Introduction to Avian Influenza

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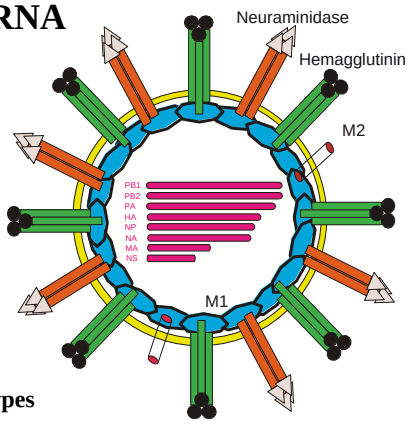
Influenza A Virus

Negative sense RNA
Single stranded
Segmented



16 Hemagglutinin subtypes
9 Neuraminidase Subtypes

Influenza Nomenclature

A/Chicken/Pennsylvania/1370/83 (H5N2)

1 2 3 4 5 6 7

1) Antigenic type
2) Isolate host of origin
3) Geographic location
4) Isolate reference
5) Year of isolation
6) Hemagglutinin subtype
7) Neuraminidase subtype

Influenza Subtypes

- 16 Hemagglutinin subtypes
- 9 Neuraminidase subtypes
- 2 Nonstructural subtypes
- Can occur in any combination
- Useful for epidemiology

What Defines a Subtype?

- Neutralizing antibody produced against one virus will neutralize all other viruses of the same subtype
- A different subtype is defined when neutralizing antibody produced for one subtype will not neutralize viruses from other subtypes
- Subtypes are defined by antigenic characteristics of the virus
- Virus isolates will occasionally cross react with more than one reference antibodies
- Hemagglutination inhibition tests provide a simple way to measure subtype differences

Natural Ecology of Avian Influenza

Mallards

Blue Wing Teal

Herring Gulls

- Avian Influenza is naturally found in wild birds
- Virus infection is not normally thought to cause disease in its natural host (Viruses are low pathogenic)
- Wild bird surveys have shown certain duck, gull, and shorebirds species are commonly infected at different times of the year
- All type A influenza viruses are thought to originate from wild birds

Isolation of Avian Influenza from Different Bird Species

- Most isolations from Anseriformes (ducks, geese, and swans) and Charadriiformes (gulls, terns, plovers, surfbirds, sandpipers, puffins)
- Within Anseriformes highest isolation rates from Mallards and other dabbling ducks
- Isolations of virus from many other Orders of birds (ex. loons, grebes, shearwaters, pelicans, herons, and coots)
- The complete host range is not known

Geographic Range of Avian Influenza

- Most reported isolations have been from North America, Europe and Asia
- A few isolations from Australia Africa, and South America
- No reported isolations Antarctica

Waterfowl Surveys

- Most hemagglutinin and all neuraminidase subtypes have been found in wild waterfowl
- The distribution of subtypes is not uniform-H6, H3, and H4 tend to predominate in North America
- Some important influenza hemagglutinin subtypes are found uncommonly in birds, including H5 and H7
- The distribution of hemagglutinin subtypes differ from year to year at the same location

Avian Influenza in Poultry

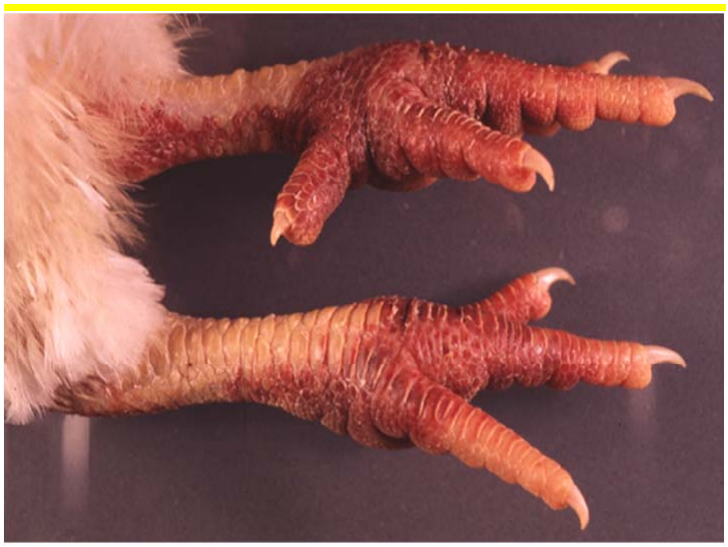
- AIV is not normally found in domestic ducks, chickens and turkeys
- Transmission of AIV from wild birds to domestic poultry species occurs commonly (ducks>turkeys> chickens)
- AIV on rare occasions may become established in chickens and turkeys and result in serious disease outbreaks
- AIV once adapted to chickens and turkeys can be difficult to eradicate

