1918 ‘Spanish’ Influenza: Lessons from the Past

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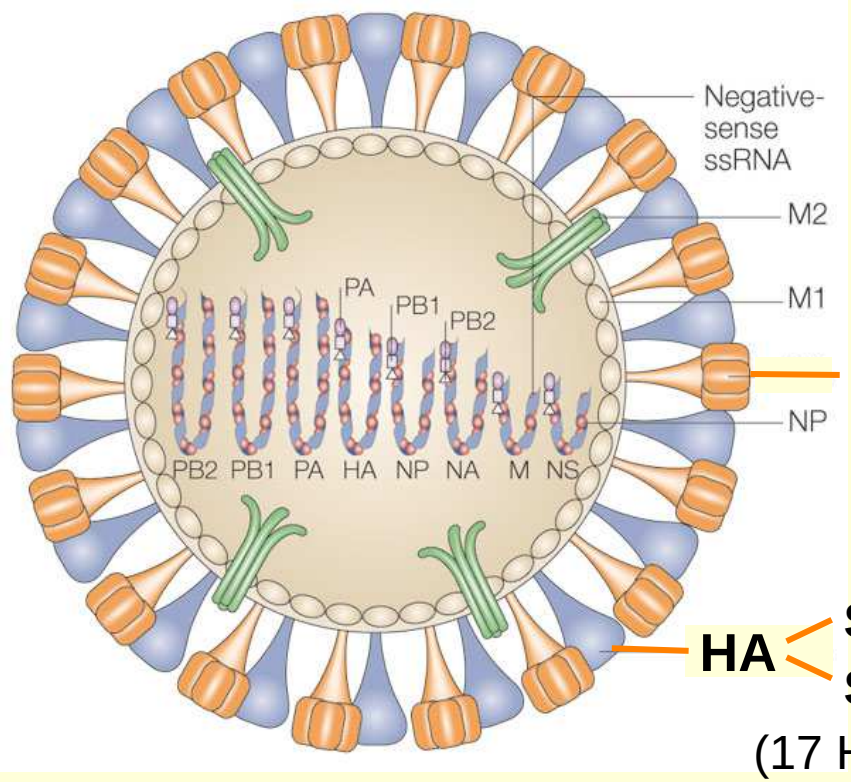
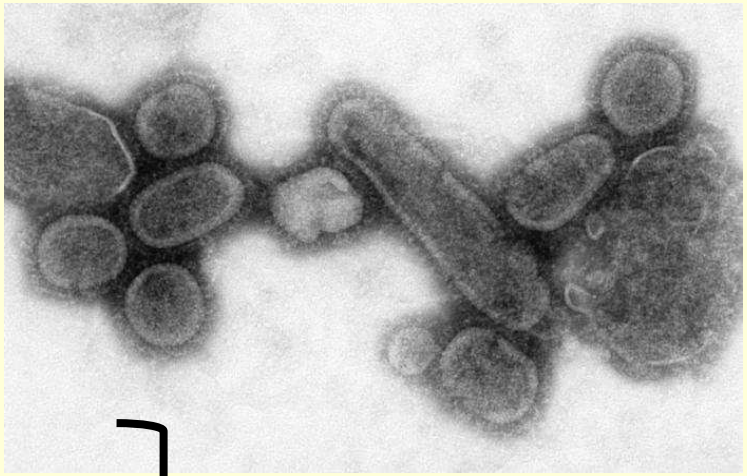
May 1, 2012

Influenza A virus (IAV)

- Family: *Orthomyxoviridae*
  - •Negative sense, segmented, single-stranded RNA genome
  - •8 segments, 12-13 ORF's





NA
(9 NA subtypes)

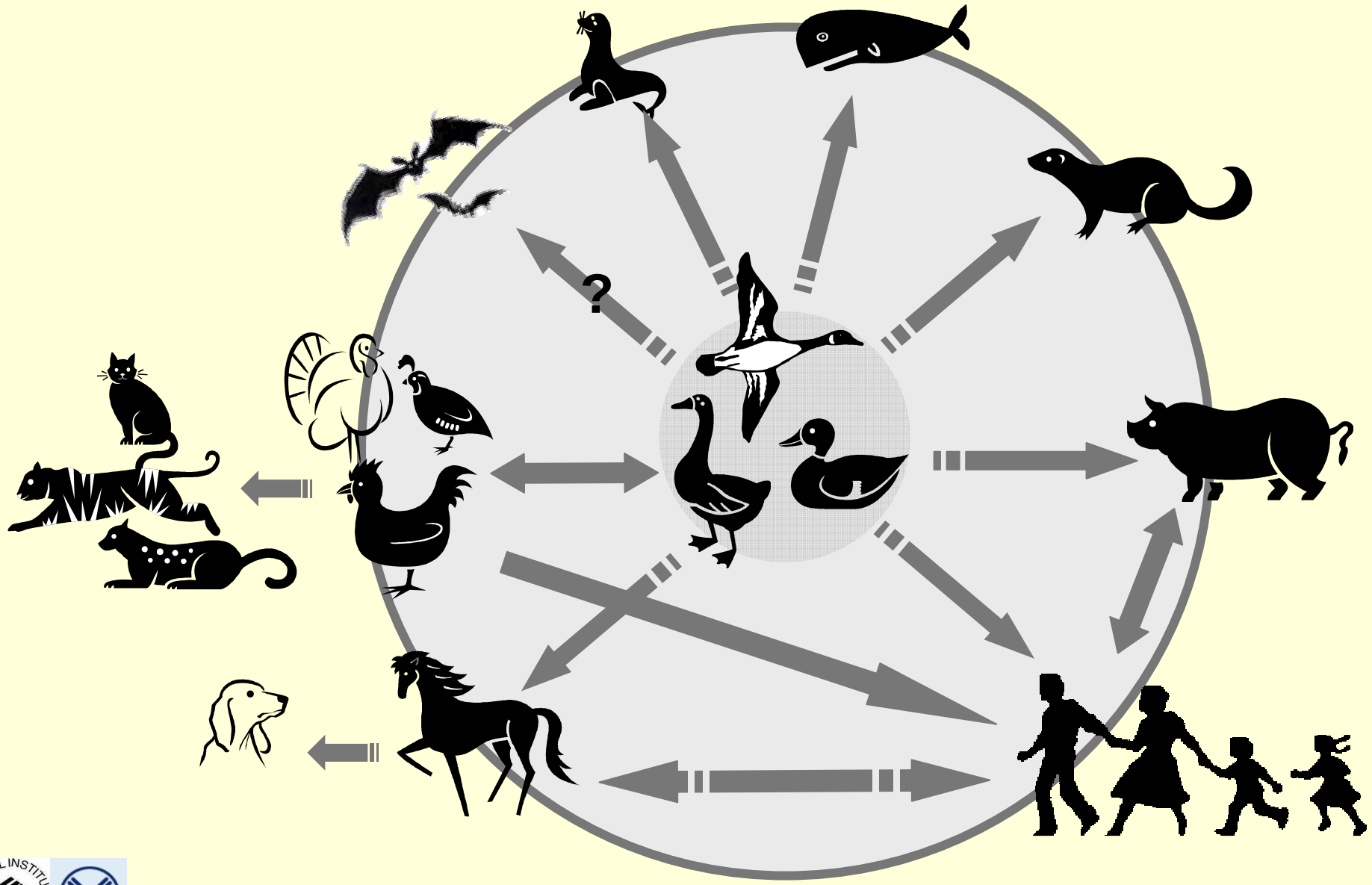
HA **SAα2-6Gal** **SAα2-3Gal**
(17 HA subtypes)

Nomenclature:
e.g., H5N1, H3N2

Modified from: Horimoto & Kawaoka (2005) Nat Rev Micro 3:591-600

"Shift and Drift"

Influenza A virus host range is quite diverse



Influenza A viruses in animals

Avian influenza:

- Many species of wild birds and domestic birds
- LPAI: H1–H16, N1–N9
- HPAI: H5 & H7 subtypes only
  - Polybasic amino acid insertion at the cleavage site
  - 25 outbreaks since 1959, 15 outbreaks since 1990

Equine influenza:

- Recognized for hundreds of years
- Historical linkages to human influenza outbreaks
- H7N7 and H3N8 subtypes
- H3N8 has adapted to dogs

Swine influenza:

- Recognized since 1918
- Different subtypes circulate:
  - H1N1, H1N2, H3N2

Influenza A viruses in humans

Annual epidemics with >200,000 hospitalizations; & up to 49,000 deaths/yr. in U.S.

Occasional and unpredictable pandemic strains with increase in illness and death

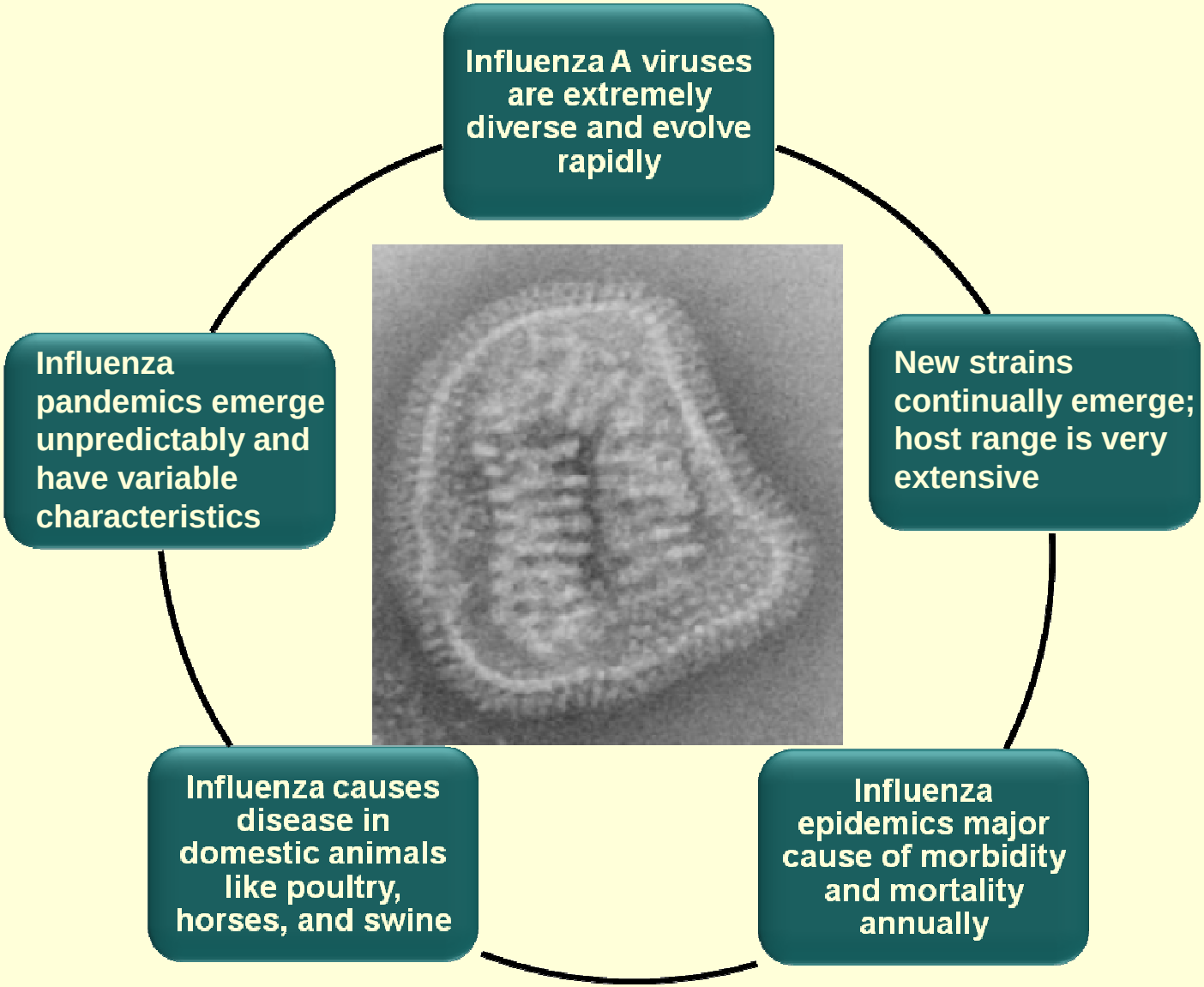
Pandemics are derived zoonotically: 1918, 1957, 1968, 2009

