Citation Key

for more information see: http://open.umich.edu/wiki/CitationPolicy

Use + Share + Adapt

{ Content the copyright holder, author, or law permits you to use, share and adapt. }

Public Domain – Government: Works that are produced by the U.S. Government. (17 USC § 105)

Public Domain – Expired: Works that are no longer protected due to an expired copyright term.

Public Domain – Self Dedicated: Works that a copyright holder has dedicated to the public domain.

Creative Commons – Zero Waiver

Creative Commons – Attribution License

Creative Commons – Attribution Share Alike License

Creative Commons – Attribution Noncommercial License

Creative Commons – Attribution Noncommercial Share Alike License

GNU – Free Documentation License

Make Your Own Assessment

{ Content Open.Michigan believes can be used, shared, and adapted because it is ineligible for copyright. }

Public Domain – Ineligible: Works that are ineligible for copyright protection in the U.S. (17 USC § 102(b)) *laws in your jurisdiction may differ

{ Content Open.Michigan has used under a Fair Use determination. }

Fair Use: Use of works that is determined to be Fair consistent with the U.S. Copyright Act. (17 USC § 107) *laws in your jurisdiction may differ

Our determination **DOES NOT** mean that all uses of this 3rd-party content are Fair Uses and we **DO NOT** guarantee that your use of the content is Fair.

To use this content you should **do your own independent analysis** to determine whether or not your use will be Fair.

Respiratory Viruses

Infectious Diseases/Microbiology Sequence Course

David J. Miller, M.D., Ph.D.

Spring 2010

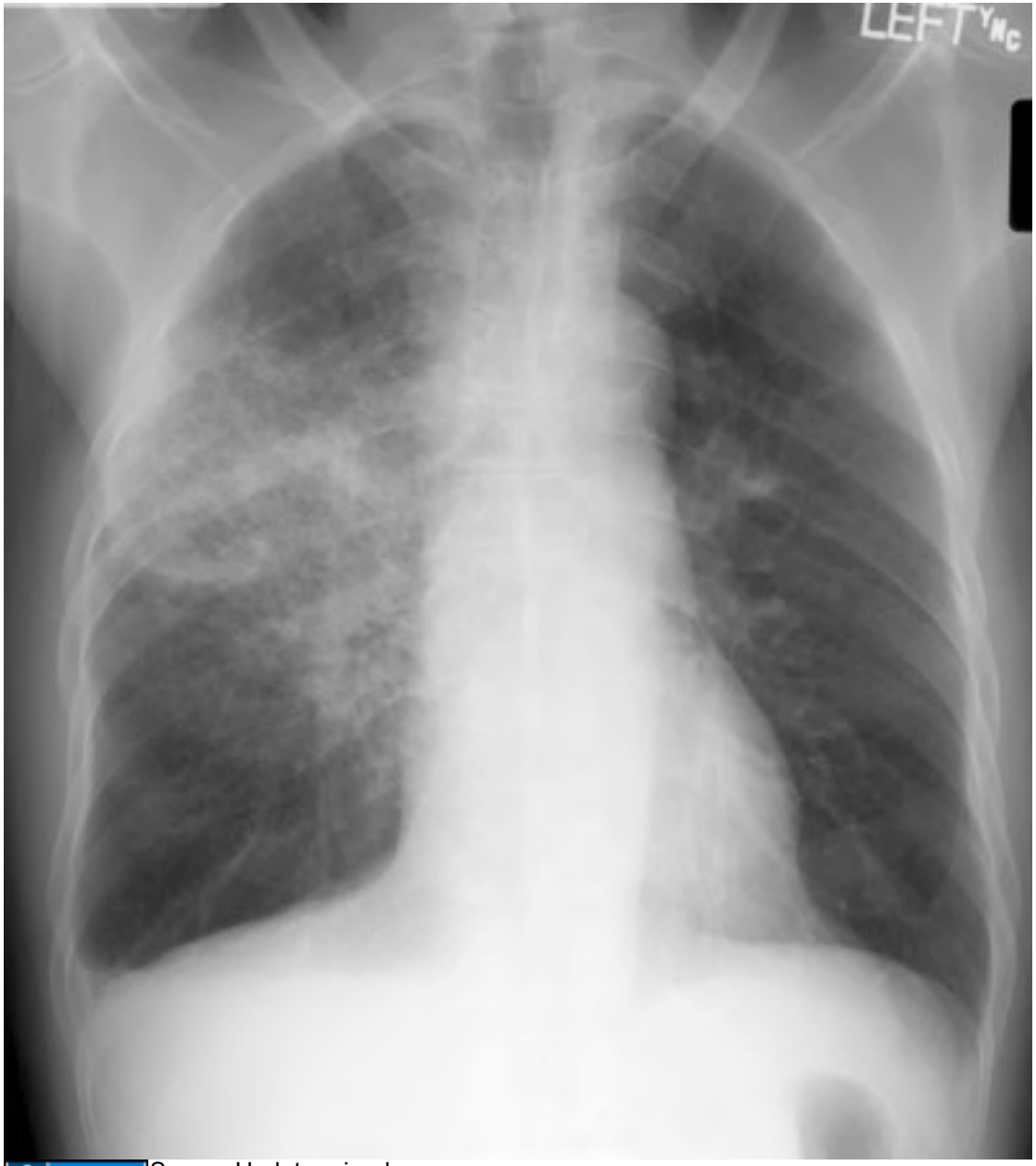
University of Michigan
Medical School

Objectives

- **Know the major respiratory viruses and their clinical presentation**
  - *influenza, rhinovirus, respiratory syncytial virus (RSV),* coronavirus, adenovirus
- **Appreciate key features of structure and replication strategies related to pathogenesis, treatment, and prevention**

Reading assignment: Schaechter's, 4th edition, chapters 32, 34, 36, and 39

30 year old generally healthy female returning from a trip to San Francisco in January sat in front of passenger on the plane who was coughing repeatedly throughout the flight. Two days later she developed fever to 39°C with shaking chills, non-productive cough, headache, and severe myalgias. Because of her severe symptoms she was bedridden for three days, but eventually fully recovered without specific treatment after a week and returned to work.



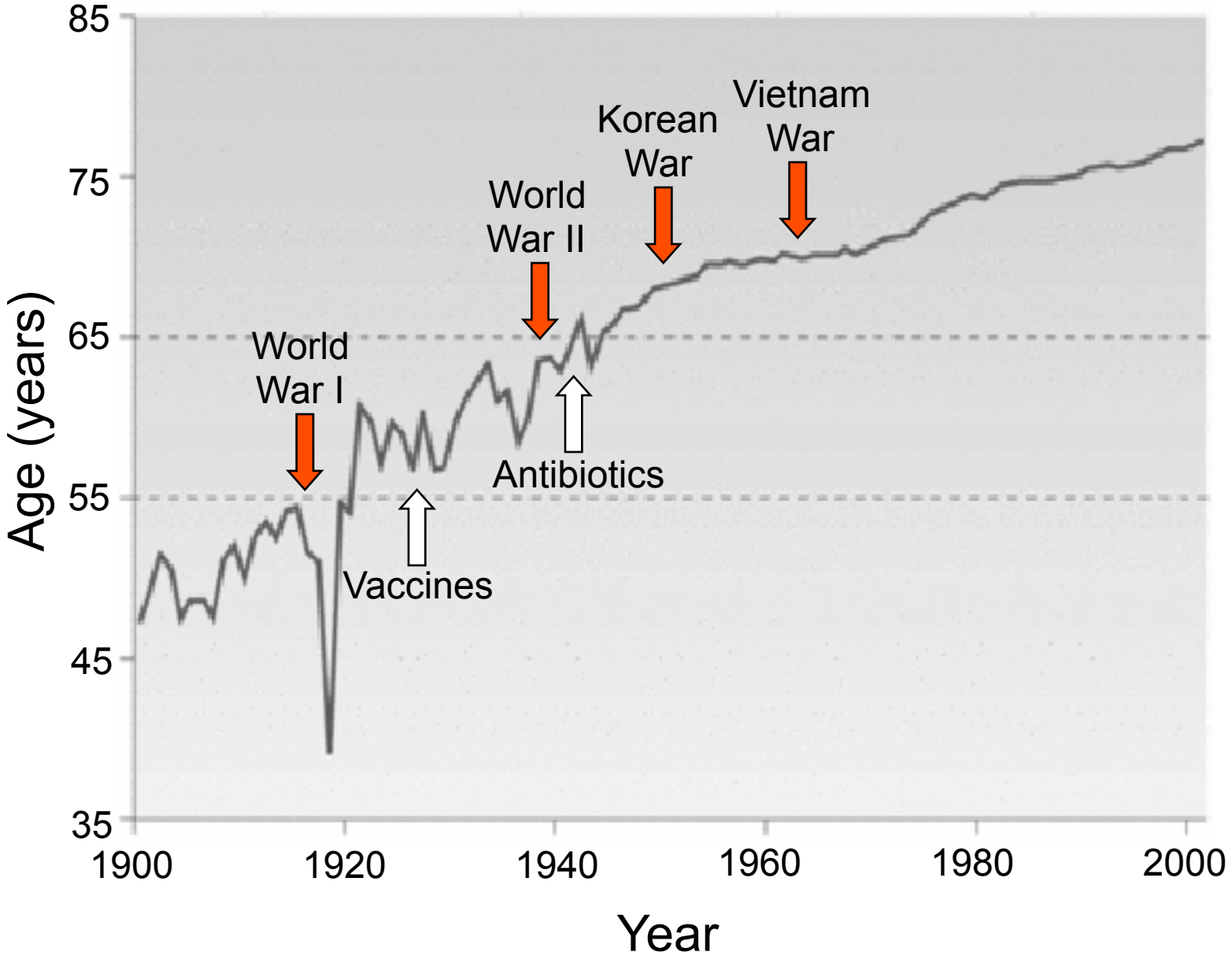
30 year old generally healthy female returning from a trip to San Francisco in *January* sat in front of passenger on the plane who was *coughing* repeatedly throughout the flight. *Two days later* she developed *fever to 39°C* with *shaking chills, non-productive cough, headache,* and *severe myalgias*. Because of her *severe symptoms* she was bedridden for three days, but eventually *fully recovered* without specific treatment after a week and returned to work.