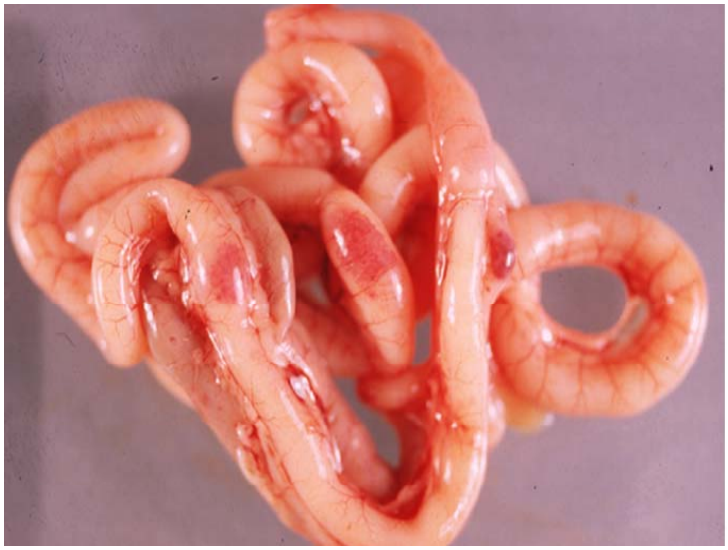
Avian Influenza: Infection and Disease

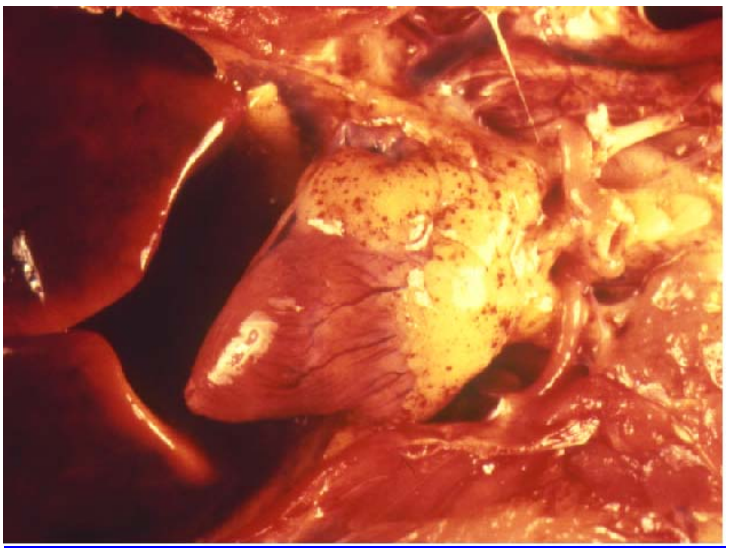
- Infection may cause a wide range of clinical signs from no disease (asymptomatic), respiratory disease, to severe disease with high mortality
- Localized Infection-mild to moderate disease
  - Intestinal-wild ducks and shorebirds, poultry
  - Respiratory-humans, swine, horses, poultry, domestic ducks, seal, mink
- Systemic Infection-high mortality
  - chickens, turkeys, other gallinaceous birds

