

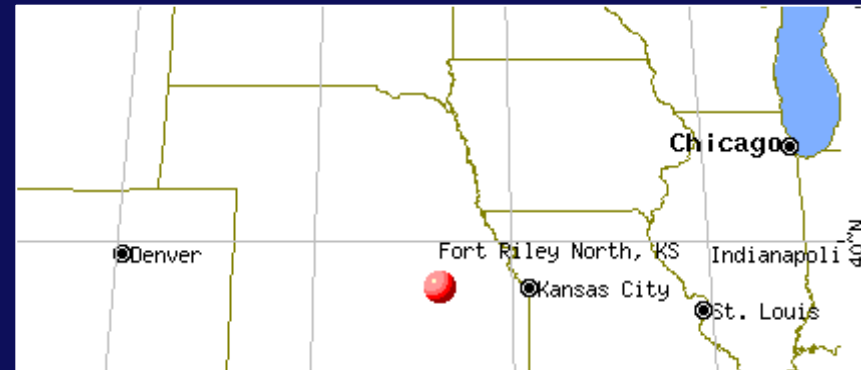


Pandemic Avian Influenza: Epidemiology, Biology and Risk Assessment

Richard P. Wenzel, MD, MSc

Case History, 1918

- March 11
 - Mess Sgt Albert Gitchell is awakened after a restless night to make breakfast for hundreds of soldiers
 - Miserable headache, fever, sore throat, muscle aches
 - Sick bay T = 39.5°C
- March 13
 - 522 men ill



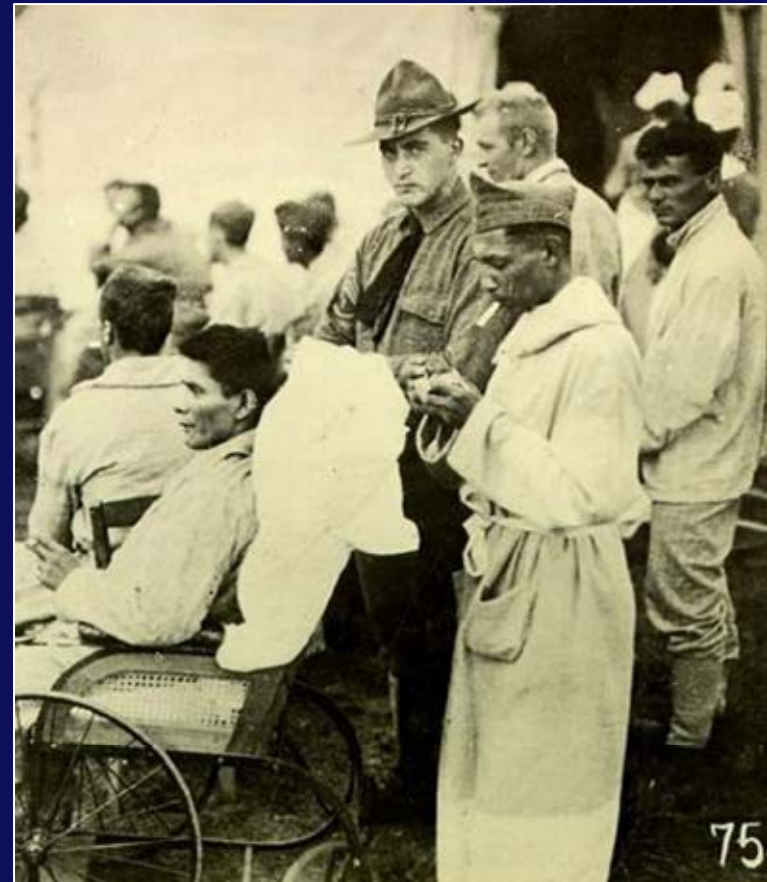
One Half of Deaths of Soldiers in Europe Due to Flu (43,000 Flu Deaths)



Staphylococcus aureus Pneumonia, 1918

- 8100 contract flu
- 1409, pneumonia (17%)
- 385 deaths (27%)
- Approx. one half of deaths (153) with *S. aureus* cults
- 92 of 153 (60%), *S. aureus* only

- Cherry-red, indigo-blue cyanosis
- No chills, localized CP
- No signs of consolidation
- Fulminating septic course
- Dirty salmon-pink sputum



56% die by day 10, 72% by day 15

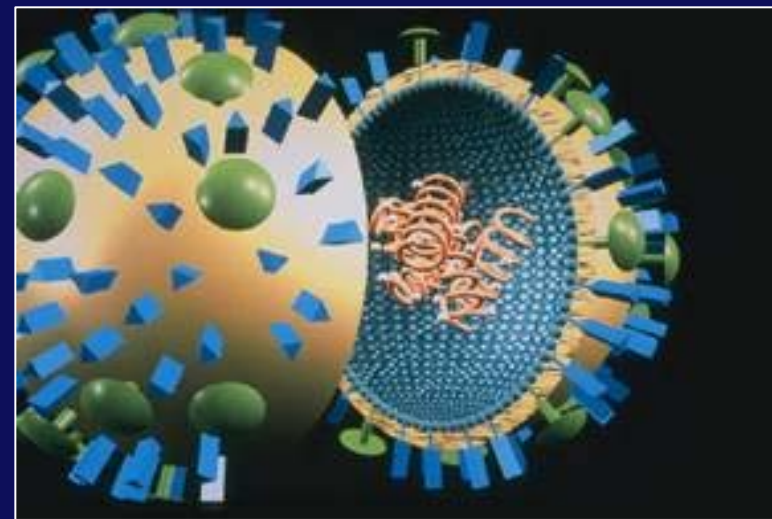
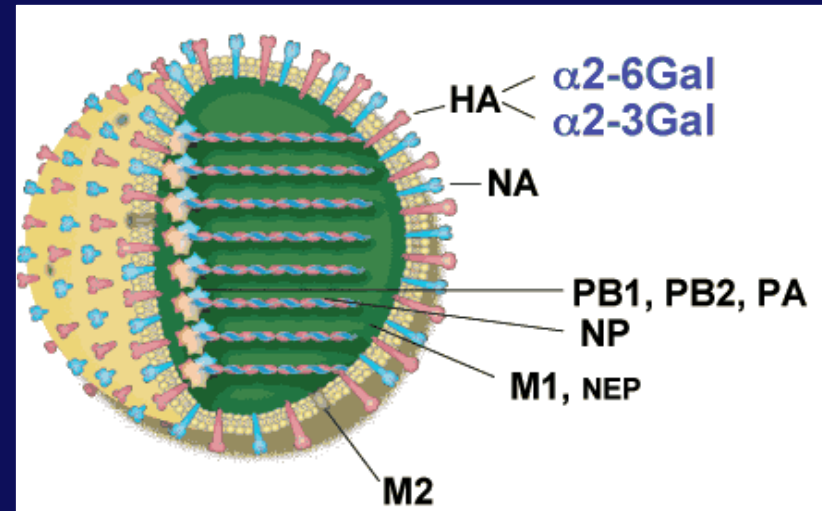
Impact of 1918-1919 Influenza (H1N1) in US

	1918-1919	1920-2005
AR	28%	10%
Mortality	2.5%	0.1%
Total deaths	675,000	36,000
Most deaths	Young adults	Older adults
Risk factors	None	Cardiopulmonary disease, pregnancy, alcoholism, renal disease

- Average life span in US fell by 10 years

Two Strategies for Influenza

1. Accumulation of point mutations with no proofreading
2. Reassortment of eight segments of RNA – “viral sex”: exchange of genes between two viruses co-infecting a host (256 possible offspring)



Creation of Pandemic Influenza: Two Tales

- 1957 (Hong Kong) – H2N2;
1968 (Asian) – H3N2
 - Coinfection with avian and human flu and reassortment of α -2,3 avian gene segments (H, N, PB1)
- 1918 (Spanish)
 - Direct bird to man and adaptation by avian strain



