Determinants of human adaptation and gain of pandemic potential

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Features of a Pandemic Influenza Virus

• Isolation from humans of a novel subtype of influenza, to which the general population has little or no immunity.

• Demonstrated ability of the virus to replicate and cause disease in humans.

• Efficient spread from person-to-person, expressed as sustained chains of transmission causing community-wide outbreaks.
Serosusceptibility to a novel influenza virus

Hancock et al NEJM 2009
Sialic Acid Receptor Distribution and Specificity

Reactivity of human respiratory tract tissues with lectins specific for sialic acid linkages

Staining for H5 Ag

Green α2,6Gal
Red α2,3Gal

Van Riel et al, Science 2006

Shinya et al, Nature 2006
Ability to replicate and cause disease in humans

HA
- Receptor specificity
  - Long chain $\alpha_{2,6}$
  - Q226L and G228S in H3, E190D and D225G in H1
  - Change in receptor specificity alone is not enough

NA
- Shortened NA stalk-reduced release
- HA-NA balance
Characteristics of avian IAV HA and NA that restrict transmission in humans

Cauldwell et al. *J Gen Virol* 2014
Ability to replicate and cause disease in humans

HA
- Receptor specificity
  - Long chain $\alpha_{2,6}$
  - Q226L and G228S in H3, E190D and D225G in H1
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PB2
- PB2 627 or 701
  - via promoter binding
  - via nuclear transport involving importin $\alpha$
- G590S+Q591R (H1N1pdm09) or T271A

PB1-F2 N66S
PA-X truncation

NS
- IFN sensitivity,
- C terminal PDZ binding
- CPSF binding
NIH airborne transmission ferret cage setup
The soft palate is an important site of adaptation and selection of transmissible influenza viruses.

Lakdawala *Nature* 2015

**Scale bars are 100μm**

**Morphology**

- **Nasopharynx**
- **Submucosal Glands**
- **Oral Cavity**

**Influenza receptors**

- Lectins: MALII: α2,3; SNA: α2,6

**Long-chain α2,6 SA**

- IHC: SC18: long chain α2,6
PB2 627 mediated host range restriction

- Interaction with NP or by affecting promoter binding
- Crystal structure of a 538-676 domain shows a unique fold with 627 in the centre of a solvent exposed surface.

A species-specific difference in host protein ANP32

627 Lysine
627 Glutamic acid

Tarendeau et al PPath 2008

Chicken

Human

Long et al Nature 2016
Mammalian adaptation of H5N1 viruses at PB2 627

VN/1203/2004 mutant K627E
Traits associated with airborne transmissibility in mammals

Schrauwen and Fouchier Emerging Microbes and Infections 2014
CDC’s Influenza Risk Assessment Tool (IRAT)

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus properties</td>
<td>Genomic variation</td>
</tr>
<tr>
<td></td>
<td>Receptor binding properties</td>
</tr>
<tr>
<td></td>
<td>Transmissibility in animal models</td>
</tr>
<tr>
<td></td>
<td>Antiviral susceptibility</td>
</tr>
<tr>
<td>Host properties</td>
<td>Population immunity</td>
</tr>
<tr>
<td></td>
<td>Disease severity</td>
</tr>
<tr>
<td></td>
<td>Antigenic relationship to vaccines</td>
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<tr>
<td>Ecology and Epidemiology</td>
<td>Human infections</td>
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<td></td>
<td>Infections in animals</td>
</tr>
<tr>
<td></td>
<td>Global distribution in animals</td>
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</table>
### Tool for Influenza Pandemic Risk Assessment (TIPRA)

#### Question 1: Likelihood of sustained human to human transmission of the virus

<table>
<thead>
<tr>
<th>Rank</th>
<th>Risk element</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Receptor binding properties</td>
<td>0.408</td>
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<tr>
<td>2</td>
<td>Transmission in animal models</td>
<td>0.242</td>
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<tr>
<td>3</td>
<td>Human infection</td>
<td>0.158</td>
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<tr>
<td>4</td>
<td>Genomic characteristics</td>
<td>0.103</td>
</tr>
<tr>
<td>5</td>
<td>Infection in animals</td>
<td>0.061</td>
</tr>
<tr>
<td>6</td>
<td>Geographic distribution in animals</td>
<td>0.028</td>
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</tbody>
</table>

