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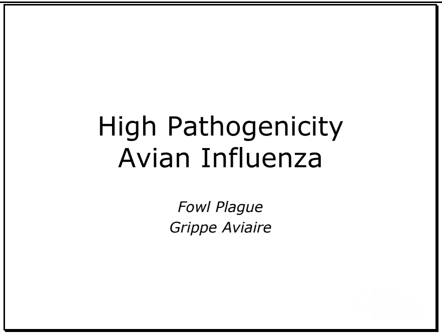
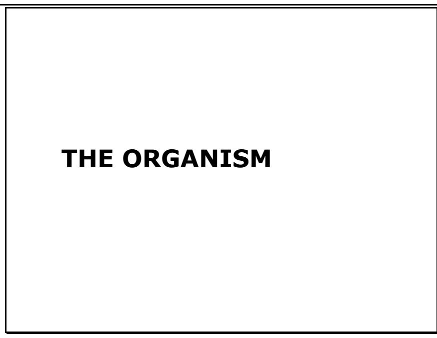


Slide 2

In today's presentation we will cover information regarding the organism that causes avian influenza and its epidemiology. We will also talk about the history of the disease, how it is transmitted, species that it affects (including humans), and clinical and necropsy signs observed. Finally, we will address prevention and control measures, as well as actions to take if avian influenza is suspected.

Slide 3



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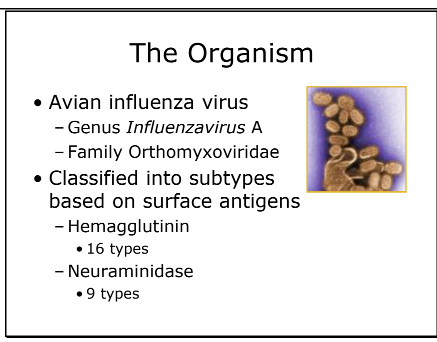


Avian influenza results from infection by viruses in the *Influenzavirus A* genus and influenza A species of the family Orthomyxoviridae. In addition to avian influenza viruses, this species includes the closely related human, equine, swine and canine influenza viruses. Influenza A viruses are classified into subtypes based on two surface antigens, the hemagglutinin (H) and neuraminidase (N) proteins. There are 16 hemagglutinin antigens (H1 to H16) and nine neuraminidase antigens (N1 to N9). These two proteins are involved in cell attachment and release from cells. They are also major targets for the immune response. Subtypes of influenza A viruses are classified into strains. Strains of influenza viruses are described by their type, host, place of first isolation, strain number (if any), year of isolation, and antigenic subtype. For example, one H5N1 virus isolated from chickens in Hong Kong in 1997 is A/chicken/Hong Kong/y385/97 (H5N1). For human strains, the host is typically omitted. Photo: CDC Public Health Image Library.

Slide 5

Pathogenicity

- High pathogenicity avian influenza (HPAI)
  - Causes severe disease in poultry
  - Contains subtypes H5 or H7
- Low pathogenicity avian influenza (LPAI)
  - Causes mild disease in poultry
  - Contains other H subtypes
    - Includes non-HPAI H5 and H7
- LPAI H5 or H7 subtypes can mutate into HPAI

Center for Food Security and Public Health, Iowa State University, 2013

Avian influenza viruses are classified as either HPAI or LPAI viruses, based on the genetic features of the virus and the severity of disease in experimentally infected chickens. Although there are exceptions (e.g., viruses that fit the genetic description of HPAI viruses but cause mild illness), HPAI viruses usually cause severe disease in poultry, while LPAI infections are generally much milder. To date, only subtypes containing H5 or H7 have been highly pathogenic; subtypes that contained other hemagglutinins have been found only in the LPAI form. H5 and H7 LPAI viruses also exist, and these strains can evolve into high pathogenicity strains. The LPAI viruses found in wild birds can be divided into Eurasian and American lineages. Although viruses occasionally cross between these two geographic regions, this is uncommon. When a subtype has become established and has circulated for a time, numerous variants may occur in the population. For example, multiple genotypes and a number of clades of Asian lineage H5N1 viruses are currently found among poultry.

