Influenza: Ecology and Continuing Evolution

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Influenza Virus

- Negative sense RNA virus
- No proof-reading during replication
- Segmented genomes
- Highly variable
- 16 HA and 9 NA subtypes
Seasonal vs Pandemic Influenza

- Interpandemic influenza: H1N1, H3N2, B
- U.S.A. ≈ 35,000 deaths per year

More deaths from interpandemic than pandemic influenza during:

- 1957 Asian pandemic
- 1968 Hong Kong pandemic
Pandemics of the Past Century

1918 Spanish 20-50 million

1957 Asian 1 million

1968 Hong Kong 0.5 million
Brevig Mission – Alaska
1918 Spanish Influenza

• Letter carrier taken home by dog team.
• Nov 15 - 20: 72 of 80 people in Brevig died.
• Children survived.
1918 Spanish Influenza

Brevig Mission – The Complete Sequence

Jeffery Taubenberger

Johan Hultin
Reconstructed 1918 Spanish Influenza

- Lethal for mice and embryonated eggs
- Marked activation of pro-inflammatory and cell death pathways
- Replicates in the absence of trypsin
- High growth in human bronchial epithelial cells

Kash et al, Nature 2006
Tumpey et al., Science, October 2005
• Wild waterfowl are the primary source of all influenza A viruses
• The viruses replicate primarily in the intestinal tract
• Two super families – one in Eurasia, one in the Americas
The Possible Pandemic

“Hit List”

H5N1
H7N7
H9N2
H2N2
Genesis of H5N1 Influenza In Asia

1996 → Goose/Guangdong/1/96 (H5N1)
1997-May → First human case in Hong Kong from live poultry markets
1997-2002 → Emergence of multiple different genotypes in Southern Asia and selection of dominant Z genotype
2003-Feb. → First human case(s) in Fujian, China
2003-2004 → Spread to Japan, South Korea, Indochina and Indonesia
2005 → Emergence of the Bar-Headed Goose Qinghai strain that spread to Europe, India and Africa
H5N1 – The Starting Point?

Live markets are *NOT* a dead end for the virus!
H5N1 Influenza Outbreak – Kowloon Park, Hong Kong

Numbers

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<th>Numbers</th>
<th>December, 2002</th>
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<tr>
<td>18</td>
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Dead: Red
Sick: Light Blue

Species:
- Puna Teal Duck
- Wood Duck
- Bar-headed Goose
- Common Pintail Duck
- Ruddy Duck
- Ringed Teal Duck
- Victoria Crowned Pigeon
- Chiloe Wigeion Duck
- Red-crested Pochard
- Rosybill Duck
- Coscoroba Swan
- Greater Flamingo
- Tufted Duck
- Bahama Pintail
- White-faced Whistling Duck
- Black-necked Swan
- Hawaiian Goose
- Brazilian Duck
H5N1 Influenza Viruses Isolated From Live Poultry Markets in Southern China 2004 - 2006

2004 - 2005

1.9% 1.8% 0.5% 0.3%

2006

4.8% 4.4% 0.8%
Spread of H5N1: 2005-2006

Poultry: 230 million
Human Cases: 258
Human Deaths: 154
The Role of Ducks

- Domestic
- Migratory

“The Trojan Horses”
Rapid Evolution of H5N1 in Ducks

- Long term shedding despite antibody
- Retain HPAI characteristics
- Antigenic drift
- Increased environmental stability
The Spread of H5N1 Virus

Initial spread to SE Asia (Thailand-Vietnam)

Humans and the integrated poultry industry?
How Pathogenic is This Virus?

A/Vietnam/1203/04 (H5N1)

- Kills chickens in less than one day
- Kills ducks in 1-2 days
- High risk of death in humans
  - Diarrhea
  - Respiratory symptoms
- High risk of death in ferrets
  - Respiratory symptoms
  - Diarrhea
  - Hind leg paralysis
Human H5N1 Cases in Vietnam

- Multiple organ dysfunction
- More virus in the lower respiratory tract
- Diarrhea in majority of patients
  - Virus isolation 1/7
  - RNA 5/7
- Detection of RNA in human serum 4/7
- Median virus load 6 days for H5N1, 2 days for H3N2
Does H5N1 Replicate and Transmit in Pigs?