Mortality Associated with Influenza Pandemics and Selected Seasonal Epidemic Events, 1918–2009.*

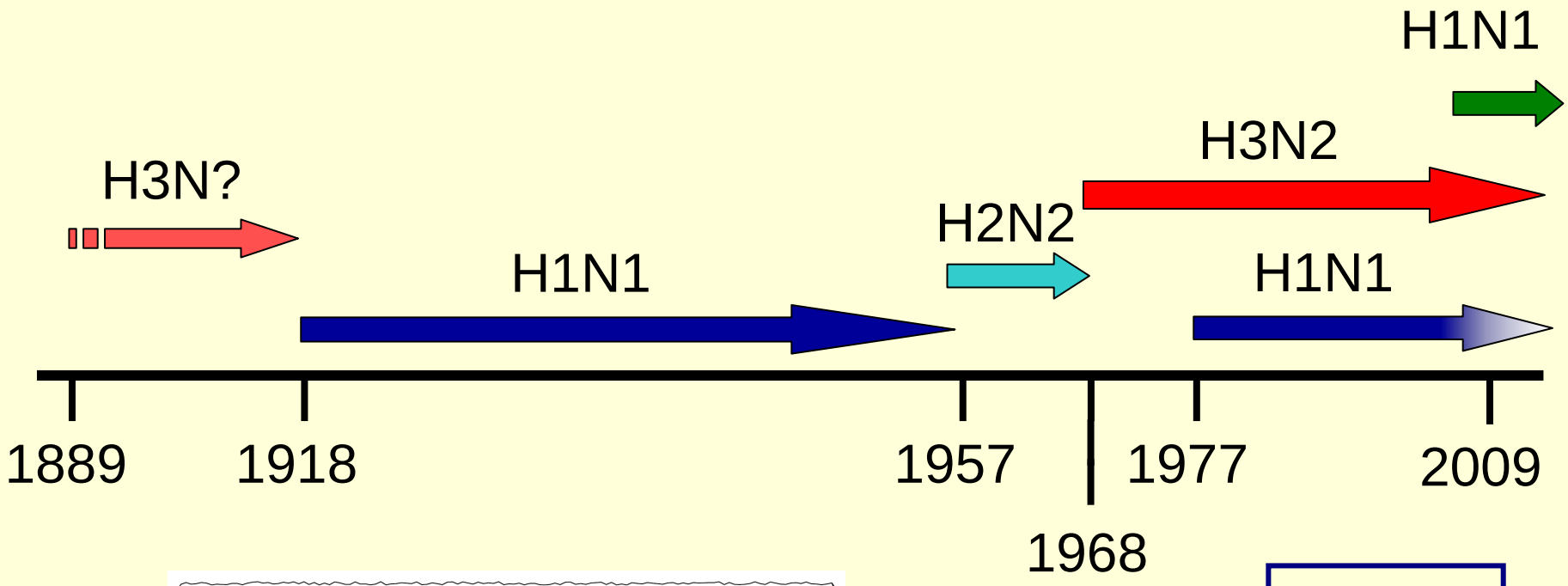
Years	Circulating Virus (Genetic Mechanism)	Excess Deaths from Any Cause *no. per 100,000 persons/yr*
1918–1919	H1N1 (viral introduction), pandemic	598.0
1928–1929	H1N1 (drift)	96.7
1934–1936	H1N1 (drift)	52.0
1947–1948	H1N1 A′ (intrasubtypic reassortment)	8.9
1951–1953	H1N1 (intrasubtypic reassortment)	34.1
1957–1958	H2N2 (antigenic shift), pandemic	40.6
1968–1969	H3N2 (antigenic shift), pandemic	16.9
1972–1973	H3N2 A Port Chalmers (drift)	11.8
1975–1976	H3N2 (drift) and H1N1 (“swine flu” outbreak)	12.4
1977–1978	H3N2 (drift) and H1N1 (viral return)	21.0
1997–1999	H3N2 A Sydney (intrasubtypic reassortment) and H1N1 (drift)	49.5
2003–2004	H3N2 A Fujian (intrasubtypic reassortment) and H1N1 (drift)	17.1
2009	H3N2 and H1N1 (drift) and swine-origin H1N1 (viral introduction), pandemic	?

Morens, *et al*. 2009 *NEJM*. 361:225-229

The influenza problem:



Human influenza A timeline



N = 4

Newsday

November 18, 2001

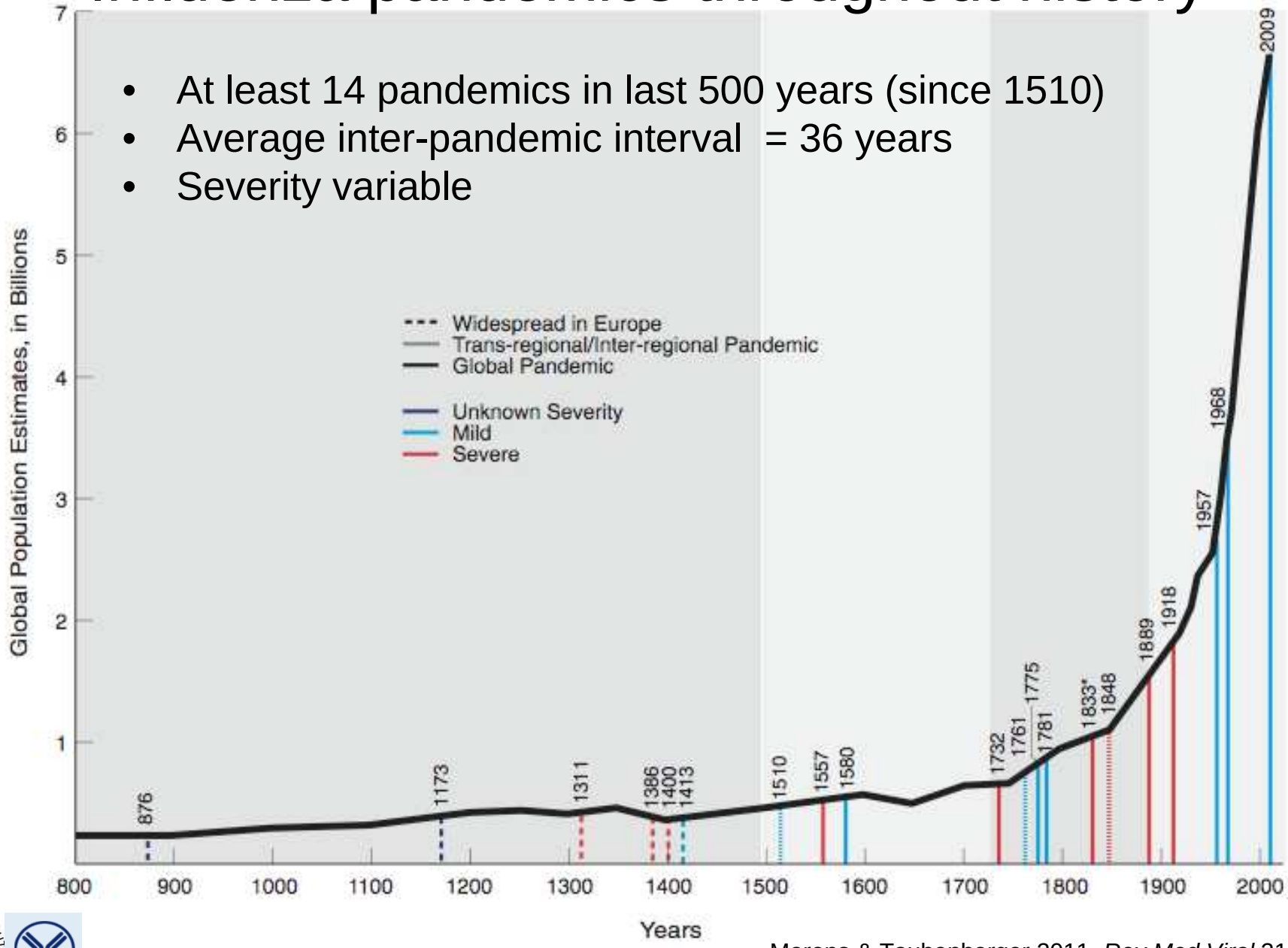
The Worst Bioterrorist May Be Nature Itself

Mortality impacts of past influenza pandemics

1918 “Spanish” flu (H1N1):	• 675,000 deaths in the U. S.
1957 “Asian” flu (H2N2):	• 70,000 deaths in the U. S.
1968 “Hong Kong” flu (H3N2):	• 30,000 deaths in the U. S.
2009 “Swine” flu (H1N1):	• 12,000 deaths in the U. S.