Slide 6

Reservoirs

- Waterfowl and shorebirds
  - Natural reservoirs for influenza A
  - Carry all known H and N antigens
    - Usually in the LPAI form
  - Predominant subtypes change periodically

Waterfowl and shorebirds, which seem to be the natural reservoirs for influenza A viruses, carry all of the known H and N antigens, usually in the LPAI form. The predominant subtypes in wild ducks change periodically. H3, H4 and H6 viruses are detected most often in North American and northern European wild ducks, but nearly all hemagglutinin and neuraminidase antigens can be found. Waders (families Charadriidae and Scolopacidae) seem to have a wider variety of hemagglutinin/neuraminidase combinations than ducks. In the eastern U.S., H1 through H12 (LPAI) viruses have been isolated from these birds; H1, H2, H5, H7 and H9-H12 viruses are particularly common. Gulls are often infected with H13 LPAI viruses, which are rare in other avian species. They can also carry H16 viruses. Limited information is available on the subtypes found in other species of birds.

Slide 7

Influenza Virus Emergence

- Avian influenza virus closely related to other influenza A viruses
  - Humans, horses, pigs, dogs
- Influenza viruses found in each species usually infect only that species

Avian influenza viruses are closely related to influenza A viruses found in humans, horses, pigs and dogs. Ordinarily, the influenza viruses found in each species infect only that species. However, occasionally a virus from one species may infect another species. Image: Structure of the Influenza A virus. CDC PHIL.

Slide 8

Antigenic Drift and Shift

- Antigenic drift
  - Small changes in influenza virus due to point mutations accumulated during virus replication
- Antigenic shift
  - Abrupt change in virus subtype
    - Genetic reassortment between subtypes
    - Direct transfer of virus
    - Re-emergence of virus

Influenza A viruses can change frequently. Strains evolve as they accumulate point mutations during virus replication; this process is sometimes called ‘antigenic drift’. An abrupt change in the subtypes found in host species is called an ‘antigenic shift.’ Antigenic shifts can result from three mechanisms: 1) genetic reassortment between subtypes, 2) the direct transfer of a whole virus from one host species into another, or 3) the re-emergence of a virus that was found previously in a species but is no longer in circulation. Antigenic drift and antigenic shifts result in the periodic emergence of novel influenza viruses. By evading the immune response, these viruses can cause influenza epidemics and pandemics.

Slide 9

Influenza Epidemics

- Epidemic requirements
  1. New influenza subtype must emerge in species with little to no immunity
  2. Virus must produce disease in that species
  3. Sustainable transmission must occur in new species
- Note: Asian lineage H5N1 has NOT met third criteria in humans

For an epidemic to occur, three requirements must be met: 1) a new influenza virus subtype must emerge in a species with little or no immunity to that subtype, 2) the virus must produce disease in that species, and 3) there must be sustainable transmission in the new species. As of January 2010, the Asian lineage H5N1 viruses have met the first two criteria in humans and some other mammals. However, efficient or sustainable transmission has not been reported in any species with the possible exception of pikas. H5N1 viruses have recently been reported to circulate among pikas in China, apparently without causing significant illness.

Slide 10

HISTORY

Slide 11

History: U.S.

- HPAI eliminated from domesticated poultry in the U.S.
- Viruses could be reintroduced
  - Imported poultry
  - Wild birds
  - Pet birds

HPAI viruses have been eliminated from domesticated poultry in many nations including the U.S. and Canada; however, these viruses can be reintroduced from imported poultry, wild birds or pet birds. It is possible for wild birds to carry HPAI viruses, but historically this seems to be rare. More often, wild birds transmit LPAI viruses to poultry, and these viruses then mutate to become HPAI viruses while they are circulating in poultry flocks. Although HPAI outbreaks can be devastating, the virus is successfully eradicated in most cases.

