Of Men, Birds, Pigs and...Flu

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Influenza types

Type A  
Potentially severe illness  
Epidemics and pandemics  
Rapidly changing

Type B  
Usually less severe illness  
Epidemics  
More uniform

Type C  
Usually mild or asymptomatic illness  
Minimal public health impact

Influenza A virus

*Family: Orthomyxoviridae*

8 Segments of negative sense single stranded RNA

- HA
- PB1, PB2, PA
- NP
- NA
- M2
- M1
- α2-6Gal
- α2-3Gal
- NEP
- vRNP

Infected cells

www.cdc.gov
A change in receptor specificity is thought to favor host switching of influenza A viruses
Type A influenza cannot be eradicated.
Reassortment is how Flu has SEX!!
Influenza “Transmissome”: molecular signatures that allow the virus to replicate throughout the host’s respiratory tract and lead to respiratory droplet transmission

A transmissible Influenza Virus is likely to require:

• Binding to SAα2,6-Gal receptors
• Balanced HA and NA (and M2?) activities
• Efficient Replication in Upper and Lower Respiratory Tract
Influenza in domestic birds

Defined as fowl plague in 1878.

Significant economic losses.

Low Pathogenic Avian Influenza Viruses – LPAIV

• Associated with outbreaks of varying intensity in domestic birds.
• Progenitors of …
  • Highly Pathogenic Avian Influenza Viruses - HPAIV
    o H5 and H7 subtypes.
    o Accumulation of basic amino acids at the HA cleavage site.
    o ~26 outbreaks since 1959, 13 since 1990
  • ~13 H7 subtype, ~13 H5 subtype
Number of human cases and deaths due to H5N1 infections/country since 1997

608 cases / 359 deaths
Number of cases and deaths due to H7 infections

Number of cases and deaths due to H9 infections
Seroprevalence of H9N2 in humans

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>% Sero-prevalence</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xinjiang, CH</td>
<td>General (64% poultry exposure)</td>
<td>1.7</td>
<td>Jia et al. 2009, <em>J Clin Vir</em></td>
</tr>
<tr>
<td>Liaoning, CH</td>
<td>General (67% poultry exposure)</td>
<td>1</td>
<td>Jia et al. 2009, <em>J of Clin Vir</em></td>
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<tr>
<td>Guangzhou, CH</td>
<td>Poultry workers</td>
<td>3-15</td>
<td>Wang et al. 2009, <em>NEJM</em></td>
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</tbody>
</table>

- 1-4% seroprevalence in the general public in China
  - Only used single antigenic strain, may be higher
- 9-15% in poultry workers in Guangdong Province
- **Cross-reaction with H2N2 antibodies or other human influenza viruses??**
Overview of H9N2 avian influenza viruses

A/guinea fowl/Hong Kong/WF10/99 (H9N2) - RGWF10

**Nature**
- Aquatic birds
- Land-based poultry

**Laboratory**
- Early isolates do not or replicate poorly (1997 and before)
- Recent field isolates replicate well (1998 and later)
- Bind preferentially to human-like sialic acid receptors

Before 1988

After 1988-1994

1999 and later

1998 and later
H9N2 geographic range

H9N2 avian influenza viruses of the G1-like lineage show the largest geographic spread.

H9 influenza viruses go where H5N1 viruses go!

Previous pandemic influenza viruses have not been associated with major disease outbreaks in birds.
Contemporary H9N2 avian influenza viruses with human-like receptor specificity (L226) infect mostly nonciliated cells in HAE cultures.
Ferrets recapitulate human infection with Influenza A viruses
Efficient direct- but not aerosol-transmission of a H9N2 isolate with human virus-like receptor specificity in ferrets

Wan et al. Plos ONE, 2008
Would an avian/human H9N2 reassortant virus show improved replication and transmission in ferrets?

Plaque morphology cannot be used as a marker of pathogenesis/transmissibility

Wan et al. Plos ONE, 2008
Efficient direct- but not aerosol-transmission of the avian/human 2WF10:6M98 (H9N2) virus in ferrets
The 2WF10:6M98 (H9N2) shows increased virulence in ferrets compared to the wt WF10.
Is it possible to generate a H9N2 virus that transmits by aerosol in ferrets? - Implications for pandemic preparedness

Adaptation of an avian/human H9N2 virus in ferrets leads to a strain that can transmit by respiratory droplets

