Origins, Ecology and Epidemiology of Pandemic and Seasonal Influenza A virus

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Systems Ecology and scale of response

Bahl et al Virol, 2009
Bahl et al PLoS Path 2013

Vijaykrishna et al Nature 2011

Su et al Nat Comm, 2016

Smith et al Nature 2009
Smith et al PNAS, 2009
Zaraket et al Nat Comm, 2015
1. Influenza virus

- Types A, B and C
- Segmented genome
- *ss RNA (single stranded, negative sense)*

**SUBTYPES**
- Haemagglutinin (HA)
- Neuraminidase (NA)
Influenza A virus

- Each segment encodes a different protein
- Smallest RNAs encode 2 proteins
- PB1-F2 in alternative reading frame of PB1
## Influenza A proteins

<table>
<thead>
<tr>
<th>Segment</th>
<th>Size (nucleotides)</th>
<th>Polypeptide</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2341</td>
<td>PB2</td>
<td>Subunit of polymerase: Host cap binding and endonuclease</td>
</tr>
<tr>
<td>2</td>
<td>2341</td>
<td>PB1</td>
<td>Catalytic subunit of polymerase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PB1-F2</td>
<td>Genomic RNA nuclear export</td>
</tr>
<tr>
<td>3</td>
<td>2233</td>
<td>PA</td>
<td>Subunit of polymerase, plays a role in replication</td>
</tr>
<tr>
<td>4</td>
<td>1778</td>
<td>HA</td>
<td>Haemagglutinin: cell receptor binding, membrane fusions.</td>
</tr>
<tr>
<td>5</td>
<td>1565</td>
<td>NP</td>
<td>Nucleoprotein: protects the RNA genome.</td>
</tr>
<tr>
<td>6</td>
<td>1413</td>
<td>NA</td>
<td>Neuraminidase: release of virus</td>
</tr>
<tr>
<td>7</td>
<td>1027</td>
<td>M1</td>
<td>Matrix protein: Budding, virion structure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>Ion channel: virus entry.</td>
</tr>
<tr>
<td>8</td>
<td>890</td>
<td>NS1</td>
<td>Anti-interferon protein. Effects on cellular RNA transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS2</td>
<td>Genomic RNA nuclear export</td>
</tr>
</tbody>
</table>
Hemagglutinin: cell entry

- Binds to sialic acid-containing receptors on the cell surface
- The major antigenic site of influenza virus
Neuraminidase: virion budding

zanamivir & oseltamivir (Tamiflu)
Evolutionary processes give rise to diversity

There are four basic mechanisms by which biological evolution takes place. These include mutation, migration, genetic drift, and natural selection.
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When we sequence a population of viruses we are observing genetic diversity resulting from those processes.
Virus nomenclature

A / Swine/ Hong Kong / 1456 / 92 [H1N1]

- Virus type
- Host of isolation
- Place of isolation
- Isolate number
- Year of isolation
- HA and NA Subtype

A / Chicken / NY / 14009 / 93 [H5N2] - Chicken isolate
A / Hong Kong / 156 / 97 [H5N1] - Human isolate
A/Sydney/05/97 [H3N2] - Human isolate
2. Ecology – Avian origin viruses with pandemic potential

**High Risk:** H5N1 & H7N9

**Intermediate Risk:** H6 & H9
Influenza Ecology

Emergence of pandemic influenza viruses

Was the 1918 flu avian in origin?

Gibbs and Gibbs, Nature 2005
Maximum clade credibility (MCC) tree of the H1 subtype of HA. (Right) Clade-specific rate distributions (in substitutions per site per year).

Michael Worobey et al. PNAS 2014;111:8107-8112

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Dated phylogenies of influenza A virus genes.

Gavin J. D. Smith et al. PNAS 2009;106:11709-11712
Reconstruction of the sequence of reassortment events leading up to the emergence of S-OIV.
1997 H5N1 incident of HK

H5N1 Gs/GD/96-like

H9N2 G1-like

H6N1 W312-like

H5N1/97

Human & Chicken

Guan et al. PNAS, 1999.
Surveillance in HK first started by Prof Ken Shortridge in 1976

Expanded to mainland China in 2002 by Prof Yi Guan with 50-70,000 birds sampled each year

Hong Kong is a world leader in the surveillance & control of avian influenza
Poultry production, global trade intensity and location of viral sampling.

Isolation of H5N1 Viruses From Live Poultry Markets in S. China

Based on numbers (%) from August 2004 to June 2005
Overview of 3 major subtypes of avian influenza A viruses in southern China

H5N1
- Gs/GD
- H5N1/97
- Gs/GD-like
- A-E, X
- B, X, Y, Z, Z*, W
- Z, Z*, V
- Z
- Z, G, QH-like, FJ-like...

