Influenza virus infectivity, pathogenicity, and transmissibility in humans

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Avian influenza A virus
- Adaptation to humans -

- Known mutations affecting infectivity and pathogenicity in humans
- Effects of these mutations on infectivity, pathogenicity and transmissibility
- Interplay between mutations
- Antiviral resistance mutations
- What are the effects of molecular/antigenic structure, strain, HP/LPAI status, virus dose, and other properties on infectivity, pathogenicity, transmission
- Contrast H5N1 and H7N7
Avian influenza A virus
- Adaptation -


**HA**
1. Virus binding, fusion and entry
2. Transcription and replication

**NS1**
3. Modulation of innate immune responses

**PB1, PB2, PA, NP**
4. Virion release

Avian influenza A H5N1 virus
- HA: Receptor specificity -

Shinya et al., Nature 440, 2006
Van Riel et al., Science 312, 2006
Van Riel et al., Am J Pathol 171, 2007
Influenza A virus
- Host differences in sialic acid receptors -

Simplification:
- Variation hosts
- Variation tissues
- Variation in sialic acids
- Not absolute

Avian influenza A H5N1 virus
- HA: Receptor specificity -

<table>
<thead>
<tr>
<th>Amino acid change</th>
<th>H5N1 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamada et al. Nature 2006</td>
<td></td>
</tr>
<tr>
<td>Q192R</td>
<td>A/Vietnam/2006/04cl0ne3</td>
</tr>
<tr>
<td>G222N</td>
<td>A/Thailand/01/2004R</td>
</tr>
<tr>
<td>G192R G222N</td>
<td>A/Vietnam/2004/04cl0ne7</td>
</tr>
<tr>
<td>N182K</td>
<td></td>
</tr>
<tr>
<td>G192R N182K</td>
<td></td>
</tr>
<tr>
<td>Stevens et al. Science 2006</td>
<td></td>
</tr>
<tr>
<td>Q229H</td>
<td>A/Vietnam/1/2004</td>
</tr>
<tr>
<td>G229S</td>
<td></td>
</tr>
<tr>
<td>G228L G228S</td>
<td></td>
</tr>
<tr>
<td>E190D</td>
<td></td>
</tr>
<tr>
<td>G228D</td>
<td></td>
</tr>
<tr>
<td>E192D G225D</td>
<td></td>
</tr>
<tr>
<td>Gambaryan et al. Virology 2006</td>
<td></td>
</tr>
<tr>
<td>S227N</td>
<td>A/Hong Kong/212/03</td>
</tr>
<tr>
<td>A134V</td>
<td>A/Hong Kong/212/03</td>
</tr>
<tr>
<td>L128V</td>
<td>A/Thailand/01(KAN-1)/04</td>
</tr>
<tr>
<td>Yang et al. Science 2007</td>
<td></td>
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<tr>
<td>S177A</td>
<td>A/Vietnam/1/2004</td>
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<tr>
<td>T193</td>
<td>A/Vietnam/1/2004</td>
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<td>S177A T193</td>
<td>A/Thailand/01(KAN-1)/04</td>
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<tr>
<td>K227E</td>
<td>A/Thailand/01(KAN-1)/04</td>
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<tr>
<td>Iyaghima et al. Plos Path 2008</td>
<td></td>
</tr>
<tr>
<td>N185S</td>
<td>A/Vietnam/1/2004</td>
</tr>
</tbody>
</table>
Avian HPAI H7N7 virus (NL)
- Polymerase activity in human cells -

PB2 E627K increased expression, independent of temperature. PA has an additive effect.

Avian HPAI H5N1 virus
- Polymerase activity -

Hatta et al., PLoS Pathogens 2007
**Influenza A virus**
- Replication temperature & host cell factors; RNPs -

- Intestinal tract 41°C
- Upper respiratory tract 33°C

De Wit, Munster et al., In preparation

**Avian influenza A virus**
- Modulation of host (innate) immune responses -

NS1: determinant of pathogenicity in mice, ferrets, chicken, human?

