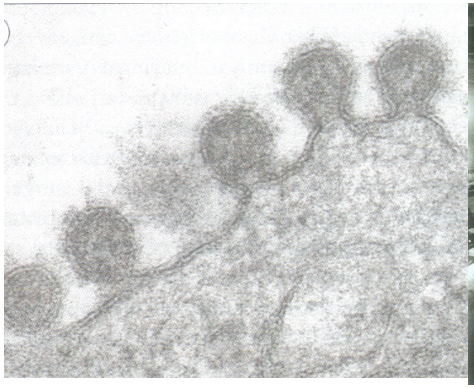
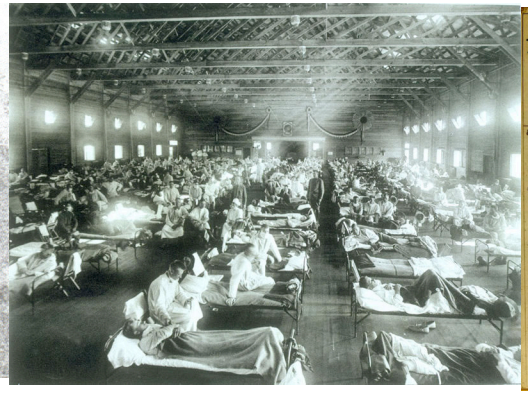
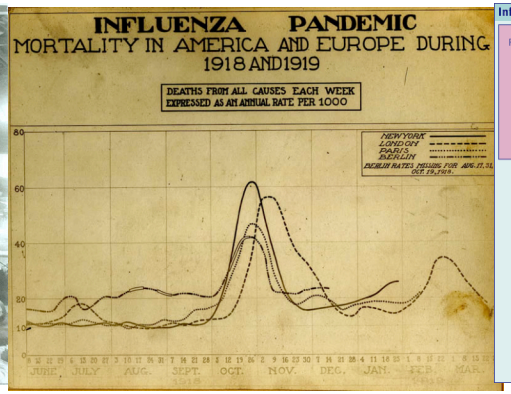
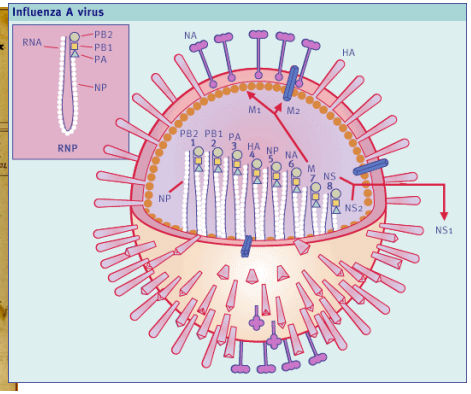