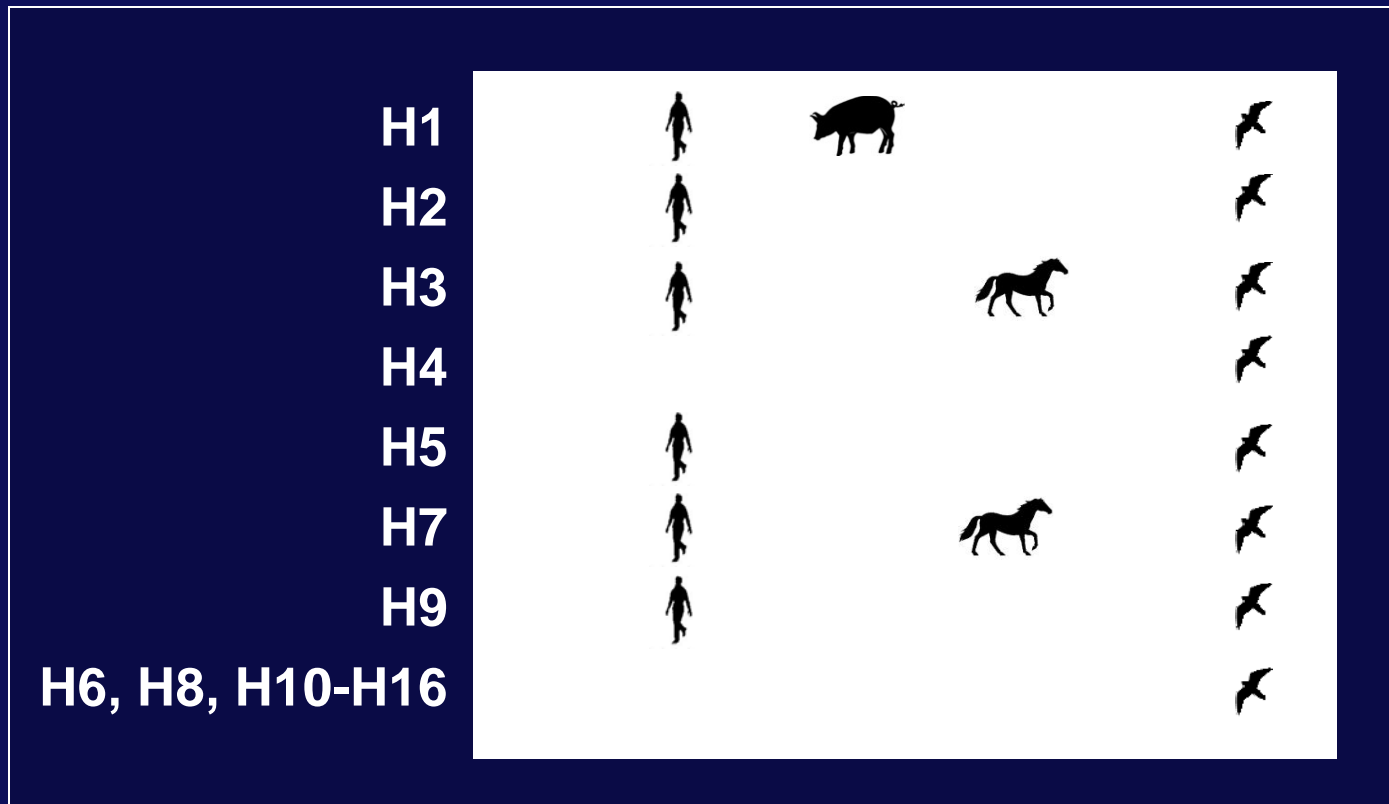
Antigenic Subtypes of Influenza A Virus Associated With Pandemic Influenza

Year	Interval Between Pandemics (y)	Designation	Extent of Antigenic Change in Surface Protein*	Severity of Pandemic/ No. of Deaths
1889	—	H2N2	?	Severe
1901	12	H3N8	H+++ N+++	Moderate
1918	29	H1N1	H+++ N+++	40-50 million
1957	39	H2N2	H+++ N+++	2 million
1968	11	H3N2	H+++ N+	1 million

*+ Minor change; ++ Moderate change; +++ Major change; - No change.

Smith DJ. *Science*. 2006;312:392-394.

Reservoir for Influenza Is in Migratory Water Fowl



Influenza Reservoirs and Transmission



Reservoir for viruses containing 16HA and 9NA subtypes



Guano
containing
virus



Domestic poultry infected



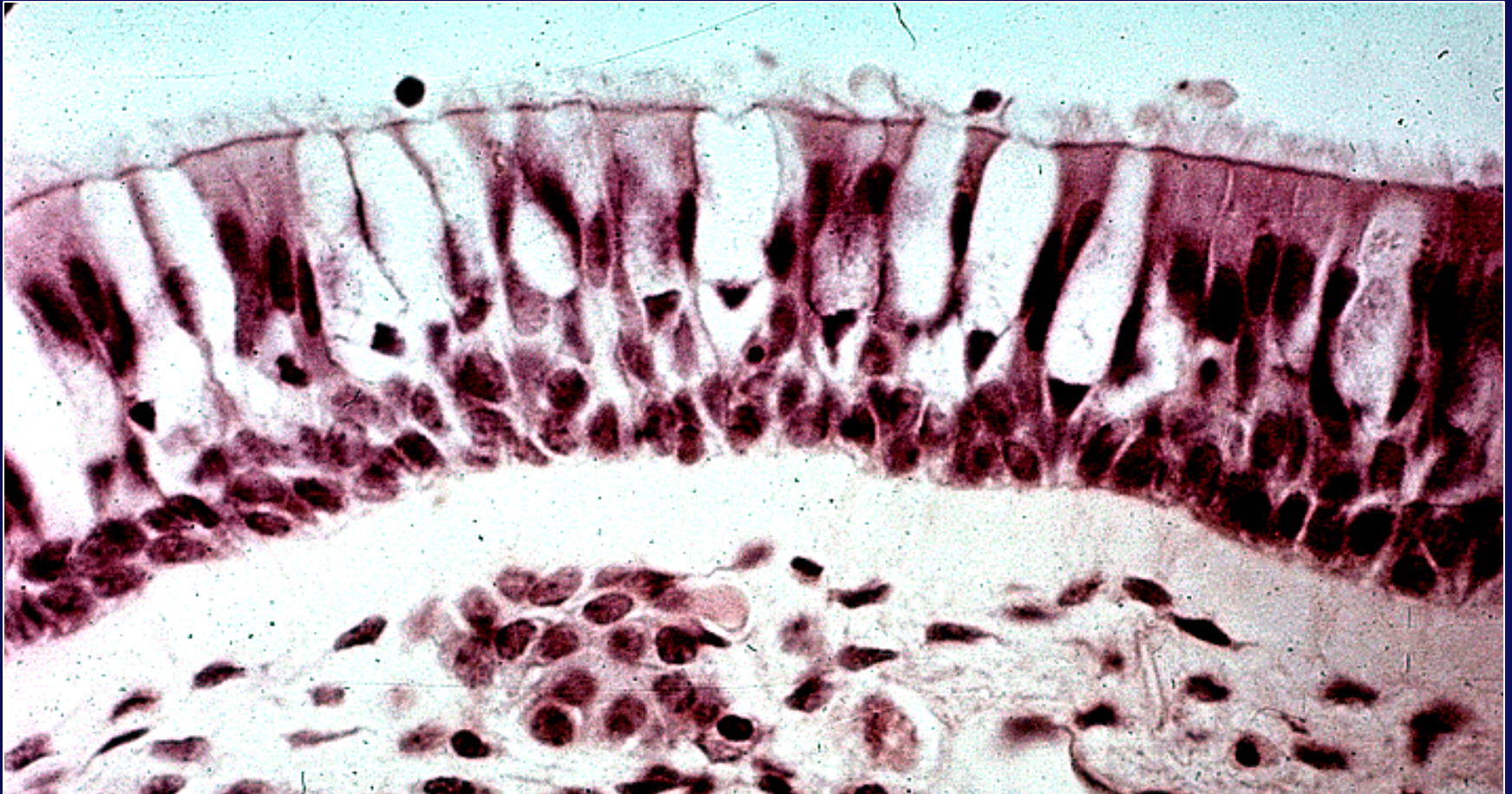
- Systemic disease with hemorrhage, edema, viral replication in endothelium¹
- Histology
 - Necrosis of the liver, pancreas, brain, lymph tissue, lamina propria
 - Viral antigen – intestine, gizzard, liver; seldom lung or trachea²