#### Question 2: Impact to the human population of sustained human to human transmission of the virus

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<thead>
<tr>
<th>Rank</th>
<th>Risk element</th>
<th>Weight</th>
</tr>
</thead>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>6</td>
<td>Human infection</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Technical experts provide point estimates & confidence scores- converted to Confidence Coefficient scores

## Direct Infection of Humans with Avian Influenza Viruses

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Subtype/pathotype</th>
<th>Cases</th>
<th>Fatalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1959</td>
<td>US</td>
<td>H7N7 HPAI</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1978-79</td>
<td>US</td>
<td>H7N7 LPAI</td>
<td>?</td>
<td>0</td>
</tr>
<tr>
<td>1996</td>
<td>England</td>
<td>H7N7 LPAI</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1997</td>
<td>Hong Kong</td>
<td>H5N1 HPAI</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>1999</td>
<td>China</td>
<td>H9N2 LPAI</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1999, 2003</td>
<td>Hong Kong</td>
<td>H9N2 LPAI</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2002-03</td>
<td>US</td>
<td>H7N2 LPAI</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>Hong Kong</td>
<td>H5N1 HPAI</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2003</td>
<td>Netherlands</td>
<td>H7N7 HPAI</td>
<td>89</td>
<td>1</td>
</tr>
<tr>
<td>2004</td>
<td>Canada</td>
<td>H7N3 HPAI</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Egypt</td>
<td>H10N7 LPAI</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2003-2017</td>
<td>16 countries</td>
<td>H5N1 HPAI</td>
<td>859</td>
<td>453</td>
</tr>
<tr>
<td>2013-2017</td>
<td>China</td>
<td>H7N9 LPAI</td>
<td>1533</td>
<td>592</td>
</tr>
<tr>
<td>2014</td>
<td>Taiwan</td>
<td>H6N1 LPAI</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2014</td>
<td>China</td>
<td>H10N8 LPAI</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2014-2017</td>
<td>China</td>
<td>H5N6 HPAI</td>
<td>16</td>
<td>6</td>
</tr>
</tbody>
</table>

Avian influenza A (H7N9) cases in humans (2013-2017)

3 amino acids that can change the receptor binding of H7N9 viruses
H7N9 virus distribution over 5 waves in China

(A)

(B)

Su Trends in Microbiol 2017
The presence of multiple basic amino acids adjacent to the HA cleavage site increases the tissue range of the virus in birds.
Is the HA multibasic site a critical virulence motif for humans?

- In mice and ferrets, the presence of the H5 HA MBS was associated with:
  - lethality
  - significantly higher virus titers in the respiratory tract,
  - virus dissemination to extrapulmonary organs
  - lymphopenia
  - significantly elevated levels of pro-inflammatory cytokines and chemokines
  - inflammation in the lungs
- In AGMs,
  - neither H5N1 nor ΔH5N1 virus was lethal and neither caused clinical symptoms.
  - The H5 HA MBS was associated with mild enhancement of replication and delayed virus clearance.

Thus, the contribution of H5 HA MBS to the virulence of the HPAI H5N1 virus varies among mammalian hosts and is most significant in mice and ferrets and less remarkable in nonhuman primates.