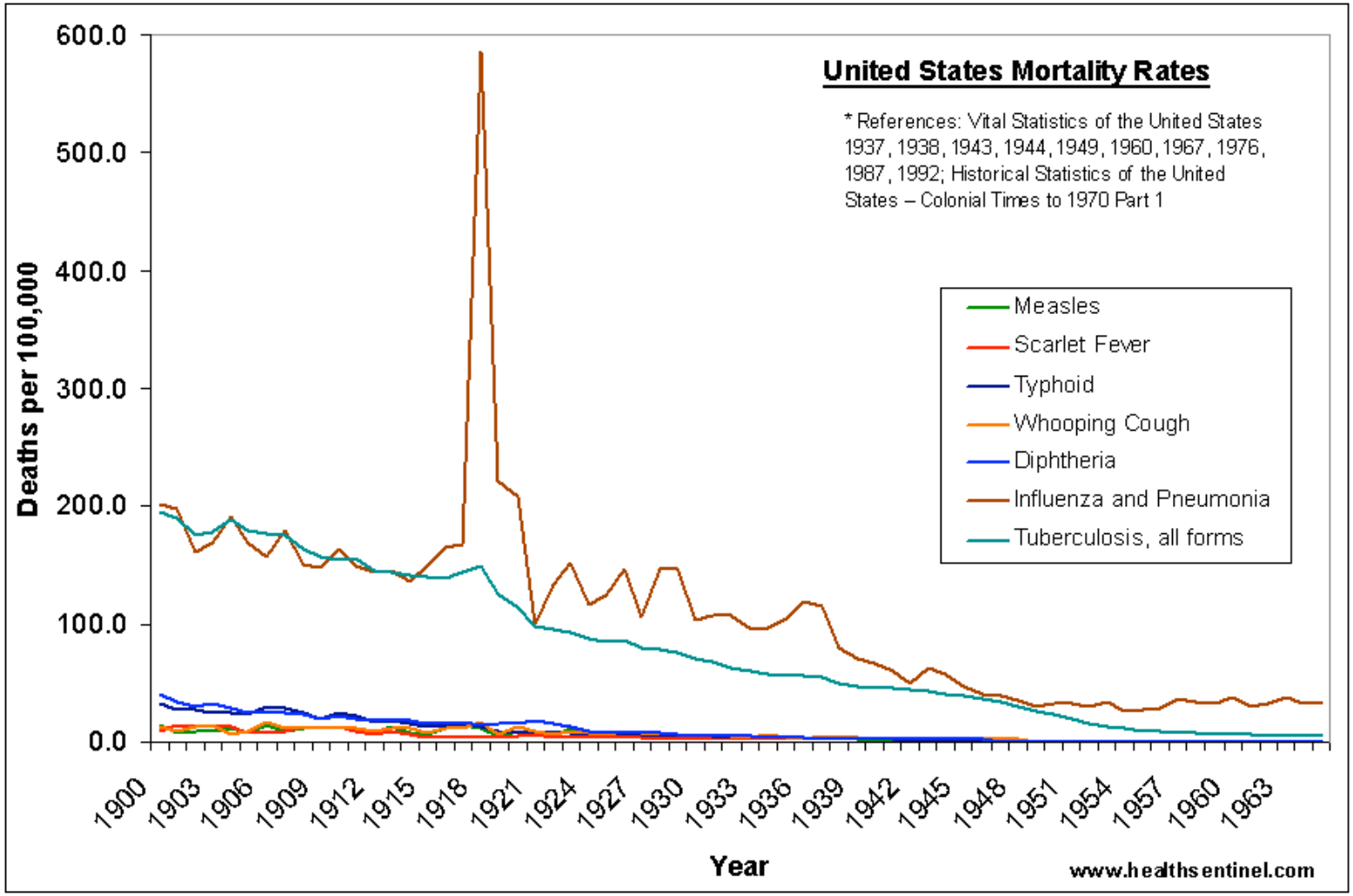




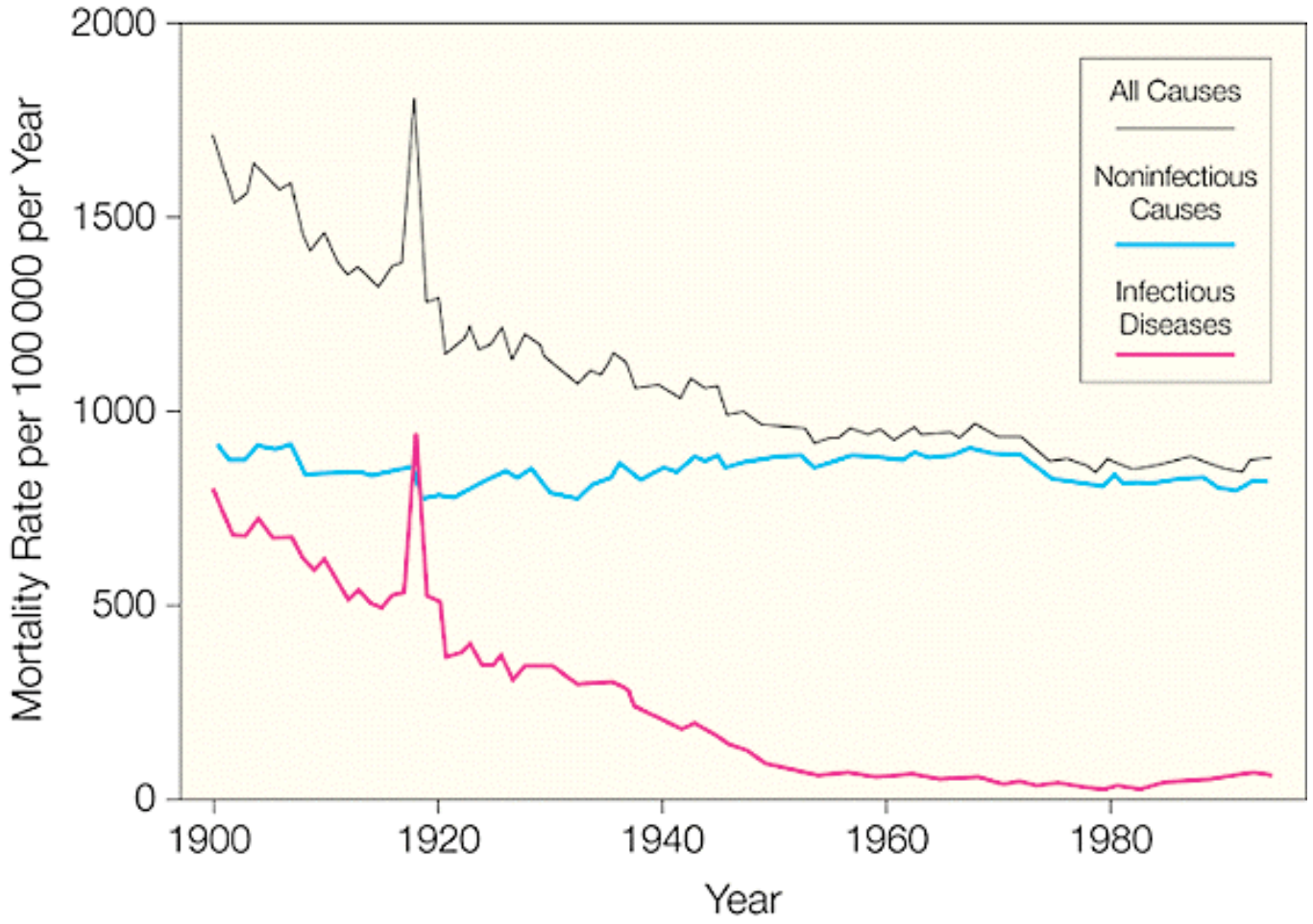
Pandemic Influenza

October 27, 2008

Death rates from major infectious diseases in the United States: 1900-1965

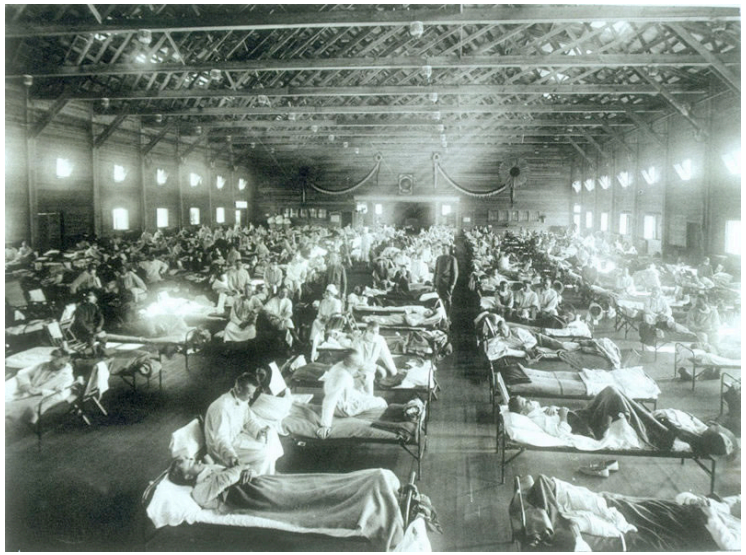


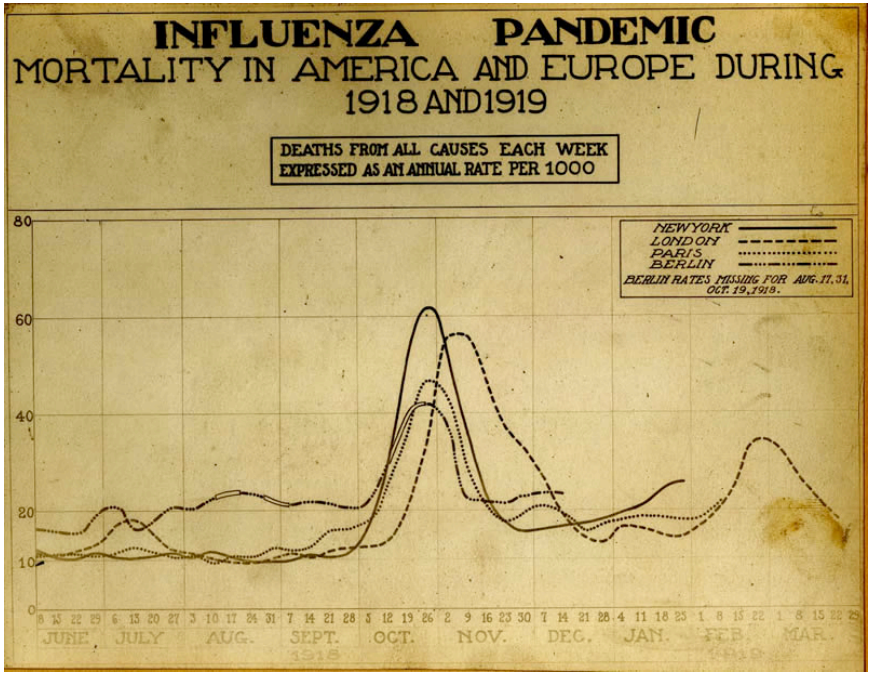
Death rates from ALL CAUSES in the United States: 1900-1994



