A FEW QUESTIONS...
DID YOU GET THE FLU THIS YEAR?
EVER HAD THE FLU IN YOUR LIFE?
EVER KNOWN SOMEONE WITH FLU?
EVER KNOWN ANYONE WITH EBOLA?
INFLUENZA BACKGROUND

HISTORY, TRANSMISSION, & STRUCTURE.
Notable Outbreaks

- 1918 Spanish Flu – H1N1
  - Affected over 20% of the world population
  - ~50 million died
  - Well over a half-million died in the U.S.
  - Mortality rates highest in 20-50 y.o.

- 1957 pandemic
  - Nearly 70,000 perished
  - First wave occurred as children went back to school
  - Experienced “second wave” in Jan-Feb 1958

- 1968 Hong Kong Flu – H3N2
  - 33,800 fatalities
  - Made a additional appearances in 1970 and 1972

- 2009 H1N1 pandemic
  - Started in the spring of 2009, 40-80 million infected
  - By the end of the outbreak over 80 million vaccinated
  - Still had as many as 18,000 deaths

1918 Spanish flu

1918 Spanish flu

SAN FRANCISCO, Oct. 28.—While scores of passersby scurried for cover, H. D. Miller, a deputy health officer, shot and severely wounded James Wisser, a horseshoer, in front of a downtown drug store early today, following Wisser’s refusal to don an influenza mask.

According to the police, Miller shot in the air when Wisser first refused his request. Wisser closed in on him and in the succeeding affray was shot in the arm and the leg.

Wisser was taken to the central emergency hospital, where he was placed under arrest for failure to comply with Miller’s order.
Transmission

The reservoir of influenza A viruses

[Image], 29 April 2015, from: http://www.aht.org.uk/skins/Default/media/flu1.gif
Transmission
Transmission

Influ-Venn-Za
Who can catch which flu?

- Pigs: a source of flu pandemics as they can be infected by bird, human & swine flu.
- Birds: known to be infected by bird flu.
- Humans: can be infected by all types of flu.

April 2013 - suspected outbreak of an avian virus may have been involved in the death of thousands of pigs outside Shanghai, China. Human fatality rate is unknown but out of 28 people infected, 9 have died.

H5N2
H5N3
H5N9
H6N9
H7N7
H7N9
H9N2
H9N2
H1N1
H1N2
H5N1
H3N2
H3N2
H3N2
H3N1
H3N2
H1N7
H1N7
H7N7
H7N7
H2N1
H2N2
H3N2
H4N5
H5N3
H5N8
H5N1
H5N1
H5N3
H5N9
H3N9
H7N9
H1N9
H1N2
H1N2
H1N1
H1N1

Influenza Types: B & C

- H5N1: The "Bird Flu" most mentioned in the media. Kills 60% of humans it infects. Direct human-to-human transmission has not been reported.
- H5N3: Lesser known "bird flu" endemic in poultry in Eurasia. Rarely seen in humans.
- H5N2: The most common variant of "Swine Flu". As "Spanish Flu" it killed 50-100 million people in 1918. The 2009-10 pandemic killed 15,000 worldwide.
- H1N1: "Common flu only found in humans. Less harmful than type A. Does not cause pandemics."
- H1N2: "Caused "Asian Flu" pandemic in 1957 then disappeared from human population. Still circulates in birds."

Sources: Centers for Disease Control, WHO
Data: http://bit.ly/1K8_influenza

Concept & Design: David McCandless
Research: Ella Holloso // Additional design: Philippa Thomas
Version 1.1 // April 2013

InformationIsBeautiful.net

Transmission
Structure

- Orthomyxoviridae
- Negative-sense ssRNA
- 8-gene “Cassette”
- HA – Hemagglutinin
- NA – Neuraminidase (Clancy, 2008)

Horimoto, T. et al. (Author). (2005). Influenza: lessons from past pandemics, warnings from current incidents. [Image], 29 April 2015, from: http://www.nature.com/scitable/content/26874/10.1038_nrmicro1208-f1_full.jpg
Structure - HA

- Glycoprotein (Clancy, 2008)
- Binds to other cells with sialic acid (Russell, 2008)
- Allows virus to bind to sites in the upper respiratory tract (Taubenberger, 2008)

Structure - NA

- Glycoprotein (Clancy, 2008)
- Cleaves the sialic acid to release progeny viruses (Russell, 2008)
- Target of common influenza antiviral treatments (Russell, 2008)

