recently emerged genetic groups (**Figure 6**) each containing a subgroup are shown:

Group	Subgroup
N125D	D94N, V250A
S128P, V199A, I295V	K163T, P271S

Phylogenetic comparison of influenza A (H1N1pdm) HA1 genes

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No change in antigenic properties of recent pandemic H1N1 viruses

Antigenic analyses of pandemic influenza A(H1N1) viruses

			Haemagglutination inhibition titre[1]					
			Post infection ferret sera					
Viruses	Collection date	Passage History	A/Cal 4/09 C4/F14/09	A/Cal 7/09 F05/10	A/Eng 195/09 F06/10	A/Auck 3/09 C4/17/09	A/Bayern 69/09 C4/33/09	A/Lviv N6/2009 C4/34/09
REFERENCE VIRUSES								
A/California/4/2009		C1,E3	1280	1280	2560	2560	1280	2560
A/California/7/2009		E8	640	1280	640	640	1280	2560
A/England/195/2009		MDCK5	1280	2560	2560	2560	1280	1280
A/Auckland/3/2009		Ex+3	1280	2560	1280	2560	640	1280
A/Bayern/69/2009		MDCK5	160	160	40	40	320	320
A/Lviv/N6/2009		MDCK4/SIAT2	640	320	80	80	640	640
TEST VIRUSES								
A/Hong Kong/2212/2010	2010-07-16	E3	2560	2560	2560	2560	1280	2560
A/Hong Kong/2200/2010	2010-07-14	E3	2560	2560	2560	2560	1280	2560
A/Cameroon/LEID/02/10/175/2010	2010-02-09	Cx/MDCK1	2560	2560	2560	2560	1280	1280
A/Cameroon/LEID/02/10/175/2010	2010-02-09	Cx/MDCK1	2560	2560	2560	2560	1280	1280
A/Switzerland/3628824/2010	2010-08-19	MDCK2	1280	1280	1280	640	640	1280
A/Cameroon/10v-2166/2010	2010-04-22	MDCK3	1280	1280	1280	2560	1280	1280
A/Cameroon/10v-2565/2010	2010-06-08	MDCK2	1280	1280	1280	1280	640	1280
A/Ghana/FS-10-3829/2010	2010-06-19	SIAT1/MDCK3	2560	1280	2560	2560	1280	2560
A/Ghana/FS-10-4249/2010	2010-08-26	SIAT1/MDCK2	640	640	640	1280	640	640
A/Johannesburg/27/2010	2010-06-30	MDCK 2	640	1280	1280	1280	640	1280
A/Dakar/034/2010	2010-03-01	MDCK3	1280	1280	2560	2560	1280	2560
A/Johannesburg/119/2010	2010-08-19	MDCK1/MDCK2	2560	2560	2560	2560	1280	2560
A/Norway/609/2010	2010-06-12	MDCK1/MDCK2	1280	2560	2560	2560	640	1280
A/Puerto Montt/11868/2010	2010-05-14	E3/E3	2560	2560	5120	5120	2560	5120
A/Niger/666/2010	2010-02-05	C1/MDCK2	1280	1280	2560	1280	1280	1280
A/Texas/03/2010	2010-06-01	MK1/MDCK3	2560	2560	2560	5120	1280	2560
A/Picardie/505/2010	2010-01-30	C1/M1	2560	2560	2560	2560	1280	2560
A/Paris/529/2010	2010-02-02	C1/M1	2560	1280	1280	1280	640	1280
A/Norway/238/2010	2010-02-23	MDCK1/MDCK2	640	640	640	1280	320	1280
A/Cameroon/10v-813/2010	2010-03-23	c2/MDCK1	160	320	160	160	320	640
A/Cameroon/10v-1090/2010	2010-04-08	c4/MDCK1	80	160	40	40	320	320
A/St. Petersburg/204/2010	2010-02-08	E2/E3	320	640	160	160	1280	1280
A/Switzerland/3628801/2010	2010-08-19	MDCK2	640	320	320	320	640	640
A/Georgia/4484/2009	2009-12-21	SIAT4	160	320	40	80	640	640
A/Belgorod/2/2010	2010-03-15	E1, E1	320	640	160	160	1280	1280
A/Ghana/FS-1974/2010	2010-04-06	SIAT3	160	320	80	160	640	640

1. < = 40

Vaccine strain

Reduced HAI titres due to cell culture selected changes in residue 155

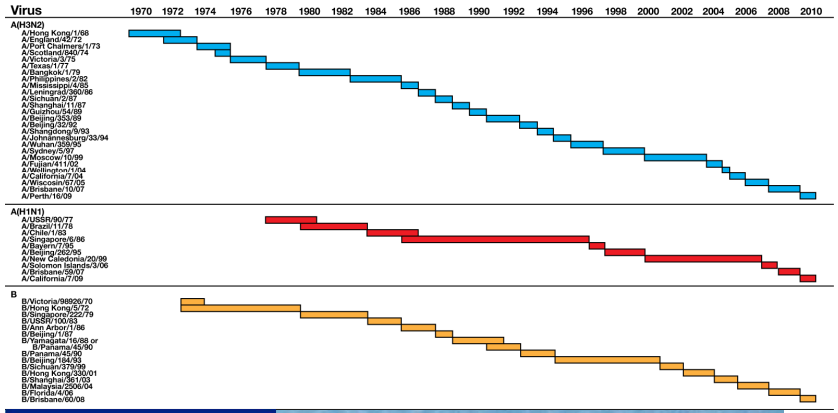
Amino acid changes in HA1 of recent H1N1 viruses