Armstrong et al., 1999, *JAMA* **281**: 61

1918 influenza epidemic: realization of a worst-case scenario

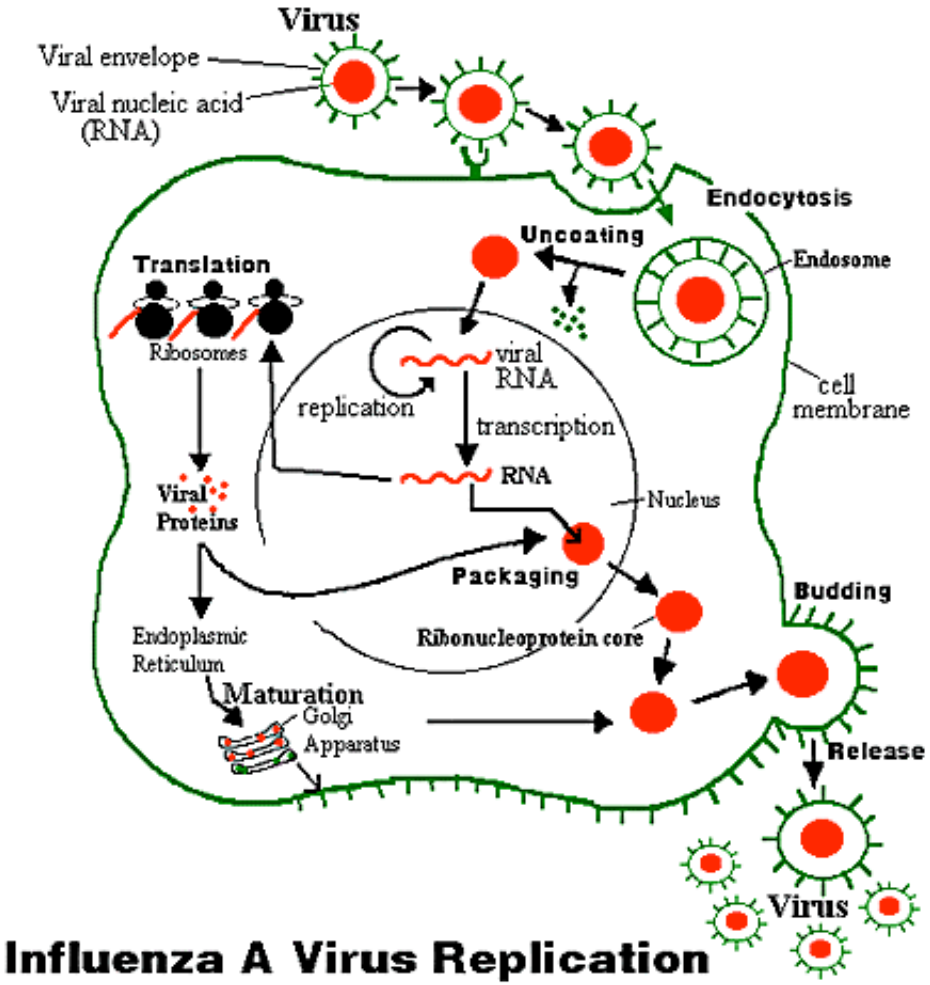




First case: Albert Mitchell, Camp Funston, KS, March 11, 1918
Up to 20% of all humans infected
20-50 million deaths worldwide, 650,000 in the US
2.5% average case mortality rate; up to 16% in some cities

Influenza A

- Pleiomorphic enveloped virus, 80-120 nm
- Orthomyxoviridae family, isolated from ferrets in 1933
- Endemic in water birds (ducks, gulls, shorebirds)
- Invasion mediated by HA protein (hemagglutinin) binding to sialic acid
  - Birds mostly α2,3 linkage to galactose, humans mostly α2,6 linkage
- Neuraminidase cleaves sialic acid links; required for viral shedding (target of Tamiflu)
- Virus also encodes an RNA-dependent RNA polymerase

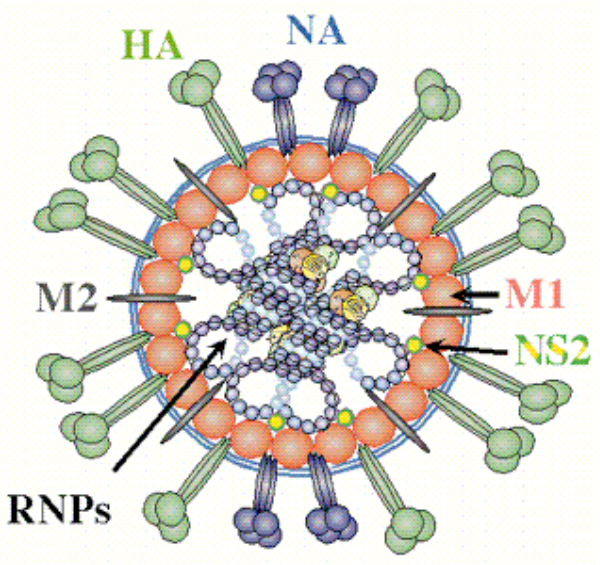


Influenza A Virus Replication

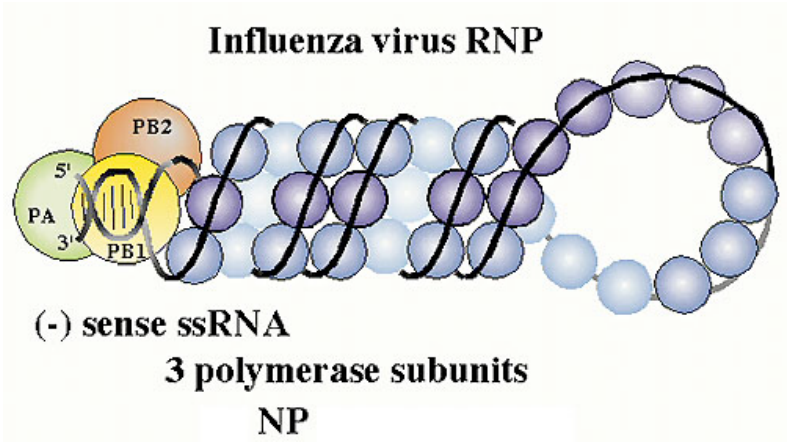
Genome has 8 RNA segments

Influenza A virus gene segments and encoded proteins

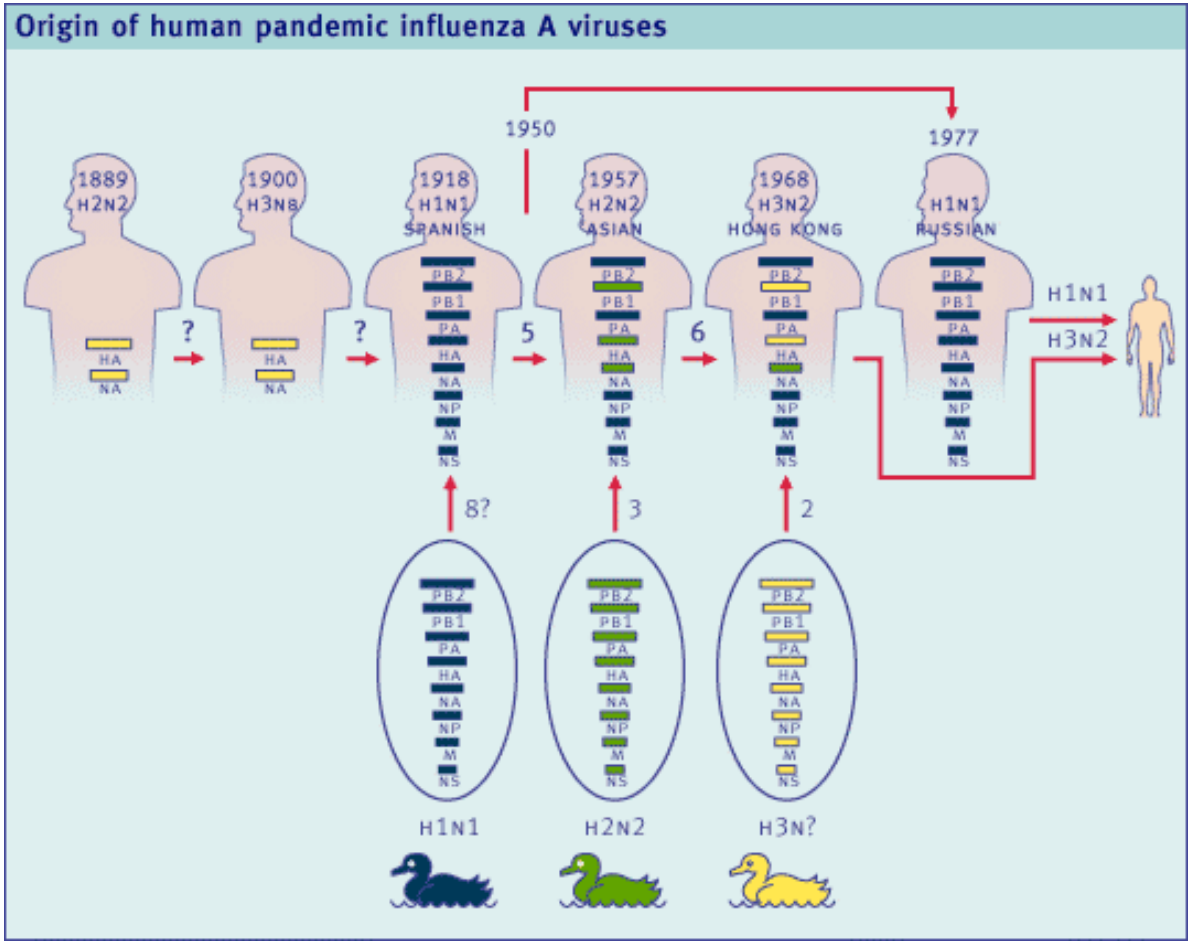
RNA segment	Nucleotides	Protein	Amino acids	Molecules per virion
1	2341	polymerase PB2	759	30-60
2	2341	polymerase PB1	757	30-60
3	2233	polymerase PA	716	30-60
4	1778	haemagglutinin HA	566	500
5	1565	nucleoprotein NP	498	1000
6	1413	neuraminidase NA	454	100
7	1027	matrix protein M1	252	3000
		matrix protein M2	97	20-60
8	890	non structural protein NS1	230	-
		non structural protein NS2	121	130-200