Slide 12

History: Asia

- H5N1 avian influenza virus re-emerged in 2003-4
  - Poultry
- About 470 human cases
  - 60% case fatality rate
- Concern remains that a severe human pandemic could occur

In 2003, HPAI viruses of the H5N1 subtype appeared among poultry in several nations in Southeast Asia. Although at times this epidemic appeared to be under control, eradication was never complete. The outbreaks continued to smolder and spread, and eventually Asian lineage H5N1 viruses reached other parts of Asia, Europe, Africa and the Middle East. The strains responsible for this epidemic appear to be unusually virulent. They have been found in many species of wild birds, which is unusual, and numerous deaths have been reported in these species. As of January 2010, these viruses have also been responsible for approximately 470 human infections, generally as the result of close contact with poultry; about 60% of these cases were fatal. There are fears that an Asian lineage H5N1 virus could eventually become adapted to humans, resulting in a severe human pandemic. Photo: CFSPH.

Slide 13

Economic Impact

- 1997: Hong Kong outbreak (H5N1)
  - $13 million for depopulation and indemnities
  - 1.4 million birds
- 2001: Hong Kong outbreak (H5N1)
  - 1.2 million birds
  - $3.8 million
- 2003: European outbreak (H7N7)
  - 30 million birds destroyed
  - $314 million

Center for Food Security and Public Health, Iowa State University, 2013

The 1997 outbreak of HP AI (H5N1) in Hong Kong live poultry market cost $13 million U.S. for depopulation and indemnities for 1.4 million birds. The 2001 outbreak, also in Hong Kong cost $3.8 million dollars and 1.2 million birds were destroyed. The 2003 European outbreak of (H7N7) strain has resulted in the destruction of 30 million birds, at the cost of $314 million. (Photo: USDA)

Slide 14

EPIDEMIOLOGY

Slide 15

Geographic Distribution

- LPAI occurs worldwide
  - Wild birds and poultry
- HPAI
  - Eradicated from domestic poultry in most developed nations
  - Epidemic ongoing in parts of Asia, the Pacific, Middle East, and Africa

Avian influenza (LPAI) viruses occur worldwide in wild birds and poultry. HPAI viruses have been eradicated from domesticated poultry in most developed nations. The Asian lineage H5N1 HPAI outbreak began among poultry in Southeast Asia in 2003. From 2003 to 2008, it spread into domesticated or wild birds in other regions of Asia as well as parts of Europe, the Pacific, the Middle East and Africa. Although some countries have eradicated the virus from their domesticated poultry, this epidemic is ongoing and worldwide eradication is not expected in the short term.

Slide 16

Morbidity and Mortality: Humans

- Asian lineage H5N1
  - High case fatality rate (60%)
  - Prevalence unknown
- H7, H9 viruses
  - Human disease reported
  - Resembles human influenza
- Antibodies to other H subtypes found in people who work with birds

The severity of zoonotic influenza seems to vary with the virus isolate. Particularly severe infections have been reported with Asian lineage H5N1 (HPAI) viruses. Most patients infected with these viruses have been young and have had no predisposing conditions. The overall case fatality rate, as of December 31, was 60%. The prevalence of human infections with Asian lineage H5N1 viruses is unknown; however, asymptomatic infections seem to be rare. Human disease has also been reported occasionally after infections with various H7 viruses and H9N2 viruses. The reported infections with LPAI H9N2 viruses have resembled human influenza, and they have not been fatal. Most infections with H7 viruses, including HPAI viruses, were limited to conjunctivitis, but influenza symptoms have also been seen. Antibodies to H4, H5, H6, H7, H9, H10 and H11 avian influenza viruses have been found in poultry workers, veterinarians and waterfowl hunters. Whether these antibodies result from productive infections, exposure to antigens or cross-reactions with human influenza viruses remains to be determined.

Slide 17

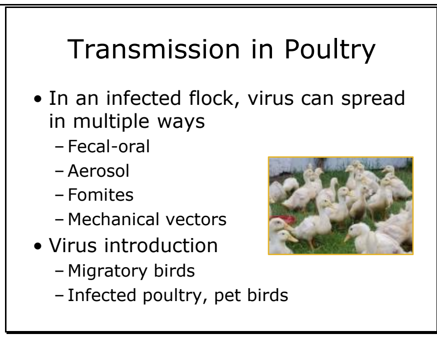
Morbidity and Mortality: Birds

- Domesticated poultry
  - HPAI morbidity and mortality rates approach 90-100%
- Wild birds
  - Typically asymptomatic
    - Some H5N1 viruses may cause death