Influenza pandemics throughout history

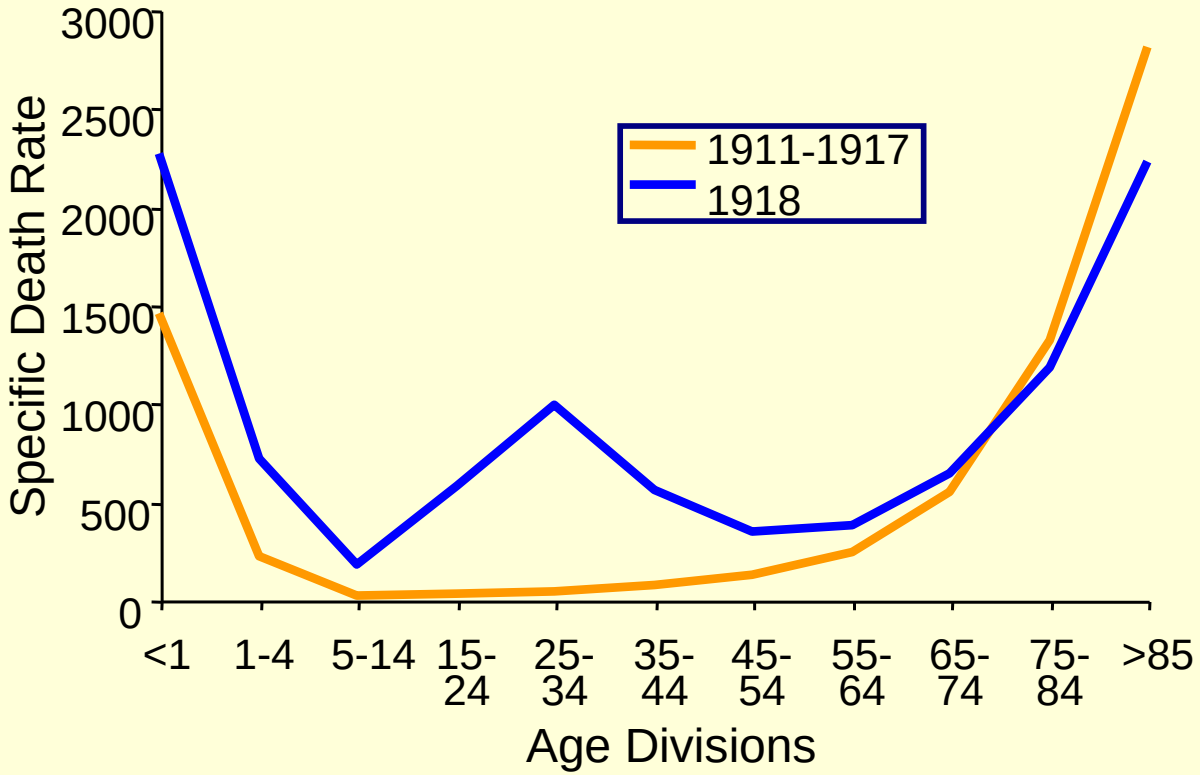
- At least 14 pandemics in last 500 years (since 1510)
- Average inter-pandemic interval = 36 years
- Severity variable



Morens & Taubenberger 2011. *Rev Med Virol* 21:262

1918 ‘Spanish’ influenza mortality

- Total deaths in the 9 months of the pandemic in 1918-1919 estimated to be 50 million
- U.S. Deaths = 675,000
- U.S. Military deaths to flu = 43,000 (out of ~100,000 U.S. Troop casualties in W.W.I.)
- Case fatality rate ~1-2%; >98% had self-limited ILI in absence of antivirals, antibiotics, vaccines, supportive care



Rationale for 1918 archaevirology project

- Naturally occurring pandemic but no direct information on pandemic virus available (no viral isolates in 1918)
- Data from virus would have important biomedical and epidemiological implications to prevent or mitigate a future pandemic, e.g.:
  - How did the pandemic strain evolve and adapt to humans? Could identified mutations be used for surveillance?
  - Why was it pathogenic, especially in young adults? Could data be used to develop novel therapeutics and vaccines?

1918 archaevirology project

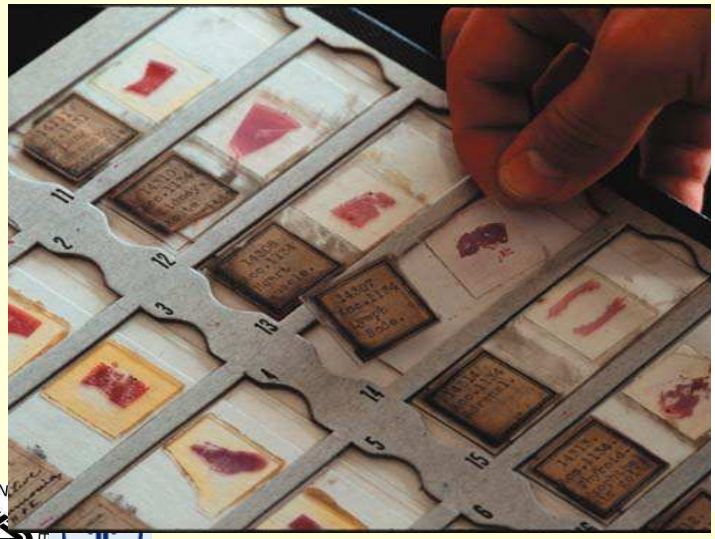
- Initiated at AFIP in 1996
- First sequences published in 1997; genome completely published in 2005 along with reconstruction of virus
- Funding:
  - Intramural funds of AFIP (1996-2006)
  - Grants from NIH, Department of Veterans Affairs, American Registry of Pathology (1996-2006)
  - Intramural funds of NIAID/NIH (2006-pres.)
- Approvals and Oversight:
  - AFIP & DoD
  - NIH, CDC, and USDA
  - NSABB
  - Reconstructed 1918 virus regulated by CDC as Select Agent since 2005, requiring oversight and enhanced BSL3 containment

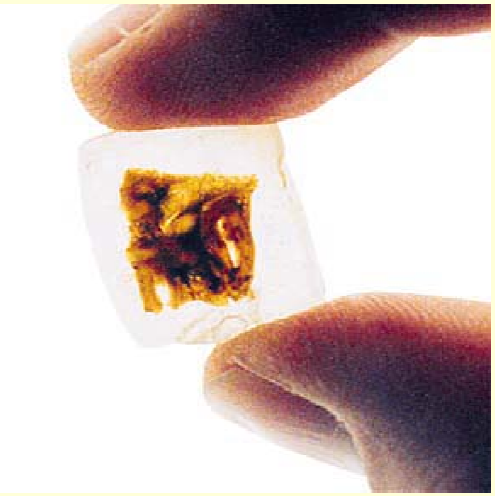
Enhanced BSL3

- Physical Security – 24hr access control and video surveillance
- Bio-surety with FBI/DoJ background checks and routine follow-up
- CDC SAP approval and oversight, IBC safety oversight
- Physical Containment:
  - Multiple levels of access control
  - Negative pressure labs
  - Class II BSCs
  - Double-sided autoclaves
- Personal protective equipment:
  - PAPRs (powered air-purifying respirators)
  - Sealed tyvek body suit
  - Outer tie-back gown
  - Double gloves & shoe covers
  - Chemical decon of PPE
- Shower-out requirement

Source of 1918 cases:

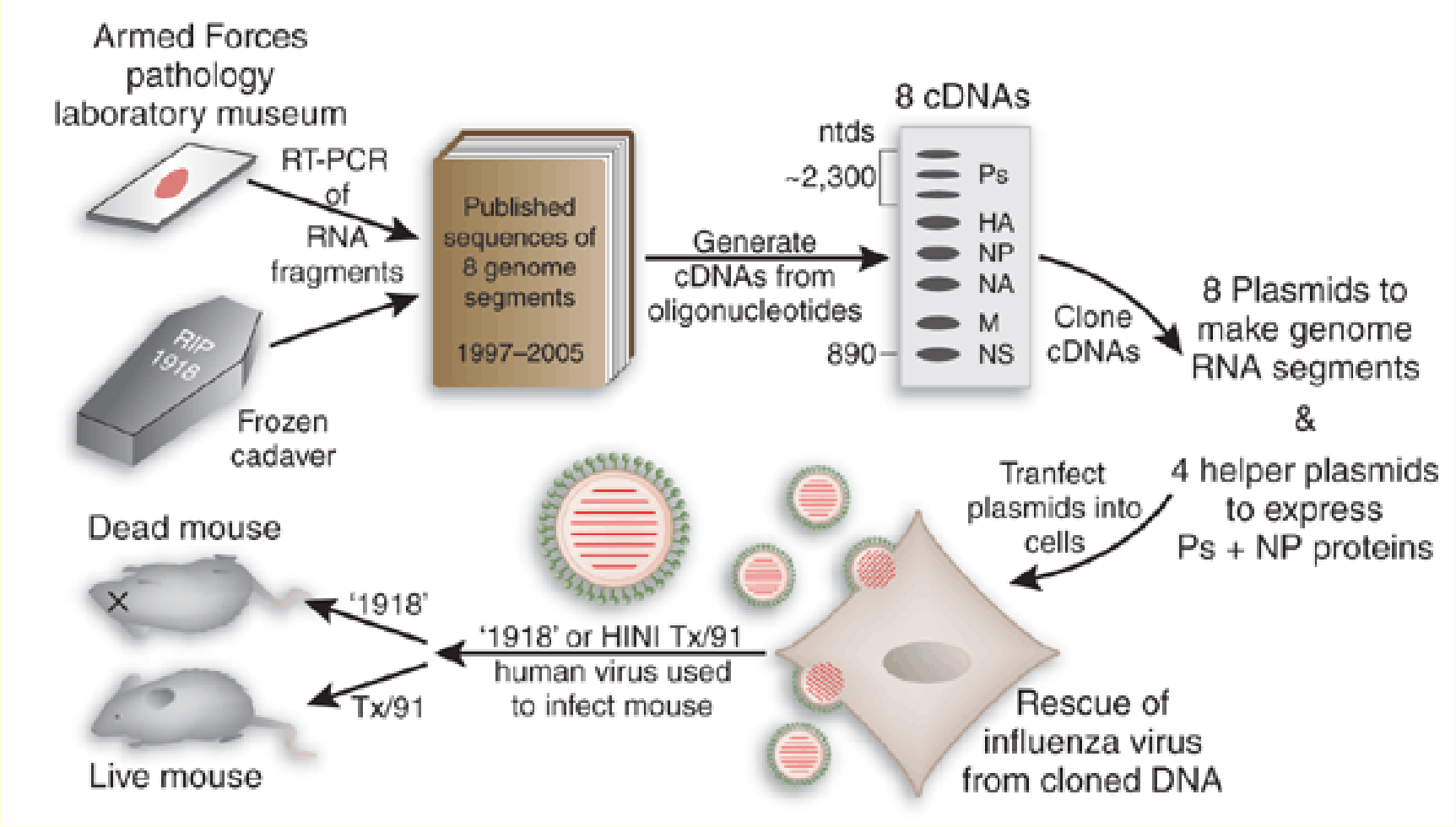
- Formalin-fixed, paraffin-embedded autopsy tissues from National Tissue Repository, AFIP
- Formalin-fixed, paraffin-embedded autopsy tissues from Royal London Hospital, UK
- Frozen, unfixed autopsy tissue from exhumation and lung biopsy in Brevig Mission, AK