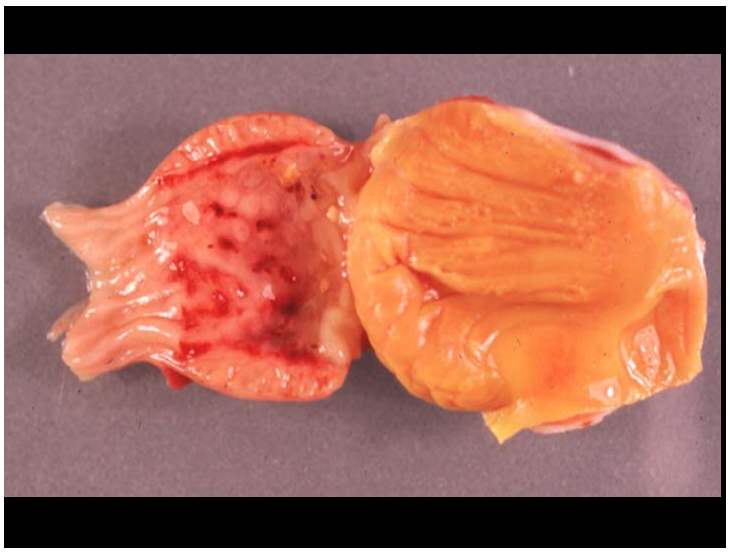
Highly Pathogenic Avian Influenza

- Systemic, rapidly fatal disease of poultry
- Only H5 and H7 subtypes are recognized to cause HPAI
- OIE List A Disease-outbreaks are reportable
- HA cleavage site critical virulence factor
- Low pathogenic H5 and H7 AI viruses can mutate into the highly pathogenic form of the virus









Emergence of HPAI

LPAI H5 or H7 virus transmitted to poultry

LPAI virus circulates in poultry with mild disease

LPAI Virus Mutates to HPAI with severe disease

History of HPAI in the Americas in the last 30 years

- HPAI is considered a foreign animal disease in the Americas
- Five HPAI outbreaks have occurred in the Americas in the 1990s
  - Pennsylvania 1983-84 (17 million birds)
  - Mexico 1994-95 (Millions of birds)
  - Chile 2002 (2 million birds)
  - Canada 2004 (17 million birds)
  - Texas 2004-Molecular definition of HPAI only (5,000 birds)

Hemagglutinin (HA) Protein

- Protein is cleaved into HA1 and HA2 subunits by host proteases
- Cleavage of HA is necessary for virus to be infectious (necessary to release fusion domain)
- HA has receptor binding site (receptor = sialic acid)
- Fusion domain becomes active when pH is lowered in endosome

Standards for Highly Pathogenic Avian Influenza

- 1) If influenza isolate kills 6 or more, out of 8, infected chickens in standard pathotyping test
- 2) Any H5 or H7 influenza virus that has multiple basic amino acids at the hemagglutinin cleavage site compatible with highly pathogenic AI
- Low Pathogenic H5 or H7 Avian Influenza H5 is notifiable to O.I.E.

Cleavage of Hemagglutinin Protein by Host Proteases

- In LPAI viruses, only trypsin-like proteases found in the enteric and respiratory tracts can cleave the HA protein-virus replication and disease is restricted
- In HPAI viruses, the HA protein can be cleaved by ubiquitous proteases found in most cells-virus can replicate systemically

H5 Hemagglutinin Cleavage Site

- For H5 LPAI waterfowl viruses, the consensus cleavage site sequence is Arg Glu Thr Arg/ Gly
- Most H5 HPAI viruses have additional basic amino acids at cleavage site
  - Mexico 1995 Arg Lys Arg Lys Thr Arg/ Gly
  - Hong Kong 1997 Arg Glu Arg Arg Arg Lys Lys Arg/Gly
- The loss of a glycosylation site was also important in the emergence of HPAI in Pennsylvania in 1983
  - LPAI PA/83 Lys Lys Lys Arg/ Gly + glycosylation at 11-13
  - HPAI PA/83 Lys Lys Lys Arg/ Gly - glycosylation at 11-13