# Molecular characteristics of a human HPAI H7N9 isolate*

<table>
<thead>
<tr>
<th>Strain</th>
<th>HA</th>
<th>NA</th>
<th>M2</th>
<th>PB2</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Guangdong/17SF006/2017 (H7N9)‡‡</td>
<td>PEVKRAKKTAR/GL</td>
<td>G186V†</td>
<td>Q226L†</td>
<td>R292K§</td>
<td>S31N¶</td>
</tr>
<tr>
<td>A/Guangdong/17SF003/2016 (H7N9)</td>
<td>PEVKRAKKTAR/GL</td>
<td>V</td>
<td>Q</td>
<td>K</td>
<td>N</td>
</tr>
<tr>
<td>A/Taiwan/1/2017 (H7N9)</td>
<td>PEVKRAKKTAR/GL</td>
<td>V</td>
<td>Q</td>
<td>K</td>
<td>N</td>
</tr>
<tr>
<td>A/Environment/Guangdong/C16283222/2016 (H7N9)</td>
<td>PEVKR</td>
<td>R/</td>
<td>Q</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>A/Fujian/3/2016 (H7N9)</td>
<td>PEIPKRAKKTAR/GL</td>
<td>V</td>
<td>L</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>A/Zhejiang/3/2017 (H7N9)</td>
<td>PEIPKRAKKTAR/GL</td>
<td>V</td>
<td>L</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>A/Netherlands/219/2003 (H7N7)</td>
<td>PEIPKRRRGL</td>
<td>G</td>
<td>Q</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

*HA, hemagglutinin; NA, neuraminidase; NAI, neuraminidase inhibitor; M2, matrix protein 2; PA, polymerase acidic; PB2, polymerase basic 2.
†Cleavage site.
‡Receptor binding site.
§Associated with NAI drug resistance.
¶Associated with amantadine resistance.
#Associated with increased virulence in mice.
**Associated with enhanced viral replication in mammalian hosts.
††Species-associated signature position.
‡‡Virus from patient in this report.

*A/Guangdong/17SF006/2017 Ke et al EIDJ 2017
An HPAI H7N9 is more virulent than the LPAI H7N9 virus in mammalian models

Virulence in animal models:
• Mice: Both HPAI and LPAI are lethal for mice; titers of HPAI>LPAI in RT; HPAI spread to the brain
• Ferrets: Both HPAI and LPAI replicated to high titers in the resp tract; HPAI cause more severe lung lesions than LPAI; both spread to the brain
• Cynomolgus macaques: Both HPAI and LPAI caused no/mild clinical signs; replicated similarly

Transmissibility in ferrets: No difference in transmissibility but some HPAI experimentally-infected and recipient animals died.

GD/3, isolated from a fatal human case treated with oseltamivir + recombinant GD/3 with and without NAI resistance mutation

Imai et al Cell Host & Microbe 2017
Caveats in pandemic risk assessment

• Difficulty in applying genetic data for risk assessment due to context (Herfst mSphere 2018) - used 3 phenotypic assays for H5N6 viruses that were associated with transmissibility of H5N1 viruses by the airborne route in ferrets:
  – receptor binding using resialylated RBCs,
  – HA acid stability
  – polymerase activity

• Difficult to get quantitative data from ferret transmission studies

• Virulence and transmissibility may not be linked - in animal models or in people (PISA)
Indicators to describe influenza severity

<table>
<thead>
<tr>
<th>INDICATOR</th>
<th>DESCRIBES</th>
<th>INFLUENCED BY</th>
<th>INFORMED BY</th>
</tr>
</thead>
</table>
| **Transmissibility** | How many people in a population get sick from influenza on a weekly basis | • Ease of movement of virus between individuals (virus shedding, viral replication, and viral binding)  
• Immunity and vaccination status  
• Age, contact patterns and health-seeking behaviour  
• Climatic factors | Routine surveillance parameters                                               |
| **Seriousness of disease** | How severely sick individual people get when infected with the influenza virus | • Virus factors  
• Host factors  
• Context (e.g. access to health care and availability of ventilators) | Hospital-based surveillance parameters                                         |
| **Impact** | How the influenza epidemic or pandemic affects the health-care system (and society) | • Public health interventions  
• Health-care use  
• Public concern | • Hospital-based surveillance  
• Vital statistics (e.g. death records)  
• School and work absenteeism                                                  |

The WHO Collaborating Centre for Reference and Research on Influenza in Melbourne is supported by the Australian Government Department of Health.