Each viral RNA segment is packaged by the nucleoprotein NP with a polymerase heterotrimer (PA, PB1, PB2) ready to go

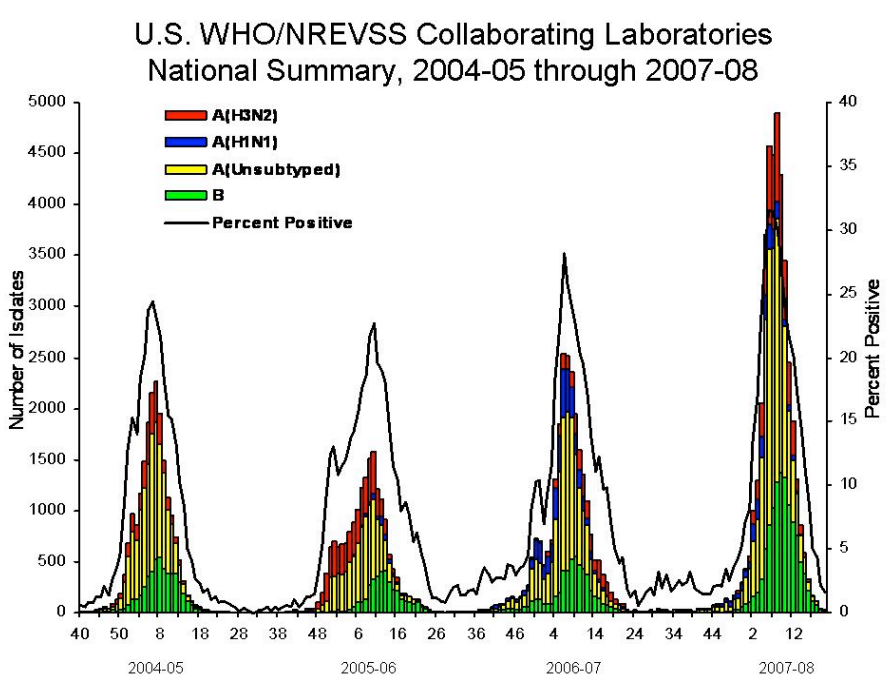


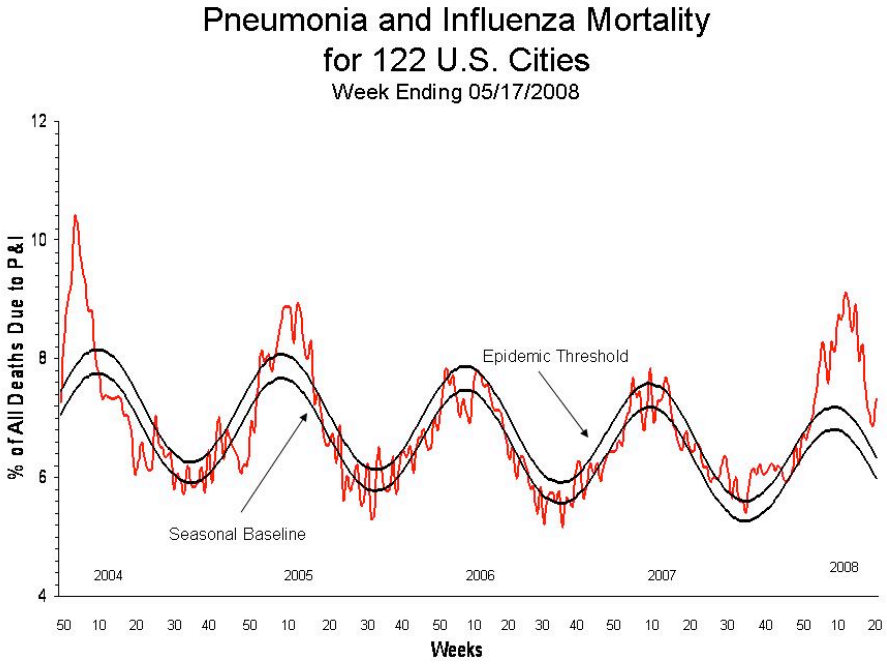
Segments reassort when distinct viruses infect the same cell



- Major antigenic determinants are HA (hemagglutinin) and NA (neuraminidase)
- 16 HA types and 9 NA types found in waterfowl
- Antigenic SHIFT is due to a new HA or NA type
- Antigenic DRIFT is due to point mutations in HA and NA
- PANDEMICS arise when human populations are immunologically naïve for a new type

Currently H3N2 and H1N1 cause most of the yearly infectious cycle





Note that influenza and pneumonia USUALLY cause ~8% of all reported deaths during the winter months

www.cdc.gov

Influenza vaccine production

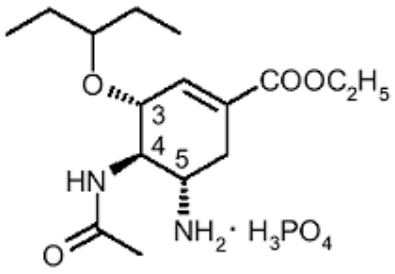
- Currently grown in embryonated chicken eggs
  - Live virus inactivated by formaldehyde treatment
  - ~ 3 eggs per dose; availability would be severely compromised with avian influenza epidemic
  - Lag time from seed strain choice to large-scale availability is 28 weeks
- Cell-based vaccine culture methods are under development
- Public confidence in vaccines is generally low
  - Swine flu - 1976
  - Compare public response to MMR vaccine

Drug treatments for influenza

Amantidine and Rimantidine

- Approved by FDA in 1976
- Active only against influenza A
- Inhibitors of M2; virus cannot escape envelope
- Widespread resistance; no longer recommended

Oseltamivir (Tamiflu) and Zanamivir (Relenza)



- Approved by FDA in 1999
- Active against both influenza A and influenza B
- Inhibitors of neuraminidase; prevent viral shedding from cell surface
- Oseltamivir delivered orally as an ethyl ester pro-drug
- Found by screening sialic acid derivatives

Can we predict which strains will emerge? Positive selection?