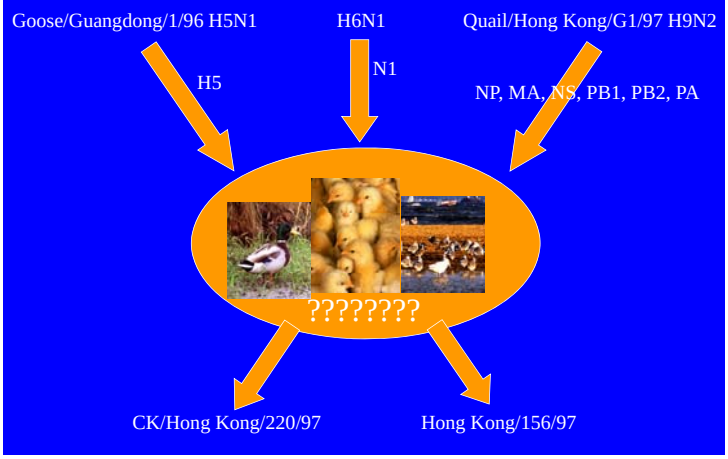
H7 Hemagglutinin Cleavage Site

- For H7 NA LPAI waterfowl viruses, the consensus cleavage site sequence is Asp Pro Lys Thr Arg/Gly
- H7 HPAI viruses have additional basic amino acids at cleavage site
  - Australia 1992 Pro Lys Lys Lys Lys Arg/ Gly
  - Australia 1994 Pro Arg Lys Arg Lys Arg/ Gly
  - Pakistan Pro Lys Arg Lys Arg Lys Arg/ Gly
  - Australia 1997 Pro Arg Lys Arg Lys Arg/ Gly
  - Italy 1999 Pro Lys Gly Ser Arg Val Arg Arg/ Gly

Reassortment of Gene Segments

- Influenza has 8 separate gene segments that encode 10 different proteins
- When a host cell is infected with two different influenza viruses, the progeny virus can be a mixture of both “parent” viruses
- Reassortment provides for increased biological variation that increases the ability of the virus to adapt to new hosts

Origins of Virulent H5N1 Influenza in Hong Kong



Influenza Host Specificity

- Influenza viruses are generally host specific
- Numerous exceptions have been documented
- Many influenza viruses can replicate in hosts other than its established host range
- Rapid adaptation, by reassortment and mutation, allows viruses (rarely) to establish new host ranges
- Replication and transmission however are required before an epidemic will occur

Methods of Control

- Stamping out-identify infected flocks and destroy them to prevent spread to other flocks
- Vaccination in conjunction with stamping out
- Vaccination only

Stamping Out

- This has been the method used in the U.S. for most foreign animal diseases including Avian Influenza
- Requires both good veterinary infrastructure and a diagnostic network
- Can be the most cost effective if outbreaks identified early
- Approach not practical when a disease is widespread in the country

To Vaccinate or Not To Vaccinate

- Vaccines will prevent clinical disease, but not infection
- Good vaccines, properly administered, can reduce virus shedding from infected birds and reduce chance of virus spread
- Vaccines will adversely affect export markets
- Costs of vaccination are not insignificant
- Bad vaccines may contribute to virus spread

Vaccination

- Proper vaccination programs must also include good surveillance, education, quarantines and animal movement controls
- Vaccination can be used to reduce the susceptible population, and when used with stamping out may be an effective tool
- Vaccination without the proper controls may reduce disease, but will not eliminate it

Do Current Vaccines Protect Against Asian H5N1 AI Virus?

Chickens vaccinated SQ 3 wks with inactivated whole AIV vaccine and IN challenged 3 wks later with $10^{6.0}$ EID_{50} of HPAIV (A/chicken/Indonesia/7/2003 [H5N1])

Group	Vaccine	Morbidity (3-4+)*	Mortality (MDT)**	Virus Isolation, 2 DPC (Log_{10} EID_{50} titer/ml)	
				oral	cloacal
1	Nobilis Hepatitis + ND Inac (Control)	$10/10^{A}$	$10/10^{A}$ (2.2)	$10/10^{A}$ (6.16 [a])	$10/10^{A}$ (5.82 [a])
2	Nobilis I.A. Inactivated H5N2 (Mexican Strain)	$0/10^{B}$	$0/10^{B}$	$5/10^{B}$ (1.23 [b])	$3/10^{B}$ (1.00 [b])
3	Nobilis Influenza, H5N2 (European Strain)	$1/10^{B}$	$1/10^{B}$ (2.0)	$6/10^{AB}$ (1.78 [b])	$3/10^{B}$ (1.53 [b])