Diagnosis?

Can this happen again?

PD-EXP Iconicphotos

Life expectancy in the United States, 1900-2001



Influenza virus

- **Yearly impact for endemic/epidemic disease (CDC estimates)**
  - >200,000 hospitalizations
  - Estimated 36,000 deaths (mortality rate <0.1%)
  - Greater than $1 billion (U.S.) economic loss

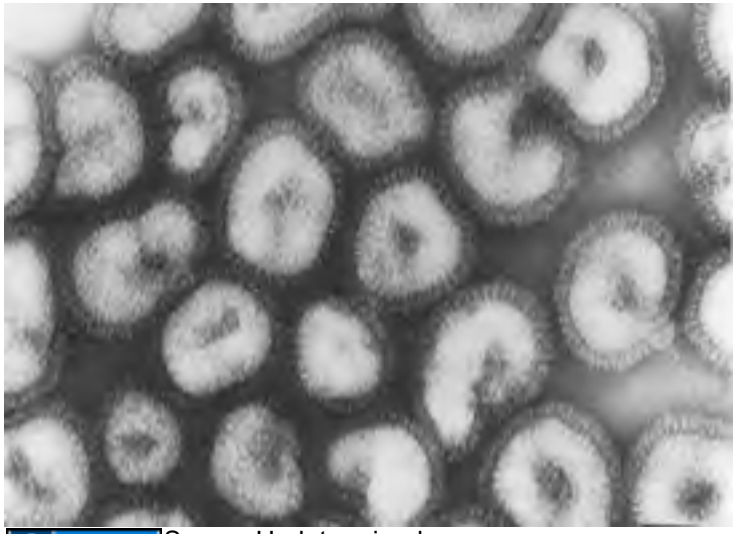
- **Estimated impact for new pandemic disease**
  - 500,000 to 700,000 hospitalizations
  - 100,000 to 200,000 deaths
  - Greater than $100 billion (U.S.) economic loss

- **2009 H1N1 pandemic (CDC estimates as of Feb. 2010)**
  - U.S. - ~57 million cases, ~250,000 hospitalizations, ~11,000 deaths
  - International - 213 countries with confirmed cases
  - Economic losses ???

Vaccine costs about $10 per person

Influenza virus

- **Family: *Orthomyxoviridae***
- **Enveloped**
- **Negative (-) strand RNA genome, 8 (7) segments**
- **Three influenza types: A, B, C**

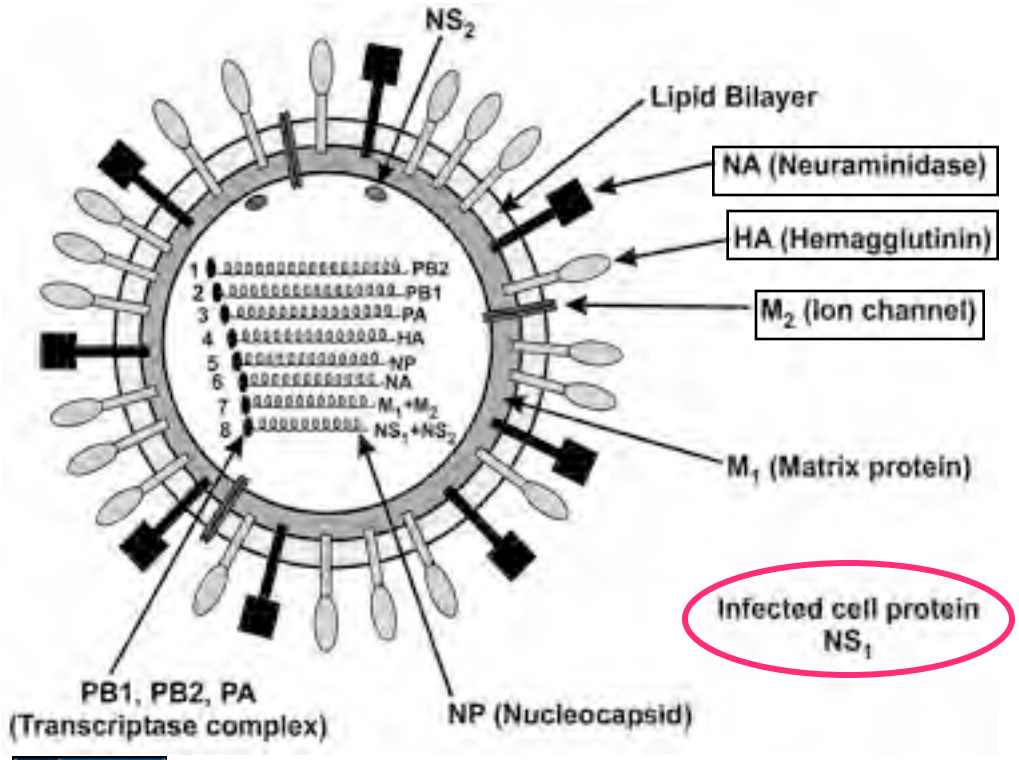


Comparison of Influenza A, B, and C Viruses

	A	B	C
Severity of illness	++++	++	+
Subtypes	Yes	No	No
Animal reservoir	Yes	No	No
Spread in humans	Pandemic	Epidemic	Sporadic
Antigenic changes	Shift, drift	Drift	Drift