H9N2
- G1-like, Ck/Bei-like
- Genotype A(0-3), B(1-16)

H6N1/N2
- W312-like
- Genotype A(1-9), B(1-8)

Year

References:
Guan Y et al, PNAS, 2002 & 2004
Chen H, et al, PNAS, 2006
Smith GJD, et al, PNAS, 2006
Cheung CL et al, J. Virol. 2007
Genesis & evolution of H5N1

Duan et al Virology 2008
Evolutionary dynamics & emergence of H5N1

Vijaykrishna et al PLoS Pathogens 2008
H5N1 emergence

• Resulted from complex ecology of farms & live-poultry markets in southern China
• Different bird species harbor different influenza A subtypes
• Markets allow interaction between different bird species
  – Aquatic poultry (goose, duck)
  – Terrestrial poultry (chicken)
  – Minor poultry (especially quail)
• AND also allow close contact of humans & infected birds
Spread of H5N1 Influenza Viruses by Migratory Birds

May 2005
Outbreak of H.P. H5N1

Jan., March 2005
5 H.P. H5N1
Outbreak of H5N1 in Migrating Birds in Western China 2005

April 30  Dead geese, neurological signs

May 4    Daily mortality over 100 geese

May 20   ~1,500 dead birds

June     ~5,000 dead birds
## Pathogenicity of H5N1 Viruses for Ducks and Geese

<table>
<thead>
<tr>
<th>Virus</th>
<th>Date of Isolation</th>
<th>Ducks</th>
<th>Geese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallard duck/JX/05</td>
<td>January/05</td>
<td>4/9</td>
<td>6/6</td>
</tr>
<tr>
<td>Bar-headed goose/QH/05</td>
<td>May/05</td>
<td>0/9</td>
<td>6/6</td>
</tr>
</tbody>
</table>
H5N2 and H5N8 virus detections in poultry and wild birds in 2014. The almost simultaneous detection of closely related viruses in Asia, Europe, and North America suggests linkage with wild bird migration via a large region in Russia.

Josanne H. Verhagen et al. Science 2015;347:616-617
3. Epidemiology – Transmission of seasonal influenza A virus

H3N2 & H1N1

Time Check?
Aerosol Transmission of Infection

- Disease transmission is classified into three routes
  - Airborne
  - Contact
  - Droplet

- Airborne transmission is defined as resulting from the inhalation of small particles, often termed droplet nuclei and considered to have diameters 5-10 μm or less.
Aerosol Transmission of Infection

- **Airborne**: aerosols become smaller by evaporation; if inhaled can travel deep into the lung
- **Droplets**: land directly on mucosal lining of nose, mouth, eyes of nearby persons (can be inhaled)
  - **Highest exposure within 3-6 feet**
- **Contact**: Aerosols/secrections contaminate surface. Transmission is through the touch of contaminated surfaces

Relative contribution of three routes varies with different agents
Aerosol Transmission of Infection
Aerosol Transmission of Infection

Smaller particles travel farther

Carried on a cloud of turbulence

Can cross a room and get sucked into air vents
Figure 1. Time-series of weekly influenza-like illness (ILI) surveillance and Google Flu Trends (GFT) search query estimates, June 2003–March 2013.

http://journals.plos.org/ploscompbiol/article?id=info:doi/10.1371/journal.pcbi.1003256
What about incidence trends in the tropics?

- Variation between countries
- Disease occurs through the year
- No obvious trends – unlike temperate regions

![Graphs showing incidence trends in various tropical countries](image-url)
What about incidence trends in the tropics?

• How are these outbreaks linked?
• Do viruses circulate locally continually emerging to cause epidemics, or do they circulate globally moving between populations?
What about incidence trends in the tropics?

- Not obvious from patterns of people getting sick (ili reports)
- Can we infer global transmission dynamics from examination of virus population?
  - Comparative genetics and ecological modeling.
  - Applied Evolutionary Biology
Migration dynamics of Seasonal influenza

(a) Migration model

(b) Latency model

Rambaut et al, Nature 2008

• Continual—but largely unidirectional—gene flow from a common source population provides the viruses that ignite each epidemic in populations of the Northern and Southern Hemispheres.

Temporally offset epidemic peaks – strong bottlenecks
Rambaut et al, Nature 2008
Russell et al., Science 2008

C A Russell et al. Science 2008;320:340-346
Russell et al., Science 2008
Figure 1. Global migration patterns of influenza A (H3N2) estimated from sequence data between 2002–2008.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000918
Evolutionary dynamics of H3N2 seasonal influenza
What tools do we have?

Source-Sink Model

Northern sink population
Southeast asian source population
Southern sink population

Source population maintains high levels of diversity without seasonal fluctuations. Seeds outbreaks in northern and southern temperate regions.
Phylogeography – transition between discrete states

• Tree trunk alternates between Japan, SEA, HK, Europe
• Within a single year, all locations occupy part of the tree backbone
Phylogeography – transition between discrete states

- No model supports persistence in SEA or HK

Source-Sink Model for seasonal influenza is not supported
Figure 2. Predictors of global H3N2 diffusion among the 14 air communities and the 15 & 26 geographic locations.

http://journals.plos.org/plospathogens/article?id=info:doi/10.1371/journal.ppat.1003932
Estimates of mean pairwise virus migration rate.
Summary

• If each population is considered to be somewhat isolated, this produces a network of flight-connected population centers with multiple epidemic peaks globally distributed at any given time.

• New York is an extremely well connected global centre. It may be that outbreaks in New York are transmitting globally during seasonal epidemics, but, only establish in other centers where suitable (i.e. other northern temperate regions). Transmission into SEA may be crucial for transmission to the southern temperate regions.