Taubenberger et al. 1997 *Science* 275:1793
Reid et al. 1999 *PNAS* 96:1651
Sheng et al. 2011 *PNAS* 108:16416

‘Resurrecting’ the 1918 influenza virus



Lamb & Jackson 2005. *Nature Med* 11:1154

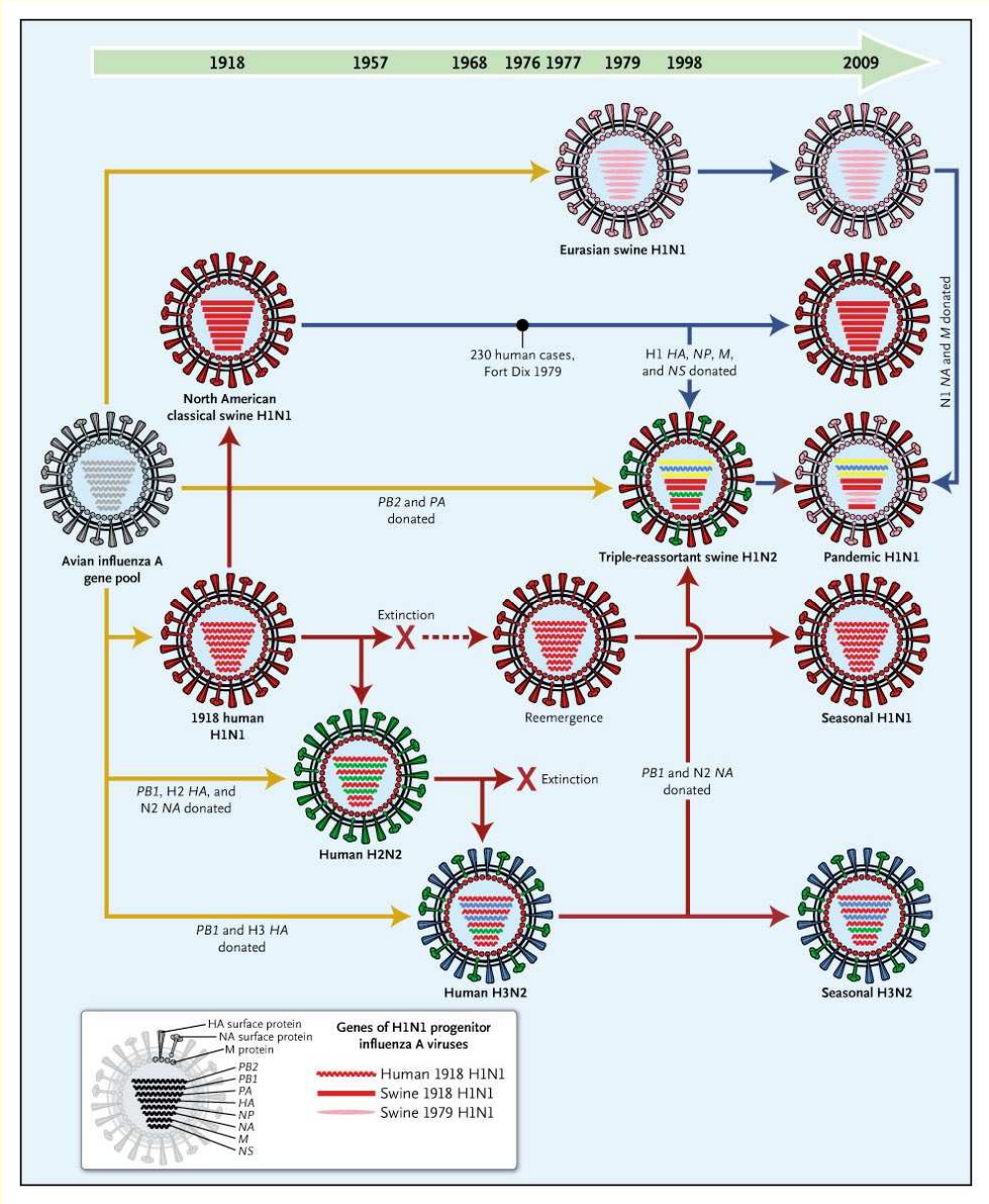
Scientific outcomes of 1918 project (1)

- New insights into influenza evolution in humans and animals
- Springboard for rapid expansion of sequenced influenza genomes
- Understanding pathology of lethal influenza infections and importance of bacterial co-infections in mortality
- Identification of viral virulence and host adaptation factors

Scientific outcomes of 1918 project (2)

- Crucial importance of host inflammatory response in disease progression
- Utility of 1918 HA for understanding receptor binding
- Use of 1918 HA for development of broadly cross-reactive influenza vaccine approaches
- Discovery of novel influenza protein encoded by all influenza A viruses

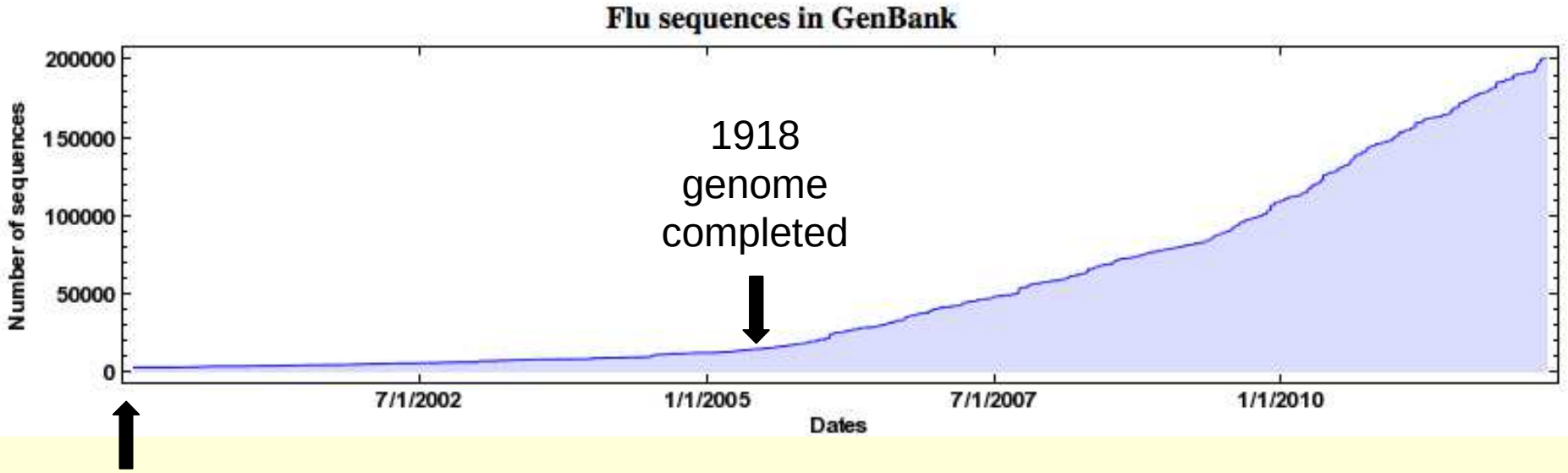
One ‘pandemic era’ since 1918:



All subsequent pandemic and seasonal viruses descended from the 1918 virus

Morens, *et al*. 2009 *NEJM*. 361:225-229

Influenza genome sequences in GenBank

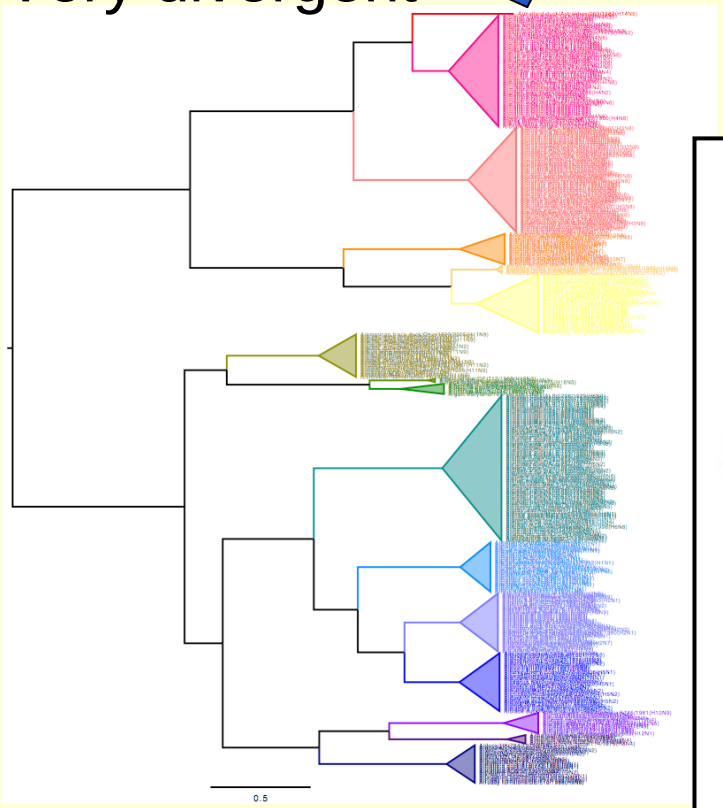


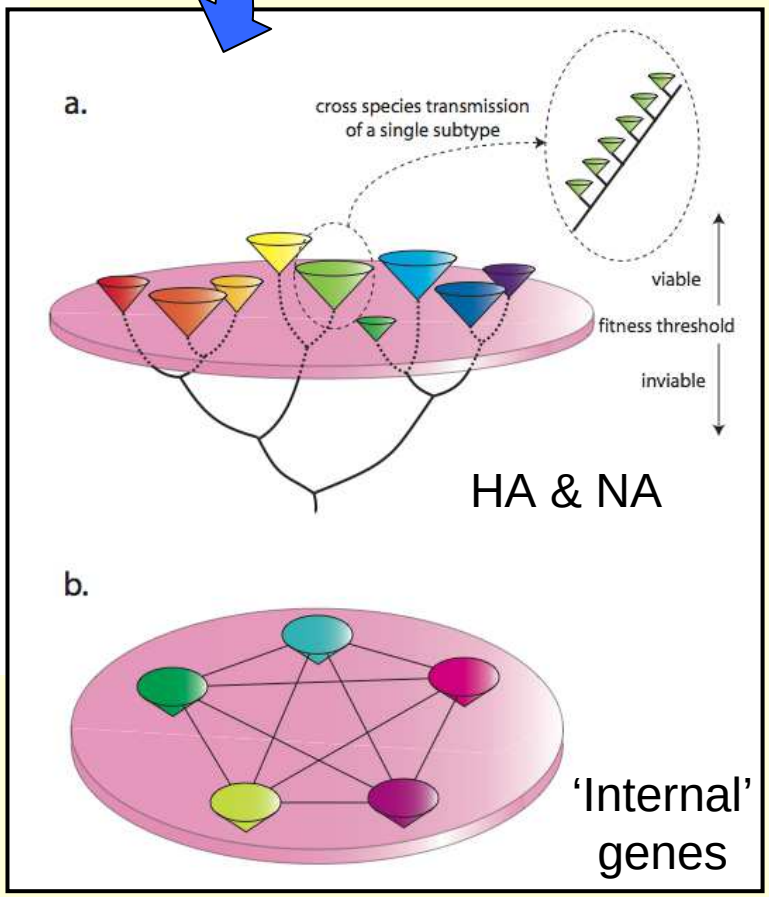
Effort to place 1918 virus in context:

- Avian influenza genomes
- Human influenza genomes

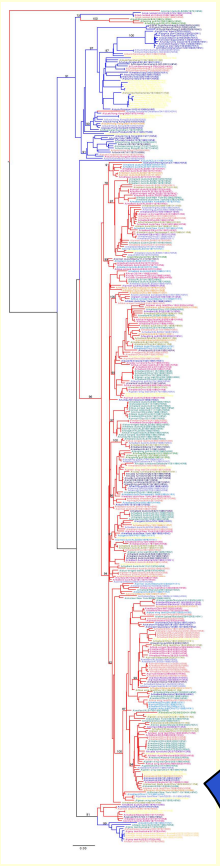
Avian influenza evolution and host switch

HA: H1-16
Very divergent



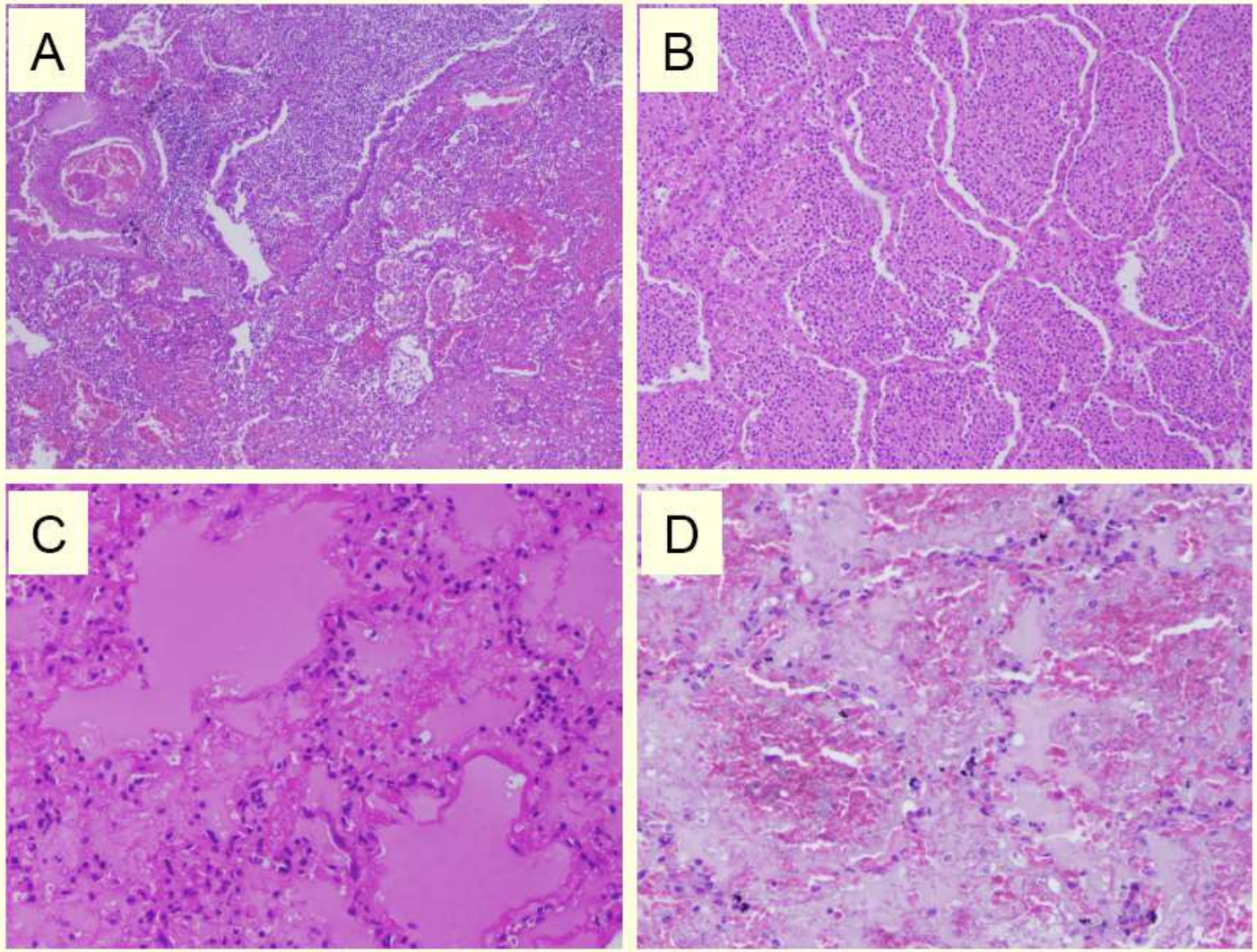


'Internal' Genes
Very conserved



Dugan et al (2008) *PLoS Path* **4**(5):e1000076.

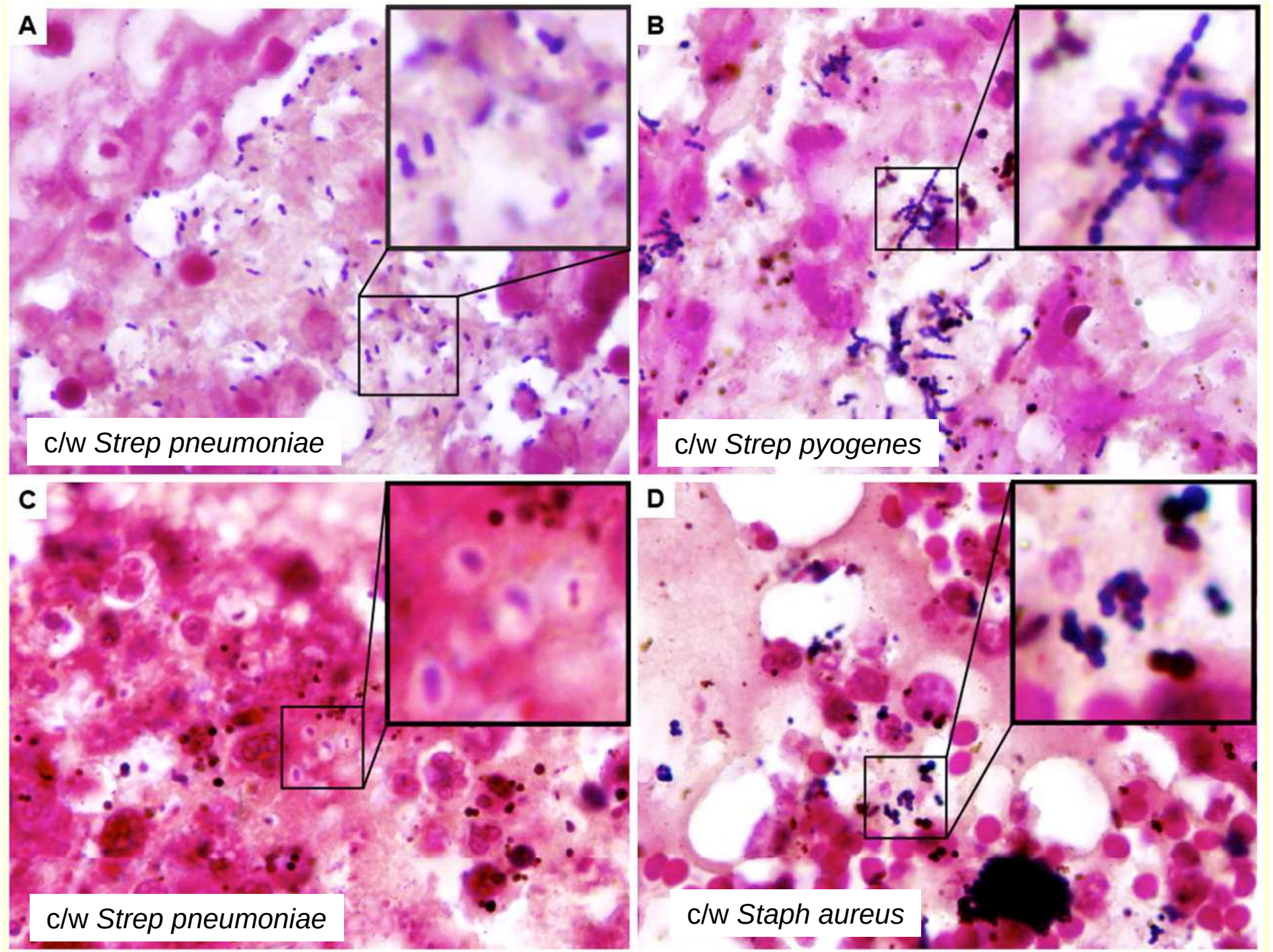
1918 autopsy pathology:
>94% cases with bacterial co-infection



Features of secondary bacterial pneumonia

Features of primary viral pneumonia

Taubenberger & Morens. 2008. Ann Rev Path **3**:499; Morens, et al. 2009 *JID.* 198:962.