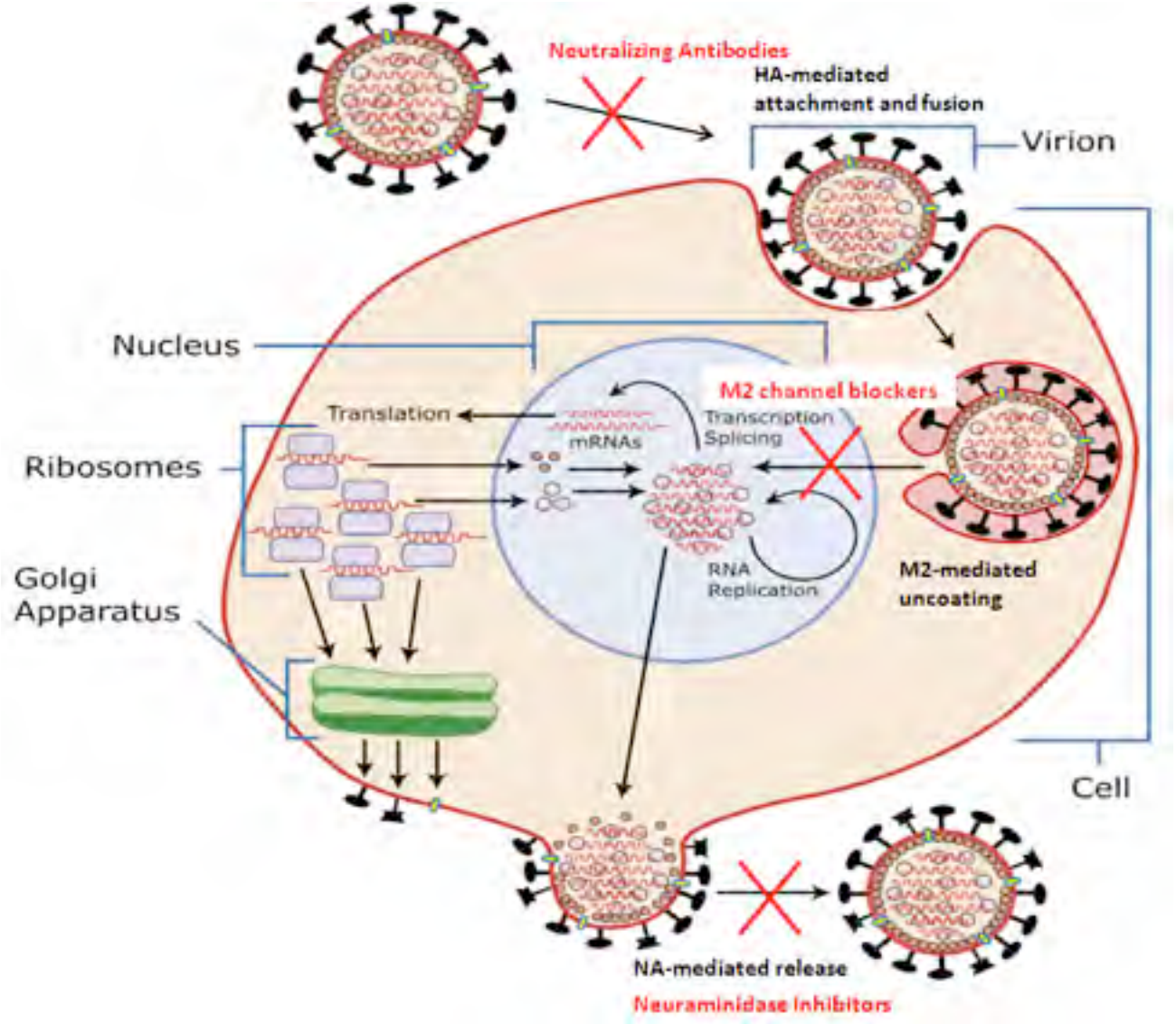
Influenza A Virus Structure

- **Hemagglutinin (HA)**
  - Receptor binding (sialic acid)
  - Membrane fusion
  - Neutralizing antibody target
- **Neuraminidase (NA)**
  - Remove sialic acid residues
  - Virion release
- **Ion channel (M2)**
  - H^+-dependent uncoating
  - Influenza A only
- **Influenza A subtypes based on HA (16) and NA (9)**
  - H1N1, H3N2
  - A/Hong Kong/8/68



PD-INCL CSB

Influenza virus life cycle

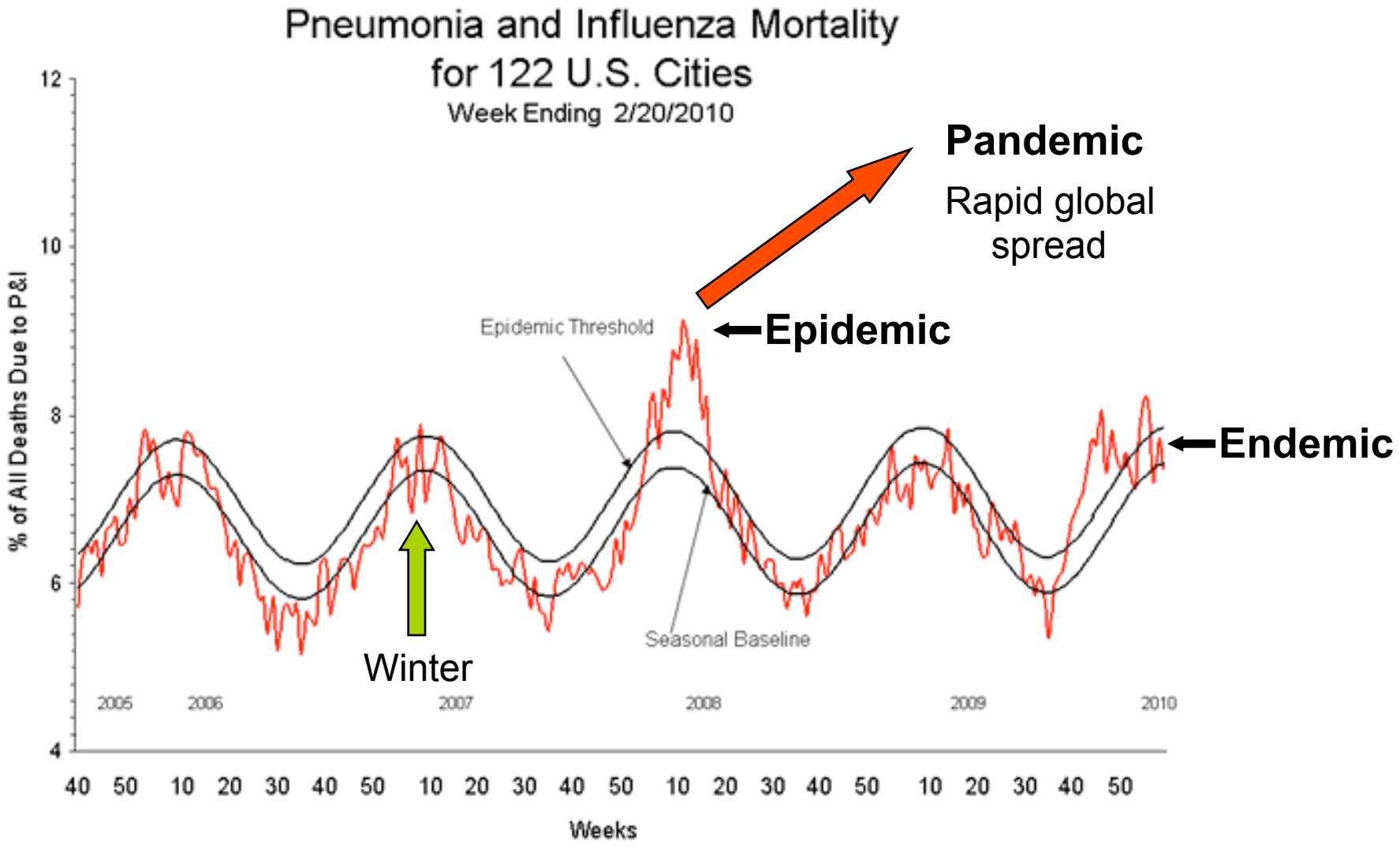


Influenza Pathogenesis

- **Direct cell lysis**
  - Primary mechanism for influenza virus
  - Upper and lower respiratory tracts
- **Role of immune response**
  - Primarily protective rather than pathogenic
  - Induces virus- and type-specific immunity
  - Virus-mediated suppression (NS1 protein)

Why was the 1918 virus so deadly?

Influenza Epidemiology



Influenza Antigenic Variation

- ***Antigenic drift***
- ***Antigenic shift***

Influenza Antigenic Variation

- **Antigenic drift**
  - Occurs with influenza A, B, and C
  - Small number of slowly occurring changes (mutations)
    - Error-prone viral RNA polymerase
  - HA changes most prominent, but can occur in any viral gene
  - Partially responsible for yearly vaccine changes
  - *MAY* result in breach of species barrier and pandemic

Health Alert Network

HAN Menu

- Home
- News & Events
- Training
- IT Infrastructure
- HAN/PHIN Jurisdictions
- Other Jurisdictions
- Advanced Practice Centers
- HAN Messages Archive

This is an official
CDC HEALTH ADVISORY

Distributed via Health Alert Network
Friday, December 19, 2008, 11:50 EST (11:50 AM EST)
CDCHAN-00279-2008-12-19-ADV-N

CDC Issues Interim Recommendations for the Use of Influenza Antiviral Medications in the Setting of Oseltamivir Resistance among Circulating Influenza A (H1N1) Viruses, 2008-09 Influenza Season

Influenza Antigenic Variation

- **Antigenic shift**
  - Influenza A only
  - Large dramatic changes that occur rapidly
  - Primarily responsible for pandemics
  - Due to gene shuffling and reassortment
  - Requirements
    - Segmented genome
    - Multiple HA and NA subtypes
    - Animal reservoir (wild aquatic birds)
    - Susceptible species for both avian and human influenza (swine)

Influenza Pandemics

1918 “Spanish influenza”
H1N1 influenza virus
Bird-to-human transmission of H1N1 virus

• All 8 genetic segments thought to have originated from avian influenza virus

1957 “Asian influenza”
H2N2 influenza virus
H2N2 avian virus – H1N1 human virus
Reassortment

• 3 new genetic segments from avian influenza virus introduced (HA, NA, PB1):
Contained 5 RNA segments from 1918