Vaccines

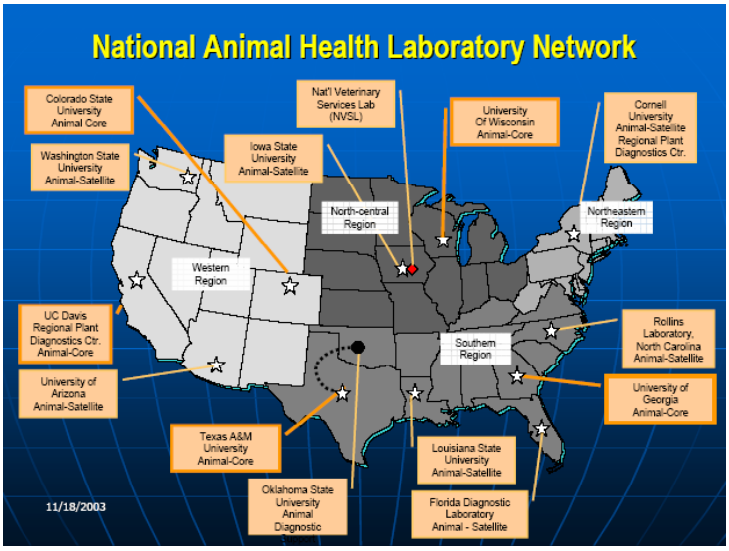
- Both the killed and fowlpox recombinant vaccines, if properly administered provide protect from clinical disease
- Vaccines will reduce shedding of challenged birds to various levels
- Concern about the quality of vaccines being used in Asia
- Concerns if vaccination is being used as an adjunct to quarantines, biosecurity, and surveillance or a replacement for it

Vaccination

- Vaccination is being used legally in Indonesia, China and Vietnam
- China plans to vaccinate all poultry in their country (2 billion birds)
- Vaccination being considered in Russia, Turkey and other countries
- Both killed whole virus vaccines and Fowlpox recombinant vaccines are being used

Control of HPAI

- Most outbreaks of HPAI are controlled through either eradication and/or vaccination
- U.S. has used eradication for HPAI outbreaks
- U.S. also has control programs for H5 or H7 LPAI because of concern of mutation to HPAI
- Strong veterinary infrastructure needed for rapid control of both LPAI and HPAI