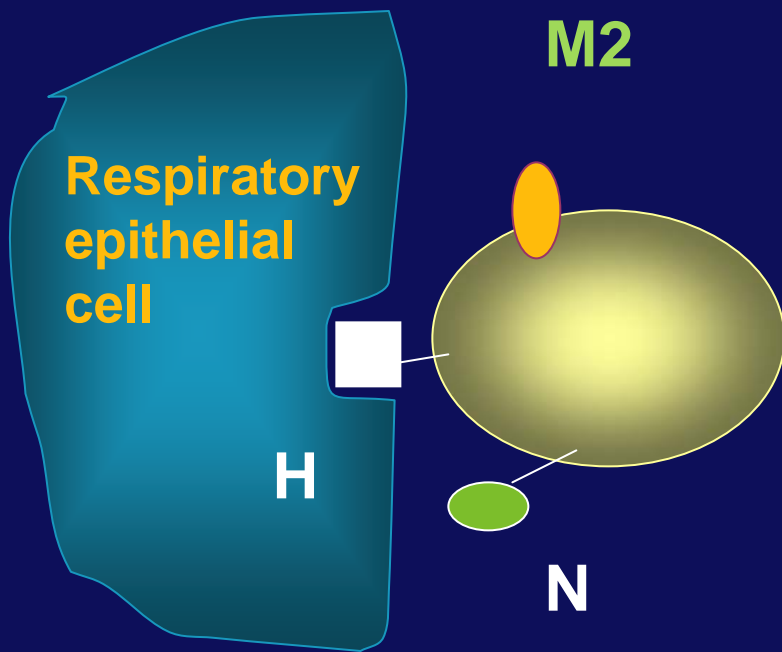
Infected mammalian hosts



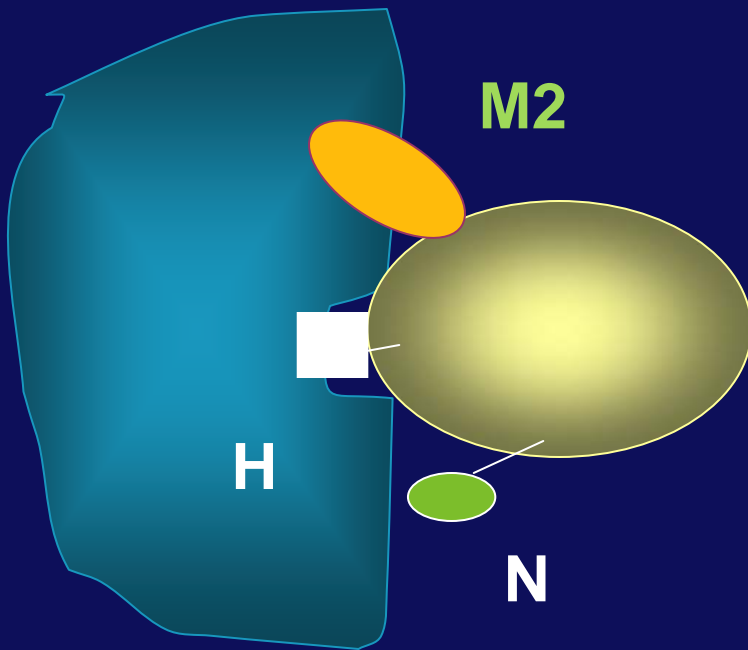
From the University of Delaware Biomedical Sciences Web Site



From the University of Delaware Biomedical Sciences Web Site (CONT'D)

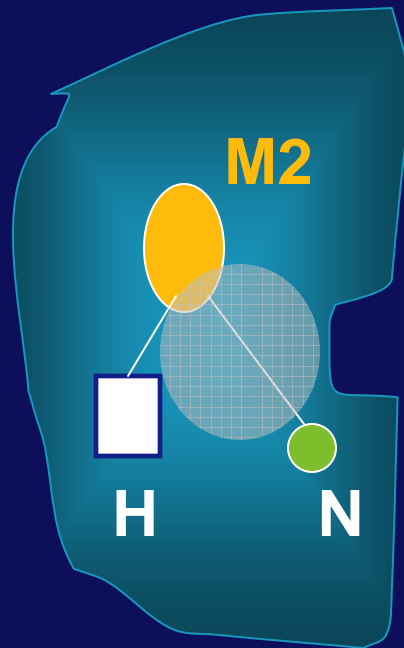


From the University of Delaware Biomedical Sciences Web Site (CONT'D)



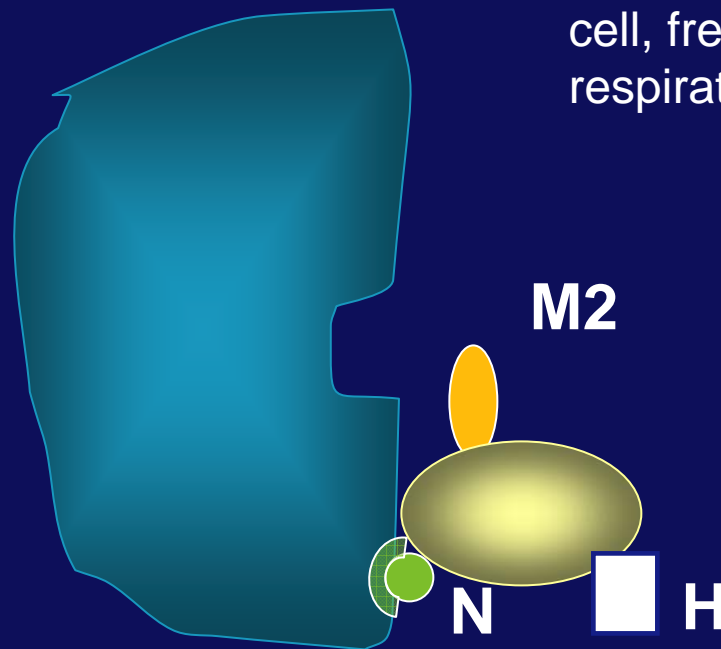
- Amantadine + rimantadine inhibit the function of M2 protein ion channels and prevent viral uncoating
- M2 matrix protein modulates pH levels via flow of ions, facilitating viral uncoating

From the University of Delaware Biomedical Sciences Web Site (CONT'D)



From the University of Delaware Biomedical Sciences Web Site (CONT'D)

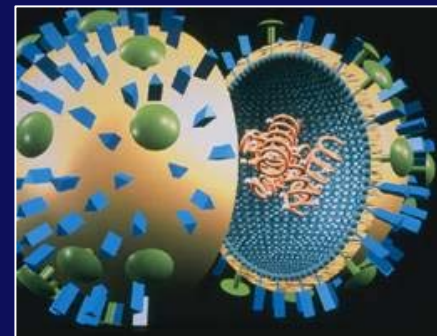
- Neuraminidase cleaves bond of virus and cell, freeing virus to enter respiratory secretions



- Neuraminidase inhibitors
 - Oseltamivir
 - Zanamivir

Influenza Pathogenesis

- Incubation period of 1-4 days
- In 50% of cases patients are asymptomatic (still shedding)
- Adults shed virus in 3-5 days
- Young children shed virus in <3 weeks
- Immunocompromised patients shed virus in >3 weeks

