Molecular Epidemiology of HPAI H5N1: Recent Update
Innovative Tools for the Analysis of Animal Health Surveillance Systems, 16-17 December 2010, Bangkok, Thailand

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Emergency Center for Transboundary Animal Diseases (ECTAD)
FAO Regional Office for Asia and the Pacific (RAP)
Acknowledgement

• National Animal Health Authorities
• International Partners
• FAO Officers
Regional Laboratory Network for Animal Influenza Diagnosis

- Regional Reference Laboratories
- Key Laboratories
- Network Laboratories
Molecular Epidemiology

• Utilizing molecular technique in epidemiology
  – Virus identification
    • polymerase chain reaction
  – Virus tracking/tracing
    • Combining genetic information with field epidemiology
  – Virus evolution and phylogenetic study
    • Monitoring changes of virus characteristic
    • Virulence factors: cleavage site, recognition of host cell receptor
H5N1 Nomenclature System
(WHO/OIE/FAO H5N1 Evolution Working Group, 2008)

• HA lineage provides a constant by which H5N1 strains can be effectively compared

• Criteria for clade designation
  – Based on phylogenetic tree topology derived from all available sequences
  – Distinct common node shared by at least 4 isolates
  – Distinct clades should have > 1.5% average distances between other clades

(Li et al., 2010)
HA clade differentiation by real-time PCR

- Real-time PCR for HA clade differentiation was developed recently.
- This could be conveniently used for quick identification of HA clade as soon as virus is detected/isolated before sending out the viruses for sequencing.

<table>
<thead>
<tr>
<th>HA clade</th>
<th>Virus code</th>
<th>Primers and probe for</th>
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<tbody>
<tr>
<td></td>
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<td>Diagnosis</td>
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(Ken Inui, FAOVN)
## Recent H5N1 HA Clades in South and Southeast Asia

<table>
<thead>
<tr>
<th>Clade</th>
<th>Description</th>
<th>China</th>
<th>Cambodia</th>
<th>Indonesia</th>
<th>Laos PDR</th>
<th>Malaysia</th>
<th>Myanmar</th>
<th>Thailand</th>
<th>Vietnam</th>
<th>South Asia</th>
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<tbody>
<tr>
<td>0</td>
<td>early progenitors; predominately 1996-2002 from Hong Kong (HK) and China (mostly avian, few human)</td>
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<td>3</td>
<td>2000-2001 from HK, China, Vietnam (all avian)</td>
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<td>4</td>
<td>2002/2003 lineage from HK and China; 2005/2006 from Guiyang Prov. (all avian)</td>
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<td>2000-2003 from China and Vietnam; 2004 lineage from Guangxi Province (all avian)</td>
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<td>2002/2004 from China; 2005/2006 from Yunnan, Hebei, Shanxi Provinces (all avian)</td>
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<td>2001-2004 from HK and China (all avian)</td>
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<td>2003-2005 from China (all avian)</td>
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<td>2002/2003 progenitors from HK; 2003-2006 from SE Asia (mixed A/H)</td>
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<td>2.1</td>
<td>2003-2007 from Indonesia (mixed avian/human) comprises at least three 3rd order subclades</td>
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<td>2.2</td>
<td>2005 progenitors from Qinghai Lake outbreak and Mongolia; 2005-2007 isolates from Eastern and Western Europe, the Middle East, and Africa (mixed avian/human)</td>
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<td>2.3</td>
<td>2003-2006 from China, HK, Vietnam, Thailand, Laos, and Malaysia (mixed avian/human)</td>
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<td>2.3.4</td>
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<td>2.4</td>
<td>2002-2005 from China (predominately Yunnan and Guangxi Provinces) (all avian)</td>
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<td>2.5</td>
<td>2003/2004 from Korea, Japan, China; 2006 lineage from Shantou Prov. (all avian)</td>
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</table>

(Mia Kim, AGAH)
2008
2010
Virus Tracking Summary 2010

- **Clade 2.1**
  - Localized in Indonesia

- **Clade 2.2**
  - Disappeared from China
  - Localized in South Asia

- **Clade 1**
  - Localized in Cambodia and Viet Nam

- **Clade 7, 4, 9**
  - Disappeared from the region

- **Clade 2.3.2**
  - Spreading into Viet Nam and Myanmar
  - Also found in Russia, S.Korea, Japan, Mongolia, Nepal, Israel, Romania and Bulgaria

- **Clade 2.3.4**
  - Localized in Viet Nam and Myanmar
  - Circulating back to China
The major HA clade of H5N1 viruses in the south of Vietnam stays as clade 1 since 2004, but the viruses are evolving.

(Ken Inui, FAOVN)
The major HA clade of H5N1 viruses in the north of Vietnam stays as clade 2-3-4 since 2007. There seems to be an introduction of new 2-3-4 virus (group B) in 2009.
Clade 2-3-2 virus was found in North Vietnam in early 2010, which is close to the recent isolates of Mongolia and Hong Kong.
Phylogeny of H5N1 isolates based on HA cds; NJ tree with 1000x bootstrapping

Root: A/goose/GD/1996

(Filip Claes, AGAH)
Recognition of Host Cell Receptor

- Human and avian influenza A viruses recognize different host cell receptor
- Mutation in HA gene can enable avian H5N1 virus to recognize human host cell receptor
  - Q192R, N182K, G139R, S227N, G228S, Q226L
- No evidence of HA mutation which may affect recognition of host cell receptor

(Stevens et al., 2006; Yamada et al., 2006)
**Virulence Determinants**

<table>
<thead>
<tr>
<th>Sequence at the HA Cleavage Site</th>
<th>CLADE</th>
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<tbody>
<tr>
<td>PQRERRRKKR/G</td>
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<tr>
<td>PQRESRRKKR/G</td>
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<tr>
<td>PQGERRRKKR/G</td>
<td>2.2</td>
</tr>
<tr>
<td>PQRERRRKR_R/G</td>
<td>2.3.2</td>
</tr>
<tr>
<td>PLRERRRRRK/G</td>
<td>2.3.4</td>
</tr>
<tr>
<td>PLREKRRRK/G</td>
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</tr>
</tbody>
</table>

- Hemagglutinin (HA) cleavage site
  - LPAI carry monobasic motif susceptible to proteases which confine replication
  - HPAI carry polybasic motif which support systemic replication

- Recent publication suggested additional determinants (Bogs et al., 2010)
- Available H5N1 isolates from 2010 posses polybasic HA cleavage site

Remark: K – Lysine, R – Arginine, S – Serine
Clade 2.3.2 HA residue analysis
HPAI in Myanmar
Molecular Surveillance

Purpose
The main priorities of molecular surveillance for HPAI are to:

• Detect outbreaks
• Detect the origin/movement pathways of viruses
• Identify changes in viral antigenicity that will reduce vaccine or diagnostic test efficacy
• Gather samples and data for broader study of influenza virus ecology
Case ID
H5, Human
02/02/2011
Location
Clinical signs

Case ID
H5, duck
28/01/2011
Location
Clinical signs

Case ID
H5, Backyard poultry
31/01/2011
Location
Clinical signs
Linking Field Epidemiology and Genetic Information

(Filip Claes, AGAH)
Conclusion

• In some areas, similar clade of H5N1 is constantly found. New clade replaced old ones in some areas

• H5N1 is still evolving.
  – The progeny viruses still retain similar characteristic i.e. virulence factor
  – May impact vaccine efficacy

• Close collaboration among national authorities and international organization is essential for monitoring of the virus distribution and characteristic