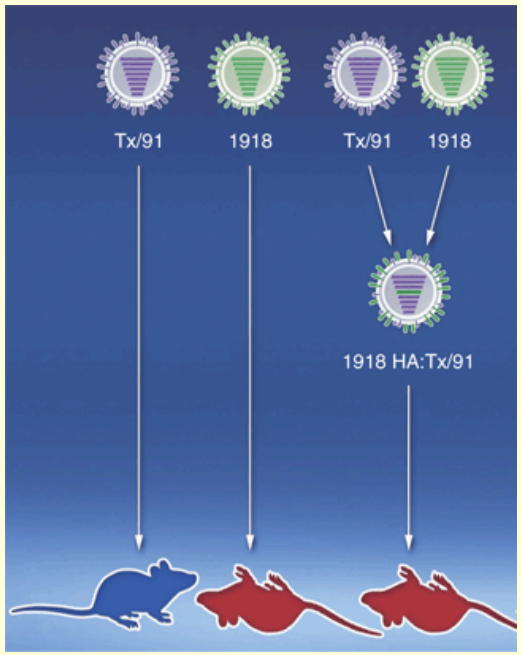

A
c/w Strep pneumoniae
B
c/w Strep pyogenes
C
c/w Strep pneumoniae
D
c/w Staph aureus

1918 host adaptation & virulence factors

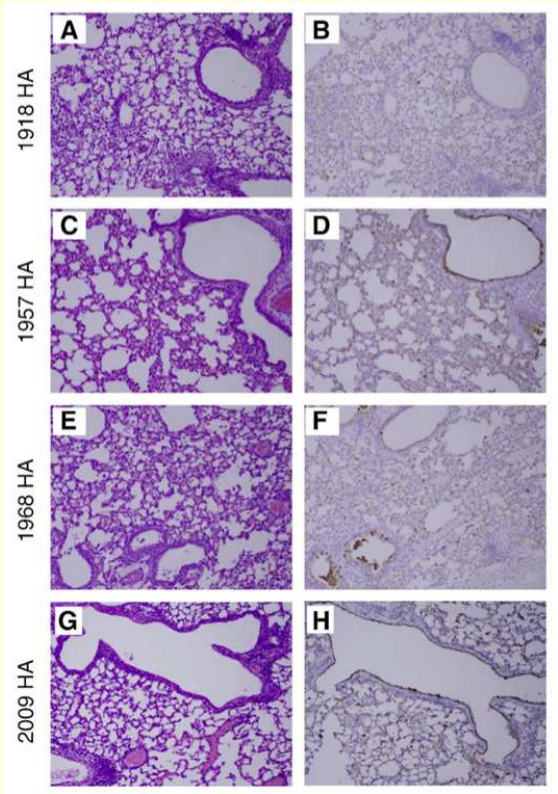
Table 1 | Amino acid residues distinguishing human and avian influenza polymerases

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

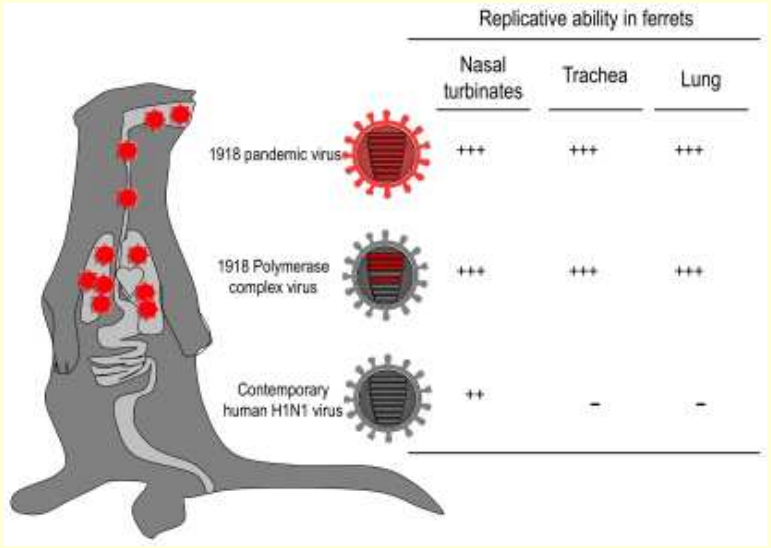
*All human H1N1 PB2 sequences have an Arg residue at position 702, except that two out of three A/PR/8/34 sequences have a Lys residue.
†The majority of avian sequences have an Asn residue at position 375 of PB1, 18% have a Ser residue, 13% a Thr residue.



HA gene

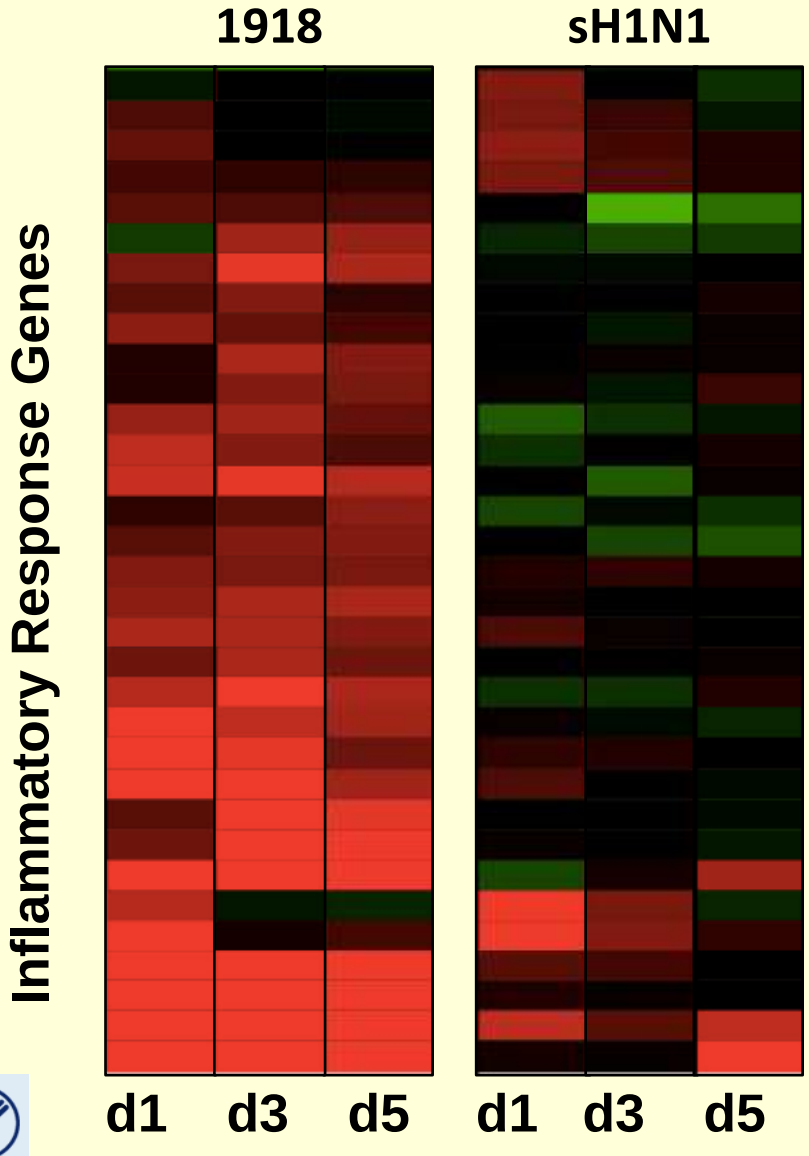


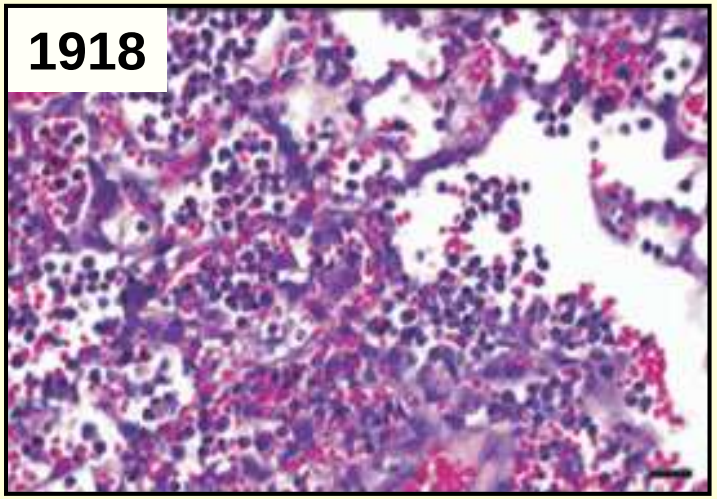
Pol genes

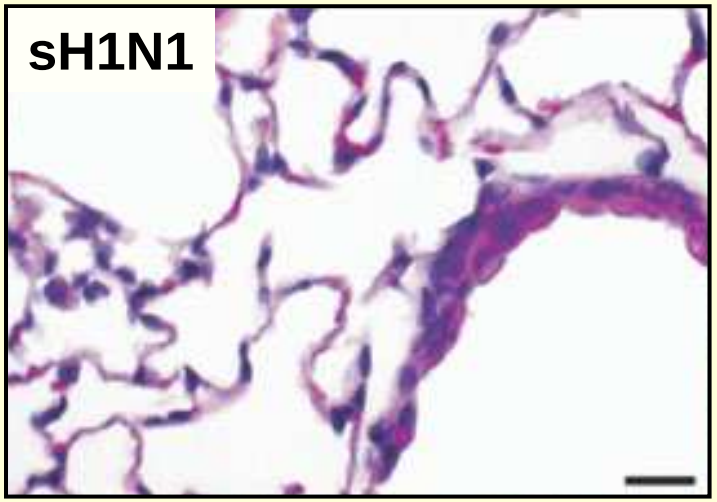


Taubenberger, *et al*. 2005 *Nature* 437:889
Wimmer, *et al*. 2009 Nature Biotech 27:1163
Qi, *et al*. 2011 *Virology* 412:426
Watanabe & Kawaoka 2011 *PLoS Path* 7:e1001218