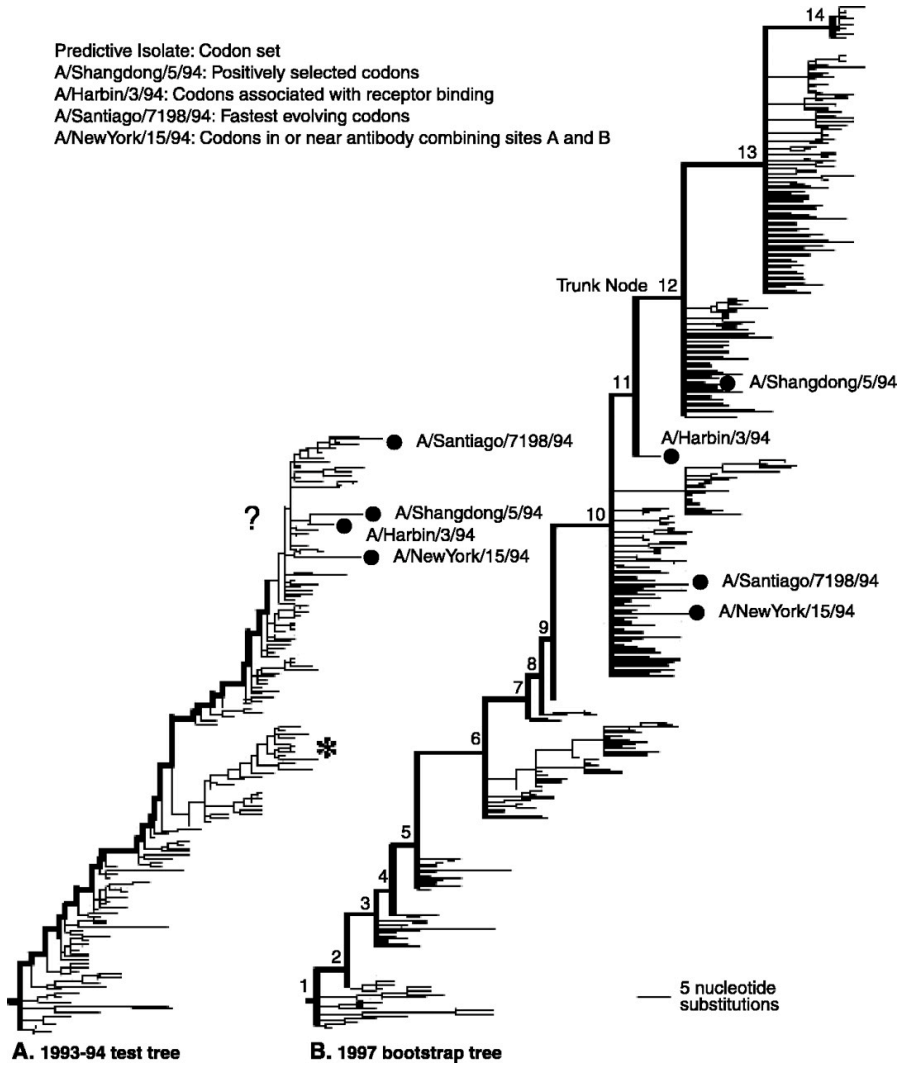


Table 1. The 18 positively selected codons in the HA1 hemagglutinin gene and their membership in alternative codon sets. R, codons associated with the sialic acid receptor binding site; A or B, codons in or near antibody combining site A or B, respectively; F, codons with rapid rates of amino acid replacement.

Codon	Codon set		
121	—	—	F
124	—	A	—
133	—	A	F
135	R	A	F
138	R	A	F
142	—	A	—
145	—	A	F
156	—	B	F
158	—	B	—
186	—	B	F
190	R	B	F
193	—	B	F
194	R	B	F
197	—	B	—
201	—	—	—
226	R	—	F
262	—	—	—
275	—	—	F

Bush et al., 1999, *Science* **286**: 1921

The next pandemic…

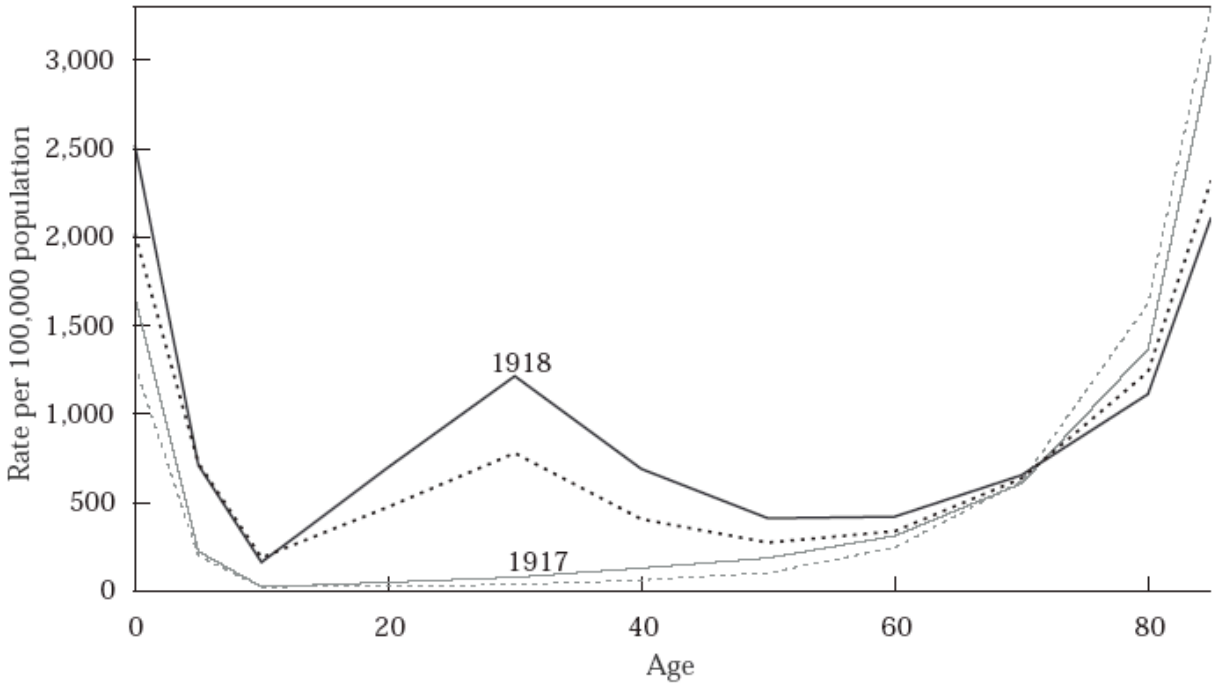
When, not if. How bad will it be?

Why was 1918 so bad?

Can we predict the next source of major antigenic shift or species crossing?