1968 “Hong Kong influenza”
H3N2 influenza virus
H3 avian virus – H2N2 human virus
Reassortment

• 2 new genetic segments from avian influenza introduced (HA, PB1):
Contained 5 RNA segments from 1918

Next pandemic influenza
Avian virus
Or
Avian virus – H3N2 human virus

• All 8 genes new or further derivative of 1918 virus

Please see: http://www.lincoln.ac.uk/dbs/images/birdflu1.jpg

Pathways for generation of virulent pandemic influenza viruses

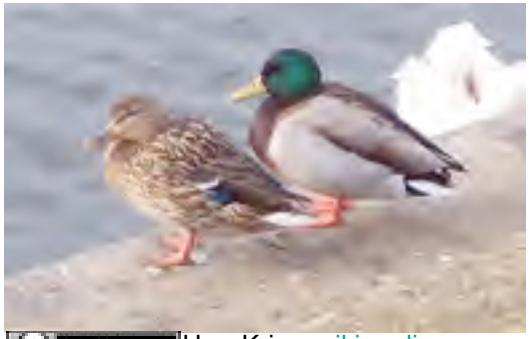
Human influenza virus

Intraspecies transmission

Reassortment

*Inter*species transmission

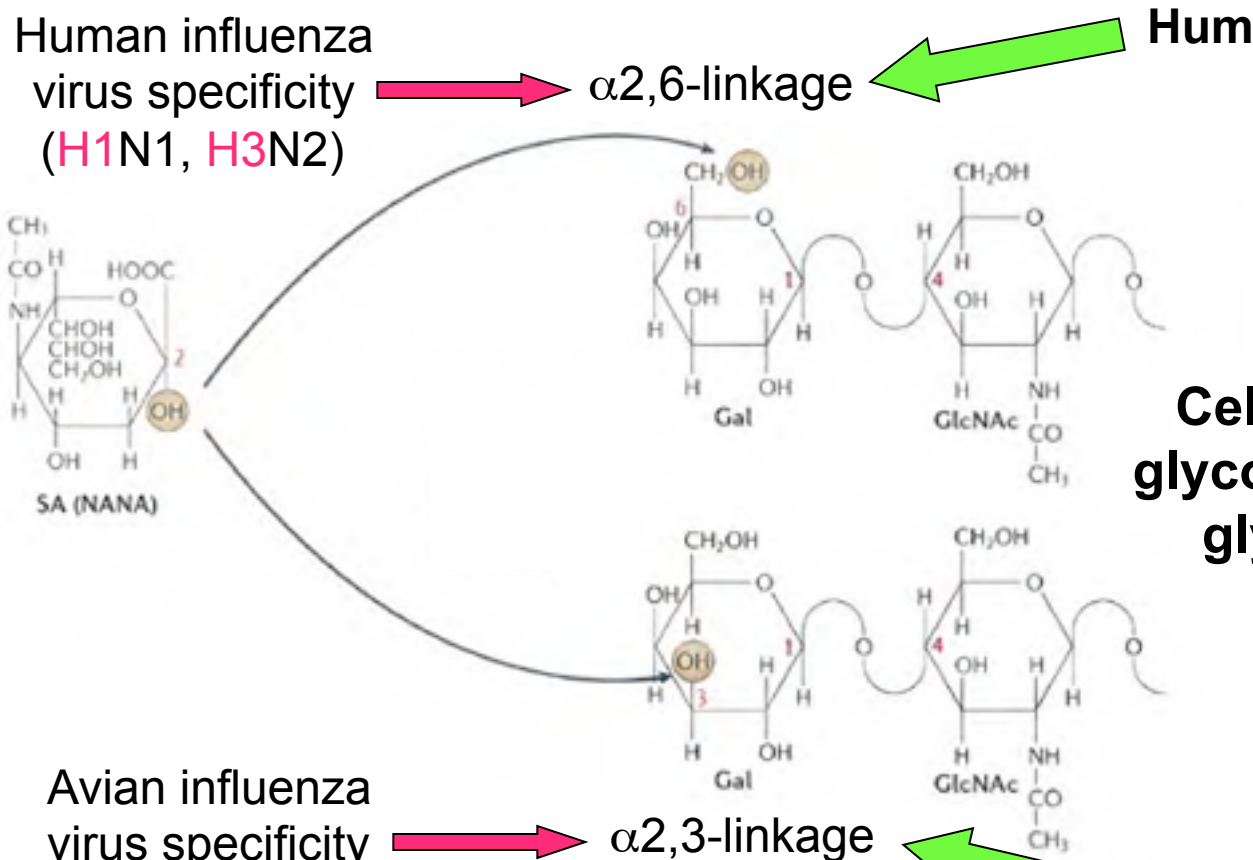
?



Intraspecies transmission

Avian influenza virus

Sialic acid linkages determines influenza virus HA receptor binding



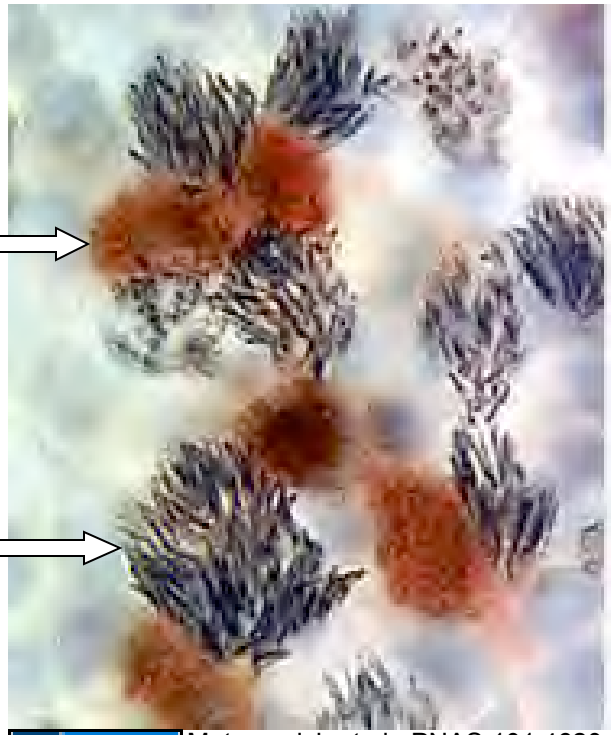
Different human airway epithelial cells can express either α2,3- or α2,6-linked sialic acid residues

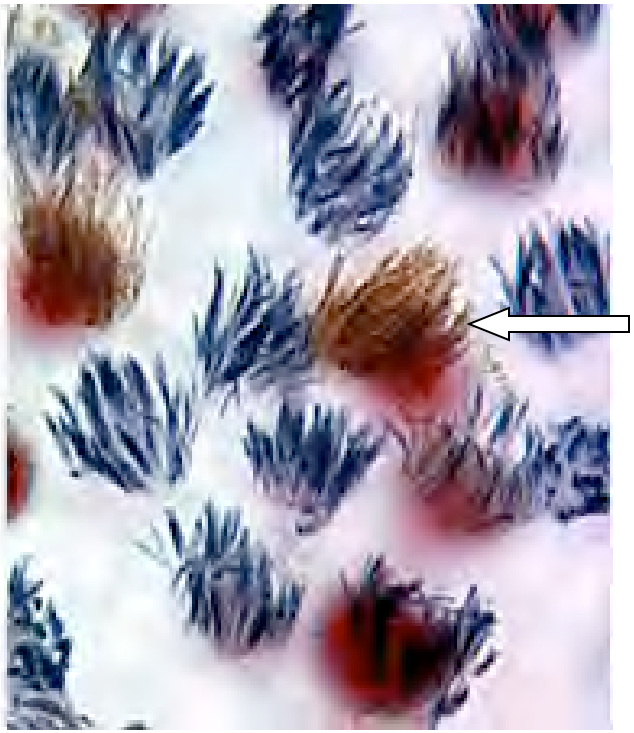
Human influenza virus (α2,6)

Avian influenza virus (α2,3)