Up-regulated inflammatory responses during 1918 infection

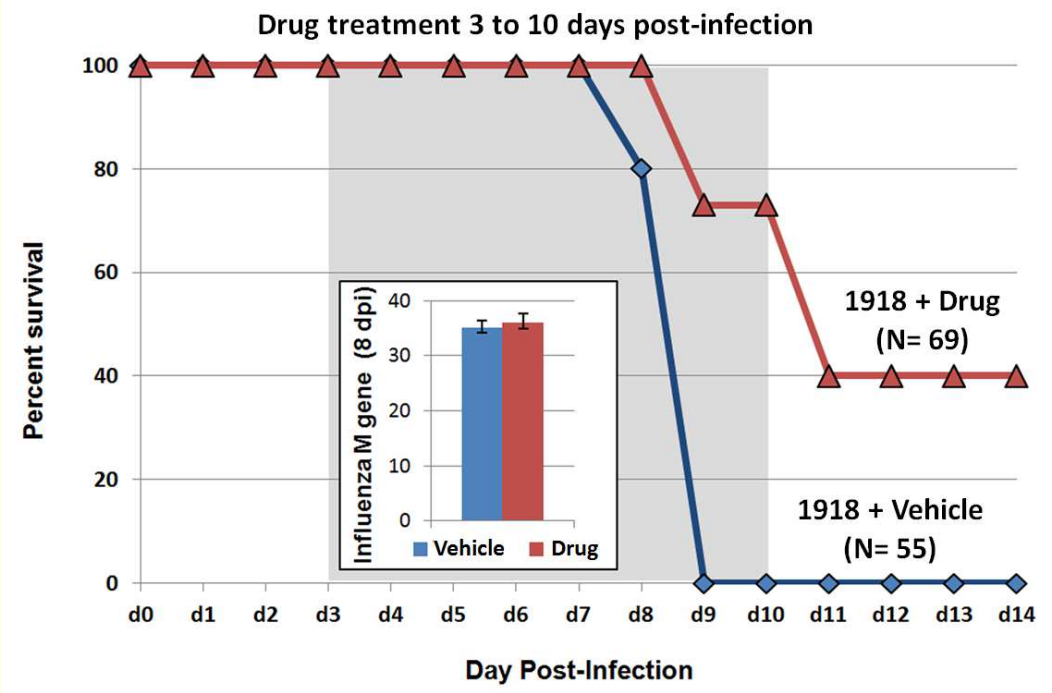


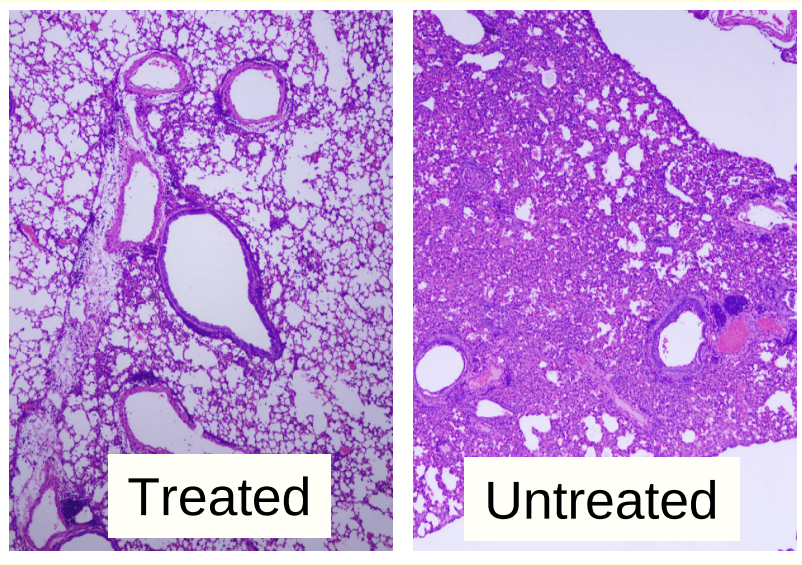




Kash, et al. 2006 *Nature* 443:578

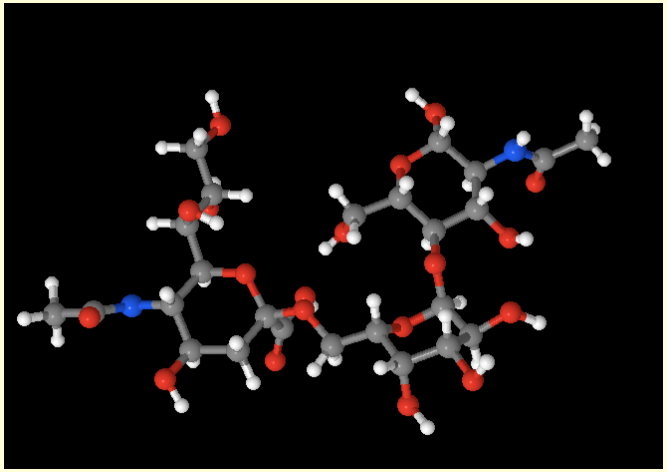
Novel anti-inflammatory drug protects mice from lethal 1918 infection





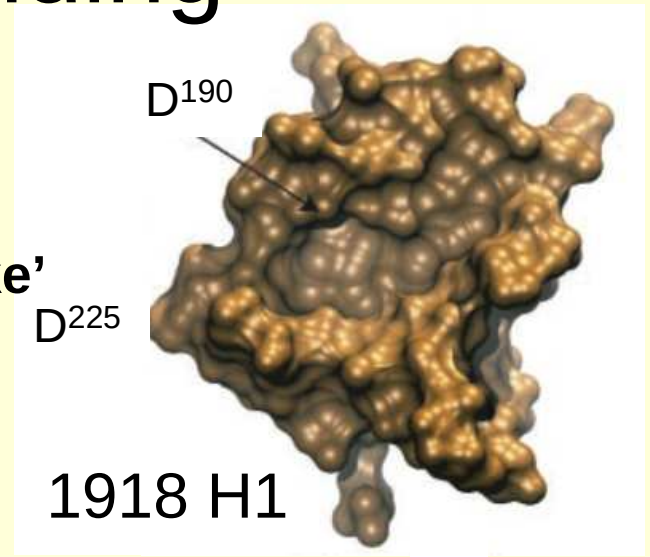
Kash, et al. 2012 In review.

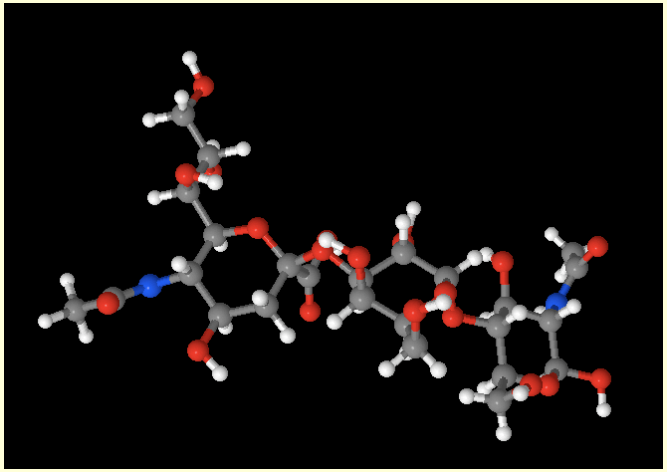
1918 HA RBD helped define sialic acid binding



Neu5Ac(α2-6)D-Gal(β1-4)GlcNAc

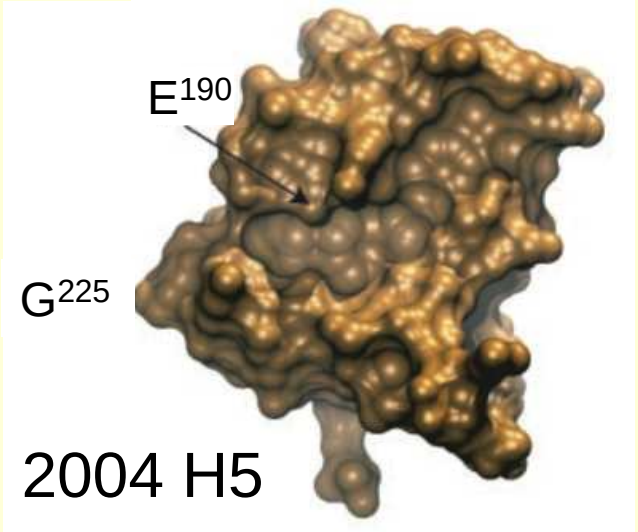
'Human-like' α2-6





Neu5Ac(α2-3)D-Gal(β1-4)GlcNAc

'Avian-like' α2-3



Stevens, J *et al*. Science (2004) 303:1866 - 1870

Natural 1918 HA receptor binding variation between cases

1918 HA partial sequences

Date of Death	Strain Name	Partial HA protein sequence (residues 172-237 of HA1 domain)[1]	Source of Sequence
		EVLVLWGVHHPPTGTDQQSLYQNADAYVSVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPG	Consensus Sequence
05/11/18	A/IA/1/1918	·····G·····	This report
06/02/18	A/IA/2/1918	·····G·····	This report
06/24/18	A/DC/1/1918	·····	This report
07/22/18	A/SC/2/1918	·····G·····	This report
09/21/18	A/NY/2/1918	·····	This report
09/26/18	A/NY/1/1918	·····G·····	Reid, et al. 1999
09/26/18	A/SC/1/1918	·····	Reid, et al. 1999
09/26/18	A/NY/3/1918	·····	This report
09/27/18	A/VA/1/1918	···R···	This report
09/30/18[2]	A/AFIP/1/1918	·····	This report
10/01/18	A/VA/2/1918	·····	This report
11/20/18[3]	A/BM/1/1918	·····	Reid, et al. 1999
11/13/18	A/London/1/1918	····S·····	Reid, et al. 2003
02/13/19	A/London/1/1919	·····I·G·····	Reid, et al. 2003

[1]Protein sequences aligned to the HA1 domain sequence of A/SC/1/1918 [GenBank Acc. No. AAD17229.1]. Dots match consensus sequence.
[2]Case was accessioned at the AFIP in conjunction with other cases from late September through early October 1918; date of death was therefore likely in this timeframe.
[3]Outbreak in Teller Mission (now Brevig Mission, AK) occurred between November 15-20, 1918; date of death was arbitrarily set at 11/20/1918 [Ref: http://www2.gi.alaska.edu/ScienceForum/ASF17/1772.html].

14 sequences: 4 from spring-summer; 10 from fall/winter:

- 3 of 4 spring-summer isolates with 222G (**'avian-like'**)
- 8 of 10 fall/winter isolates with 222D (**'human-like'**)
- 3 novel RBD region polymorphisms

Sheng, *et al*. 2011 *PNAS* 108:16416.

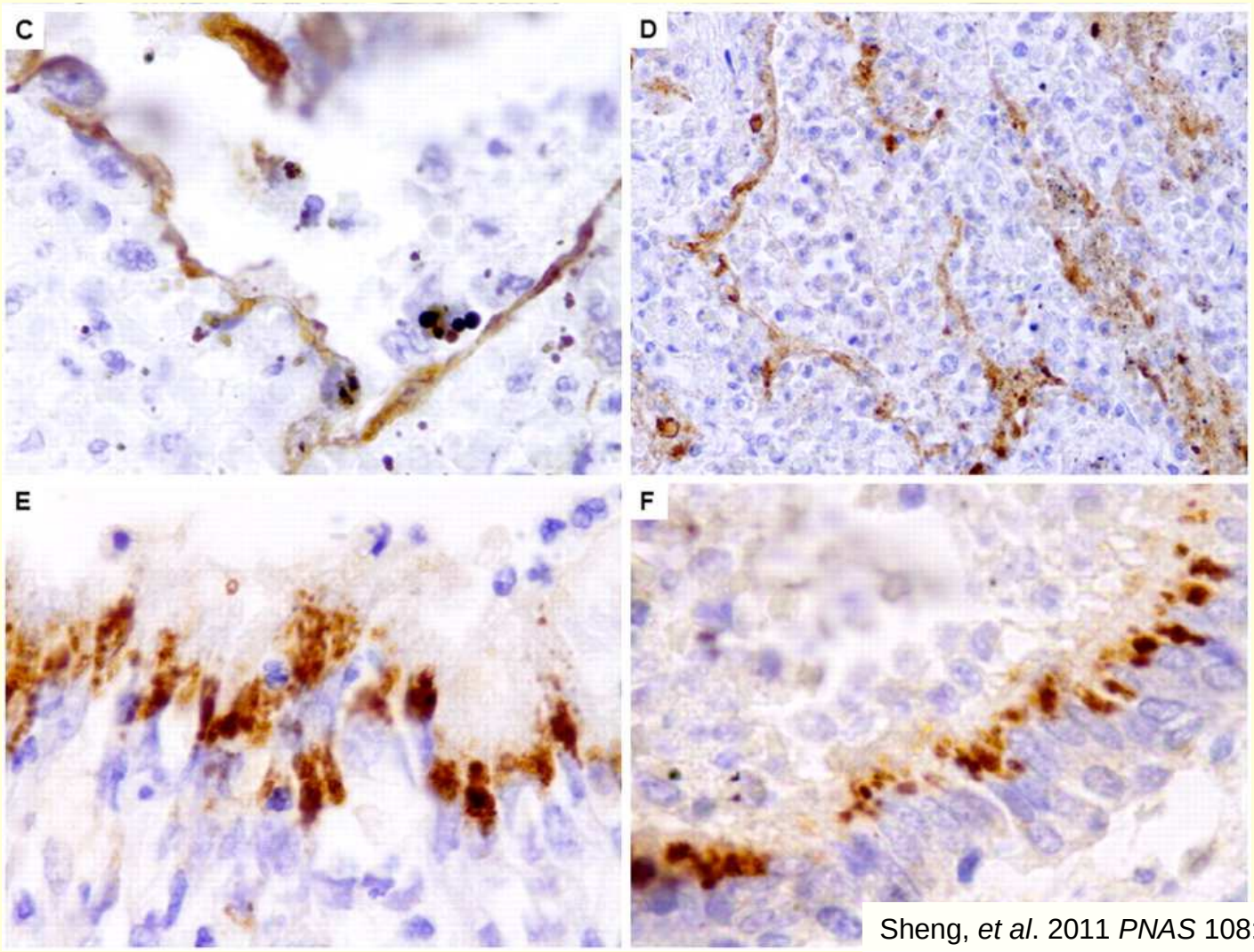
1918 HA receptor binding specificity and tropism