Glu-92: resistance to TNF, IFNs (pigs) (Seo et al., 2002, 2004)
Ser-42: IFN antagonism (mice) (Jiao et al., 2008)
Ala-149: IFN antagonism (chicken) (Li et al., 2006)
Phe-103 & Met-106: mRNA processing (Twu et al., 2007)
C-term PDZ: pathogenicity, not IFN (mice) (Jackson et al., 2008)

PB1-F2: determinant of pathogenicity in mice?
Ser-66: replication, pathogenicity, cytokine secretion (Conenello et al., 2007)
Avian HPAI H5N1 virus
- NA; stalk deletion -

19-25 amino acids deletion in stalk region of Neuraminidase of H5N1 influenza A virus may be required for the adaptation of influenza viruses from wild aquatic birds to domestic chickens (Matrosovich et al., 1999 J Virol 73:1446-55)

Influenza A H7N7 virus
- Determinants of infectivity in human cells -

<table>
<thead>
<tr>
<th>Virus</th>
<th># amino acid substitutions in Gene Segment</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Netherlands/219/03</td>
<td>A/Netherlands/219/03 (FC)</td>
<td></td>
</tr>
<tr>
<td>1 (PB2)</td>
<td>5 (S701V297I,R355K,Q563R,E627K)</td>
<td>Replication</td>
</tr>
<tr>
<td>2 (PB1)</td>
<td>1 (F666L)</td>
<td></td>
</tr>
<tr>
<td>3 (PA)</td>
<td>3 (I335S,A143T,K416R)</td>
<td>Replication</td>
</tr>
<tr>
<td>4 (HA)</td>
<td></td>
<td>Receptor binding</td>
</tr>
<tr>
<td>5 (NP)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6 (NA)</td>
<td>4 (N308S,A346V,T442A,P458S)</td>
<td>Particle release</td>
</tr>
<tr>
<td>7 (MA)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8 (NS)</td>
<td>1 (V137I)</td>
<td></td>
</tr>
</tbody>
</table>

Sequence as compared to A/Chicken/Netherlands/1/03
### Influenza A H5N1 virus
- Cynomolgus macaques -

<table>
<thead>
<tr>
<th>#2 Lung, HE</th>
<th>#2 Lung, αNP</th>
<th>#2 Bronchus, HE</th>
<th>#2 Bronchus, αNP</th>
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</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>


### Influenza A H5N1 virus
- Pathogenesis in Cynomolgus macaques -

- Severe necrotizing broncho-interstitial pneumonia
- Necrotic lesions in kidney, liver, spleen and lymph nodes
- High virus titers in respiratory tract
- No evidence for virus replication in heart, brain, spleen (virus titration, immunohistochemistry)

ARDS & MODS may be caused by severe lesions and virus replication in lungs alone

Avian influenza A (H5N1) in felids
- Experimental infection of domestic cats -


Avian influenza A (H5N1) virus
- Extra-respiratory spread in man? -

Cats
- Ganglioneuritis in intestines
- Encephalitis, virus in brains
- Hepatitis in cats

Humans
- Diarrhea, virus in feces
- Encephalitis, virus in CSF
- High liver enzymes

Fatal Avian Influenza A (H5N1) in a Child Presenting with Diarrhea Followed by Coma

Merce H. de Jueng, M.D., Ph.D., Bach Van Gare, M.D., Phan To Quy, M.D.,
Vo Minh Han, M.D., Trat Tan Thanh, M.Sc., Nguyen Bach Hua, M.D.,
Manuel Held, Ph.D., Le Thi Phuong, M.D., Pham Hoa Vanh, M.D.,
Nguyen Van Yen-Chau, M.D., Tran Tinh Hien, M.D., Du Quang Hu, M.D., Ph.D.,
and Henning Kruse, M.D., Ph.D.
H7N7 in The Netherlands
- Pathogenesis in mice; fatal case vs. conjunctivitis

![Graph showing bodyweight loss and survival rates over days after infection for Conjunctivis virus and Fatal case virus](Munster, de Wit, et al. J. Inf. Dis. (2007))

Human influenza A H7N7 virus pathogenicity
- Pathogenesis in mice; PB2 E627K

![Graphs comparing bodyweight and survival rates for various virus combinations](Munster, de Wit, et al. J. Inf. Dis. (2007))
Human influenza A H7N7 virus pathogenicity
- Systemic replication in mice: PB2 and HA -

Black: wildtype viruses
A: Conjunctivitis case (CC)
B: Fatal case (FC)

Gray: reassortants
C: CC+PB2 of FC
D: CC+PA of FC
E: CC+HA of FC
F: CC+NA of FC
G: CC+NS of FC

White: point mutants
H: CC + PB2 E627K
I: FC + PB2 K627E


Human influenza A H7N7 virus pathogenicity
- Pathology in mice -

Human influenza A H5N1 virus pathogenicity
- virulence in mice -

Avian influenza A virus
- Transmission models -

Lack of transmission of H5N1 avian–human reassortant influenza viruses in a ferret model