1918 death rate unusually high in males and people ages 20-40

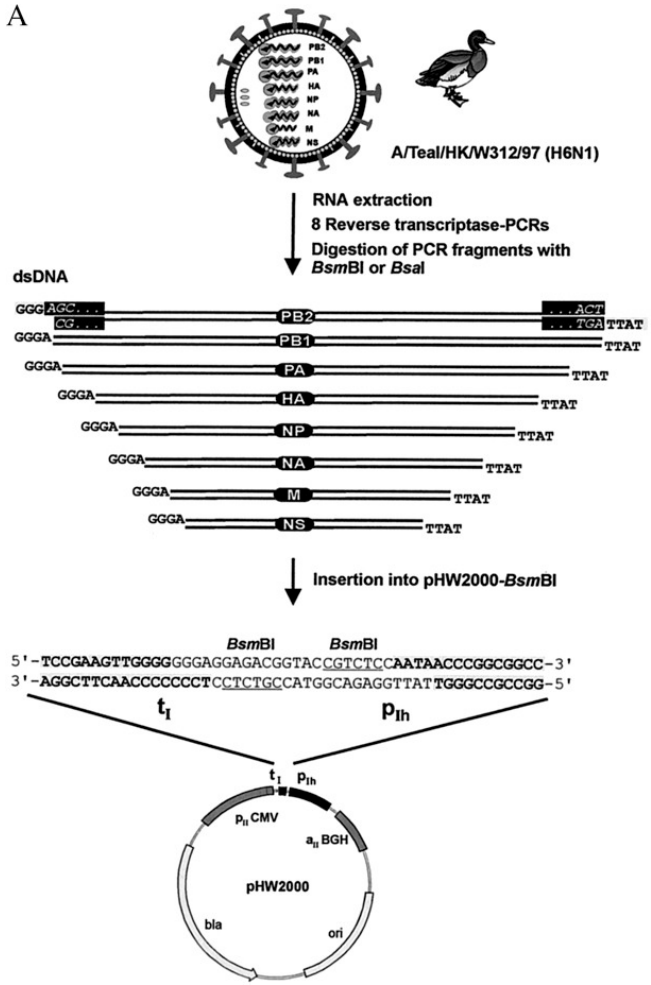
FIGURE 1 Age-specific death rates for influenza and pneumonia combined, males (solid) and females (dotted), 1917 and 1918

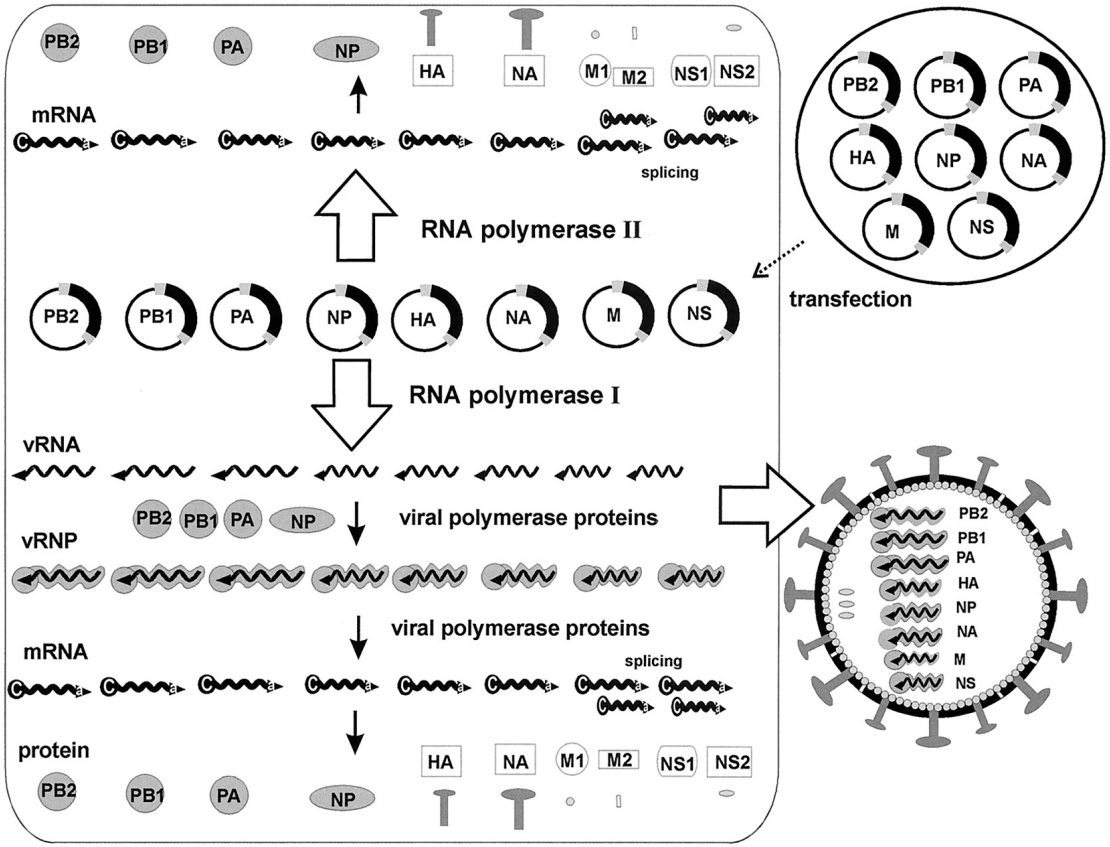


SOURCE: US Department of Health, Education, and Welfare 1956.

Noymer and Garenne, 2000, Pop. Dev. Rev. 26: 565

1918 flu recovered from US Army pathology samples and one person buried in the Alaska permafrost