In domesticated poultry (particularly chickens), HPAI viruses are often associated with morbidity and mortality rates that approach 90-100%. Any survivors are usually in poor condition and do not begin laying again for several weeks. Ducks and geese are clinically unaffected by many HPAI viruses. Avian influenza virus infections in wild birds are typically asymptomatic; however, some of the Asian lineage H5N1 viruses have resulted in high mortality rates in wild birds. Morbidity and mortality rates in passerine and psittacine birds have varied with the species. Photo: Danelle Bickett-Weddle, CFSPH.

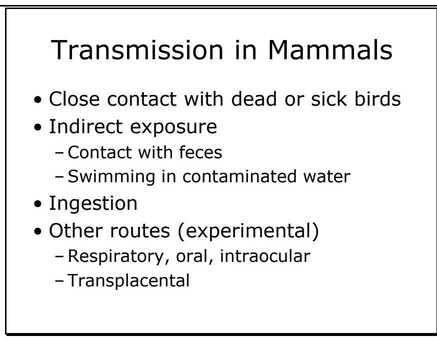
Slide 18	**Morbidity and Mortality: Mammals** • Antibodies found – Cats and dogs • Fatalities reported – Captive tigers, leopards • Ferrets, mice – Severity of disease varies • Pikas (China), pigs – No evidence of significant illness	Asian lineage H5N1 viruses have been reported in a variety of mammalian species. Antibodies have been found in cats and dogs. Fatal cases have been reported among captive tigers and leopards in Thailand, but captive leopards, tigers, Asiatic golden cats and lions at a wildlife rescue center in Cambodia all recovered after an illness lasting 5-7 days. In experimentally infected ferrets and mice, the severity of the clinical signs varied with the specific isolate and the route of inoculation (intranasal or intragastric). Interestingly, there is no evidence that HPAI H5N1 viruses are causing significant illness among infected pikas in China. Although Asian lineage H5N1 viruses have been reported in pigs, severe disease does not seem to occur in this species.
Slide 19	**TRANSMISSION**	
Slide 20	**Transmission in Wild Birds** • Influenza virus shed in feces, saliva, nasal secretions • Fecal-oral – Predominant mode of transmission • Other possible modes – Fecal-cloacal – Respiratory	In birds, avian influenza viruses are shed in the feces as well as in saliva and nasal secretions. The feces contain large amounts of virus, and fecal-oral transmission is usually the predominant means of spread in wild bird reservoirs. Fecal-cloacal transmission might also be possible. Fecal transmission is facilitated by the persistence of avian influenza viruses in aquatic environments for prolonged periods, particularly at low temperatures. Respiratory transmission of LPAI viruses is thought to be unimportant in most wild birds; however, it is possible that is might play a role in some species, particularly those that live on land. Some recent isolates of Asian lineage H5N1 (HPAI) viruses have been found in higher quantities in respiratory secretions than the feces. This suggests that, at least in some wild birds, these strains may no longer be transmitted primarily by the fecal-oral route.
Slide 21	**Influenza Virus Survival** • Virus persistence in aquatic environments – Weeks to months – Preferred conditions • Low temperatures, brackish water – May survive indefinitely when frozen • Virus persistence in feces – Weeks to months	The survival of avian influenza viruses in the environment is influenced by temperature, pH, salinity and the presence of organic material. These viruses, which are often transmitted between birds in feces, may persist for relatively long periods in aquatic environments. They appear to survive best at low temperatures and in fresh or brackish water rather than salt water. Various avian influenza viruses were reported to survive for four weeks at 18°C (64°F). One recent study suggested that H5 and H7 HPAI viruses may survive for shorter periods in water than LPAI viruses; however, they still persisted in fresh water for 100 days or more at 17°C (63°F) and for approximately 26-30 days at 28°C (82°F). Avian influenza viruses might survive indefinitely when frozen. A few studies have examined virus persistence in feces. In one study, LPAI viruses (H7N2) persisted for up to two weeks in feces and on cages. These viruses could survive for up to 32 days at 15-20°C (59-68oF), and for at least 20 days at 28-30°C (82-86oF), but they were inactivated more quickly when mixed with chicken manure. In other studies, LPAI viruses were reported to survive for at least 44 or 105 days in feces.

