Highly pathogenic avian influenza H5N1
How well are we doing?

HPAI outbreaks by continent: excludes Indonesia

Human cases by country over time

FAO EMPRES
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Countries affected</th>
<th>Disease</th>
<th>Cases / deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7N7, H7N2, H7N3</td>
<td>USA, UK, Canada, Netherlands</td>
<td>Conjunctivitis, ILI, ARDS/Death</td>
<td>99 cases / 1 death</td>
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<tr>
<td>H9N2</td>
<td>Hong Kong, S. China</td>
<td>ILI</td>
<td>12 cases / 0 deaths</td>
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<tr>
<td>H5N1</td>
<td>15 countries</td>
<td>ILI, LRI, ARDS</td>
<td>518 cases / 306 deaths (CFR 59%)</td>
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</tbody>
</table>
Humans and Chicken

H5N1 Gs/GD/96-like

H9N2 G1-like

H6N1 W312-like

PB2
PB1
PA
HA
NP
NA
M
NS

PB2
PB1
PA
HA
NP
NA
M
NS

PB2
PB1
PA
HA
NP
NA
M
NS

Xu, X et al., *Virology*, 1999
Guan, Y et al., *PNAS*, 1999
Hoffmann, E et al., *J. Virol.* 2000

Humans and Chicken

Not detected after 1997
H5 HA

What is driving antigenic diversity of HPAI H5N1?

Duan L et al JVirol 07
H5N1: crossing species barriers

Genetic markers of virulence and transmissibility?
Haemagglutinin

- Connecting peptide -RRKKR-
  - Cleavability by ubiquitous proteases → dissemination
  - Associate with virulence in mice *(Hatta et al 2001)*

- Receptor binding: α2-3 → α2-6
  - Increased 2-6 binding: Asn182Lys; Gln192Arg (Seen in some human isolates from Vietnam, Azerbaijan, Iraq). *(Yamada et al 2006)*
  - HA A134T affects receptor binding and virulence in ferrets *(Imai et al 2010)*

**Antigenic variation:**
- Need to update human vaccine candidates
- Challenge for animal vaccines as well?
PB2

- **Glu627Lys:**
  - associated with mammalian adaptation and virulence (Subbarao et al 1993; Hatta et al 2001)
  - Replication at lower temp in respiratory tract epithelium (Massin et al 2001)
  - In avian H5N1: Not typically seen, except for clade 2.2
  - In human H5N1:
    - Clade 2.2 (with 627Lys): no increase in virulence, in fact apparently lower mortality rates (e.g. Egypt)?
    - Seen in some human isolates of non-clade 2.2 viruses (De Jong et al 2006, Le et al 2010)
  - Other mammals (e.g. Tigers) (Amonsin et al 2006)

627Glu disrupts basic surface patch on PB2
PB2

- **Asp701Asn:**
  - Seen in some human strains, an alternative adaptation to 627Lys (*De Jong et al. 2006, Le et al. 2010*)
  - Increased mouse virulence (*Li et al. 2005*)

- **591Lys or Arg:**
  - Compensates for lack of 627Lys in mammalian adaptation (*Yamada et al. 2010*)
  - 591: Structural proximity to 627
  - (Pandemic H1N1 does not have 627Lys but has 591Arg)
NS1

- Interferon antagonist, interacts with RIG-I, inhibits TRIM-25 mediated RIG-I CARD ubiquitination
- Asp92Glu (Seo et al 2002):
  - increases H5N1 resistance to IFN
  - Increased virulence for pigs
- C terminal PDZ ligand domain X-S/T-X-V
  - Associated with mouse virulence, independent of IFN antagonism (Obenauer et al 2006; Jackson et al 2008).
  - 15nt deletion (263-277) affects pathogenicity for mice and chicken (Long et al 2008). Found in two virus isolates from pigs (Zhu et al 2008)

PB1-F2

- Localises to mitochondria and causes apoptosis.
  - PB1-F2 66Ser associated with high pathogenicity in mice. Conenello et al 2007
Transmissibility in ferrets