‘Avian-like’ (α2-3/α2-6 SA)

‘Human-like’ (α2-6 SA)

Alveolar cells

Respiratory epithelial cells

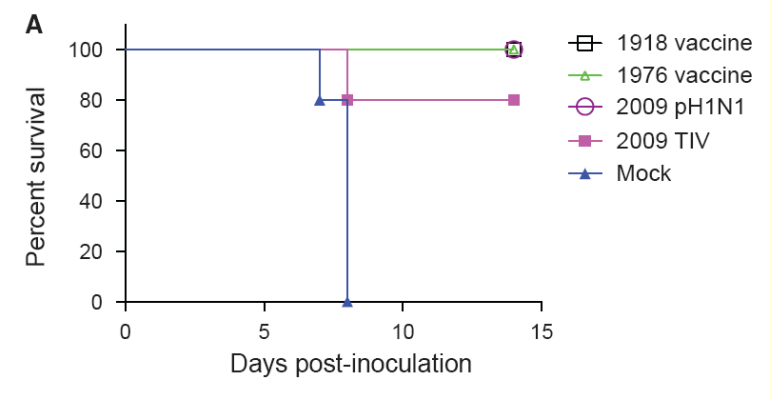


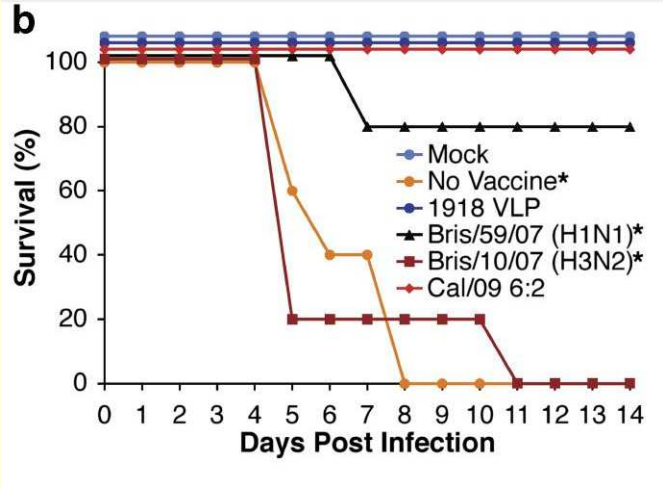
Sheng, *et al*. 2011 *PNAS* 108:16416.

1918 influenza virus antiviral sensitivity and vaccine cross-Protection

1918 virus fully sensitive to all four approved antiviral drugs:

- Ion channel blockers:
  - Amantadine
  - Rimantadine
- Neuraminidase inhibitors:
  - Oseltamivir
  - Zanamivir

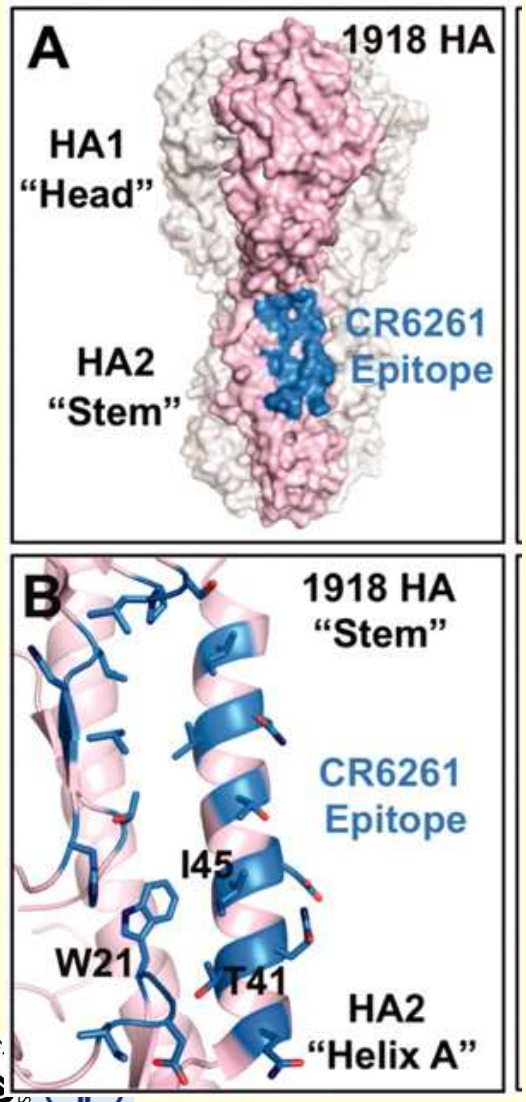


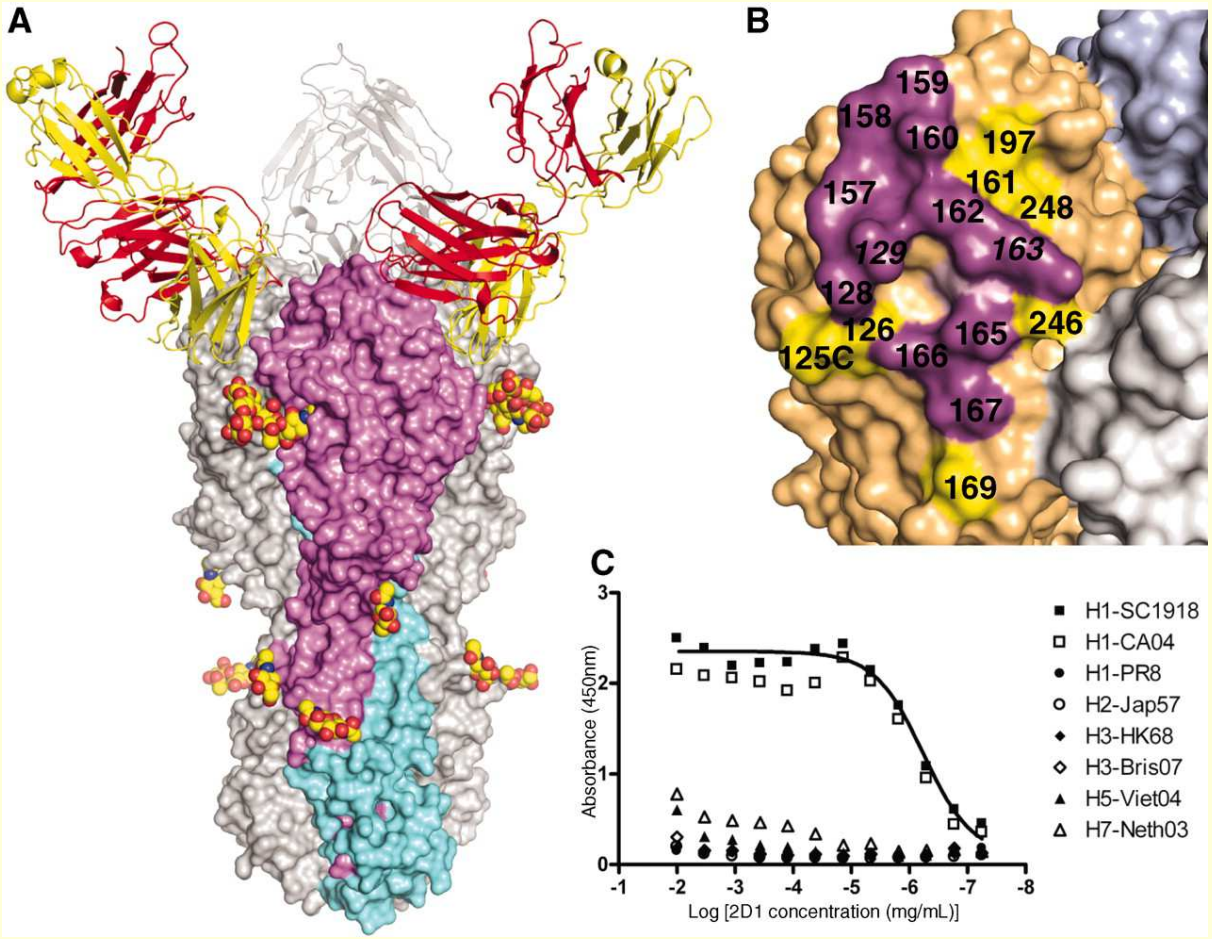


Both 2009 pH1N1 and seasonal H1N1 vaccines protect mice against 1918

Tumpey, *et al*. 2002 *PNAS* 99:13849
Tumpey, *et al*. 2004 *PNAS* 101:3166
Medina et al. 2010 Nature Commun 1:28
Easterbrook, et al. 2011 IRV. E-pub Jan. 25, 2011

Use of 1918 HA structure to develop monoclonals & broadly reactive vaccines





Xu, *et al*. 2010 *Science* 328:357.
Fleishman, *et al*. 2011 *Science* 332:816.

1918 virus genome used to identify a novel influenza protein

- Novel ORF encoded by influenza A viruses
- Encodes a non-structural protein that alters the host inflammatory response, including down-regulation of MHC class I genes
- Novel target for therapeutics development and for vaccine effectiveness

Jagger, *et al*. 2012 In review.

Conclusions

- 1918 virus project has significantly enhanced our understanding of influenza pathobiology
- 1918 virus sequence has been utilized in hundreds of published studies
- 1918 genome continues to provide useful data about influenza