Virological characteristics of public health concern (what are we worried about)

Ron Fouchier and David Swayne

2nd FAO-OIE-WHO Joint Scientific Consultation
Influenza and other Emerging Zoonotic Diseases at the Human Animal Interface
"Past Experiences - New Paradigms - For Future Threats"
Verona, Italy, April 2010

The questions:

What do we know about:
1. Markers/motifs/characteristics (e.g. receptor binding preference, antigenic information) that could indicate zoonotic or "HPHI" capacity (e.g. what is stopping the canine H3 virus from infecting people)

2. Mutations important to human pathogenicity/ transmissibility, compared to those important to swine, canine, feline, etc.

3. Gene constellations that are worrisome in terms of emergence and PH impact (i.e. why wasn't the SIV triple reassortant been more zoonotic)
Markers/motifs/characteristics that could indicate zoonotic or "HPHI" capacity


1. Virus binding, fusion and entry

2. Transcription and replication

3. Modulation of innate immune responses

4. Virion release

Common features from:
1918 H1N1
1957 H2N2
1968 H3N2
1997 H5N1
2003 H7N7

Markers/motifs/characteristics that could indicate zoonotic or "HPHI" capacity


1. Virus binding, fusion and entry

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Common features from:
1918 H1N1
1957 H2N2
1968 H3N2
1997 H5N1
2003 H7N7
Only one thing predictable about influenza: it’s unpredictability

**Introduction of Virulence Markers in PB2 of Pandemic Swine-Origin Influenza Virus Does Not Result in Enhanced Virulence or Transmission**


**Adaptive strategies of the influenza virus polymerase for replication in humans**

Andrew Methle and Jennifer A. Doudevsky, July 21, 2016 | PNA | December 15, 2009 | vol. 104 | no. 30

**DOGMA: only H5/H7 are HPAI**

(not true in the lab; why in the field?)

Wood et al H10N5 and H10N4 (1996) and Brugh (1992) H4N8 viruses were highly lethal on IVPI testing, but not on IN testing

Munster et al., submitted
DOGMA: Flu emerging from avian reservoirs, can infect poultry, pigs, horses, marine mammals. Pigs are mixing vessel for infection of humans

BUT:
Is it that simple?

H5N1:
- Poultry
- Wild birds (>100 species)
- Human
- Pig
- Leopard
- Tiger
- Domestic cat
- Owston's palm civet
- Stone marten
- Mink
- Dog
- Pika
- Etc Etc
Problem: genetic (and phenotypic) diversity

DOGMA: $\alpha_{2,3}SA/\alpha_{2,6}SA$ determine host range

Over-simplification: Variation hosts
- Variation tissues
- Variation in sialic acids
- Not absolute
On H5N1 receptor binding; it looks simple and strict

Shinya et al., Nature, 2006
v. Riel et al., Science, 2006

On H5N1 receptor binding; but single aa substitutions cause big changes

Chutinimitkul et al., J Virol 2010
On receptor binding; Binding ≠ replication ≠ transmission

2009 pH1N1 receptor binding; D222G

Observed in 1918 strains of Spanish flu (receptor change; 2,3 SA)

Detected upon passage in eggs (CDC, April-May); avian receptor binding

Detected in severe/fatal cases in NL (June; Groningen, November; Zwolle)
Detected in high proportion of severe/fatal cases in Ukraine (December)

Norway: 11/61 severe cases, 0/205 mild cases (p<0.001)
A. Kilander et al., Eurosurveillance 15 (2010)

Hong Kong: 9/219 severe cases, 0/239 mild cases (p=0.002)
G.C. Mak et al., Eurosurveillance 15 (2010)
2009 pH1N1 receptor binding; D222G
Do animal models tell the truth?

Ferret:
- Weight loss
- Shedding throat
- Shedding nose

Mice:
- Weight loss
- Survival
- Lung titers
  - WT
  - D222G
  - D222E

Predominant receptor on duck colon:
- N-Glycolyl-neuraminic acid: NeuGca2,3Gal
- N-Acetyl-neuraminic acid: NeuAca2,3Gal
2009 pH1N1 receptor binding; D222G

Submucosal glands

WT
D222G

Trachea Bronchus Bronchiole Alveoli Alveolar MΦ

Submucosal glands
Sulfated sialic acid 6-O-Su-3'-SLN?
Chutinimitkul et al, submitted

Receptor binding; glycan arrays

18SC ➔ 222D
18NY ➔ 222G

The questions:

<table>
<thead>
<tr>
<th>What do we know about:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Markers/motifs/characteristics (e.g. receptor binding preference, antigenic information) that could indicate zoonotic or &quot;HPHI&quot; capacity (e.g. what is stopping the canine H3 virus from infecting people)</td>
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<td>We know a lot (HA, NS1, PB2, etc). But flu is not predictable (yet). Behavior in new hosts hard/impossible to predict; continuous threats</td>
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<td>2. Mutations important to human pathogenicity/ transmissibility, compared to those important to swine, canine, feline, etc.</td>
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<td>Pathogenicity is relatively easy to study. Animal models may be imperfect Determinants of transmission still unknown</td>
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<td>3. Gene constellations that are worrisome in terms of emergence and PH impact (i.e. why wasn't the SIV triple reassortant more zoonotic)</td>
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<td>Genotype-phenotype predictions are hard (context dependence) SIVs have been zoonotic throughout history (but transmission…..)</td>
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Future directions

| 1. Continue studies on markers/motifs/characteristics of zoonotic/HPHI influenza; we know a lot, but knowledge is still incomplete |
| 2. We know flu is zoonotic; but focus on transmission. |
| 3. Receptor binding studies need impulse; which receptors are where, in which species? Role in binding vs. replication vs. transmission. Biological relevance of array glycans unknown. |
| 4. Link genotypes to phenotypes. The future is in sequencing, but only if we can infer phenotypes. |

Challenge the dogma’s (Further) integrate veterinary-human influenza research communities

Break international barriers to facilitate research; role for WHO/FAO/OIE