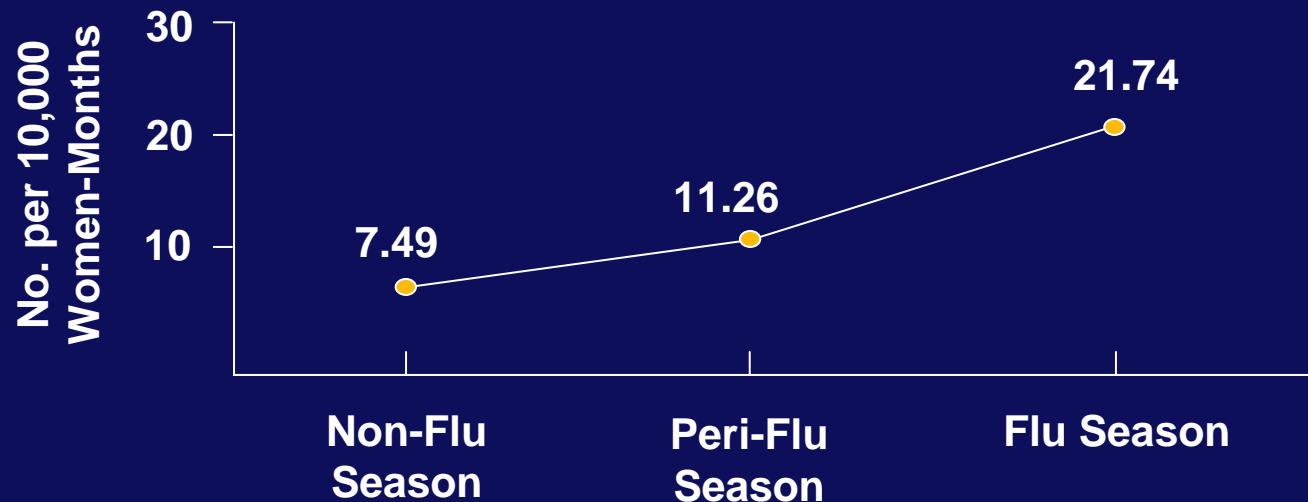


alveoli
if particle
<10 mm

large
airways
if particle
>10 mm



Third-Trimester Hospitalizations



- One half of morbidities attributable to flu
- Of 10,000 third-trimester women with no risk factors, 25 will be hospitalized due to influenza-related morbidities during average flu season
- Odds ratio for third-trimester cardiopulmonary events 4.7

Droplets and Droplet Nuclei in Influenza: Volunteer Studies

- Droplet administration
 - Milder disease in volunteers; required larger inoculum
- More likely to cause infection of lower respiratory tract

Droplet Nuclei

>10 mm

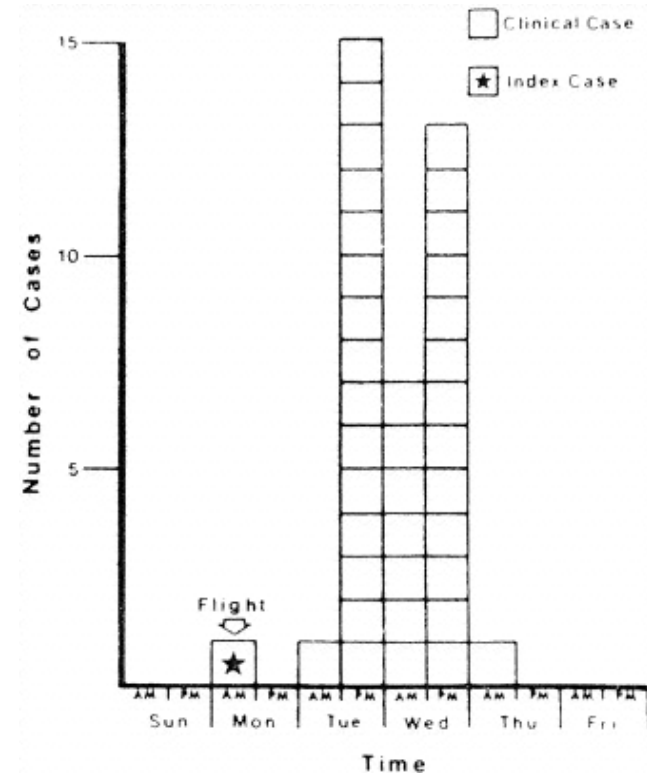
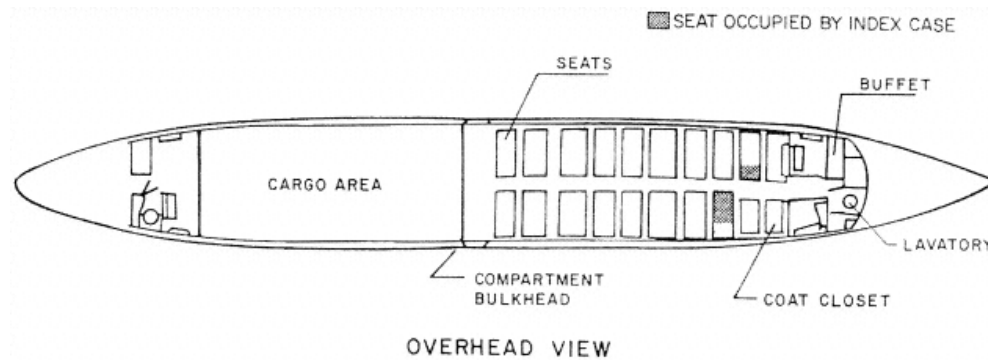


<10 mm



Evidence for Airborne Transmission of H3N2 Influenza

Boeing 737



Airborne Spread of Asian Influenza (H2N2), 1957-1958



UV light
AR (serologic)
2% of 209 patients
2nd wave – no cases

Test Building
Main Building
Livermore, CA



No UV light
AR (serologic)
19% of 396 patients
2nd wave – epidemic of flu

Control Building
Neighboring Building

Avian Influenza (H5N1)

- 1997 – First cases in Hong Kong, killing 6 of 18 who were exposed to geese, chickens
- Until then, experts believed that avian flu had to pass via pigs to humans!
- 1.5 million chickens and ducks slaughtered and outbreak ended



1996 – Goose/Guangdong/1/96/H5N3;
then reassortment of goose x quail
x duck to H5N1

- **February 2003 – Two cases in Hong Kong; 1 died**

Outbreak in Italy of H7N1 Virus, 1999-2001

- H7N1 mutated over the course of 9 months to great virulence; 13 million birds destroyed



Altman L. *The New York Times*. January 20, 2004:F6.

H7N7 Avian Flu in the Netherlands, 2003

- >1000 infected, one veterinarian dies
- 59% household contacts infected
- Conjunctivitis prominent
- Risk factor: bird at home
 - Mask/goggles *did not* protect
 - Oseltamivir *did* protect
 - But only 25% cullers, 50% farmers used



Take your pills. Many of those exposed to infected chickens did not take antiviral drugs, the study found.

Avian Influenza A (H5N1): 10 Confirmed Cases in Vietnam

- Mean age: 13.7 years (range, 5-24 years)
- 9 of 10 had direct contact with poultry (mean, 3 days prior)
- All had fever (38.5-40°C), shortness of breath, cough
- 7 of 10 had diarrhea
- Respiration: 55 BPM (range, 20-70 BPM)
- All had rales
- Mean laboratory values:

Total leukocyte count: 2100 mm³

Hemoglobin: 12.5 g/dL

Platelet count: 75,500 mm³

BPM = breaths per minute.



8 of 10 (80%) died

Avian Influenza A (H5N1): 10 Confirmed Cases in Vietnam (CONT'D)

- Median time to death: 9 days
- 20 confirmed cases of human infection in Vietnam/Thailand; 18 of 20 (80%) died
- Direct contact with poultry contributed to human transmission
- No *obvious* person-to-person transmission
- No cases among (unprotected) health care workers or families
- 4 of 5 who were given oseltamivir died
- In vitro resistance to amantadine/rimantadine
- 6 of 7 who were given steroids died
- RT-PCR is more sensitive than rapid tests



Viral RNA in most fecal samples

RT-PR = reverse transcriptase polymerase chain reaction.

Tran TH, et al. *N Engl J Med.* 2004;350:1179-1188; World Health Organization. Available at: http://www.who.int/water_sanitation_health/emerging/h5n1background.pdf. Accessed June 20, 2006.

Histories of Patients With H5N1 and Their Contact With Poultry

- Mass culling
- Plucking/ preparing birds
- Handling cock fights
- Playing with ducks
- Consumption of raw duck blood



- “Live chickens can be sold for \$3.20, while the government only pays 63 cents for destroyed chickens”

Bird Flu Is Back, Raising Fear of Spread Among Humans

Resurgence in Asia Called 'Time Bomb'

By KEITH BRADSHER

YEN NGHIA COMMUNE, Vietnam, Aug. 27 — A small house here by a river covered with dark green spinach plants is quieter now, the cries of an 11-month-old girl no longer heard.