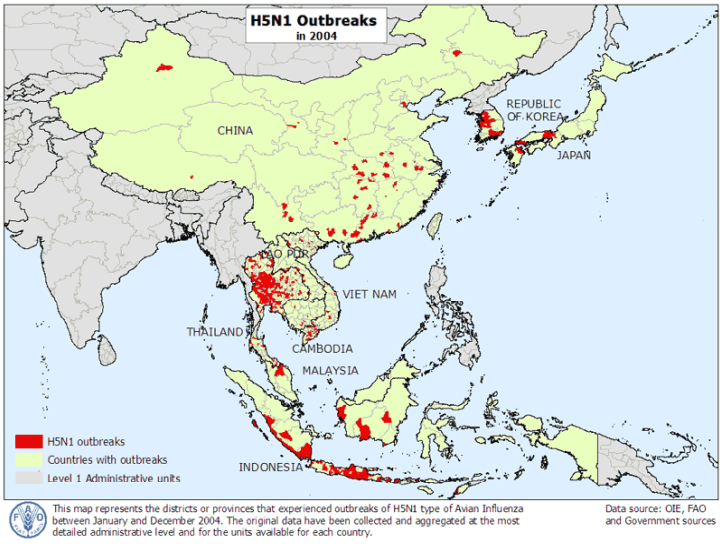


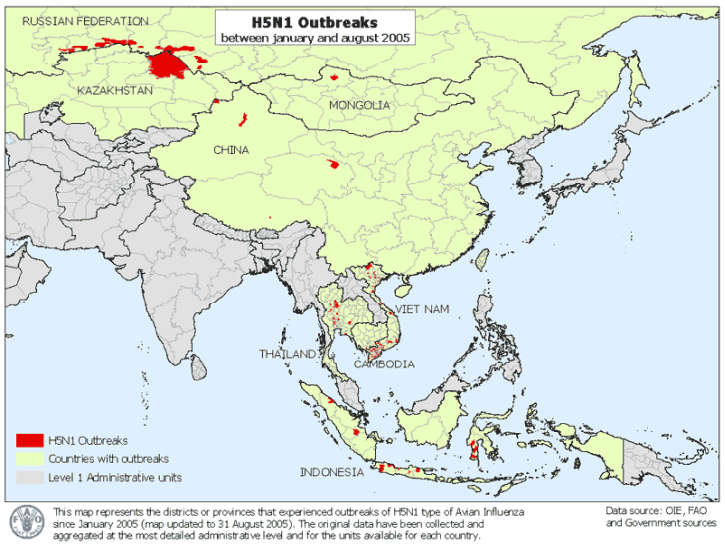
H5N1 Asian "Bird Flu"

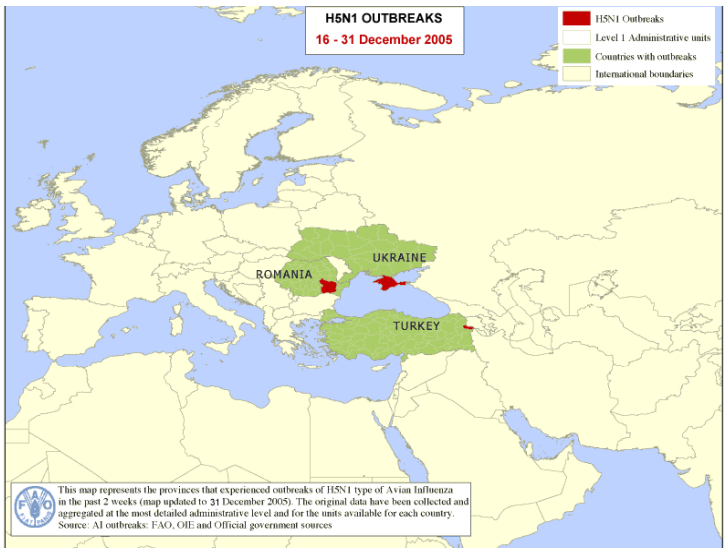
- The HPAI H5N1 Asian lineage was first detected in China in 1996 with the Goose/Guangdong/1/96 isolate
- This isolate had a unique multi-basic aa cleavage site and was highly pathogenic for chickens
- 1997 Hong Kong poultry and human H5N1 viruses had same H5 gene but different internal genes
- 1999 Hong Kong goose viruses were most similar to Guangdong/96 virus
- 2001 Korean quarantine station isolate (from China) 4 genes like Guangdong/96 including HA and four unique genes
- 2001 Hong Kong H5N1 viruses with 5 distinct combinations of genes observed (same HA)

H5N1 Epizootic

- The virus started spreading more widely at the end of 2003
- Has spread to at least 40 different countries, including European and African countries
- Virus is changing in its ability to cause disease in ducks and wild birds
- There are H5N1 viruses with different biological properties







Differences in Species Susceptibility

- All the H5N1 viruses tested are highly pathogenic for chickens-killing rapidly (1-2 days by I.V. route)
- Differences in domestic duck pathogenicity
  - Historically HPAI viruses can infect but do not kill ducks (including Asian H5N1)
  - Starting in 2002 some H5N1 viruses from Hong Kong were highly pathogenic for ducks
  - Some recent viruses may cause high mortality in ducks
- Other species
  - Little work done with other species-Hong Kong 97 viruses was generally lethal only for gallinaceous birds

Role of Wild Birds

- Many species of wild birds have been shown to be susceptible to infection
- Isolates primarily from dead or dying animals
- Some isolates from predator or carrion eating birds (falcons, crows)
- Most of these wildbird infections are thought to occur from spillover from infected poultry
- Only recently has strong epidemiologic evidence shown that migratory birds are likely spreading virus within a country or between countries

Conclusions

- HPAI H5N1 is endemic in certain countries in S.E. Asia
- The virus is present in wild birds and it may be a source of transmission to poultry
- The virus has shown the ability to change and infect new species
- Control in the short term is unlikely
- Vaccination likely to be used widely in the region as a control method
- More international support will be needed to control the problem

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