H5N1 virus or reassortants of H5N1xH3N2

- No evidence of aerosol Tx
  - Maines et al 2006
  - Yen et al 2007
  - Jackson et al 2009
Reassortment between avian H5N1 and human seasonal influenza: retain mouse virulence

<table>
<thead>
<tr>
<th>Parental origin of genes in reassortant viruses</th>
<th>Virus</th>
<th>$LD_{50}$</th>
<th>$MID_{50}$</th>
<th>Weight Loss (%)</th>
<th>MST</th>
<th>Virus titer</th>
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<tr>
<td>TH04 WT</td>
<td>1.8</td>
<td>1.5</td>
<td>19.8</td>
<td>6</td>
<td>7.1±0.8</td>
<td>1.9±1.2</td>
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<tr>
<td>r6(H5N2)</td>
<td>2.2</td>
<td>1.4</td>
<td>≥ 25.0</td>
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<td>8.7±1.1</td>
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<tr>
<td>r1/3/5/7/8</td>
<td>2.5</td>
<td>1</td>
<td>17.2</td>
<td>7</td>
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<td>r1/3/7</td>
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<td>1.5</td>
<td>21.8</td>
<td>7</td>
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<td>WY03 WT</td>
<td>&gt; 6.0</td>
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<td>0</td>
<td>&gt;14</td>
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</table>

63 reassortants rescued by reverse genetics
Some with avian H5 and N1 have high lethality in mice

Chen et al 2008
Reassortment by co-infection of H5N1 x pH1N1

- M2 defective viruses grown in MDCK cells stably expressing M2
- 33 different “genotypes”
- 15% parent H5N1; 85% reassortants
- Some reassortants have better growth kinetics that parental viruses in humal lung epithelial cell lines
- Compatibility in gene segments between the two viruses

Octaviani et al 2010
H5N1 infection in swine

- Low sero-prevalance in swine (clade 1) (*Choi et al 2004*)
- Experimental infection: leads to infection but not transmission (clade 1) (*Choi et al 2004, Lipatov 2008*)
- Swine H5N1 from China (*Zhu et al 2008*).
- Indonesia: (*Nidom et al EID 2010*)
  - isolation rates: ranged from 0-61% in different farms and years.
  - NT serology done in one year, though very low titres (4-16).
  - Swine viruses from diverse geographical regions seem very closely related. (e.g. E Java, N Sumatra, Banten in 2006). Swine viruses are being shipped across country in swine?
  - Most swine viruses bind \(\alpha_2-3SA\), but one isolate have dual binding to both \(\alpha_2-3\) and \(\alpha_2-6SA\) and associated with change HA Ala134Ser. (NB Ser134 is never seen in avian viruses)

- Evidence of pdmH1N1 infecting pigs and reassortment with other swine viruses e.g, *Vijaykrishna et al 2010*
Questions

- Virus evolution and antigenic change
  - Drivers of genetic reassortment, virus fitness, antigenic change

- Control the panzootic in poultry
  - Epidemiological drivers of its maintenance in poultry
  - Critical control points
  - Vaccines / vaccine escape
  - Food safety
  - Wild birds: victim or vector (e.g. clade 2.2; clade 2.3.2)

- Transmissibility in humans:
  - What are virus genetic and host determinants of transmissibility?
  - Paradox: high exposure / low infection rate, but family clusters / high disease severity: Host-genetics? Host resistance?
  - Better sero-epidemiology tools

- Risk-behaviors and perceptions, KAP studies, changing behavior
  - (Ly et al 2007; Fielding et al 2007)

- Environmental virus contamination / stability
  - Vong et al 2008; Indirani et al 2010

- Pathogenesis / disease severity in humans?
  - Role of virus and host response
  - Will human transmissibility be associated with loss of virulence? How complete?
  - Animal models need to mimic human pathology (neuro-tropism vs lung pathology)