What have we learned from H5N1?

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H5N1 is the first HPAI virus

• Which has persisted for over 13 years
• For which extensive vaccination has been applied
• Has spread to three continents
• Has infected an unpredictable number of animal species, including humans
• Is evolving into lineages and sublineages
• Has been mentioned by most political leaders of the world
Review of H5N1 1997-2010

What was expected?

What was a surprise

What was expected and what was a “surprise” on.....

- Persistence and circulation in the animal reservoir
- Host range and host-adaptive mutations
- Evolution and reassortment
- Antigenic variation
- Risks for human infection
Things we expected could influence persistence

Host range – expected

- Birds: wide range of orders (14) infected by H5N1 viruses, over 130 species

- Extensive circulation in birds facilitates the perpetuation of H5N1 in poultry, which is the main source of infection to non-avian species

- Infection in animals may also occur via swill-feeding/predation other species, usually through carcasses of infected birds
This was a surprise

<table>
<thead>
<tr>
<th>Species</th>
<th>European Union - Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2006</td>
</tr>
<tr>
<td>Podiceps nigricollis</td>
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</tr>
<tr>
<td>Podiceps cristatus</td>
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</tr>
<tr>
<td>Aythya sp.</td>
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</tr>
<tr>
<td>Tachybaptus ruficollis</td>
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</tr>
<tr>
<td>Cygnus olor</td>
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</tr>
<tr>
<td>Buteo buteo</td>
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</tr>
<tr>
<td>Larus sp.</td>
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</tr>
<tr>
<td>Ciconia ciconia</td>
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</tr>
<tr>
<td>Cygnus cygnus</td>
<td>44</td>
</tr>
<tr>
<td>Cygnus sp.</td>
<td>4</td>
</tr>
<tr>
<td>Fulica atra</td>
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<tr>
<td>Branta canadensis</td>
<td>2</td>
</tr>
<tr>
<td>Anser anser</td>
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</tr>
<tr>
<td>Anas platyrhynchos</td>
<td>34</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>478</strong></td>
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</tbody>
</table>

"Wild birds" – difference in clinical signs and implications for surveillance

Lethal for some species – “passive surveillance”

Non-lethal for some species – “active surveillance”

Tracheal vs cloacal shedding
Host range – unexpected

Natural infections in:
- **Humans**: Hong Kong 1997, first report of fatal infection in humans with avian viruses
- **Carnivores**: tigers and leopards in Asia (Thailand) (Amosin et al. 2006, Thiry et al. 2007).
- **Domestic cats**: in Asia and Europe (Songserm et al. 2006, Mittelheiter et al. 2006).
- **Asian palm civets**: Vietnam (Robertson et al. 2006).
- **Dogs**: Thailand (Songersen 2006; Beeler et al. 2009).
- **Pigs**: China 2001 and 2003 (Fujian province) (reviewed by Neumann & Kawaoka 2010).
- **Stone marten**: Germany 2007 (Kopfleish et al. 2007).
- **Donkey**: Egypt 2010 (Moneim et al., 2010).

Experimental infection in mice (Gao et al. 2007), ferrets (Zitzow et al., 2002), monkeys (Kuiken et al. 2003) and cattle (Kalthoff et al., 2008).

Host range – unexpected

- Very limited natural infection in pigs
H5N1 host-adaptive mutations and pathogenicity

1. Changes in the receptor specificity (HA): introduction of the human type residues at position 226 and 228 of HA confers the ability to recognize alpha 2-6 (human influenza receptors) in addition to alpha 2-3 (avian influenza receptors) sialic acid receptors to H5N1 viruses (reviewed by Neumann & Kawaoka 2010).

2. Changes in the polymerase complex (i.e. PB1 and PB2 genes): adaptation of the virus in mammals (enhancement of viral polymerase activity).

• Glu627Lys in PB2: found in most mammalian isolates, in Qinghai lake descendents, and in isolates from ostriches (!) – recognized as a major determinant for H5N1 HPAIV pathogenicity in mice (Hatta et al. 2001) and as a major determinant of efficient replication of H5N1 viruses in the mammalian respiratory tract (Hatta et al. 2007).

• Asp701Ans in PB2: associated with virulence in mice, found in some human virus isolates.

3. NS1 molecular determinants of pathogenicity:

✓ PDZ ligand domain at the C-terminus (ESEV), E227K mutation modulate viral pathogenicity in the mouse model (Jackson et al., 2008).

✓ PB1-F2

✓ a proapoptotic factor (through the binding of mithochondrial membrane)

✓ a Ser at position 99 of PB1-F2 confers high pathogenicity to H5N1 in mice (Conenello et al., 2007).