Slide 22



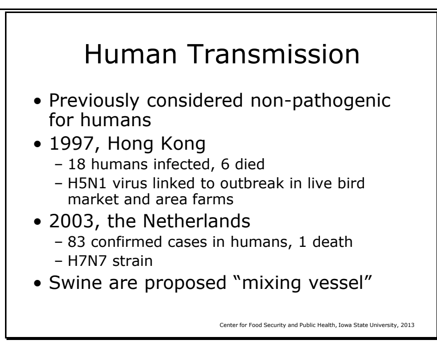
Once an avian influenza virus has entered a poultry flock, it can spread on the farm by both the fecal–oral route and aerosols, due to the close proximity of the birds. Fomites can be important in transmission, and flies may act as mechanical vectors. HPAI viruses have also been found in the yolk and albumen of eggs from infected hens. Although these eggs are unlikely to hatch, broken shells could transmit the virus to other chicks in the incubator. In countries where HPAI has been eradicated from domesticated poultry, influenza viruses can be introduced into flocks by migratory waterfowl or shorebirds, as well as by infected poultry, pet birds or fomites. Migrating birds, which can fly long distances, may exchange viruses with other populations at staging, stopover or wintering sites. Wild birds usually carry only the low pathogenicity form of avian influenza viruses. Once they are introduced into poultry, these viruses reassort and/or mutate to produce HPAI viruses. The Asian lineage HPAI H5N1 strains appear to occur regularly in wild birds, although their importance in transmitting these viruses to poultry is controversial.
Photo: Pam Zaabel, CFSPH.

Slide 23



Some avian influenza viruses can be transmitted to mammals by direct or indirect contact. Close contact with dead or sick birds seems to be the principal way this virus is spread to humans, but a few cases may have resulted from indirect exposure via contaminated feces, and swimming in contaminated water is theoretically a source of exposure. Ingestion of H5N1 viruses has been reported in naturally infected housecats, other felids and dogs; experimentally infected cats, pigs, ferrets, mice and foxes; and rarely in humans. Similarly, leopards and tigers in zoos, as well as some housecats, were apparently infected when they ate raw birds. Experimental studies suggest that Asian lineage H5N1 viruses can be transmitted to mammals by the respiratory, oral and intraocular routes; however, all routes have not been reported in each species. Transplacental transmission of avian influenza viruses is not well studied in mammals. As of January 2010, little or no host-to-host transmission of H5N1 viruses has been seen in mammals, with the possible exception of pikas.

Slide 24



AI viruses were once thought to be nonpathogenic for humans until 1997 when 18 people were infected and six people died from a highly pathogenic H5N1 strain avian influenza virus in Hong Kong. The virus was linked to birds in a live bird market and on farms that were experiencing an outbreak of HP AI. An outbreak in the Netherlands in early 2003 resulted in 83 cases of confirmed Avian Influenza in humans. A 57 year old veterinarian who visited a poultry farm affected by the H7N7 strain died in April of acute respiratory distress syndrome and H7N7 was isolated from the patient. Swine have been proposed as a "mixing" vessel for co-infection by influenza viruses from birds and influenza viruses from mammals which develop into new strains (reassortments) that have the ability to infect people and other mammals. Sporadically cases of the transmission of entire AI viruses to humans have occurred but such cases are very rare compared to the hundreds of millions of human infections by human-adapted influenza viruses that occur each year and during pandemics.

