The Next Influenza Pandemic:
Remembering the Past & Planning for the Future

Jeffery K. Taubenberger, M.D., Ph.D.
The Next Influenza Pandemic
Can It Be Predicted?

Jeffery K. Taubenberger, MD, PhD
David M. Morens, MD
Anthony S. Fauci, MD

Although most experts believe another influenza pandemic will occur, it is difficult to predict when or where it will appear or how severe it will be. Neither is there agreement about the subtype of the next pandemic influenza virus. However, the continuing spread of H5N1 highly pathogenic avian influenza A (HPAI) among poultry on several continents, associated with an increasing number of severe and fatal human infections, has raised the pandemic stakes.\(^1\) Genetically and antigenically divergent H5N1 HPAI strains appeared in 1997 and have been spreading globally since 2003.\(^2,3\) To date, epizootics in approximately 60 countries have caused a reported 291 human cases with 172 deaths.\(^4\)

Reassortment with other avian influenza viruses.\(^5\) It is not yet clear which of these changes is associated with lethality in wild birds or with pathogenicity and transmissibility in poultry and other species. Asymptomatic endemic H5N1 HPAI circulation in domestic ducks maintains a pool of pathogenic viruses to which poultry are continually exposed,\(^8\) suggesting that the current H5N1 situation will likely persist.

There are limited data indicating whether any H5N1 influenza strain is evolving in the direction of human adaptation. Some H5N1 viruses exhibit a change in the polymerase protein complex PB2 that has been associated with increased H5N1 virulence in mice and ferrets, and adaptation of other avian influenza viruses to humans.\(^9,12\) It remains unclear, however, whether this or any other mutation is associated only with increased mammalian virulence or provides an independent evolutionary advantage in birds.

The pathogenicity of influenza viruses for their different hosts is related to complex viral and host factors and re-
No! (Not yet, at least)

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Understanding Influenza Backward

David M. Morens, MD
Jeffery K. Taubenberger, MD, PhD

The novel 2009 influenza A(H1N1) pandemic virus has been an unexpected trigger for pandemic preparedness plans in the United States and elsewhere. It is appropriate to ask how the novel virus might behave epidemiologically in coming months, including the possibility of multiple recurrences or “waves.” Spring circulation of the novel virus in the Northern Hemisphere at the end of the 2008-2009 influenza season inevitably has led to comparisons with events in 1918-1919, which in some settings were preceded and followed by outbreaks of respiratory illnesses. Some also believe that the 1918 pandemic began with a premonitory “herald wave,” a term related to an old hypothesis, which influenza and dengue fever appeared to have supported, that as new viruses begin to circulate in human populations they inevitably acquire mutations that increase transmissibility and virulence.2

Largely seasonal postpandemic influenza mortality peaks recognized in many large cities between 1890 and 1894.7 What happened in 1918 was quite different. A recent tendency to refer to any influenza-like illness in the first 8 months of 1918 as “the spring wave” has altered the use of this term. Importantly, no viruses from the 1918 spring outbreaks or the summer wave have yet been identified. Many investigators working in and since 1918 have cited evidence for or against “spring waves” and their protection against later pandemic waves. However, such data are potentially confounded by inability to discern whether protection, or lack thereof, was associated with spring or summer infectious agents, which could have been different, and by the possibility of nonspecific short-term influenza cross-protection elicited by a different spring virus (had 1 or more circulated).

What is most puzzling is that during the 1918 pandemic, different countries had anywhere from 0 to 3 waves or occurrences, the course and timing of which varied greatly. Most of the world had 2 occurrences, one around October-
1918 ‘Spanish’ Influenza  Mortality

- Total global deaths in the 9 months of the pandemic in 1918-1919 estimated to be 50-100 million‡,*

- U.S. Deaths = 675,000

- Flu deaths in Philadelphia in October 1918 = 10,959. Total flu deaths = 15,785

- U.S. Military deaths to flu = 43,000 (out of ~100,000 U.S. Troop casualties in WWI)

*Perspective: ~37 million AIDS fatalities in the last 36 years
Global Influenza Mortality in 1918 Underestimated

Studies of population size suggests that 1918 flu mortality in India was at least 14 million

US Soldiers with 1918 Influenza, Ft. Riley, KS
1918 Influenza Pandemic Waves
Death Registry, Oregon 1918-19
Unique 1918 Age-Specific Mortality

Viboud, et al. 2013 JID 207:721
Influenza A virus

⭐ Family: Orthomyxoviridae

- Negative sense, segmented, single-stranded RNA genome
- 8 segments, at least 12-13 ORF’s