<table>
<thead>
<tr>
<th></th>
<th>Infected</th>
<th>Contact</th>
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<tbody>
<tr>
<td>Vietnam/1203/04</td>
<td>❌</td>
<td>0</td>
</tr>
<tr>
<td>Ck/Vietnam/C-58/04</td>
<td>❌</td>
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<tr>
<td>Dk/TH/D4AT/04</td>
<td>❌</td>
<td>0</td>
</tr>
<tr>
<td>Gs/TH/G7CS/04</td>
<td>❌</td>
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</table>
Expanding Host Range for Influenza

H5N1 in Thailand

Experimental transmission in domestic cats

Kuiken et al, Science 2004
VACCINES
Potential Value of Seasonal Influenza Vaccine for H5N1

NewCaledonia/20/99 (H5N1)

Vietnam/1203/04 (H5N1)

3 weeks

50% survival

Sandbuile, Webby 2006
Studies on r.g. H5N1 Vaccines in Ferrets

• r.g. Hong Kong/213/03 (H5N1)
• Complete protection from homologous challenge
• Cross protection from challenge with A/Vietnam/1203/04
  – Virus shedding reduced
  – Weight loss
  – No virus in brain
  – No disease signs
**Control Strategies: H5N1 Poultry**

**Vietnam: The ongoing experiment.**

- October 2005 → 170 million doses of H5N1 poultry vaccines used.
- November 2005 → Present -- No human cases of H5N1
Antivirals
## Sensitivity of Human H5N1 Influenza Viruses to Amantadine

<table>
<thead>
<tr>
<th>Year</th>
<th>Virus</th>
<th>Sensitivity</th>
<th>Amino acid change on M2 protein</th>
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<tbody>
<tr>
<td>1997</td>
<td>A/HK/156/97</td>
<td>Yes</td>
<td>Ser31</td>
</tr>
<tr>
<td>2003</td>
<td>A/HK/213/03</td>
<td>No</td>
<td>Ser31→Asn</td>
</tr>
<tr>
<td></td>
<td>A/Vietnam/1203/04</td>
<td>No</td>
<td>Ser31→Asn</td>
</tr>
<tr>
<td>2004</td>
<td>A/Vietnam/1194/04</td>
<td>No</td>
<td>Ser31→Asn</td>
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</table>
Reduction of A/Vietnam/1203/04 (H5N1) Virus Replication after Prophylactic Treatment of Mice with Oseltamivir
# The Current Situation

<table>
<thead>
<tr>
<th>Current Human Situation</th>
<th>Humans</th>
<th>New human cases in:</th>
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<tbody>
<tr>
<td></td>
<td># infected: 258</td>
<td>Indonesia:</td>
</tr>
<tr>
<td></td>
<td># deaths: 154</td>
<td>► Family clusters of H5N1</td>
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<tr>
<td></td>
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<td>► Antigenically and genetically different viruses in Indonesia, China, Europe</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Poultry</th>
<th>H5N1 causing asymptomatic infection in ducks but killing swans/geese</th>
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<tbody>
<tr>
<td>Wild birds</td>
<td>Are the highly pathogenic H5N1 viruses being perpetuated in migratory birds?</td>
</tr>
<tr>
<td>Antigenic diversity</td>
<td>What is driving the selection of variants?</td>
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</table>
Will H5N1 Acquire Transmissibility?

Avian source → H5N1 → Infected human → Human virus → Pandemic virus

Steps:
1. Reassortment
2. Mutation
VIVERO

LAMB + GOAT

Webster Live Chicken

HALAL

1167 - POULTRY, KANG, GOAT, CHICKEN, TURKEN, GUINEA, RABBITS, DUCKS, PALOMAS, GUINEAS, HENS, PIGEONS

AL-
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