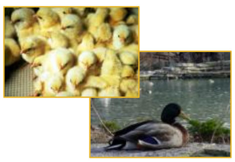
Slide 25

DISEASE IN ANIMALS

Slide 26

Species Affected

- Wild birds
  - Waterfowl
  - Shorebirds
- Cage birds
  - Passerines
- Poultry
- Mammals
  - Pigs, horses, mink, cats, dogs, ferrets, stone martens, palm civets, and others



Avian influenza viruses mainly infect birds. In wild species, these viruses are especially common among birds that live in wetlands and other aquatic environments. Waterfowl and shorebirds appear to be the natural reservoirs for the influenza A viruses, and carry all of the known subtypes. Important reservoir hosts include ducks, geese, swans, gulls, terns and waders. The vast majority of viruses found in birds are LPAI; HPAI viruses are usually detected mainly in poultry. Among cage birds, most avian influenza virus infections have been recorded in passerine birds. Psittacine birds are rarely affected. Some strains of avian influenza viruses may occasionally cause disease in mammals including pigs, horses, mink, cats, dogs, ferrets, stone martens, palm civets, marine mammals and other species. The Asian lineage H5N1 (HPAI) viruses seem to have a particularly broad host range. Photos: Chicks (USDA); Duck (Clint May, CFSPH).

Slide 27

Incubation in Animals

- Poultry: 1-7 days
- Disease control purposes
  - 21-day incubation period used
  - Accounts for virus transmission dynamics
- Incubation period for mammals thought to be short

The incubation period in poultry is one to seven days. A 21-day incubation period, which takes into account the transmission dynamics of the virus, is used for an avian population in the context of disease control. The incubation period for avian influenza viruses in mammals is also thought to be short.

Slide 28

Disease in Poultry

- Highly virulent
- Clinical signs
  - Sudden death
  - Systemic disease
  - Drop in egg production
  - Neurological signs
  - Depression, anorexia, ruffled feathers
  - Combs swollen, cyanotic
  - Conjunctivitis and respiratory signs
- Most birds in an affected flock die

In contrast to LPAI viruses, which usually cause asymptomatic infections, mild respiratory disease or decreased egg production and other reproductive signs, the HPAI viruses are highly virulent. HPAI viruses can cause severe infections in some species of birds on a farm while leaving others unaffected. The clinical signs are variable. Sudden death of large numbers of birds is a common presentation. Systemic signs, and in some cases, respiratory signs, may be noted in chickens, turkeys and other gallinaceous birds. The birds can be markedly depressed, with decreased feed and water consumption, and ruffled feathers. Sinusitis, lacrimation, cyanosis of the head, comb and wattle, edema of the head, and green to white diarrhea may be present in some poultry. In addition, there can be coughing, sneezing, blood-tinged oral and nasal discharges, ecchymoses on the shanks and feet, neurological disease, decreased egg production, loss of egg pigmentation and deformed or shell-less eggs. However, none of these clinical signs is pathognomonic, and sudden death can occur with few other signs. Most of the flock usually dies. [Top photo shows congested and edematous comb and wattles; Bottom photo shows swollen and reddened (hemorrhages) shanks. Photos from David Swayne, USDA]

Slide 29

Disease in Wild Birds

- Disease often subclinical
  - Some strains cause illness
- Clinical signs
  - Minimal in ducks and geese
  - Swans may be found dead
- Experimental infections
  - Gulls, passerines, psittacines

Avian influenza is often subclinical in wild birds, but some strains can cause illness and death. Clinical signs are minimal in ducks and geese infected with most HPAI viruses. Swans have been severely affected by H5N1 viruses; these birds are generally found dead. Symptomatic infections with H5N1 viruses have also been reported in experimentally infected gulls and passerine or psittacine birds.

Slide 30

H5N1 Infections in Mammals

- Felids
  - Fatal infections
  - Respiratory distress
- Other mammals
  - Dogs
  - Palm civets
    - Neurological, respiratory, CNS, and liver disease

Both symptomatic and subclinical Asian lineage H5N1 virus infections have been seen in felids. Although fatal infections have been reported in some housecats, little is known about the clinical signs after natural exposure in this species. Fatal infections have also been reported in some captive tigers and leopards. Some of these animals exhibited respiratory distress, serosanguineous nasal discharge, high fever and neurological signs before death. Other mammals may also be affected by Asian lineage H5N1 viruses. A dog that ate infected poultry developed a high fever, with panting and lethargy, and died the following day. Captive palm civets had neurological signs, with evidence of interstitial pneumonia, encephalitis and hepatitis at necropsy.