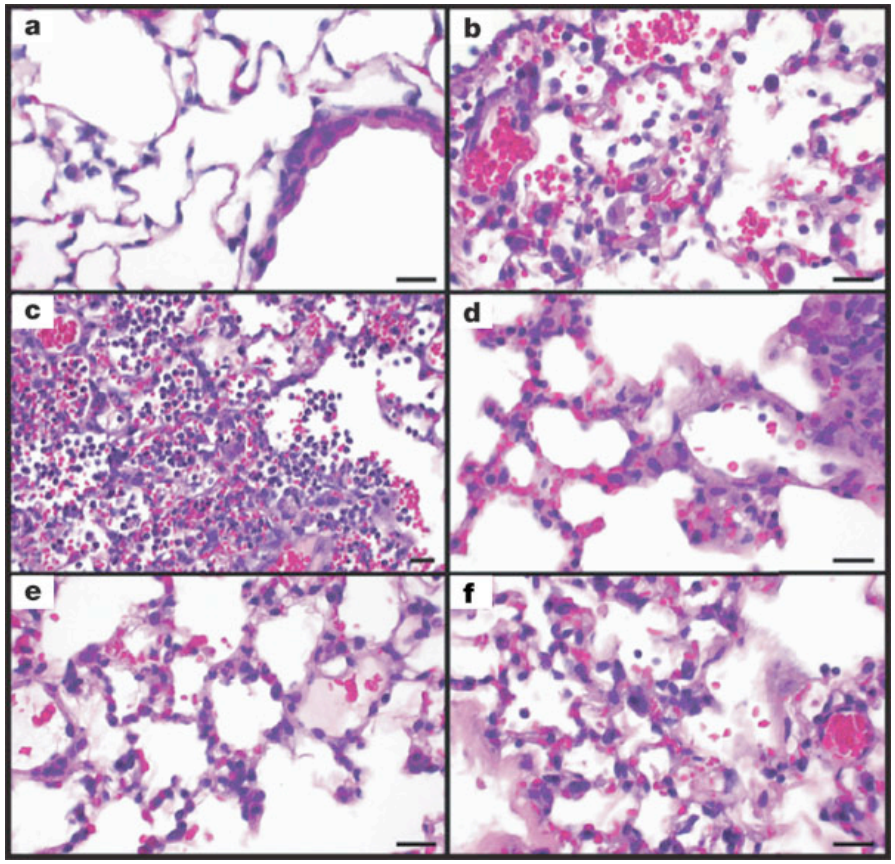


Recombinant expression strategy:
Hoffmann et al., 2000, PNAS 97:6108

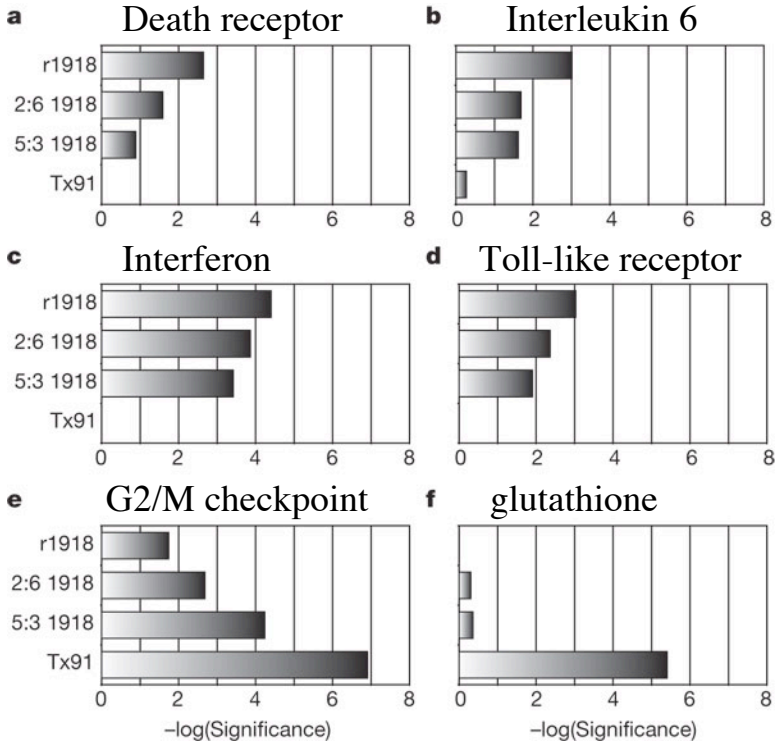
Properties of the reconstructed 1918 virus in mouse infections

a: regular influenza

b-f: recombinant 1918



Microarray analysis for inflammation-associated gene expression



Kash et al., 2006, *Nature* **443**: 578

Only 10 aa changes in polymerase subunits consistently distinguish human from avian

Table 1 | Amino acid residues distinguishing human and avian influenza polymerases

Gene	Residue no.	Avian	1918	Human H1N1	Human H2N2	Human H3N2	Classical swine	Equine
PB2	199	A	S	S	S	S	S	A
PB2	475	L	M	M	M	M	M	L
PB2	567	D	N	N	N	N	D	D
PB2	627	E	K	K	K	K	K	E
PB2	702	K	R	R*	R	R	R	K
PB1	375	N/S/T†	S	S	S	S	S	S
PA	55	D	N	N	N	N	N	N
PA	100	V	A	A	A	A	V	A
PA	382	E	D	D	D	D	D	E
PA	552	T	S	S	S	S	S	T

*All human H1N1 PB2 sequences have an Arg residue at position 702, except that two out of three A/PR/8/34 sequences have a Lys residue.
†The majority of avian sequences have an Asn residue at position 375 of PB1, 18% have a Ser residue, 13% a Thr residue.

PB2: 5 changes, found rarely in avian lineages but occasionally in high pathogenicity avian strains (HPAI) H5N1, H7N7, or H9N2 that infected humans

PB2: Lys627 crucial for high pathogenicity

PB1: replaced by reassortment in both 1957 and 1968; replicative advantage?

PB1: Asn375 to Ser found in swine and equine as well as human isolates

Tradeoffs between function and antigenicity?

Taubenberger et al., 2005, *Nature* **437**: 889

Questions

Can this work help us to predict the next pandemic?

What else do we need to know?

Pandemics require virulence plus transmissibility; how can we study transmissibility?

What are the dangers of this project?

Should this project have been approved by the NSABB? Should the sequences have been published?

How should we be preparing for the next influenza pandemic?

H5N1: The next big thing?

Highly pathogenic avian influenza (HPAI): communicated directly from birds to humans but not (so far) communicated among humans (except maybe sometimes)