Nasal washes used to infect new set of ferrets

Sorrell et al. PNAS, 2009
Five amino acid changes occurred during adaptation of the 2WF10:6M98 virus in ferrets.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Origin</th>
<th>Amino Acid Position</th>
<th>Parent</th>
<th>P8</th>
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<th>P10</th>
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<tr>
<td>PB2</td>
<td>Human</td>
<td>374</td>
<td>L</td>
<td>L</td>
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<td>PB1</td>
<td>Human</td>
<td>No changes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
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<tr>
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<td>nd</td>
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<tr>
<td>HA</td>
<td>Avian</td>
<td>HA1 189</td>
<td>T</td>
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</table>

<sup>a</sup> No amino acid changes detected between the parent and either the RCP10 or the RCP10₂ viruses. <sup>b</sup> nd, sequencing not done. <sup>c</sup> Bold and italicized letter denotes more prominent residue at particular amino acid position based on electropherograms of sequencing profiles.
The P10 and 2WF10:6M98 viruses show improved LRT replication in ferrets compared to the wt WF10.
The P10-2WF10:6M98 (H9N2) virus shows consistent aerosol transmission in ferrets
The P10-2WF10:6M98 (H9N2) virus shows consistent aerosol transmission in ferrets
An amino acid change on the tip of the HA protein, near the RBS, is crucial for respiratory transmission.

**Position T189A: RBS and antigenic profile**

**Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus**

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Contributed by D.R.P., designed research; E.M.S., H.W., Y.A., and H.S. performed research; E.M.S., H.W., and D.R.P. analyzed data; and E.M.S., H.W., and D.R.P. wrote the paper.

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**Compatibility of H9N2 avian influenza surface genes and 2009 pandemic H1N1 internal genes for transmission in the ferret model**

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Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved June 10, 2011 (received for review May 19, 2011)
Epidemiology of SIVs in North America since 1918

Variations in the Hemagglutinin of the 2009 H1N1 Pandemic Virus: Potential for Strains with Altered Virulence Phenotype?

Jianqiang Ye,1,2 Erin M. Sorrell,1,2 Yibin Cai,1,2 Hongxia Shao,1,2 Kemin Xu,1,2 Lindomar Pena,1,2 Danielle Hickman,1,2 Haichen Song,3 Matthew Angel,1,2 Rafael A. Medina,4,5,6 Balaji Manicassamy,4,5,6 Adolfo Garcia-Sastre,4,5,6 and Daniel R. Perez1,2

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This article has been cited by other articles in PMC.

Fitness of Pandemic H1N1 and Seasonal influenza A viruses during Co-infection

Evidence of competitive advantage of pandemic H1N1 influenza versus seasonal influenza

Daniel Roberto Perez,* Erin Sorrell,† Matthew Angel,‡ Jianqiang Ye, Danielle Hickman,1 Lindomar Pena,† Gloria Ramirez-Nieto,‡ Brian Kimble,† and Yonas Araya†

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Accepted August 25, 2009.

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How does the 2009 Pandemic change how we view H9s?

A/Netherlands/602/09 (H1N1)
A/Guinea Fowl/Hong Kong/WF10/99 (H9N2)
A/Ferret/Maryland/P10-UMD/08 (H9N2)
H9N2:H1N1pdm reassortants can be transmitted by respiratory droplets in ferrets.
Summary H9N2 viruses – Risk Assessment

Contemporary H9N2 avian influenza viruses with human-like receptor specificity infect mostly nonciliated cells in cultures of human airway epithelium (HAE).

L226 containing H9N2 viruses show efficient direct - but not aerosol - transmission in ferrets. No signs of disease.

An avian/human H9N2 reassortant shows improved replication in ferrets with no detectable respiratory droplet transmission.

Respiratory droplet transmission in ferrets of an avian/human H9N2 virus requires few amino acid changes with major implications in replication, virulence and antibody recognition profiling.

H9 HA in the context of N2 or N1 NA is compatible with respiratory transmission when the internal genes are derived from the H1N1pdm virus.
Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai1, Tokiko Watanabe1,2, Masato Hatta1, Subash C. Das1, Makoto Ozawa1,3, Kyoko Shinya4, Gongxin Zhong1, Anthony Hanson1, Hiroaki Katsura5, Shinji Watanabe1,2, Chengjun Li1, Eriyo Kawakami2, Shinya Yamada5, Maki Kiso5, Yasuo Suzuki6, Eileen A. Maher1, Gabriele Neumann1 & Yoshihiro Kawaoka1,2,3,5