Reassortment dynamics - expected

Since its emergence in 1996, Gs/GD virus lineage underwent extensive genetic reassortment with avian viruses from terrestrial and aquatic birds, to generate many different reassortant viruses (or genotypes) between 1997 and 2007 and producing both persistent (such as genotypes Z and V) and transient genotypes (Zhao et al., 2008).
Reassortment dynamics - unexpected

- Intra-H5N1 reassortment is a common event, (Nigeria, China, Indonesia, Vietnam, Japan, Thailand) and generate viruses that may become dominant and replace parental strains.
- Only two inter-subtypic reassortment events (both in China)

For comparison – H9N2

Phylogenetic relationships of the NS genes of H9N2 avian influenza viruses isolated from poultry in Pakistan.

NS gene sequences were compared with closely related H5, H7 and H9 viruses. **Blue arrow indicate H9N2 virus from Pakistan containing the NS gene similar to highly pathogenic H7N3**, while **red arrow** indicate the H9N2 virus from Pakistan containing the **NS gene similar to highly pathogenic H5N1** (Iqbal et al., 2009).
Why doesn’t it reassort successfully with other subtypes, but only with other H5N1 viruses?

- Viral fitness?
- Site of replication (trachea/vs cloaca)?
Antigenic diversity and evolution

- Expected: many examples of drift in other species
- Never occurred before with a HPAI virus
- Implementation of extensive vaccination campaigns

INDONESIA vs EGYPT

<table>
<thead>
<tr>
<th>Introduction of only one clade:</th>
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<tbody>
<tr>
<td>Egypt: clade 2.2.1 (since 2005)</td>
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<tr>
<td>Indonesia: clade 2.1 (since 2003)</td>
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The clade has evolved locally creating new sublineages both in Egypt and Indonesia. Given the geographic characteristic of Indonesia and the long period of circulation of the H5N1 viruses (since December 2003), the genetic evolution of the strain into distinct subclades (Takano et al., 2009) is more evident in this country compared to Egypt.

The virus is endemic both in Indonesia and Egypt and continued to cause sporadic zoonotic transmission to humans:
- Indonesia: 163 cases since 2005
- Egypt: 109 cases since 2006

Indonesian clade has never been detected outside the country (Takano et al., 2009). Egyptian clade has been detected only in Israel and Gaza (Cattoli et al., 2009)

Vaccination has been applied in both countries
Is it relevant?

• Animal health – only in countries that vaccinate (as updating of vaccines is necessary)
• Public health – **YES** for pre-pandemic vaccine preparedness
  – Indonesia: 163 cases since 2005
  – Egypt: 109 cases since 2006

Human exposure - expected

• Direct and indirect contact with: sick birds, healthy (presumably infected!) birds, live bird (wet)markets
Human exposure - unexpected

• No (documented) case of human infection linked to contact of humans with other animals (e.g., cats and dogs)

HUMAN CASES OF AVIAN INFLUENZA H5N1

Genetic sequences of human H5N1 viruses isolated in China, Vietnam, Indonesia and Egypt are closely related to poultry isolates, suggesting that H5N1 human infection is directly associated with outbreaks in poultry.

The case fatality rate of HPAI H5N1 viruses in humans is more than 50% (Neumann et al., 2009).

In total (data until 16 April 2010) 493 human infections with 292 fatalities were reportedly caused by HPAI H5N1, the highest number of human fatal infections occurring in Indonesia (135), Viet Nam (59), Egypt (34), China (25) and Thailand (17) (WHO timeline of major events; cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO).

HA sequences of HPAI H5N1 detected in humans belonged to 4 of the 10 described H5N1 clades (Babakir-Mina et al., 2009):

- clade 0: Hong Kong, 1997
- clade 1: Thailand and Vietnam, 2003-2005
- clade 2: subclade 2.1: Indonesia, since 2005
  - subclade 2.2: Africa and Middle East, since 2006
  - subclade 2.3.4: China and Vietnam, since 2005
- clade 7: only 1 sequence from China, 2007

The clades detected in humans were clades extensively circulating in poultry in those countries.
In conclusion

• Very wide host range
• Not likely to reassort with other subtypes
• Likely to reassort within H5N1
• Only handful of genetic markers of pathogenicity known
• Antigenic variation relevant in countries that vaccinate poultry for AH and PH prevention/control strategies
• Human exposure caused by contact with birds

Expected

• Coordinated interventions are necessary in the animal reservoir to reduce the risk of human infection
Unexpected

H5N1, a “rare” disease of poultry until 2000, would be able to draw more resources and attention than any other contemporary animal or zoonotic disease.