H5N1 - 1997 H9N2 - 1999 H7N2 - 2002

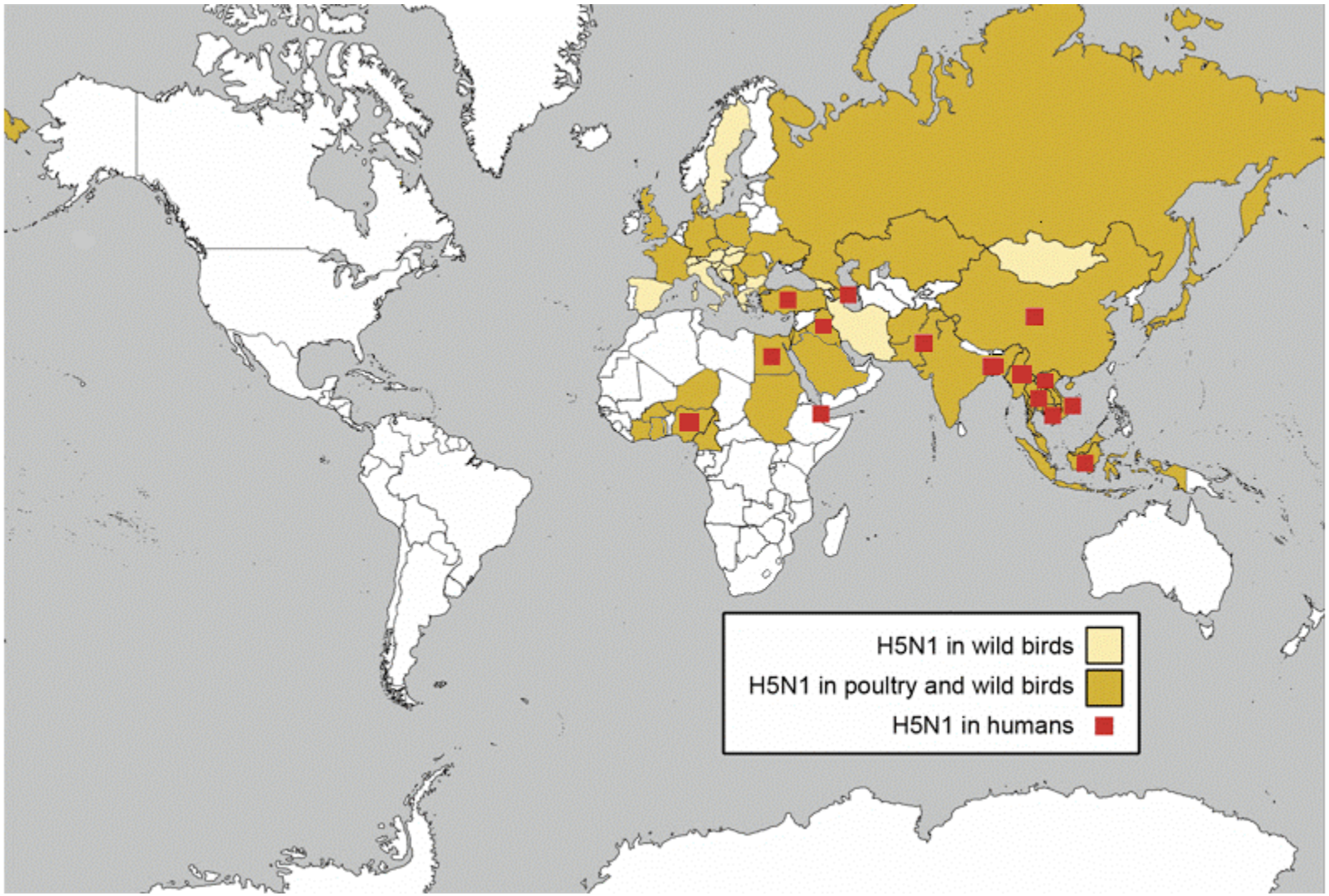
H7N7 - 2003 H7N3 - 2004 H10N7 - 2005

H5N1 returns most years

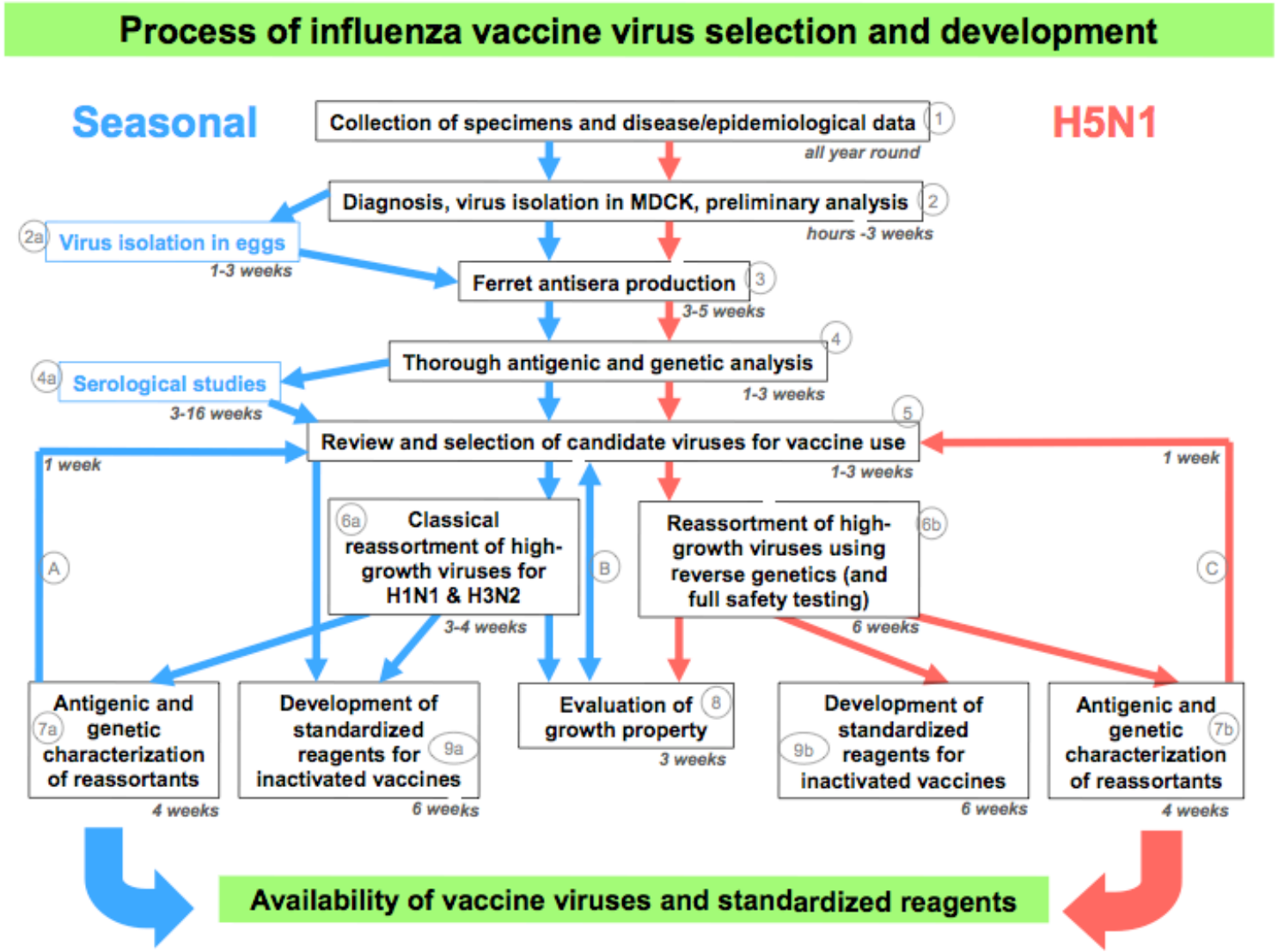
- Cumulative laboratory-confirmed cases through September 2008: 387
- Cumulative deaths: 245

www.who.int

H5N1 spread - October 2008



22-40 weeks to an H5N1 vaccine



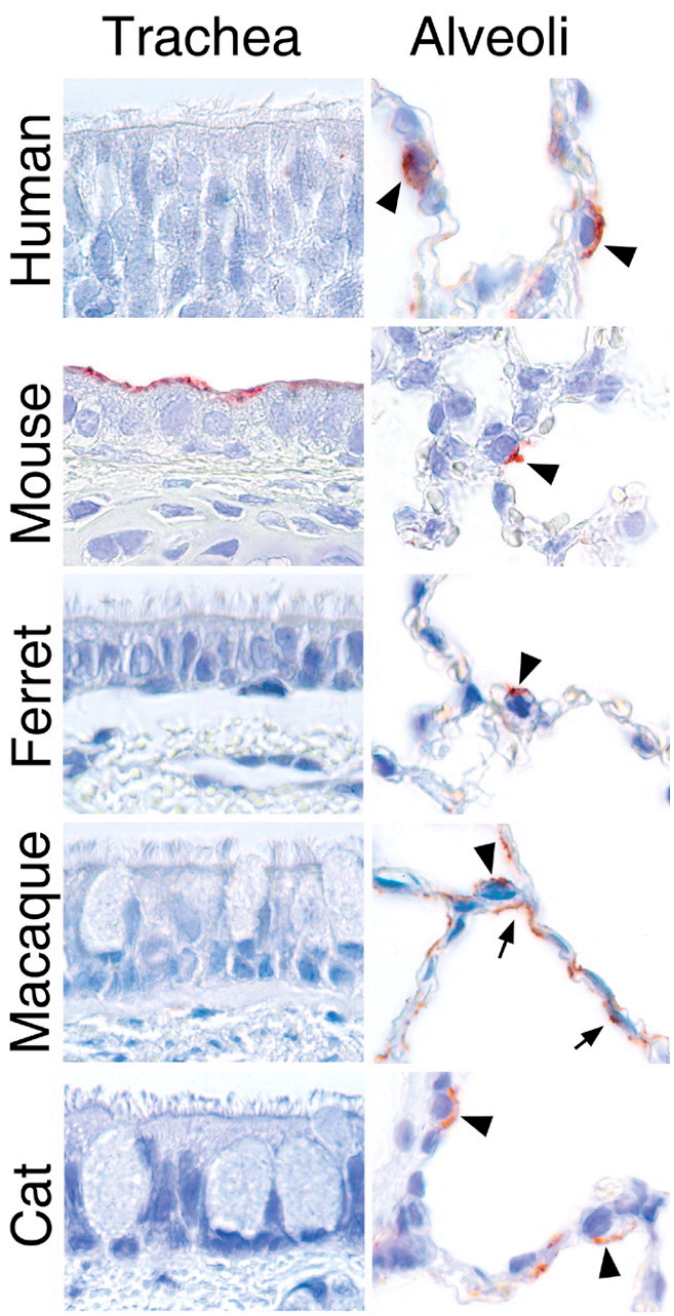
How is H5N1 (or HPAI) different from normal human influenza?

Human, normally SA-α-2,6-Gal

Avian, normally SA-α-2,3-Gal

Fixed H5N1 binds to alveolar macrophags and type II pneumocytes; consistent with severe alveolar damage in human patients

Note ferrets and cats are better models for human infection than mice



van Riel et al., 2006, *Science* **312**: 399

Shinya et al., 2006, *Nature* **440**: 435

Wednesday papers:

Paper 1: Stevens J, Blixt O, Chen LM, Donis RO, Paulson JC, Wilson IA. "Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity." J Mol Biol. 2008 Sep 19;381(5):1382-94

Paper 2: Morens DM, Taubenberger JK, Fauci AS. "Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness." J Infect Dis. 2008 Oct 1;198(7):962-70.