Slide 31

H5N1 Infections in Mammals

- Susceptible to infection with all subtypes of avian influenza A
  - Called a "mixing vessel"
  - Receptors for both avian and human influenza virus
- Mild or asymptomatic
  - Mild respiratory disease
  - Few other clinical signs

Pigs have also been infected with avian influenza viruses. One of the concerns with this, is that pigs have receptors for both avian and human influenza viruses (as well as swine influenza viruses). Pigs can serve as "mixing" vessels for these various subtypes and serve as a site for genetic reassortment (or "mixing") of these viruses. The alterations may develop a novel virus more virulent or transmissible than the original subtypes. Asian lineage H5N1 infections in pigs appear to be mild or asymptomatic. Mild respiratory signs including coughing, fever and transient anorexia were observed in some experimentally infected pigs. Photo: Pam Zaabel, CFSPH.

Slide 32

H5N1 Infections in Mammals

- Experimental infections
  - Foxes: asymptomatic
  - Ferrets: mild to severe disease
  - Mice: clinical signs variable
  - Cattle: asymptomatic

Experimental infections have been established in foxes, ferrets, mice and cattle, although no naturally infected animals have been reported. Some infected foxes developed a fever but no other clinical signs; however, lung lesions were reported at necropsy. In ferrets, the syndromes ranged from very mild upper respiratory infections to severe, fatal disease; the pathogenicity varied with the specific isolate and the route of inoculation (intranasal or intragastric). The clinical signs in severe cases included high fever, extreme lethargy, anorexia, weight loss, respiratory disease, diarrhea and neurological signs. Similarly, infections in mice varied with the isolate and the route of inoculation (respiratory or intragastric). Cattle inoculated with high titers of H5N1 viruses isolated from infected cats remained asymptomatic but could transiently shed virus. Photo: Pam Zaabel, CFSPH.

Slide 33

Other Avian Influenza Infections in Mammals

- Ferrets
  - Experimentally infected with both LPAI and HPAI avian influenza viruses
- Seals, pilot whales
  - Outbreaks of pneumonia
  - Co-infections may have increased severity of clinical signs

Ferrets have been infected experimentally with a few LPAI or HPAI avian influenza viruses. Infections with influenza A viruses, apparently of avian origin, have been associated with outbreaks of pneumonia in seals and disease in a pilot whale. Experimental infections with these viruses were milder or asymptomatic, suggesting that co-infections may have increased the severity of the clinical signs. In the single known case in a whale, the clinical signs were extreme emaciation, difficulty maneuvering and sloughing skin.

Slide 34

Communicability: Birds

- Avian influenza viruses readily transmitted between birds
- Viral shedding
  - Begins 1-2 days after infection
  - Last up to 36 days (chickens) or 72 days (turkeys)
- Birds-to-mammal transmission uncommon

Avian influenza viruses are transmitted readily between birds. Virus shedding can begin as early as 1 to 2 days after infection. Most chickens shed LPAI influenza viruses for only a week, but a minority of the flock can excrete the virus in the feces for up to two weeks, and shedding for as long as 36 days has been reported in experimentally infected birds. Turkeys may excrete some avian influenza viruses for up to 72 days. Waterfowl are often infected subclinically, and ducks can shed these viruses for up to 30 days. Transmission from birds to mammals seems to be uncommon.

Slide 35

Communicability: Mammals

- Cats
  - Shed virus by third day post-infection (experimental)
  - Naturally infected cats excrete virus sporadically and for < 2 weeks
  - Shed from intestinal and respiratory tracts
- Horizontal transmission not observed in cats or other mammals

Cats that were experimentally infected with the Asian lineage H5N1 virus shed the virus by the third day post-inoculation, and were able to infect two sentinel cats in close contact. Naturally infected, asymptomatic cats appeared to excrete virus only sporadically, and for less than two weeks. Horizontal transmission was not observed in this instance. Cats appear to shed avian influenza viruses from the intestinal tract as well as the respiratory tract. Limited animal-to-animal transmission was reported among tigers in a zoo. Dogs, foxes, pigs and cattle can also shed HPAI viruses, but horizontal transmission has not been reported. Sustained or prolonged transmission has not been reported with Asian lineage H5N1 viruses in any of these mammals. However, the recent isolation of H5N1 viruses from pikas suggests that these viruses may be maintained in this population.