Non-ciliated cell

Ciliated cell

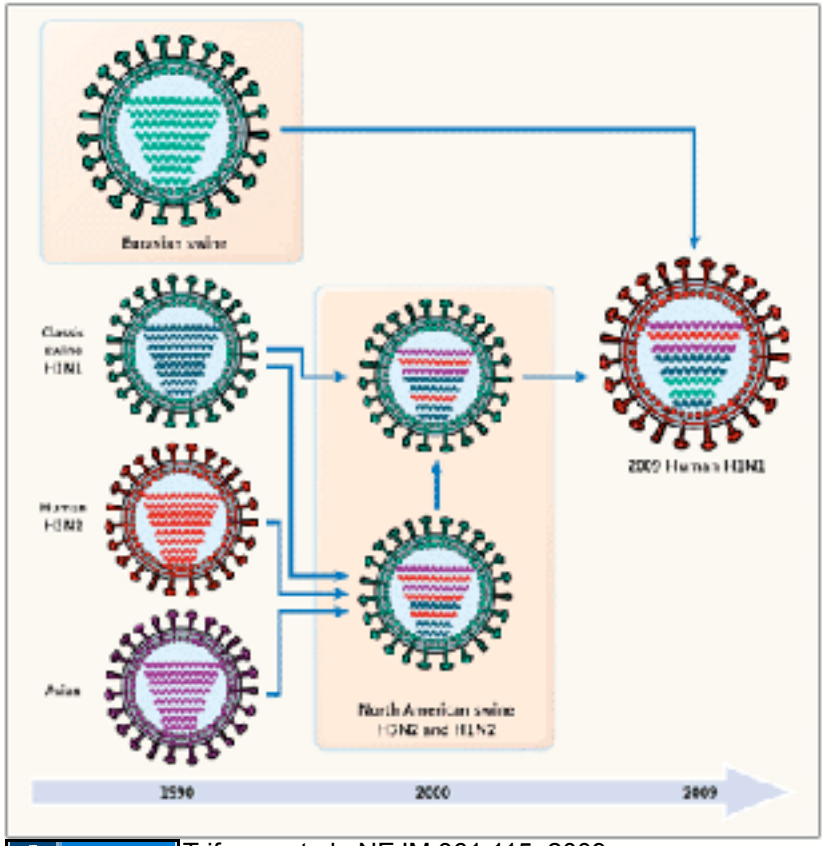




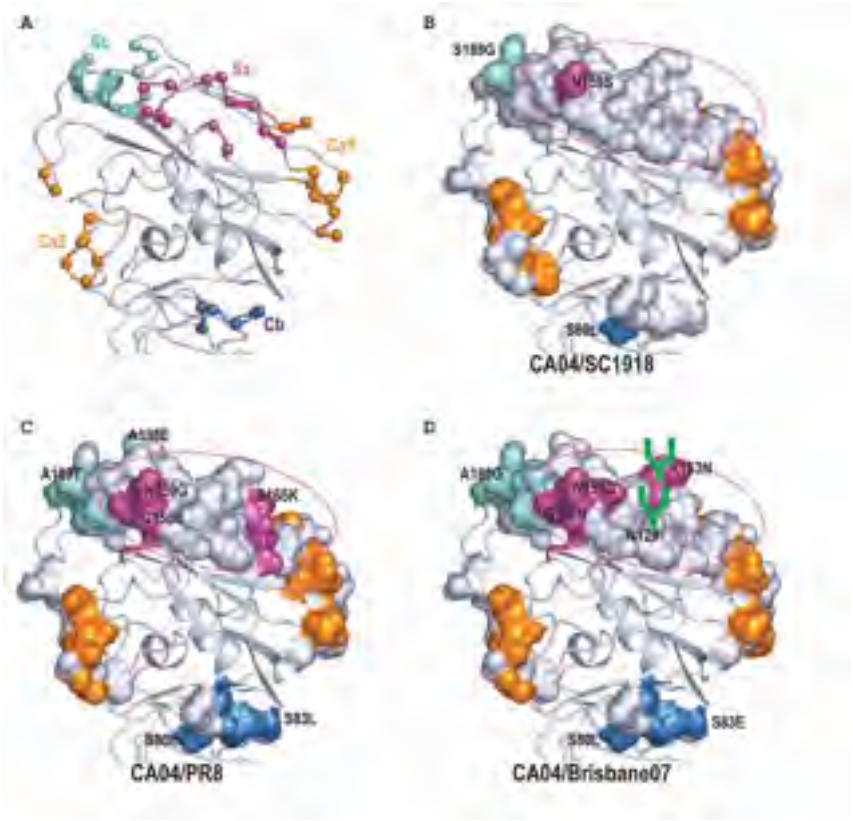
Ciliated cell

PD-INCL Matrosovich et al., PNAS 101:4620, 2004

Origin of 2009 pandemic H1N1 influenza strain "quadruple reassortant"



PD-INEL Trifonov et al., NEJM 361:115, 2009



PD-INEL Xu et al., *Sciencexpress,* 25 March 2010
Wei et al., *Sci. Transl. Med.* 2, 24ra21, 2010

Influenza Clinical Manifestations

- **Transmission**
  - Airborne droplets
- **Primary symptoms**
  - Acute onset fevers, chills, headache, myalgias
  - Non-productive cough
  - Potentially severe even in generally health patients

PD-GOV James Gathany, CDC Public Health Image Library #11162

- **Complications**
  - Young and elderly most susceptible
  - Viral and secondary bacterial pneumonia
  - Encephalitis
  - Reye syndrome (aspirin use)

Influenza Diagnosis, Treatment, and Prevention

- **Diagnosis**
  - Clinical suspicion
  - Virus detection (nasal swab)
  - Culture, antigen (DFA), or genome (RT-PCR) detection

- **Treatment**
  - Symptomatic
  - M2 channel blockers
    - Amantidine and rimantadine
  - Neuraminidase inhibitors
    - Oseltamivir (oral) and zanamivir (inhaled)
    - *Peramivir (IV) – CDC issued EAU in late fall, 2009*
  - NO ANTIBIOTICS (unless concern for bacterial superinfection present)

- **Prevention**
  - Prophylaxis
  - Vaccination

TRUE or FALSE?

There is very small yet scientifically validated link between childhood vaccination and autism.

Pre-vaccine annual U.S. cases/deaths

Table 1

Vaccine-Preventable Diseases, 2009[a]

Adult	Age 7-18 Years	Age 0-6 Years	Pre-vaccine annual U.S. cases/deaths
Tetanus	Tetanus	Tetanus	1,000/200
Diphtheria	Diphtheria	Diphtheria	150,000/15,000
Pertussis (whooping cough)	Pertussis (whooping cough)	Pertussis (whooping cough)	200,000/10,000
Measles	Measles	Measles	3,500,000/500
Mumps	Mumps	Mumps	200,000/100
Rubella	Rubella	Rubella	60,000/rare
Varicella (chickenpox)	Varicella (chickenpox)	Varicella (chickenpox)	4,000,000/100
Influenza	Influenza	Influenza	
Pneumococcal diseases	Pneumococcal diseases	Pneumococcal diseases	
Hepatitis A	Hepatitis A	Hepatitis A	
Hepatitis B	Hepatitis B	Hepatitis B	
Meningococcal diseases	Meningococcal diseases	Meningococcal diseases	
Herpes zoster (shingles)	Polio	Polio	25,000/2,000
HPV (cervical cancer)	HPV (cervical cancer)	HIB	20,000/1,000
		Rotavirus	(500,000 deaths worldwide)

[a] For specific dosing criteria based on age, medical condition, and other conditions, refer to the ACIP recommended immunization schedules.[3,4]

ACIP: Advisory Committee on Immunization Practices; HIB: Haemophilus influenzae *type b; HPV: human papillomavirus.*

Source: References 3, 4.

Smallpox (total 300-500 million deaths during 20th century worldwide)

Influenza Vaccines

- **Three general types**
  - Killed virus (TIV – trivalent inactivated influenza vaccine)
  - Live virus (LAIV – live attenuated influenza vaccine)
  - Genetically engineered, subunit (in development)

- **Very effective (70-80%)**
  - ***Dependent*** *on virus match (only 40% in 2007-2008)*

- **Contain three different viruses**
  - 2 influenza A subtypes, 1 influenza B strain
  - 2008-2009 vaccine: A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006
  - 2009-2010 vaccine: A/Brisbane/59/2007 (H1N1), and A/Brisbane/10/2007 (H3N2), *B/Brisbane/60/2008*
  - 2010-2011 vaccine: *A/California/7/2009 (pandemic H1N1), A/Perth/16/2009-like (H3N2),* and B/Brisbane/60/2008

- **Changes (possible) every year**

- **Should be administered yearly**

Influenza Vaccine Recommendations – Children (prior to 2010)

- ***All* children aged 6 months to 18 years (new recommendation for 2008-2009 season)**

- **High priority populations**
  1. Very young (age 6 months to 4 years)
  2. Chronic pulmonary, cardiovascular, renal, hepatic, hematologic, or metabolic disorders

     Includes children with asthma and diabetes
  3. Immunosuppression
  4. Aspiration risk (e.g. seizure disorder, spinal cord injury)
  5. Long-term aspirin therapy
  6. Chronic care facility residents
  7. Anticipated pregnancy during influenza season