GEIG
Groupe d'Expertise et d'Information sur la Grippe

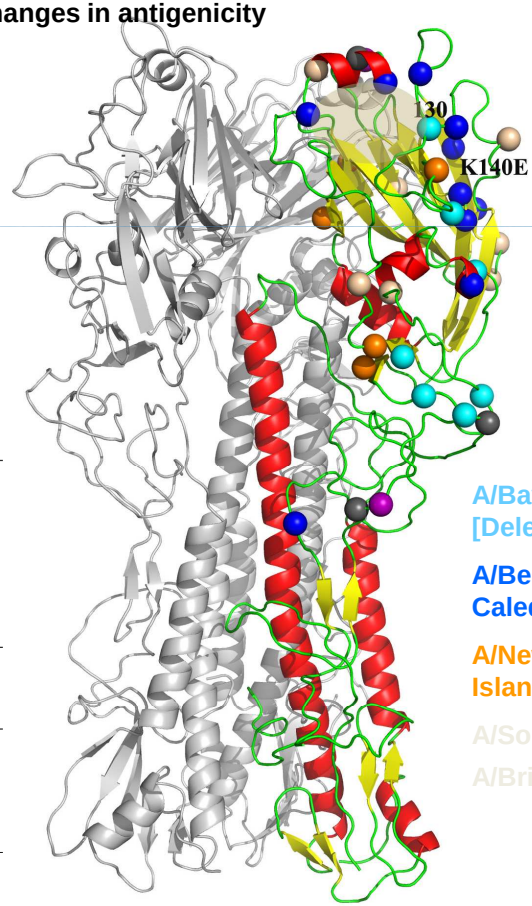
23e Rencontres du GEIG
Retours d'expériences sur la pandémie H1N1
jeudi 25 et vendredi 26 novembre 2010

Antigenic change in A(H1N1) less frequent than for A(H3N2) or B viruses

Changes in Influenza Vaccine Composition, 1970-2010



Single amino acid changes responsible for major changes in antigenicity



A/Bayern/7/95 – A/Beijing/262/95 (8) [Deletion of residue 130]

A/Beijing/262/95 – A/New Caledonia/20/99 (10)

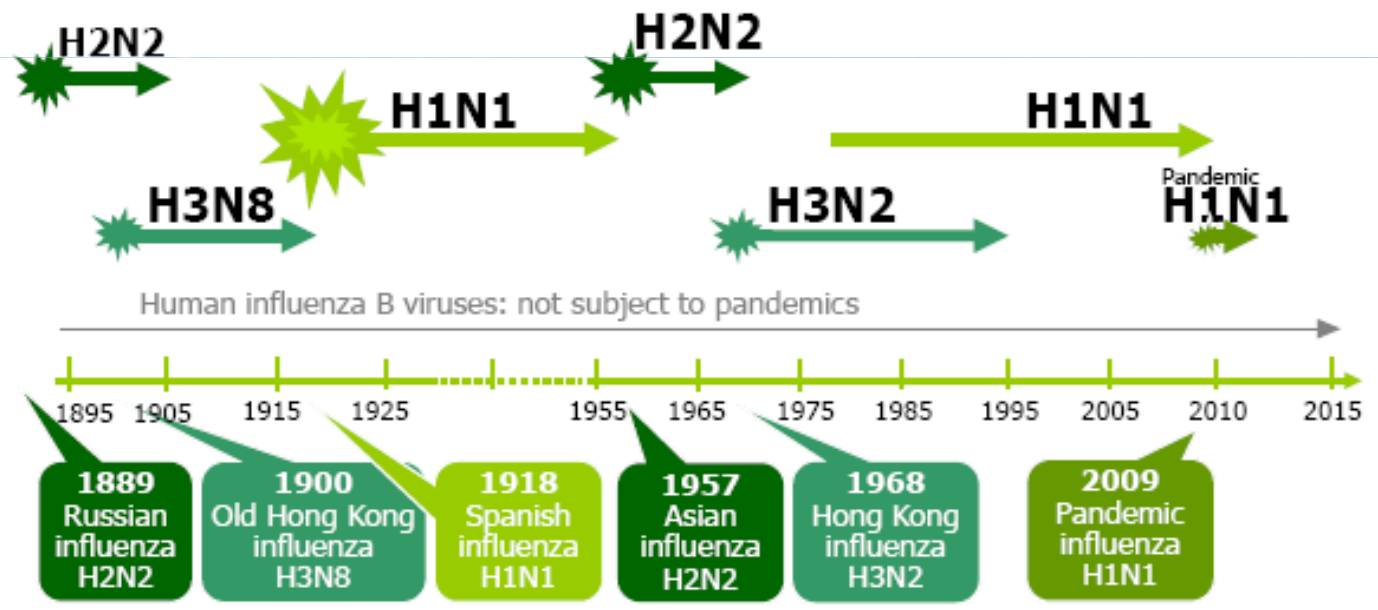
A/New Caledonia/20/99 – A/Solomon Islands/3/06 (6) [K140E change]

A/Solomon Islands/3/06 – A/Brisbane/59/07 (4)

MRC National Institute for Medical Research

Recirculation of 'human' subtypes of influenza A viruses??

Figure 1: Previous pandemics and inter-pandemic (seasonal) influenza



From Masato Tashiro

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