HA $\alpha 2-3Gal$

SA $\alpha 2-6Gal$

NA

(11 NA subtypes)

(18 HA subtypes)

“Shift and Drift”

Influenza A viruses in humans

- Yearly outbreaks with up to 80,000 deaths in U.S.
- Occasional and unpredictable pandemic strains with increase in illness and death

Deaths: 12,000 – 56,000
Hospitalizations: 140,000 – 710,000
Cases: 9,200,000 – 35,600,000

**MY FLU ALGORITHM**

Do you feel like you've been hit by a train?
- Yes → Have you been hit by a train?
  - Yes → You have been hit by a train
  - No → You do not have the flu
- No → You do not have the flu
Influenza virus evolution is extremely rapid

H3 HA gene

Antigenic Drift


Antigenic Shift (Intrasubtypic Reassortment)

Antigenic Drift Necessitates Continual Updating of Annual Influenza Vaccine Strains

A. Significant Antigenic Changes

- Annual epidemic influenza causes up to 500,000 hospitalizations and up to 56,000 deaths in the U.S.
- Overall seasonal vaccine effectiveness over the past 10 years has ranged from 10 to 56%, with a mean of 40%, lower in at-risk populations
Influenza A Virus Host Range Quite Diverse
Avian Influenza A Virus Diversity

Darwin circa 1860

Influenza A Virus Host Switch
Human Influenza A Timeline

- 1889: "Russian" Flu
- 1918: "Spanish" Flu
- 1957: "Asian" Flu
- 1968: "Hong Kong" Flu
- 1977: H3N2
- 2009: "Swine" Flu (pH1N1)

H5N1, H9N2, H7N9? N = 4
Mortality Impact of 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 in History

• ~14 pandemics in last 500 years
• Average interpandemic period ~36 years

Hunting for the 1918 Influenza Virus

- Concept of viruses as infectious agents still new in 1918
- No isolates of virus made during pandemic
- Influenza A viruses first isolated from pigs in 1930 and from humans in 1933
1918 Influenza Autopsy Cases

Johan Hultin, M.D.
1918 Lung Pathology

Primary Viral Pneumonia: DAD with edema, alveolitis, thrombi

Taubenberger & Morens 2008 Ann Rev Path 3:499
Morens, Taubenberger & Fauci 2008 JID 198:962
Kuiken & Taubenberger 2008 Vaccine 26(S4):D59
1918 Lung Pathology
Secondary Bacterial Pneumonia and Repair

Taubenberger & Morens 2008 Ann Rev Path 3:499
Morens, Taubenberger & Fauci 2008 JID 198:962
Kuiken & Taubenberger 2008 Vaccine 26(S4):D59
1918 H1N1 Autopsy Study

A) c/w Strep pneumoniae

B) c/w Strep pyogenes

C) c/w Strep pneumoniae

D) c/w Staphylococcus
1918 H1N1 Autopsy Study

Analysis of 68 fatal 1918 pneumonia cases

Viral Antigen Distribution

US Army P&I Admissions

US Army P&I Deaths

68 P&I fatal cases in series

9 spring-summer cases

Sheng et al. 2011 PNAS 108:16416
Since 1918 all pandemic and seasonal influenza viruses descended from the 1918 virus.

All influenza mortality in last 100 years ultimately due to one viral introduction.

Concept of ‘pandemic era’

Seasonal Vs. Pandemic Influenza Mortality

- Seasonal flu 1960-1967: 9%
- Seasonal flu 1971-1976: 8%
- 1977 pandemic: 4%
- 2009 pandemic: 3%
- 1957 Pandemic: 9%
- 1968 pandemic: 9%
- Seasonal flu 2012-2016: 6%

75% of mortality
Lessons Learned

- Pandemics are unpredictable in their origin, timing, and severity.
- The age-specific “W” mortality pattern of the 1918 pandemic remains unelucidated.
- The 1918 pandemic epidemic ‘waves’ were not uniform in character or timing.
- Concept of ‘pandemic eras’
- Almost all human cases of influenza in last 100 years ultimately due to a single founder virus in 1918.
- In general, most influenza mortality collectively occurs in seasonal influenza not in pandemic influenza years.
Influenza Pathogenicity

- Host Factors
- Viral Factors
- Bacterial Factors

R I P
Influenza Pathogenicity

- Host Factors
- Bacterial Factors
- Viral Factors
1918 Influenza Pathogenesis

Pandemic HA Virulence Factors

- Isogenic viruses containing pandemic HA’s cause severe disease
- 1918 > 1957, 1968, or 2009
- Seasonal H1 or H3 bearing viruses did not cause severe disease

Qi, et al. 2011 Virology 412:426-34
1918 HA is the main virulence factor in pathogenicity in mice, ferrets, NHP.
1918 virus has a very avian-like genome.
Avian H1 HAs did not attenuate 1918 virus, and share virulence with 1918.
1918 virus virulence therefore likely not pandemic virus-specific but inherited from a low path avian H1 ancestor.