The girl died this month of a strain of avian influenza, or bird flu, that has alarmed health officials around the world. She appears to have contracted the disease from Muscovy ducks, but scientists warn that, if the virus develops the ability to spread easily from person to person, it could kill huge numbers of people.

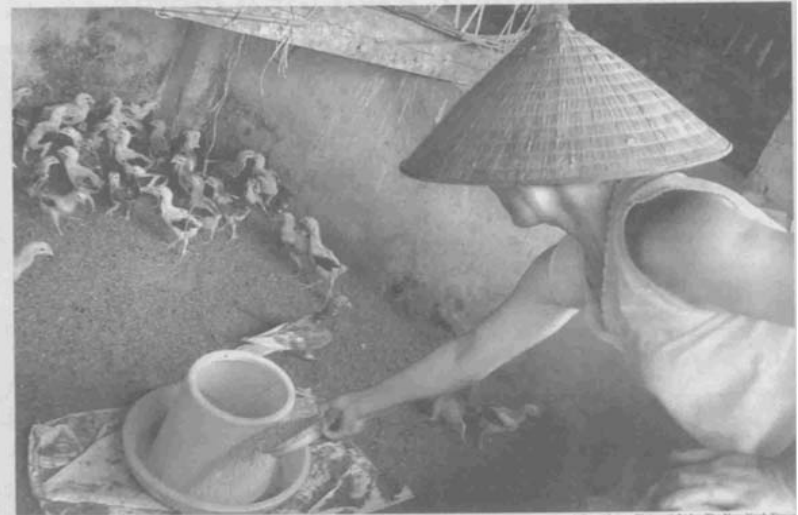
Citing bird flu as well as a longer-term concern about other diseases, Bush administration officials on Wednesday released a national pandemic preparedness plan aimed at limiting American deaths if a global outbreak occurs.

"I think what we're concerned about is looking at what's going on in Asia right now with avian flu, and a very big worry that this is statistically a time bomb ticking," said Dr. Julie L. Gerberding, the director of the federal Centers for Disease Control and Prevention.

Experts at international health organizations say they do not know whether the disease will start spreading from person to person. But they say that the virus appears to be evolving rapidly and is already deadly in birds and, in lab tests, in mammals like ferrets.

No vaccine is available, although manufacturers are preparing one for initial safety tests this winter. Tamiflu, a costly antiviral medicine, only appears effective if taken a day or two after the onset of symptoms. The virus may also develop a resistance to Tamiflu, although that has not been documented yet.

"As much as we know, there is much more we do not know," said Anton M. Rychener, who is in charge of the United Nations Food and Agricul-



Julian Abramson Waterfront by The New York Times

Le Thi Huyen fed her chickens at Yen Nghia Commune. She said fear spread after a baby died of bird flu.



The New York Times

An 11-month-old girl died of bird flu in Yen Nghia Commune.

ture need to try to halt the spread of the disease by slaughtering healthy domesticated birds here, he said.

But Ms. Huyen said that the girl's family had kept 30 Muscovy ducks, all of which fell ill and died shortly before the girl did. The commune strongly encouraged but did not require the family's closest neighbors to kill their chickens, she said.

Some families handed over their chickens to be burned alive in a special pit. But others quickly ate their chickens, Ms. Huyen said. Doctors contend it is extremely unlikely someone could contract the disease by eating infected chickens after they have been well cooked, but warn that preparing chicken, especially a live infected chicken, may be hazardous, because the cook may come in contact with nasal secretions or fe-

week ago that the virus strain had been detected in pigs on at least two farms in Fujian Province in South eastern China. Chinese officials described the cases as the first documented in pigs.

Dr. Nam and Dr. Nguyen Tien Dung, who runs the virology department at the National Institute of Veterinary Research in Vietnam, said in separate interviews in Hanoi that at the time of the human deaths and culls of chickens last spring, no cases of bird flu had been found in pigs in Vietnam. Workers are collecting more samples to be sent to Hong Kong for tests, they added.

But Mr. Rychener of the Food and Agriculture Organization said swabs of pigs in Vietnam had tested positive for avian flu for months. He said he had publicly stated this in

Influenza A Virus

Family: Orthomyxoviridae

Negative-Sense Single-Strand RNA Genome

- 10 AA changes in polymerase proteins distinguish 1918 strain; all found in H5N1 strains, but not yet together

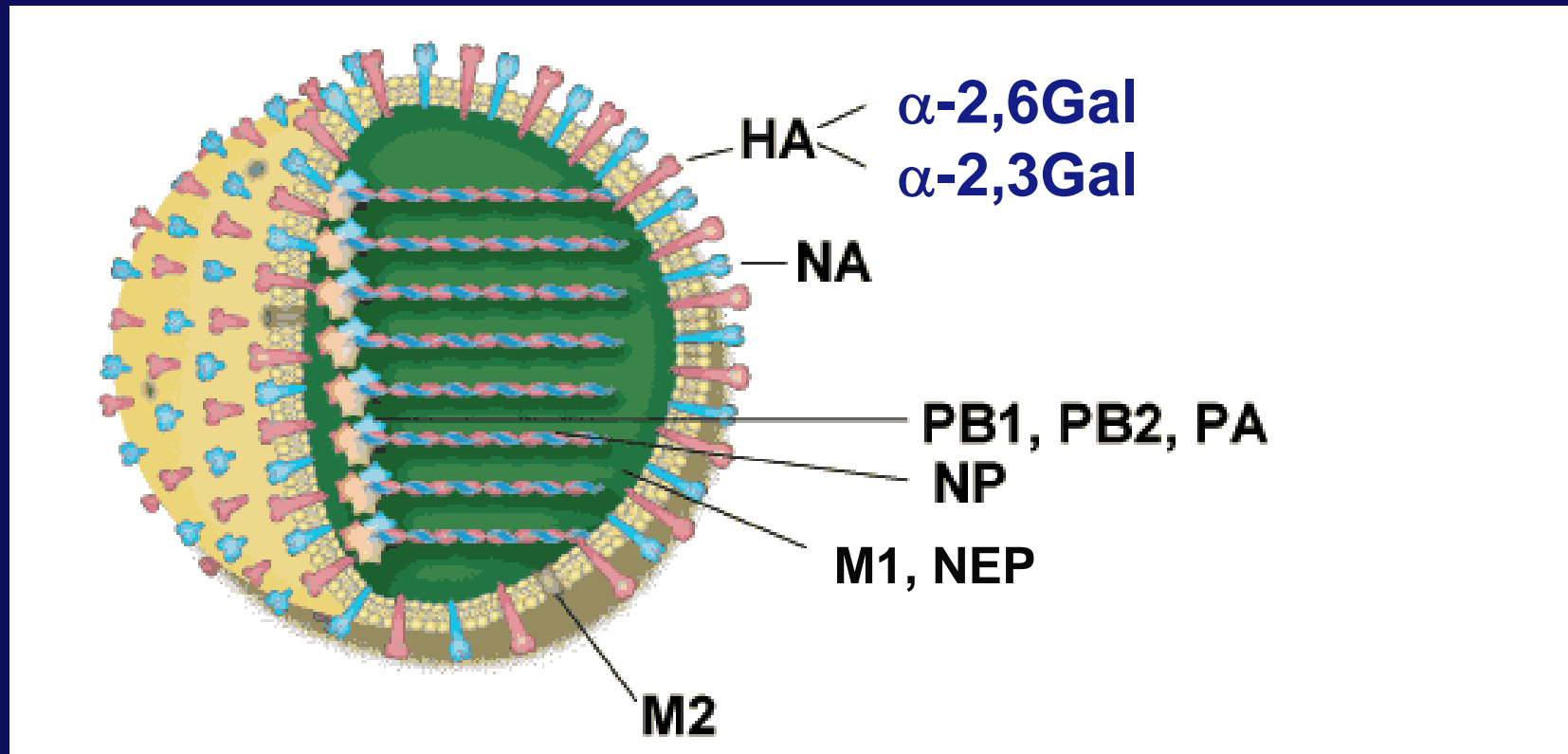
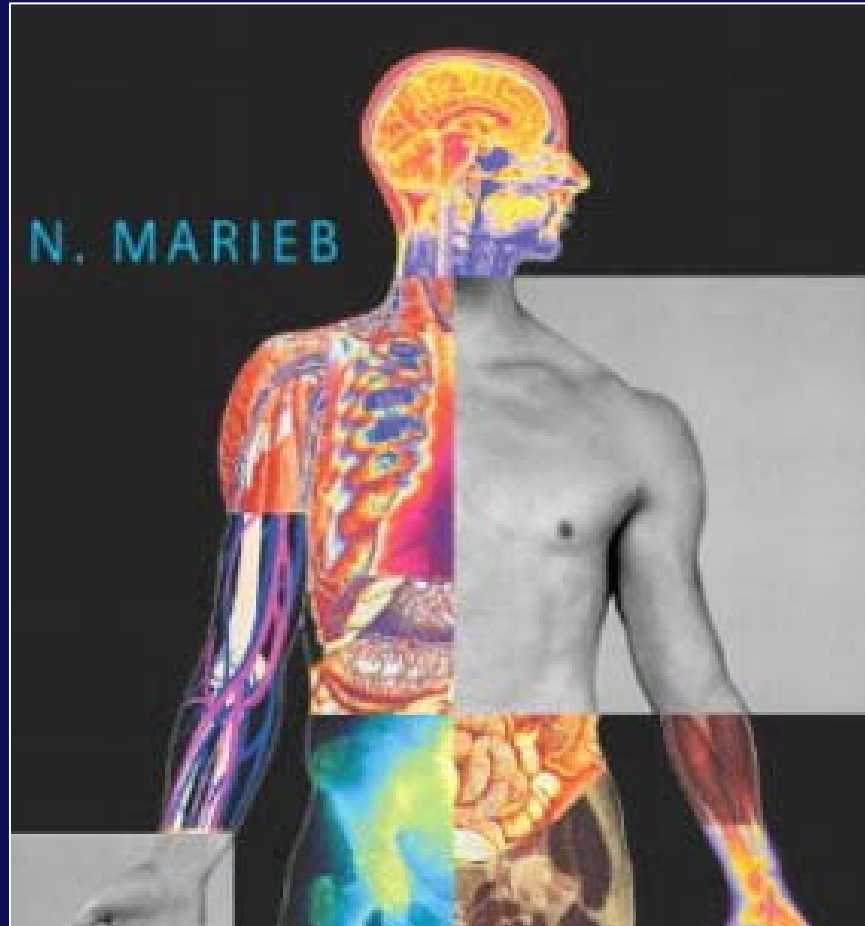