[Image], 29 April 2015, from: http://upload.wikimedia.org/wikipedia/commons/f/f1/Neuraminidase_from_1918_influenza.gif
Structure - Variability

- High error rate in transcription polymerases leads to drift in progeny
- Drift causes new challenges to host
- Allows quick evolution of the viral genome (Clancy, 2008)

[Image], 29 April 2015, from: http://education.expasy.org/images/RNA-transcription.jpg
Structure - Variability

- Reassortment is a genetic mixtape
- It allows different varieties of influenza to swap genes (Clancy, 2008)
- Occurs most dramatically in poultry markets (“Avian Influenza in Birds,” 2015)
- Can occur in you!

[Image], 29 April 2015, from: http://www.blouinartinfo.com/sites/default/files/20130214mixtape1-promo1.png
Reassortment

A flu virus contains eight gene segments. The goal is to combine the desired HA and NA genes from flu strain 1 with genes from flu strain 2, which grows well in eggs and is harmless in humans.

1. Flu strains 1 and 2 are injected into a fertilized chicken egg.

2. The genes from flu strain 1 multiply and mix with the genes from flu strain 2, forming as many as 256 possible gene combinations.

3. Researchers search the many combinations for the flu strain that contains the HA and NA genes from flu strain 1 and genes from flu strain 2 that ensure that it is able to grow efficiently in eggs.

4. This new reassortant flu strain and two other flu strains will make up next year’s vaccine.

[Image], 29 April 2015, from: http://www.niaid.nih.gov.SiteCollectionImages/topics/flu/Reassortment_HiRes.jpg
Reassortment

Reassortment History of Human Pandemic Influenza Strains

Reassortment

Genetic Evolution of H7N9 Virus in China, 2013

The eight genes of the H7N9 virus are closely related to avian influenza viruses found in domestic ducks, wild birds and domestic poultry in Asia. The virus likely emerged from “reassortment,” a process in which two or more influenza viruses co-infect a single host and exchange genes. This can result in the creation of a new influenza virus. Experts think multiple reassortment events led to the creation of the H7N9 virus. These events may have occurred in habitats shared by wild and domestic birds and/or in live bird/poultry markets, where different species of birds are bought and sold for food. As the above diagram shows, the H7N9 virus likely obtained its HA (hemagglutinin) gene from domestic ducks, its NA (neuraminidase) gene from wild birds, and its six remaining genes from multiple related H9N2 influenza viruses in domestic poultry.

Live Poultry Markets

- Breeding zone for flu
- High concentrations of different species of birds
- Transmitted through respiratory secretions and intestinal excretions
- Normally wild birds are unaffected by flu, but domesticated specimens are susceptible

(“Avian Influenza in Birds,” 2015)
Etiology/Sx

- Etiology
  - Not all ILI is flu A
  - Flu A, B, C infect humans
  - Parainfluenza
  - RSV
  - *Mycoplasma pneumoniae*
  - Outbreaks usually occur in the winter
    (Taubenberger, 2008)

- Sx
  - Fever, malaise, sore throat, cough
  - Acute symptoms typically last 7-10 days
  - After contact, virus readily replicates for approximately 6 days
Flu Testing in AL

Emerging Infectious Diseases

- Influenza PCR
- Malaria PCR
- Norovirus PCR
- Dengue PCR
- *B. pertussis* PCR
- *N. meningitidis* PCR
- *H. influenzae* PCR
- Bio-Terrorism Response (LRN), 13+ Assays
- Bacterial sequencing

- Norovirus sequencing
- Pulsed Field Gel Electrophoresis
- Lyme (*B. burgdorferi*) ELISA
- West Nile ELISA
- Film Array Respiratory Panel (Viral and Bacterial)
- CDC Referral services
- New test development
Equipment - Extraction

- Qiagen QIAcube
- 12 samples per run
- 20 min set up, 40 min run time
- Membrane filtration
- Automates a gold standard manual extraction
Equipment - Extraction

- Roche MagNA Pure Compact (2)
- 8 samples per run
- 5 min set up, 30 min run time
- Magnetic bead separation
- Pre-packaged reagents
Equipment - Extraction

- Roche MagNA Pure LC (3)
- 32 samples per run
- 10 min set up, 90 min run time
- Magnetic bead separation
- Bulk reagents
Equipment – PCR

