Outbreak of Influenza A virus 
(H5N1) in Bhutan

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• Prior to the introduction into Bhutan, HPAI H5N1 virus was having a 
  major impact on the poultry sector in the neighboring countries in 
  South Asia.
• During early 2006, H5N1 virus infection in poultry was reported for 
  the first time in India and Pakistan (February 2006) and Myanmar 
  and Afghanistan (March 2006).
• Bangladesh and Nepal reported their first H5N1 infection in poultry 
  during March 2007 and January 2009, respectively.
• Reoccurrence of the disease was reported in Afghanistan (2007 and 
  2009), Bangladesh (2007 - continuing), India (2007, 2008, 2009 and 
  early 2010), Pakistan (2007 and 2008) and Nepal (early 2010).
• Bhutan reported the first HPAI H5N1 infection in free-range village 
  chickens in Chukha district on 25 February 2010.

Timeline of H5N1 Avian Influenza outbreaks in 
South Asia and neighboring countries

<table>
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<tr>
<th>Year</th>
<th>Country</th>
<th>Date</th>
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<tr>
<td>2005</td>
<td>China (May)</td>
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<td>2006</td>
<td>India (Feb)</td>
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<td>2006</td>
<td>Pakistan (Feb)</td>
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<td>2007</td>
<td>Bangladesh (Mar)</td>
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<td>2009</td>
<td>Nepal (Jan)</td>
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<td>2010</td>
<td>Bhutan (Jan)</td>
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Background information

• Mortality in free range chickens was reported in Chukha Dist., Royal Govt. of Bhutan from 14 to 16th Feb., 2010.

• The chickens which died were tested +ve for H5 AIV by Flu Detect (Synbiotics Corp., US) & Colloidal gold card test (Ani. Gen. Inc., Korea) at Nat. Cen. for Ani. Health, Bhutan.

• Active surveillance was carried out from 14 Feb. to 14th March, 2010, and 3 confirmed outbreaks were detected in Rinchending, Pasakha and Burkhey in Chukha dist.

• Ten cloacal /tracheal swabs including the +ve samples collected from the whole carcass during the initial outbreak were submitted to HSADL, Bhopal, India on 22nd Feb., 2010.

• Five human infections have been reported in South Asia (WHO, 18 Octo., 2010). Of these, 1 was fatal. 3/5 occurred in Pakistan (1death), 1 in Bangladesh and 1 in Myanmar.

Confirmation of H5N1

• HA subtyping: one-step RT PCR and real time RT PCR targeting matrix and HA genes.
• NA subtyping: one-step RT PCR.
• Virus isolation was carried out in 10-day old SPF chicken eggs.
• Confirmation of H5 subtype: hemagglutination inhibition test using H5-subtype specific anti-serum.

Five viruses could be isolated
Bhutan H5N1 virus Pathogenicity Test

• Pathogenicity of one isolate (A/chicken/Bhutan/248009/2010) was evaluated in eight 6-week old AIV antibody negative chickens, and all the birds died on 2nd day of the experiment.

• The IVP index was calculated as 2.88/3.00. Thus the Bhutan virus was classified as highly pathogenic for chickens.

H5N1 in South Asia-Phylogeny

As per the WHO nomenclature, they are included in clade 2.2 of “Qinghai-Like viruses.”

The isolates from Bhutan (red triangle) clustered with contemporary viruses from Bangladesh (Blue Triangle) and India (purple Triangle).

The Bangladesh/Bhutan/India isolates of 2007-2010 groups with viruses from the Middle East (yellow squares; 2.2 clade)

Dubey et al (2010) under publication
• Isolates from neighbouring Myanmar (green triangle) groups with viruses from clade 7 and clade 2.3.4.
• According to FAO, the H5N1 virus outbreaks in poultry in Nepal was due to two distinct clades- clade 2.2 (2009) and clade 2.3.2 (2010)

**H5N1 in South Asia – Phylogeny contd..**

**H5N1 in South Asia- molecular signatures**

• The isolates possessed multiple basic amino acids at their HA cleavage site, indicating high virulence of the virus to chickens.
• Amino acid Lysine at position 627 of the PB2 protein, highlights the risk to mammals.
• Vast majority of the isolates are sensitive to influenza drugs presently available in the market.
• Bhutan isolates are sensitive to the influenza drugs available in the market.
• There were reports of the emergence of drug-resistant H5N1 virus mutants in West Bengal outbreaks during 2008 (oseltamivir-resistance) and 2010 (amantadine-resistance).
Discussion

• The close genetic relationship of Bhutan isolates with Bangladesh and Indian isolates in all 8 gene segments is indicative of epidemiological link between the viruses.

• However, the way(s) of introduction of H5N1 avian influenza virus in Bhutan remains unclear.

Illegal/Unsupervised/Unchecked trade of poultry & poultry products

• Sharing of borders between countries (India, Bhutan and Bangladesh), illegal movement of birds is likely to be one of the most important factor for virus transmission.

• As per the livestock statistics 2008, Bhutan has very less poultry population (total 0.2m) compared to the populous neighboring countries, but poultry meat and eggs are important components in the Bhutanese diet, hence, legal or illegal movement of live domestic poultry can not be ruled out.

Discussion contd..

Other Factors:

• Compensation policy.
• Topography of the area near boundary.
• Hygienic and sanitary practices.
• Density of poultry population and food habits.
• Veterinary education and infrastructure.
• Regional co-operation, Diagnostic capacity and Technical collaboration.
Bhutan was declared free from HPAI H5N1 on 18 May 2010.

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