CDC/ACIP. Prevention and control of seasonal influenza with vaccines. MMWR 58 (RR-8), 2009; http://www.cdc.gov/mmwr/pdf/rr/rr5808.pdf

Influenza Vaccine Recommendations – Adults (prior to 2010)

- ***Anyone* who wants it**
  - **Exceptions:** egg allergies and previous adverse reactions to vaccine

- **High risk populations**
  1. Persons aged ≥ 50 years
  2. Chronic pulmonary, cardiovascular, renal, hepatic, hematologic, or metabolic disorders
     Includes children with asthma and diabetes
  3. Immunosuppression
  4. Aspiration risk (e.g. seizure disorder, spinal cord injury)
  5. Chronic care facility residents
  6. Anticipated pregnancy during influenza season
  7. Health care workers
  8. Close contacts/caregivers of children < 5 years and adults ≥ 50 years and patients with high risk medical conditions

CDC/ACIP. Prevention and control of seasonal influenza with vaccines. MMWR 58 (RR-8), 2009; http://www.cdc.gov/mmwr/pdf/rr/rr5808.pdf

Influenza Vaccine Options

- TIV (inactivated) approved for *ALL* patients

- LAIV (attenuated) exceptions
  - Persons age <2 or ≥ 50 years
  - Children 2-4 years old with history of possible reactive airway disease
  - High risk for influenza-related complications
  - Caregivers of severely immunosuppressed patients (e.g. BMT)
  - Pregnancy

CDC/ACIP. Prevention and control of seasonal influenza with vaccines. MMWR 58 (RR-8), 2009; http://www.cdc.gov/mmwr/pdf/rr/rr5808.pdf

Influenza Vaccine Recommendations

CDC Newsroom Press Release February 24, 2010

Centers for Disease Control and Prevention
Your Online Source for Credible Health Information

Press Release

For Immediate Release: February 24, 2010
Contact: CDC Division of Media Relations (http://www.cdc.gov/media) (/nchs/pressroom/)
(404) 639-3286

A panel of immunization experts voted today (February 24, 2010) to expand the recommendation for annual influenza vaccination to include all people aged 6 months and older. The expanded recommendation is to take effect in the 2010 – 2011 influenza season. The new recommendation seeks to remove barriers to influenza immunization and signals the importance of preventing influenza across the entire population.

The Advisory Committee on Immunization Practices (ACIP) (http://www.cdc.gov/vaccines/recs/acip/) , which advises the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov) on vaccine issues, voted on the new recommendation during its February 24, 2010 meeting in Atlanta. The vote took place against a backdrop of incremental increases in the numbers and groups of people recommended for influenza vaccination in years past, and lessons learned from the world's still ongoing first flu pandemic in 40 years.

Prior to today's vote, ACIP recommendations for seasonal influenza vaccination – which focused on vaccination of higher risk persons, children 6 months through 18 years of age and close contacts of higher risk persons – already applied to about 85 percent of the U.S. population.

Discussion at the ACIP meeting focused on the value of protecting all people 19 to 49 years of age, who have been hard hit by the 2009 H1N1 pandemic virus, which is likely to continue circulating into next season and beyond. Another reason cited in favor of a universal recommendation for vaccination is that many people in currently recommended "higher risk" groups are unaware of their risk factor or that they are recommended for vaccination. The ACIP discussion also recognized the practicality and value of issuing a simple and clear message regarding the importance of influenza vaccination in the hopes that this would remove impediments to vaccination and expand coverage. Finally, new data collected over the course of the 2009 H1N1 pandemic indicates that some people who do not currently have a specific recommendation for vaccination may also be at higher risk of serious flu-related complications, including those people who are obese, post-partum women and people in certain racial/ethnic groups.

42 year old generally healthy male student returned from Christmas break and developed acute onset of clear rhinorrhea, mild sore throat, and low grade fevers. He had very minimal cough and no myalgias, and felt well enough to return to classes. Symptoms spontaneously resolved within a week.

Mcfarlandmo, flickr

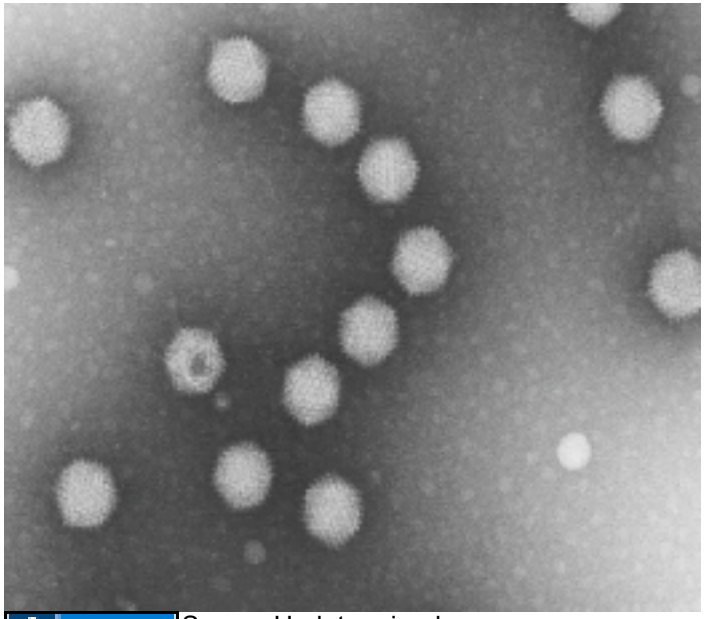
729:512, flickr

42 year old generally healthy male student returned from *Christmas break* and developed *acute onset* of *clear rhinorrhea, mild sore throat,* and *low grade fevers*. He had *very minimal cough and no myalgias*, and *felt well enough* to return to classes. Symptoms *spontaneously resolved* within a week.

Diagnosis?

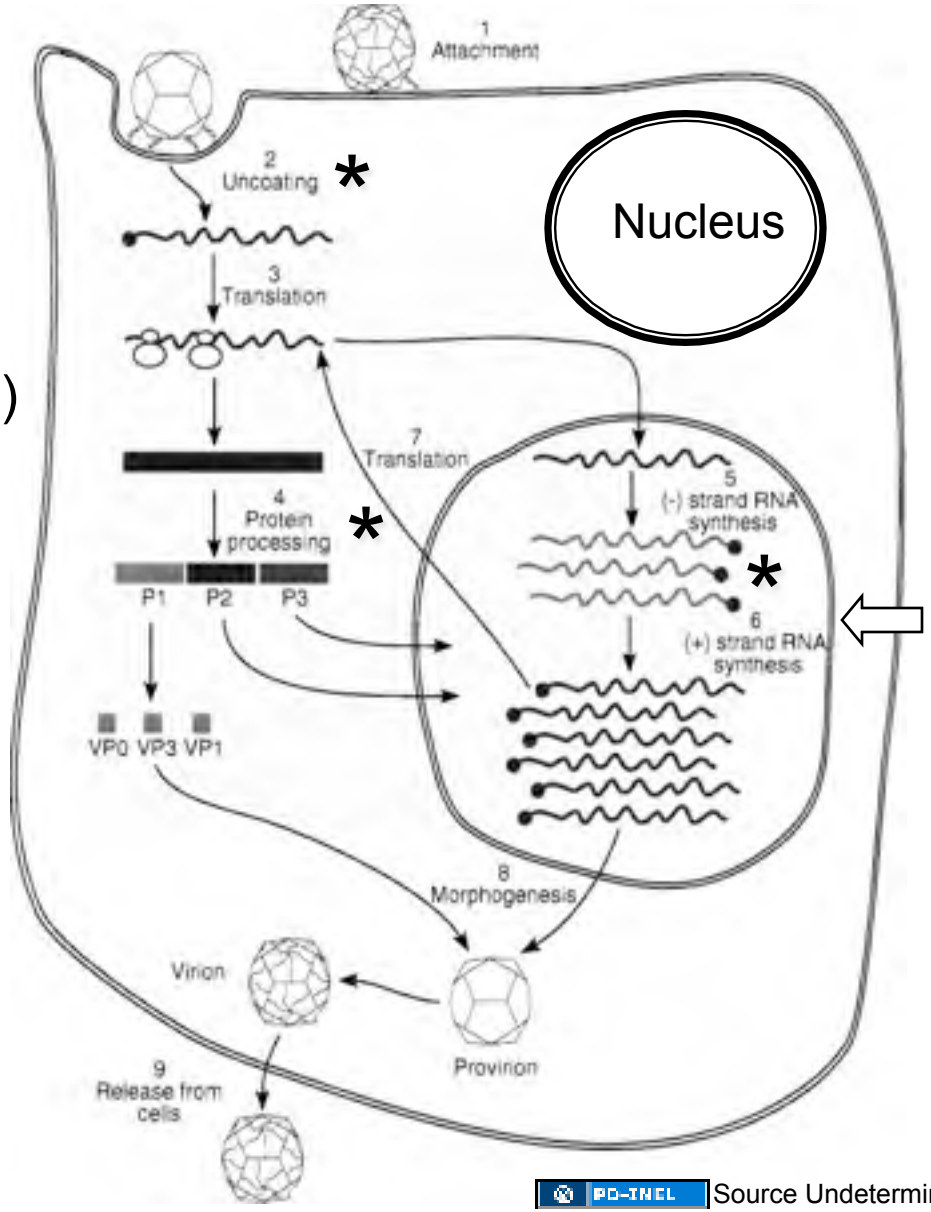
Rhinovirus

- **Family: *Picornaviridae***
  - Other members: coxsackie viruses, poliovirus, hepatitis A virus
- **Non-enveloped**
- **Non-segmented positive (+) strand RNA genome**
- **> 100 *serotypes* known**