Image courtesy of the University of Maryland Avian Influenza Virus Program.

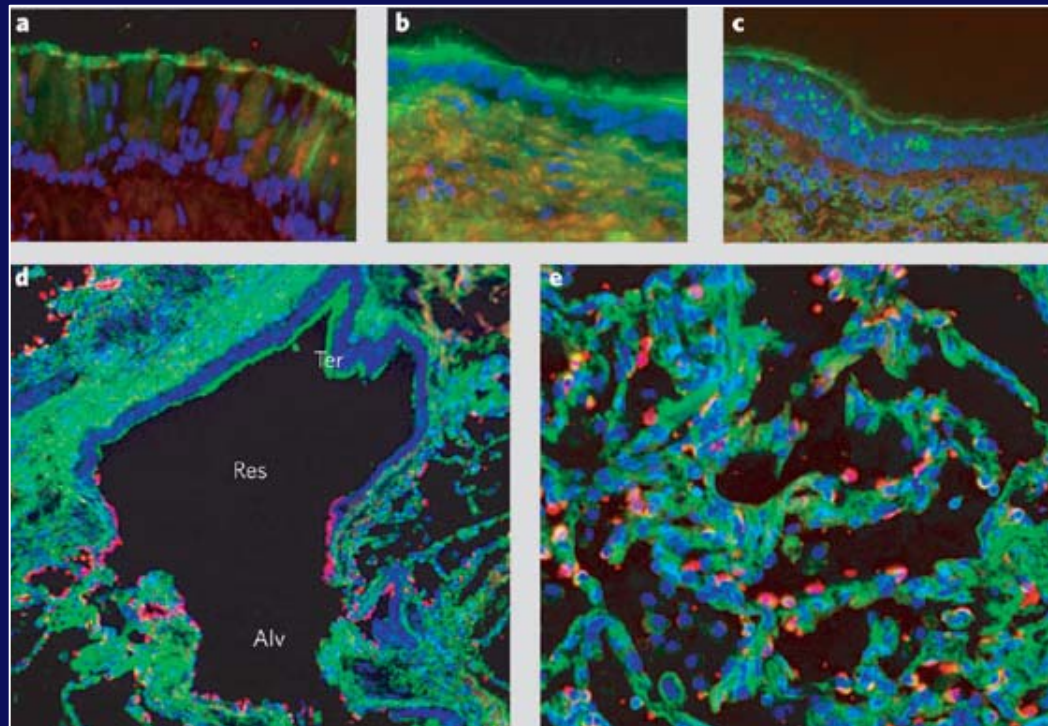
Kimoto H, et al. *Nature*. 2005;437:898-901.

Tropism of Avian Influenza Viruses and Associated Symptoms, 2004-2005

- Ocular and lacrimal duct epithelial cells have α -2,3-linked sialic acid
- NOTE: α -2,3 \rightarrow α -2,6 switch requires one- or two-point mutation in hemagglutinin
- Nonciliated epithelial cells – most have α -2,6-linked sialic acid



- H7 strains
- Conjunctiva
- Nasal mucosa
- Rhinitis (0%-60%)
- Large pulmonary airways, H5
- Cough, 88%
- Pulmonary infiltrates, 88%
- Respiratory failure, 80%
- Gastrointestinal tract, H5
- Diarrhea, 40%
- Abdominal pain, 23%
- Detection of RNA in feces



(a) Nasal mucosa; **(b)** Paranasal sinuses; **(c)** Bronchus; **(d)** Bronchiole; **(e)** Alveolus.

Res-respiratory bronchiole (adjacent to alveoli); Ter-terminal bronchiole (distal to alveoli); Alv-alveolus;

Green-reaction with *Sambucus nigra* lectin, indicating the presence of sialic acid linked to galactose by an α -2,6 linkage (SA α -2,6 Gal); Red-reaction with *Maackia amurensis* lectin, indicating the presence of SA α -2,3 Gal. Cells were counterstained with DAPI (4',6-diamidino-2-phenylindole).

Shinya K, et al. *Nature*. 2006;440:435-436.

Interspecies Transmission of Influenza: Limited by Chemistry



- Human strains of HA: bind to sialic acid residues linked to galactose via α -2,6 linkage

HA
 α -2,6

Epithelial
cell

Human
trachea

HA
 α -2,3

Avian strains
bind via α -2,3 linkage

Human alveoli
and conjunctiva



Epithelial cells
contain both α -2,6
and α -2,3 linkage

- Reassortment in a pig may create pandemic flu
- Mutation of avian flu strain – may adapt to humans

Reactive Hemophagocytic Syndrome

- Risdall – Proliferation of hemophagocytic histocytes in hematopoietic organs in some cancer patients¹
- Henter – Cytopenia, frequent pancytopenia coagulopathy, impaired liver function, death; underlying malignancy, EBV²
- Akashi, Imashuku – Cytokine driven^{3,4}
- Potter – Influenza A; single report among three children with acute leukemia⁵

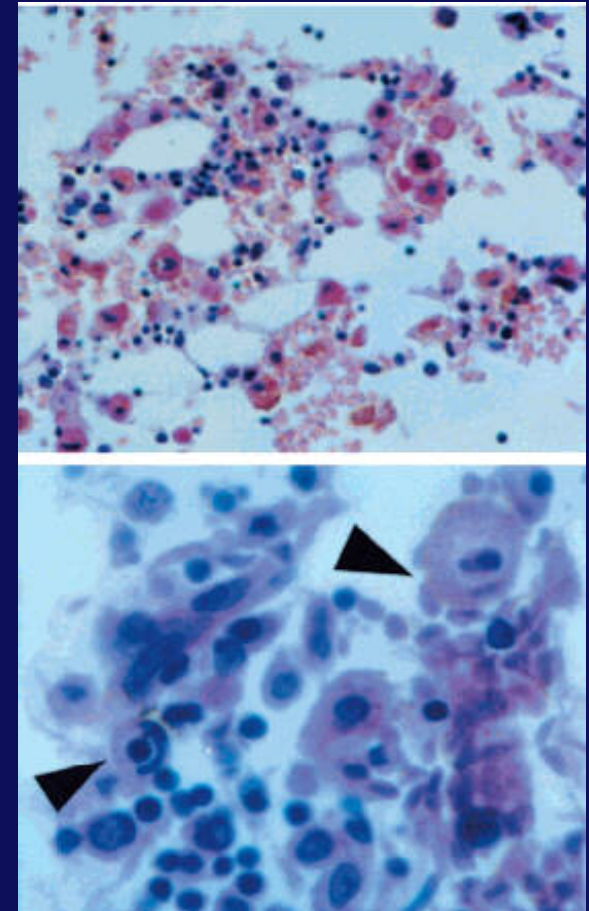
EBV = Epstein-Barr virus.