What about other low path avian influenza (LPAI) HA subtypes?

What about other LP Avian HA Subtypes?

Qi, et al. 2014 MBio. 5:e02116-14
Pathogenic viruses:
- H1, H6, H7, H10, H15
- Lung titers did not correlate with pathogenicity
Structural/ Functional Relationship of Pathogenic Avian HA Subtypes?

H1, H6, H7, and H10 inflammatory responses similar to the 1918 virus

Qi, et al. 2014 mBio. 5:e02116-14
NHBE Culture – Cytopathicicy Correlates with Mouse Pathogenicity

Qi, et al. 2014 MBio. 5:e02116-14
Recent AIVs causing severe zoonotic infections have included HA subtypes H6, H7, H10.
Zoonotic Avian Influenza Infections and the Risk of a Future Pandemic

- **H5N1**: 860 documented cases, 454 deaths
  - Reported CFR 53%
  - WHO, 2003-2017, as of December 2017

- **H7N9**: 1623 confirmed with 620 deaths
  - Reported CFR 38%

- Problems associated with current vaccine strategies:
  - Zoonotic viruses continue to evolve, requiring updating stockpiled pre-pandemic vaccine stocks
  - Epizootic outbreaks often do not result in pandemics, and emergence of pandemic viruses cannot yet be predicted
# H7N9 avian influenza cases

![Graph showing H7N9 cases and deaths over time](image)

<table>
<thead>
<tr>
<th>H7N9 Protein</th>
<th>Codon Substitution</th>
<th>Avian H7N9 Consensus</th>
<th>Human H7N9 Consensus</th>
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<td>1, 2, 3, 6, 8, 13, 14, 15, 16, 20</td>
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</table>

Xiao, et al. 2018 3:e00462-18
Influenza Pathogenicity

Host Factors

Viral Factors

Bacterial Factors
Upregulated Inflammatory Responses During 1918 Infection

Type I IFN response

Inflammatory mediators

Cell stress responses

1918

sH1N1

Treatment with a Catalytic ROS Scavenger Decreases Lung Damage and Increases Survival

EUK-207: organometallic SOD/catalase mimetic

1918 Pandemic Influenza Virus

Daily from day +3 to day +10

H&E

Pathology

ROS damage

Anti-8-oxo-2'-deoxyguanosine

Kash et al. 2014 FRBM 67:235-47
Influenza Pathogenicity

- Host Factors
- Viral Factors
- Bacterial Factors
Viral/Bacterial Coinfection is Associated with Loss of Airway Basal Epithelial Cells

Kash et al. 2011 mBio 2:e00172
pH1N1+SP infection associated with loss of basal cells and absence of re-proliferation and repair of airway epithelial cells.

Viral damage to and loss of airway epithelial cells may expose basal epithelial cells to bacteria leading to the death of these progenitor cells, limiting re-proliferation.
1918 Viral & Streptococcus pneumoniae Co-infection alter bacterial gene expression

Model of Inflammation and Pulmonary Thrombosis during 1918 & SP Co-Infection

1918 autopsies
- Marked F3 staining
- Thrombus formation

1918 Pneumonia Case with Prominent Erythrocyte Sickling

DNA sequence of the hemoglobin beta gene from the 1918 FFPE lung tissue showed **Glu6Val hemoglobin S mutation**, 4 years before term “sickle cell anemia” described

Sheng, et al. 2010. EID 16:2000-1
Lessons Learned

- 1918 pathogenesis is multifactorial involving the interplay of viral virulence factors, host inflammatory response, and secondary bacterial infections.

- 1918 virulence likely not a pandemic specific mutation but a phenotype observed with influenza viruses expressing certain avian HA subtypes in a mammalian host (H1, H6, H10, H15).

- Future pandemics viruses with one of these subtypes may share features and severity with the 1918 virus.