Rhinovirus life cycle

- **Cellular receptor *(species barrier)***
  - ICAM-1 (90%)
  - VLDL receptor (10%)
- **Entirely cytoplasmic**
- **Replication most efficient at 33°C**



RNA replication factory

* Antiviral drug targets

Rhinovirus Pathogenesis

- **Minimal direct virus-induced cell damage**
  - Primarily upper respiratory tract
- **Role of immune response**
  - Inflammatory response correlates with symptoms
  - Responsible for COPD and asthma exacerbations
  - Induces *serotype*-specific immunity

Rhinovirus Clinical Manifestations

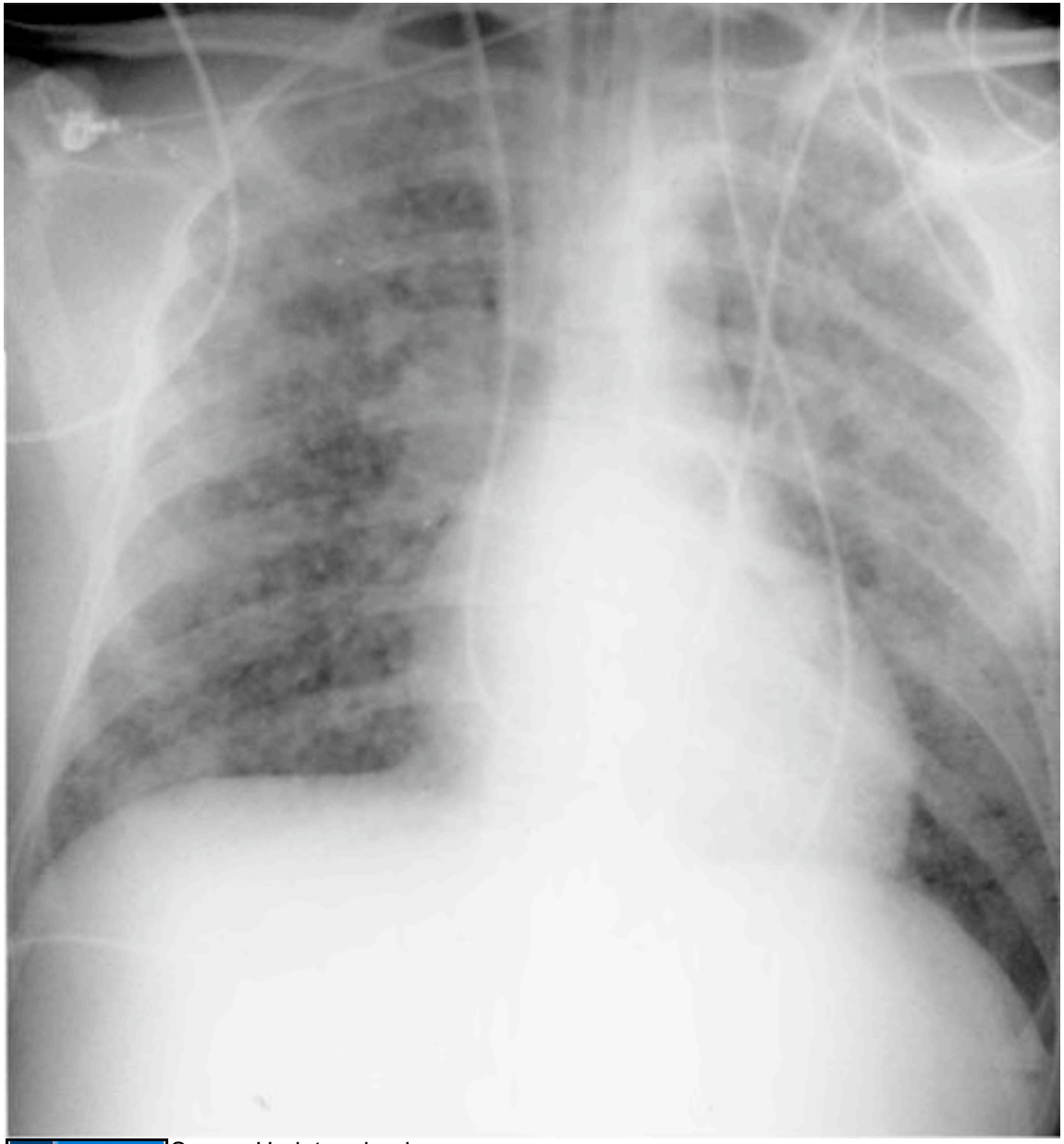
- **Transmission**
  - Aerosol (sneezing)
  - Direct transmission (fomites)
- **Primary symptoms**
  - Rhinorrhea, sore throat, minimal cough, low grade fever
  - Generally mild
- **Complications**
  - Asthma/COPD exacerbations

Rhinovirus Diagnosis, Treatment, and Prevention

- **Diagnosis**
  - Clinical suspicion
- **Treatment**
  - Symptomatic
  - Enormous market for “alternative” medications
  - NO ANTIBIOTICS
- **Prevention**
  - Vaccine development unlikely (mild disease, serotypes)

“Wash your hands!”

83 year old male nursing home resident with history of coronary artery disease, hypertension, and emphysema in April developed low grade fever, nasal congestion, and a non-productive cough. There were numerous other residents with similar symptoms over the past month. His cough progressed over two weeks and was keeping his roommate awake at night. He was eventually taken to the hospital when he began having trouble breathing, and despite being given numerous antibiotics he died within a week due to respiratory failure.



83 year old male nursing home resident with history of coronary artery disease, hypertension, and emphysema in *April* developed *low grade fever, nasal congestion*, and a *non-productive cough*. There were numerous *other residents with similar symptoms* over the past month. His *cough progressed over two weeks* and was keeping his roommate awake at night. He was eventually taken to the *hospital* when he began having trouble breathing, and despite being given numerous antibiotics he *died* within a week due to respiratory failure.

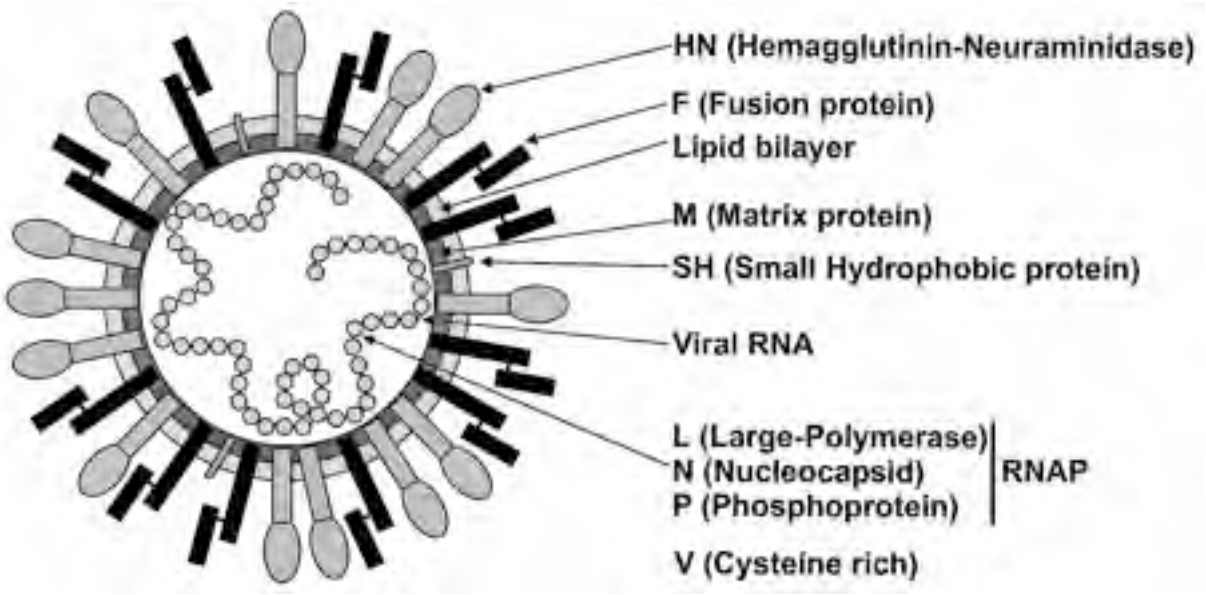
Diagnosis?