1. Risdall RJ, et al. *Cancer*. 1979;44:993-1002; 2. Henter JI, et al. *Semin Oncol*. 1999;18:29-33; 3. Akashi K, et al. *Br J Haematol*. 1994;87:243-250; 4. Imashuku S, et al. *Br J Haematol*. 1996;93:803-807; 5. Potter MN, et al. *J Clin Pathol*. 1991;44:297-299.

Pathology of H5N1

- Diffuse alveolar damage – extensive hemorrhage
- Intestinal fibrosis
- Hepatic central lobular necrosis
- Lymphoid depletion
- ATN
- Hemophagocytic syndrome (hemophagocytic lymphohistiocytosis)
 - Most frequent finding

Premortem
↑ **SIL-2R**,
IL-6,
IFN- γ early
↑ **TNF- α late**

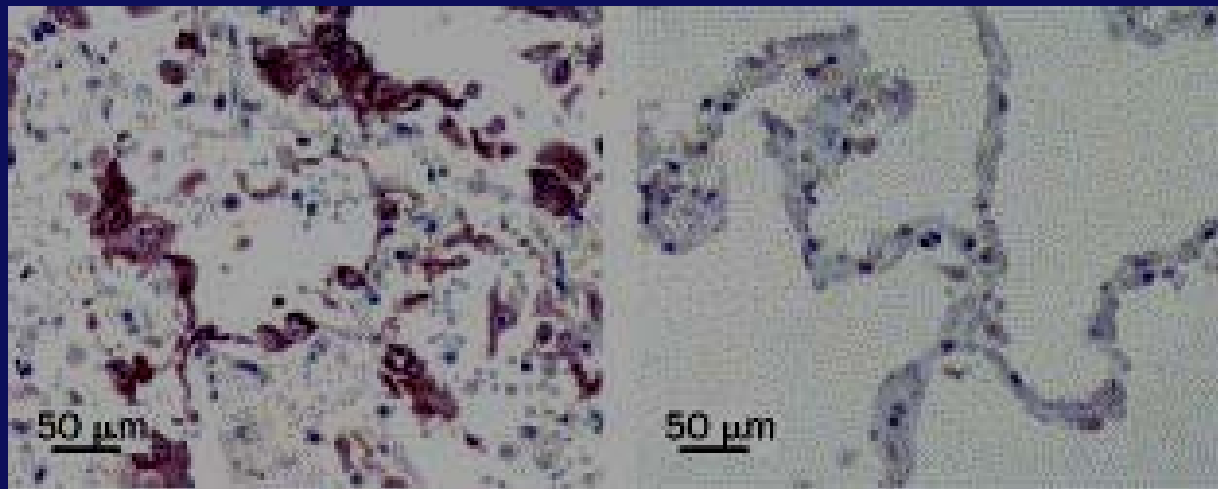


ATN = acute tubular necrosis; IFN = interferon; IL = interleukin; SIL = soluble interleukin; TNF = tumor necrosis factor.

To KF, et al. *J Med Virol*. 2001;63:242-246.

Pathology and Biology of H5N1

- Immunohistochemical staining for TNF- α in lung tissue; noninfective illness stains made with monoclonal antibody SC-7317 at a dilution of 1/10 with antigen retrieval
 - 33-year-old man with H5N1 pneumonia (left panel) shows greater staining of alveolar epithelial cells than does control subject (right panel)



Influenza – Will Statins Protect?

- Retrospective large database analysis: Dutch patients (60,000) on statins in 1996 and 2003 outbreaks
 - 26% reduction of pneumonia

Q: anti-inflammatory effect?

Enhanced Virulence of Influenza A With Hemagglutinin of 1918 Spanish Flu Strain

- Reversed genetics: generation of infectious viruses from cloned complementary DNA; genes of 1918 flu used to produce viruses without Hsp
 - All viruses with Hsp
 - High titers in lungs; great inflammation; high cytokine generation
 - Morbidity → death
 - Nsp did not create virulence
 - Control viruses – not pathogenic

Array of viruses



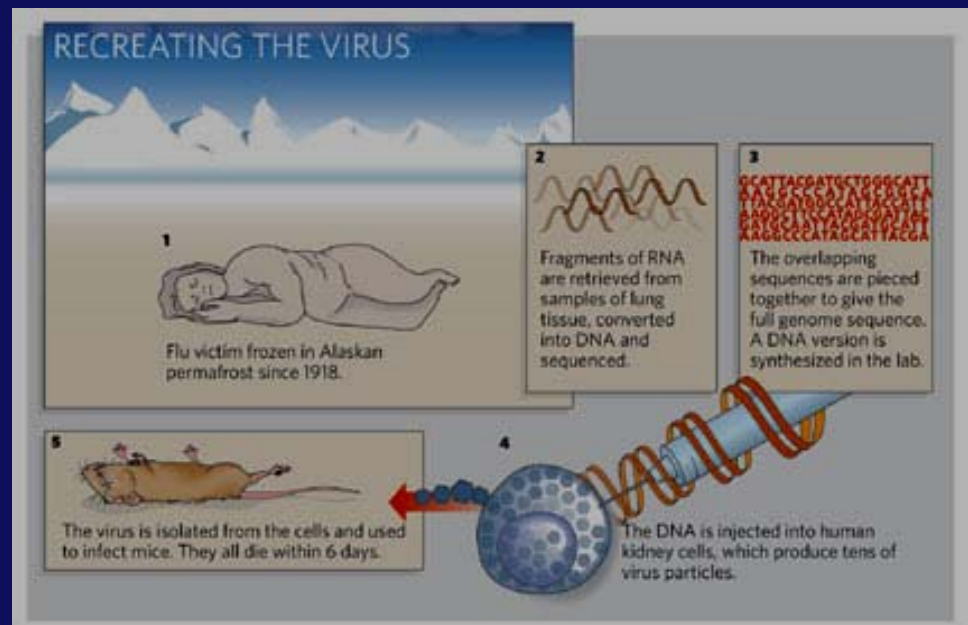
Nose of mice inoculated with virus

Polymerase Genes of 1918 Influenza Virus and H5N1

- Trimeric polymerase: PB1, PB2, PA
 - 10 AA changes differentiate 1918 flu strain from subsequent flu strains
 - Many of 10 found in circulating H5N1
 - Single AA change in PB2
 - Important for mammalian adaptation
 - Distinguish highly pathogenic H5N1 in mice
 - Found in the single human death from H7N7 in Netherlands
- Data suggest that such changes enhance viral replication and increase pathogenicity

1918 Influenza Strain: How Virulent Is the Flu?

- **50 times** as many virus particles are released from human lung cells 1 day after infection with the 1918 virus as are released after exposure to a contemporary strain called the Texas virus
- **13% of body weight** is lost by mice 2 days after infection with 1918 flu; weight loss is only transient in mice infected with the Texas strain
- **39,000 times** more virus particles are found in mouse lung tissue 4 days after infection with 1918 flu than are found with the Texas virus
- **All mice died** within 6 days of infection with 1918 flu; none died from the Texas strain