- Applied Biosystems 7500 Fast Dx
- Up to 19 specimens per run (A/B screen)
- 5 min set up, 100 min run time
- rtRT-PCR
- Reverse transcriptase, Real-time, polymerase chain reaction
- Find the needle in the haystack
What is PCR?
What is rtRT-PCR

**RT**
- Real-time
- Fluorophore added to primers, called a probe
- Utilizes a camera
- Camera detects fluorescence
- Picture taken after every cycle

**rt**
- Reverse transcriptase
- Enzyme added to mix
- Occurs first in the 7500
- Converts ssRNA(-) to cDNA
- Required for PCR to work
Other Flu Tests

- Hemagglutination Inhibition Assay
  - Called HI test
  - Can detect antigenic drift

- Pyrosequencing
  - Targeted sequencing
  - Used to detect antiviral resistance in flu

- Neither of these available at ADPH

- Samples sent by us to the CDC regularly for surveillance testing
Surge capacity

- What happens in an outbreak/epidemic/pandemic?
- Lab flooded with specimens
- 2011 Booz-Allen-Hamilton study
  - 11 personnel
  - Normal operations: 102 full panel, 227 screen only
  - Maximum capacity: 192 full panel, 301 screen only
  - Both personnel and testing algorithms have changed since this study.
Flu Epidemiology

- Flu coordinator: Miranda Daniels
- Compiles data and tracks flu across the state
  - ILInet providers
    - Report all ILI pts
    - Sent to CDC
  - SpeciNet
    - Submits specimens on regular basis
    - Tested at BCL
- Coordinates with other epidemiologists in other states
Right Size Workshop

- Population: 4.849 Million (U.S. Census Bureau, 2014)
  - Outbreak with 20% involvement (1918 Spanish)
  - 969,800 sick
  - Test them all? Would take nearly 9 years!
  - How many need to be tested in any given year?
  - How many need to be tested to find drifted or reassorted viruses?

- Right Size is tool for states to be 95% confident in what is circulating and novel event detection
- Based on statistical analysis of flu patterns and state population, integrated with the national outlook
- More testing is not necessarily better
Right Size Workshop

- How many need to be tested for surveillance only?
  - Baseline – 135 per week
  - Peak season – 314 per week

- This does not have to happen solely at the BCL

- NREVSS data may be integrated to meet data needs
  - Currently three hospitals reporting to NREVSS
  - NREVSS data + BCL testing volume meets the statistical requirement for testing
Right Size Workshop

- How many need to be tested for novel event detection?
  - 1:4 – 1/wk off-season
  - 1:200 – 10/wk-shoulders
  - 1:700 – 32/wk-peak
- Specimens are not pre-screened
- Must be performed at BCL
- Positives must be subtyped
- Specimens also used for vaccine selection

Since the start of the season, influenza A (H3N2) viruses have predominated nationally; however since early March, the proportion of influenza B viruses has been increasing. During week 15, 88% of all influenza positive specimens reported were influenza B viruses, and influenza B viruses predominated in all 10 regions.
Specimens

- **Types**
  - Nasopharyngeal swabs
  - Nasopharyngeal aspirate/wash
  - Throat swabs
  - Nasal Swabs

- **Synthetic swabs only, no Ca-alginate or cotton**

- **Plastic or aluminum shafts, no wood**

- **Collect within 72 hours of onset and ship ASAP, store in refrigerator**

- **Specimen must be received within 7 days of collection**

- **DO NOT FREEZE SPECIMEN**
Packaging and Shipping

- Place sample swab into VTM
- Break shaft at break-point
- Place in sealable bag with absorbent material
- Place in appropriate, insulated shipping container
- Don’t forget a FROZEN cold pack in the container
- Place the requisition form in a separate sealable bag or space from the specimen
- Close, seal, and appropriately label the container
- Ship the specimen, DO NOT SHIP ON FRIDAY
Web Portal

- Online system for ordering tests and receiving results
- Allows real-time results receipt via email
- Easy to set up, contact:
  - Ron Howard
  - 334-260-3409
  - ron.howard@adph.state.al.us
We Want You!

- We need high-quality specimen submitters
- The sample you submit today could be tomorrow’s vaccine
- You could be the first facility to detect a new flu virus
- Do your part to protect public health
References