Respiratory syncytial virus (RSV)

- **Family: *Paramyxoviridae***
  - Parainfluenza virus, human metapneumovirus
  - Measles (rubeola) and mumps viruses
- **Enveloped**
- **Non-segmented negative (-) strand RNA genome**
  - No reassortment
- **Two major groups (A and B)**

RSV Structure

- **G protein (HN)**
  - Hemagglutinin and neuraminidase
  - Receptor binding (target unknown)
  - Group determinant
- **F protein**
  - Promotes virus-cell and cell-cell fusion
  - Candidate vaccine target
  - Target for palivizumab (preventive monoclonal antibody)



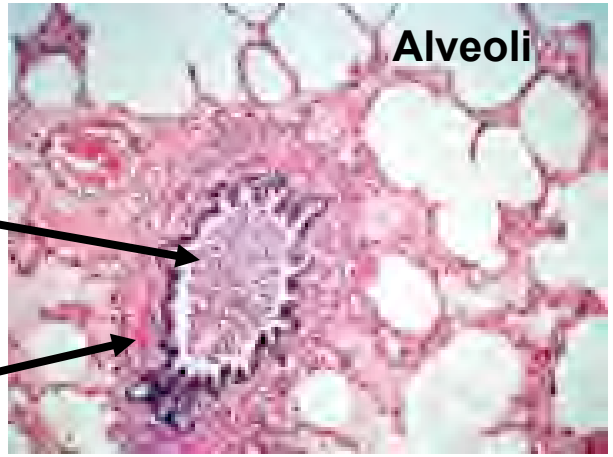
RSV Pathogenesis

- **Extensive direct virus-induced damage**
  - Primarily epithelial cells in lower respiratory tract
- **Intense inflammatory response**
  - Skewed inflammatory response (T_H2-like) may contribute to severe disease
  - Induces only partially effective immunity

Hallmark of RSV pathology is “bronchiolitis”

Bronchiole obstruction with mucus and necrotic cells

Peribronchiole inflammation



PD-INEL Source Undetermined

RSV Clinical Manifestations

- **Transmission**
  - Aerosol (sneezing)
  - Direct transmission (fomites, contagious secretions)
  - Highly infectious and ubiquitous (~100% children infected by 2 yo)

- **Primary symptoms (mild to severe)**
  - URI (rhinorrhea, sore throat, minimal cough, low grade fever)
  - Bronchitis (cough)
  - Bronchiolitis (wheezing, dyspnea)
  - Pneumonia (severe dyspnea, tachypnea, hypoxemia)

- **High risk groups for complications**
  - Premature infants
  - Cardiopulmonary disease
  - Immunocompromised patients

RSV Diagnosis, Treatment, and Prevention

- **Diagnosis**
  - Clinical suspicion
  - Culture, antigen (DFA), or genome (RT-PCR) detection

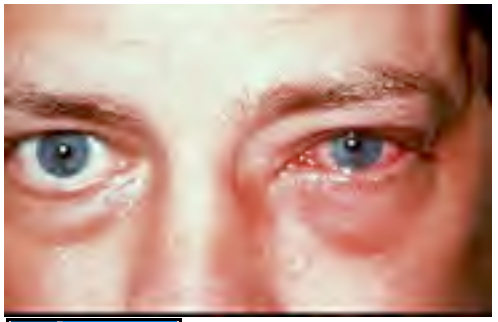
- **Treatment**
  - Symptomatic
  - Ribavirin of questionable utility
  - NO ANTIBIOTICS

- **Prevention**
  - Passive immunization (Palivizumab)
    - EXPENSIVE (~$77,000 to prevent one hospitalization annually)
  - Live attenuated vaccine development underway
    - Deaths associated with inactivated vaccine in 1960's

Other respiratory viruses

- **Coronaviruses (e.g. SARS)**
  - Enveloped, positive (+) strand RNA viruses
  - Typically cause mild respiratory tract symptoms

- **Adenoviruses**
  - Non-enveloped DNA viruses
  - Many (~50) serotypes
  - Associated with mild URI symptoms, pharyngoconjunctivitis, and GI disease
  - Can cause severe pneumonia



PD-INEL Source Undetermined

Table I

Ten Commandments of Antimicrobial Use

I	I am the Lord of Antimicrobial Agents. Do not holdeth Antipyretics before me.	Antimicrobial agents are not antipyretics. If you wish to treat a fever do so, but not with antimicrobials.
II	Thou shalt remember the History and physical and keep it Holy.	A good history and physical examination will be very useful in the diagnosis of infection.
III	Thou shalt not bear false witness upon the location of the host.	You need to distinguish between community- and hospital-acquired infections.
IV	Thou shalt not forget the little things.	Ask about travel, jobs, pets, immunizations, other people with the same symptoms, etc.
V	Thou shalt knoweth thy neighbors.	Certain organisms cause infections in certain organ systems.
VI	Thou shalt useth what worketh, and thou shalt not covet thy apothecary's new agents without a good reason.	Use antimicrobial agents with proven efficacy for the suspected or known infection.
VII	Thou shalt remembereth primum non nocerum.	When given a choice, avoid toxicity.
VIII	Thou shalt treateth what thou findeth.	When the infection is established and sensitivities known, try to use the narrowest spectrum agents.
IX	Thou shalt not killeth thy own pharmacy budget.	After choosing for high efficacy and lowest toxicity, consider cost as a variable and try to aim low.
X	Thou shalt study thy adversary.	Learn about the natural history of what you are treating and how it responds to therapy.

Additional Source Information

for more information see: http://open.umich.edu/wiki/CitationPolicy

Slide 6: Source Undetermined

Slide 8: Iconicphotos, http://iconicphotos.wordpress.com/2009/05/19/1918-spanish-flu/

Slide 9: Source Undetermined

Slide 11: Source Undetermined

Slide 12: David Miller

Slide 13: Computational Systems Biology Lab, http://camd.yonsei.ac.kr/1160

Slide 14: YK Times, Wikimedia Commons, http://commons.wikimedia.org/wiki/File:Virus_Replication_large.svg, CC:BY-SA, http://creativecommons.org/licenses/by-sa/3.0/deed.en

Slide 16: Source Undetermined

Slide 19: Centers for Disease Control and Prevention, http://www.cdc.gov/

Slide 22: Nevit Dilmen, Wikimedia Commons, http://commons.wikimedia.org/wiki/File:Crowd_04379.JPG, CC:BY-SA, http://creativecommons.org/licenses/by-sa/3.0/deed.en; titanium 22, Flickr, http://www.flickr.com/photos/nagarazoku/20867122/, CC:BY-SA, http://creativecommons.org/licenses/by-sa/3.0/deed.en; Herr Kriss, Wikimedia Commons, http://commons.wikimedia.org/wiki/File:The_pair_of_ducks.jpg, CC:BY-SA, http://creativecommons.org/licenses/by-sa/3.0/deed.en

Slide 23: Source Undetermined

Slide 24: Matrosovich et al., PNAS 101:4620, 2004

Slide 25: Trifonov et al., NEJM 361:115, 2009; Xu et al., Sciencexpress, 25 March 2010, Wei et al., Sci. Transl. Med. 2, 24ra21, 2010

Slide 26: James Gathany, CDC Public Health Image Library #11162

Slide 29: U.S. Pharmacist, http://uspharmacist.com/content/d/feature/i/783/c/14501/

Slide 34: Centers for Disease Control and Prevention, http://www.cdc.gov/

Slide 36: mcfarlandmo, Flickr, http://www.flickr.com/photos/mcfarlandmo/4014611539/, CC:BY, http://creativecommons.org/licenses/by/2.0/deed.en; 729:512, Flickr, http://www.flickr.com/photos/tummysak/5716692/, CC:BY-NC, http://creativecommons.org/licenses/by-nc/2.0/deed.en

Slide 38: Source Undetermined

Slide 39: Source Undetermined

Slide 44: Source Undetermined

Slide 47: Source Undetermined

Slide 48: Source Undetermined

Slide 51: Source Undetermined

Slide 52: Source Undetermined