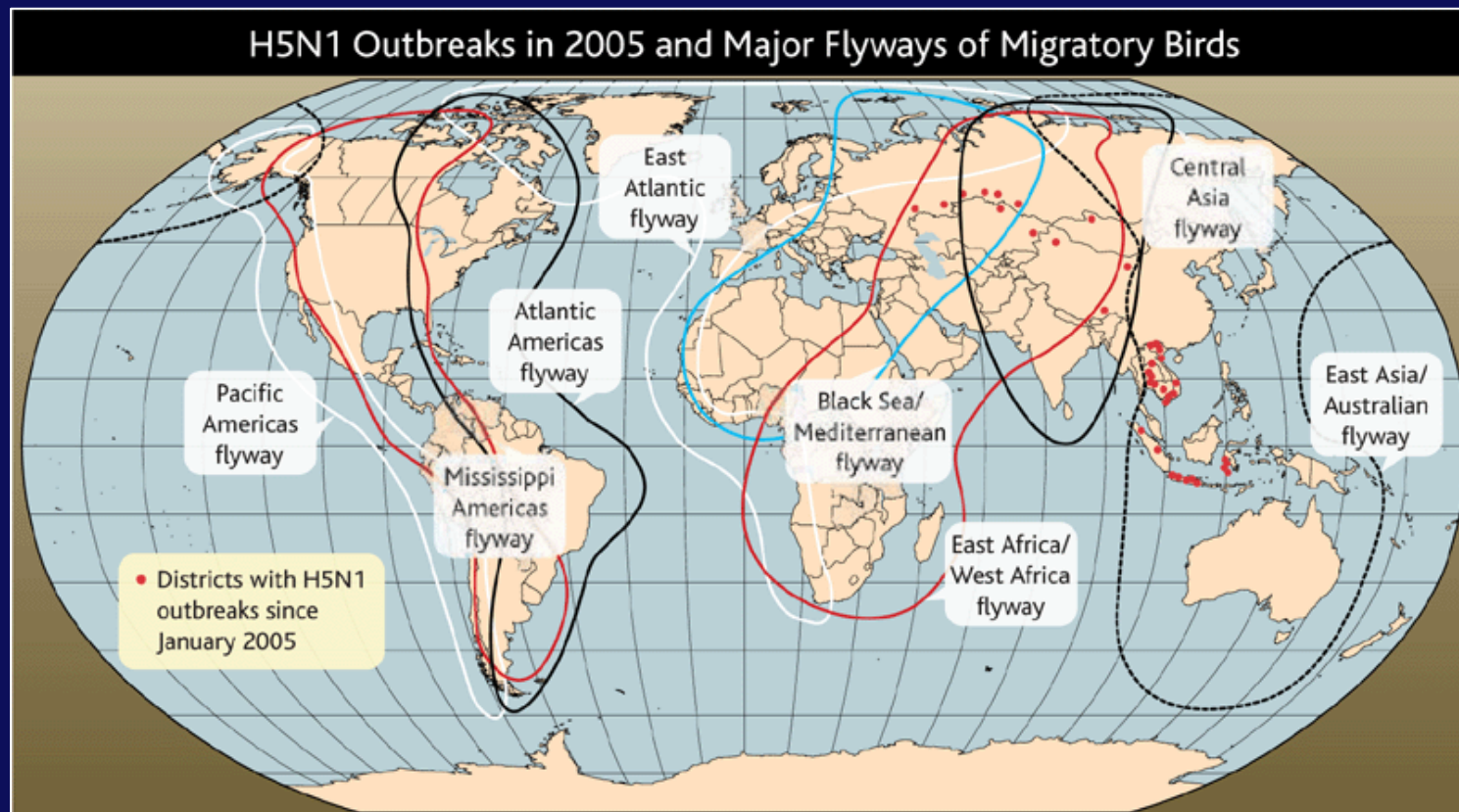


How Pathogenic Is H5N1?

- Killed >100 million domestic birds
 - Chickens in <1 day
 - Ducks (not all) in <2 day
- High risk of death among ferrets
 - Respiratory symptoms include diarrhea, paralysis
- Tigers/leopards die if infected
- Experimental transmission in domestic cats
- High risk of death among humans associated with diarrhea (diarrhea seen in minority in 1918)

Migrating Birds and the Trajectory of Avian Influenza

Guano of infected birds – 10^9 viral particles/gram



“No scientific basis for culling wild birds to control outbreaks....”

Illicit Bird Importation Introduces H5N1

- Oct 18, 2004: Two crested eagles seized at Brussels International Airport
- Thai resident kept in open-hand luggage in overhead
- Birds asymptomatic (H5N1); euthanized
- 25 closest passengers given oseltamivir
- Traveler → isolated 4 days + given oseltamivir
- Veterinarian → bilateral conjunctivitis; given oseltamivir



Bird Flu Virus May Be Spread by Smuggling

- “H5N1 is robust enough to survive in frozen meat, feathers, bones and cages”



Rosenthal E. *The New York Times*. April 15, 2006:A1.

Human-to-Human Infection by Bird Flu Virus Is Confirmed

By ELISABETH ROSENTHAL
International Herald Tribune

ROME, June 23 — An Indonesian who died after catching the A(H5N1) bird flu virus from his 10-year-old son represents the first confirmed case of human-to-human transmission of the disease, a World Health Organization investigation of an unusual family cluster has concluded the agency said Friday.

The W.H.O. investigators also discovered that the virus had mutated slightly when the son had the disease, although not in any way that would allow the virus to pass more readily among people.

"Yes, it is slightly altered, but in a way that viruses commonly mutate," said Dick Thompson, a spokesman for the agency in Geneva. "But that didn't make it more transmissible or cause more severe disease."

The greater importance of the finding, researchers from the World Health Organization and the Centers for Disease Control and Prevention in the United States to document that the virus almost certainly was passed from person to person. In previous cases where human-to-

and Prevention in the United States to document that the virus almost certainly was passed from person to person.

In previous cases where human-to-



For the first time, the passage of the disease is clearly traced.

might mutate to acquire that ability, setting off a devastating pandemic.

More than 200 people have contracted bird flu worldwide, almost all of them after very close contact with infected birds.

International health officials have been in Indonesia for much of the past month, investigating a family outbreak that affected seven relatives in Kubu Sembilang, a remote village in the mountainous Karo district of Sumatra. Six of the seven died, and one is still hospitalized.

Although Indonesia has been hit by outbreaks of bird flu among poultry, the family on Sumatra had no known direct contact with sick birds, although the first to die was a woman who sold vegetables in a market that also sold birds.

But scientists have long suspected that A(H5N1), though an avian virus, could also spread between people in rare cases, if there was prolonged close contact.

The family members in the cluster had a banquet in late April when the vegetable merchant was already ill and coughing heavily. Some spent the night in the same room with him and some nursed sick relatives.

The first five family members to fall ill had identical strains of A(H5N1), one found in animals in Indonesia. But that virus had mutated slightly in the sixth victim, a child, and he apparently passed the mutated virus to his father, who cared for him in a hospital without proper protection, said Dr. Tim Uyeki, an American epidemiologist on the W.H.O. team.

Dr. Thompson said there was no evidence that the mutated virus had been better adapted to human infection. To the contrary, the agency has been following 54 relatives and neighbors for a month and none has caught it. "So we know it is not more easily transmitted," he said.

No evidence for viral adaptation for increased transmission

Preparing for a Pandemic: Build an Infrastructure to Respond to Multiple Threats

- Stockpile available antivirals, vaccines
- Invest in research on vaccines targeting *all* strains
- End international trade in wild birds
- Develop surge capacity for ICUs, negative air pressure rooms, respirators
- Design flexible, nimble response teams
- Accelerate EMR, links to labs, pharmacies nationally
- Design a program with accountable leadership and communication

The Likelihood of Pandemic H5N1 Influenza: Fitting the Puzzle Together

Proportion of droplets <10 mm (airborne)

Most of 10 mutations of polymerase noted in 1918-1919

Mutation to allow HA to link avidly to α -2,6 sialic acid

Coinfection in pig or other mammal to create reassortment

Postscript: The Current Threat of Pandemic Avian Influenza

	Annual Influenza	Avian Influenza
Deaths globally	250,000/year	100/year
Deaths US	36,000	None yet

- Get the annual influenza vaccine!
- It is safe to eat cooked eggs, chicken and turkey