REPORT

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

Sander Herfst,1 Eefje J. A. Schrauwen,1 Martin Linster,1 Salin Chutinimitkul,1 Emmie de Wit,1a Vincent J. Munster,1a Erin M. Sorrell,1 Theo M. Bestebroer,1 David F. Burke,2 Derek J. Smith,1,2,3 Guus F. Rimmelzwaan,1 Albert D. M. E. Osterhaus,1 Ron A. M. Fouchier1†
Transmissible H5N1 viruses: Chronology of events

- September 2011, Prof. Ron Fouchier of the Erasmus Medical Center (Rotterdam, Netherlands) reported at a public scientific meeting the results of genetically modifying influenza A (H5N1) viruses transmissible through the air between ferrets.
- Similar research at the University of Wisconsin-Madison, USA under Dr. Yoshihiro Kawaoka except that the transmissible viruses are reportedly not lethal to ferrets.
- Manuscripts submitted to the journals Science and Nature, respectively…
  - Both sets of studies funded by the US National Institutes of Health (NIH) and came under review by the U.S. National Science Advisory Board for Biosecurity (NSABB) prior to publication.
Localization of amino acid changes identified in this study on the three-dimensional structure of the monomer of VN1203 HA (Protein Data Bank accession 2FK0)\textsuperscript{15}. 

\textbf{a} Modelled human receptor

- K193N
- N158D
- 190-helix
- N186K
- G228A
- S227N
- Q226L
- G225E
- M230L
- E231G
- V152I
- A242T

\textbf{b} Modeled human receptor

- Q226L
- N224K
- T318I
- N158D
- Fusion peptide

Serial passaging in ferrets
- Sequence analysis -

H103: forms part of the trimer interface

T156: part of a N-linked glycosylation sequon

Herfst et al., Science (2012)
Transmissible H5N1 viruses: Interlocking issues

- A voluntary moratorium on the research has been declared by the groups capable of undertaking such research. (Still in effect)
- Public concern that laboratory-modified H5N1 viruses could accidentally cause an influenza pandemic;
- The need for assessment of the balance of public health risk and benefit in research;
- Appropriate laboratory biosafety requirements of research on the evolution of pathogens to become more virulent or transmissible;
- The need and ability to revise risk assessments concerning H5N1 viruses;
- A potential threat to the new pandemic influenza preparedness framework for influenza virus and benefit sharing;
- and academic freedom to publish.
“Current epidemiologic evidence indicates that, once transmitted into a human host, H5N1 viruses may result in more severe disease in humans than other subtypes of influenza.”

“Listing influenza viruses that contain an HA from the goose/Guangdong/1/96 lineage as an HHS select agent will ensure that the focus of regulation will also be on the potential impact of these viruses on human health as well as agriculture.”
“Designating HPAI containing an HA from the Goose/Guangdong/1/96 lineage an HHS select agent, in addition to its status as a USDA select agent, may help to ensure that HPAI strains that have the greatest potential for major direct effects on human health will be regulated with a focus on protection of human health.”
The HHS/CDC’s Intrigovernmental Select Agents and Toxins Technical Advisory Committee (ISATTAC) recognized …

- That study of the Goose/Guangdong/1/96 lineage-derived viruses could lead to significant public health benefits for understanding pandemic influenza, improved diagnostics, and the development of more effective countermeasures.
- Therefore, the risks posed by these viruses need to be weighed against any adverse impact that a regulation will have on legitimate research.
Establishment of a Docket and Request for Specific Input on Certain Topics

- Should these viruses be regulated as Tier 1 HHS select agents?
- Should special precautions (i.e., safety and containment measures) be considered when working with diagnostic specimens suspected of containing HPAI H5N1 influenza viruses containing the HA from the Goose/Guangdong/1/96 lineage (i.e., any precautions versus none at all, precautions beyond those usual for clinical samples and/or laboratory microbes, etc.)?
- Should special precautions (i.e., safety and containment measures) be considered when working with strains of HPAI containing the HA from the Goose/Guangdong/1/96 lineage that have been shown to be transmissible between mammals beyond those recommended for non-mammalian transmissible HPAI?
The Potential for Respiratory Droplet–Transmissible A/H5N1 Influenza Virus to Evolve in a Mammalian Host
Herfst et al. set
Number of nucleotide mutations in HA necessary for aerosol transmission

Asia - avian

3 mutations
4 mutations
5 mutations

Africa and Middle East - avian

Mostly from Egypt (have E627K)

Japan, Mongolia, Nepal 2009-2011 (do not have E627K)

Russell et al., Science (2012)
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Ron Fouchier

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