Contemporary North American influenza H7 viruses possess human receptor specificity: Implications for virus transmissibility

The guinea pig as a transmission model for human influenza viruses
Avian influenza A virus
- Transmission models -

A million ways to destroy a car  Only one way to build one, properly

Avian influenza A H5N1 virus
- Neuraminidase inhibitor resistance -

![Graph showing complementary DNA levels over days since admission](image)
Influenza A H1N1 virus
- Neuraminidase inhibitor resistance -

- Lackenby et al., Euro Surveill. 2008

Avian influenza A H5N1 virus
- Neuraminidase inhibitor resistance -

- Yen et al., J. Virol. 2007
A/H5N1 virus
- Genetic variation -

Source: www.who.int
www.offlu.net

Avian influenza
- Transmissibility of H7N7 versus H5N1? -

<table>
<thead>
<tr>
<th>Year</th>
<th>Subtype</th>
<th>Location</th>
<th>No. confirmed cases</th>
<th>Illness</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>H7N7(H4)</td>
<td>USA</td>
<td>3 (0)</td>
<td>Conjunctivitis</td>
<td>[2]</td>
</tr>
<tr>
<td>1997</td>
<td>H7N7(L)</td>
<td>UK</td>
<td>1 (0)</td>
<td>Conjunctivitis</td>
<td>[4]</td>
</tr>
<tr>
<td>1999</td>
<td>H5N1(L)</td>
<td>Hong Kong</td>
<td>2 (0)</td>
<td>EFLP</td>
<td>[26]</td>
</tr>
<tr>
<td>2003</td>
<td>H7N7(H4)</td>
<td>Netherlands</td>
<td>89 (1)</td>
<td>Conjunctivitis, ILI, pneumonia</td>
<td>[8,9]</td>
</tr>
<tr>
<td>2004</td>
<td>H5N1(L)</td>
<td>Egypt</td>
<td>2 (0)</td>
<td>ILI</td>
<td>[10]</td>
</tr>
<tr>
<td>2004</td>
<td>H7N3(H3)</td>
<td>Canada</td>
<td>2 (0)</td>
<td>Conjunctivitis, ILI</td>
<td>[2]</td>
</tr>
<tr>
<td>2006</td>
<td>H5N1(H4)</td>
<td>USA</td>
<td>1 (0)</td>
<td>ILI</td>
<td>[13]</td>
</tr>
<tr>
<td>2007</td>
<td>H7N3(L)</td>
<td>UK</td>
<td>4 (0)</td>
<td>Conjunctivitis, ILI</td>
<td>[5]</td>
</tr>
<tr>
<td>1997-2006</td>
<td>H5N1 (H6)</td>
<td>Eastern hemisphere</td>
<td>400 (247)</td>
<td>ILI, pneumonia, diarrhea, encephalopathy, etc.</td>
<td>[16,13-17]</td>
</tr>
</tbody>
</table>

De Wit et al., Vaccine (2008)

Olofsson et al., Lancet Inf Dis (2005)
Avian influenza virus adaptation
- interplay between genes and gene products -

Avian influenza A virus
- Infectivity, pathogenicity, transmissibility in humans

- Determinants of infectivity & pathogenicity of avian influenza viruses
  - HA/NA; host cell receptors
  - Polymerase genes; body temp & cellular factors
  - NS; innate immunity
- Multiple mutations, in several genes, some may act in concert
- Pathogenicity often linked to efficient replication, sometimes other factors
- Pathogenicity may vary in different models (humans?)
- Basic cleavage site is a determinant of infectivity/pathogenicity in mammals
- No good clues to determinants of transmission;
  - Animal models available
  - Multiple gene products in right conformation needed
- Strain & dose-dependent differences in infectivity, pathogenicity, transmission?
- Mutations affecting neuraminidase inhibitor resistance may occur without loss of infectivity or pathogenicity in animal models
Avian influenza A virus
- Infectivity, pathogenicity, transmissibility in humans

Future work:
- Receptor studies HA and NA, with focus on natural receptors
- Glycan arrays; integrate with biology
- Other determinants of infectivity, pathogenicity (consider H5N1 lineages)
- Pathogenesis in humans
- Pathogenesis in mammalian models (multiple)
- Host-specific factors affecting replication (e.g. polymerase complex)
- Transmission studies (ferrets and guinea pigs)
- Alternatives for antivirals in outbreak management

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