- Future pandemics may be dependent on how long H1N1 and H3N2 viruses circulate.
Human Influenza Challenge Studies at NIH Clinical Center

- VPES human influenza challenge model
  - Healthy adult volunteer, in-patient study (min 9 days)
  - GMP-manufactured wild-type IAVs
  - 2009 pandemic H1N1 and 2012 H3N2 IAVs
  - Other challenge viruses in production (H1s, H3s, Bs)
  - >400 participants challenged to date

- Phase I and II challenge studies
  - Basic pathogenesis and correlates of protection
  - Completed vaccine and therapeutic antibody trials
  - VPES universal vaccine candidate Phase I testing in 1 year
Serologic Correlates of Protection

<table>
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<th>Binary Endpoints</th>
<th>HAI</th>
<th>NAI</th>
<th>Stalk Titer</th>
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<td>Reduction in +/- shedding</td>
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<th>Continuous Disease Severity Measures</th>
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<th>Stalk Titer</th>
<th>Independent Predictor</th>
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<td>Symptom Severity (FluPRO)</td>
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<td>NAI, not Stalk or HAI titer</td>
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Influenza Challenge Study – Symptoms and Shedding

Patients shedding 3-4 log\(_{10}\) virus on day 2 with very few symptoms

Influenza Challenge Study – Symptoms and Shedding

Patients shedding $3-4 \log_{10}$ virus on day 2 with very few symptoms.

Biomarker discovery: diagnostic and prognostic mRNA, miRNA, or proteomic markers.

PBL Transcriptome Analysis – Prognostic Biomarker Discovery

Symptoms and Shedding

Days post inoculation

Kash, et al. In review - Confidential
Human PBL Expression of Genes Predicting Illness Severity at D2

Kash, et al. In review - Confidential
Improved Influenza Vaccines

- Universal influenza vaccines could:
  - Offer pre-pandemic protection against all influenza A viruses (H1-H16), or
  - Protect against seasonal viruses, or
  - Protect against both
Broadly Protective Influenza Vaccines

• **Concept:** Non-infectious vaccines presenting a mixture of avian influenza hemagglutinins would induce broad cross-protection without need for antigenic matching to specific strains.

• **Proof of Concept:** A vaccine cocktail (H1, H3, H5, H7) provides extremely broad cross-protection against most or all influenza A viruses.
Experimental Vaccine is Strongly Protective

100% protection against 10x LD$_{50}$ 1918 H1N1 (Intrasubtypic challenge)

Percent survival

PBS

VLP

No vaccine

VLP

Schwartzman, *et al.* mBio. 2015; 6:e01044
Experimental Vaccine is Broadly Protective

100% Protection against subtypes *not* in the vaccine (e.g., 10x LD$_{50}$ 1957 H2 pandemic, avian H10, H11, & H15)

Tetravalent Influenza Vaccine Provides Broad Protection

Vaccine induces antibodies to HA head, HA stalk, NA, and elicits T cell responses

100% survival, including broad heterosubtypic cross-protection

0% survival

Schwartzman, et al. mBio. 2015; 6:e01044
Park, et al. Unpublished
Tetravalent Vaccine Efficacy in Ferrets

- Challenge with antigenically mismatched H1, H3 viruses
- Rapid clearance of virus from nasal and lung tissues
  - 10,000-100,000 fold reductions in viral titer
- Prevention of pneumonia

Mock

Vaccinated
Lessons Learned

- Influenza Pathogenicity is a complex of viral, host, and secondary bacterial factors.
- 1918 virulence is shared with circulating avian influenza viruses.
- Studying viral pathogenesis and host responses in humans is critically needed for rational universal vaccine design.
- Influenza challenge models are ideal for detailed studies of immune and molecular correlates of disease and protection and are ideal models to evaluate new vaccines and drugs in phase II trials.
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Alison Han, MD
Dana Neitzey
Susan Reed

Pathogenesis Group
John Kash, PhD
Sharon Fong, BS, RLATG
Jae-Keun Park, DVM, PhD
Qi Li, PhD
Mitchell Ramuta
Luz Angela Rosas, MS
Zong-Mei Sheng, MD, PhD
Stephanie Williams
Yongli Xiao, PhD
Xingdong Yang, PhD
Kathie Walters, PhD (ISB)
Kelsey Scherler (ISB)

NIH Collaborators
Richard Davey, NIAID DCR
Anthony Fauci, NIAID Director
Peter Jahrling, NIAID IRF
Rodney Levine, NHLBI LB
David Morens, NIAID, OD
Cecile Viboud, FIC

Non-NIH Collaborators
Felice D’Agnillo, FDA
Paul Digard, Univ. Edinburgh
Susan Doctrow, Boston Univ
Maryna Eichelberger, FDA
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Emanuel Petricoin, GMU

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NIH National Institute of Allergy and Infectious Diseases
DARPA Biological Advanced Research and Development Agency
BARDA Biomedical Advanced Research and Development Authority
Bill & Melinda Gates Foundation