Slide 36

Post Mortem Lesions: Birds

- Chickens and turkeys
  - Swollen sinuses
  - Edematous comb and wattle
  - Subcutaneous edema
  - Petechiae
    - Trachea
    - Lungs
    - Proventriculus

The lesions in chickens and turkeys are highly variable and resemble those found in other systemic avian diseases. Birds that die peracutely and young birds may have few or no lesions. In other cases, the sinuses may be swollen, and the comb and wattle are often edematous, hemorrhagic, congested and/or cyanotic. There may be subcutaneous edema on the head and neck, edema and diffuse subcutaneous hemorrhages on the feet and shanks, fluid (which may contain blood) in the nares and oral cavity, and congestion, swelling and hemorrhages of the conjunctivae. Hemorrhagic tracheitis can be seen in some birds; in others, the tracheal lesions may be limited to excess mucoid exudate. The lungs may be reddened from hemorrhages and congestion, and they may exude fluid when cut. Petechiae may be noted throughout the abdominal fat, on serosal surfaces and on the peritoneum, and they can sometimes be found in the muscles. Hemorrhages may also be seen on the mucosa and in the glands of the proventriculus, beneath the lining of the gizzard, and in the intestinal mucosa. The kidneys can be severely congested and they are sometimes plugged with urate deposits. Additional post mortem lesions may be observed. [Photos show (top) multiple hemorrhages on the mucosal surface of the proventriculus – Photo from Dr. David Swayne, USDA; (bottom) facial edema – Photo from Dr. Corrie Brown].

Slide 37

Sampling

- Before collecting or sending any samples, the proper authorities should be contacted
- Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease
- HPAI samples may be zoonotic

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. Some isolates of the avian influenza virus may be zoonotic; samples should be collected and handled with all appropriate precautions.

Slide 38

Post Mortem Lesions: Mammals

- Multiple lesions possible
  - Pulmonary edema, pneumonia
  - Conjunctivitis
  - Cerebral, renal and splenic congestion
  - Multifocal hepatic necrosis
  - Hemorrhages in the intestinal serosa, lymph nodes, perirenal tissue and/or diaphragm
  - Severe hemorrhagic pancreatitis

Pulmonary edema; pneumonia; conjunctivitis; cerebral, renal and splenic congestion; multifocal hepatic necrosis; hemorrhages in the intestinal serosa, lymph nodes, perirenal tissue and/or diaphragm; and severe hemorrhagic pancreatitis have been reported in naturally infected cats. The lungs were also affected in experimentally infected cats, with multiple to coalescing foci of pulmonary consolidation. In naturally infected tigers and leopards, the gross lesions included severe pulmonary consolidation and multifocal hemorrhages in multiple organs including the lung, heart, thymus, stomach, intestines, liver and lymph nodes. Bloody nasal discharge, severe pulmonary congestion and edema, and congestion of the spleen, kidney and liver were reported in a naturally infected dog. Pulmonary lesions including interstitial pneumonia have been reported in some experimentally infected pigs.

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Differential Diagnosis

- Virulent Newcastle disease
- Avian pneumovirus
- Infectious laryngotracheitis
- Infectious bronchitis
- Chlamydia
- Mycoplasma
- Acute bacterial diseases
  - Fowl cholera, *E. coli* infection

Center for Food Security and Public Health, Iowa State University, 2013

Differentials for highly pathogenic avian influenza include Newcastle disease virus, which is clinically indistinguishable from HPAI; avian pneumovirus and other paramyxoviruses, infectious laryngotracheitis, infectious bronchitis, chlamydia, mycoplasma and other acute bacterial diseases including fowl cholera and *E. coli* infections.

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Diagnosis

- Clinically indistinguishable from virulent Newcastle Disease (END)
- Virus isolation
  - Oropharyngeal, tracheal, and/or cloacal swabs
  - Feces
  - Organ samples
- Virology and serology necessary for definitive diagnosis
  - AGID, ELISA, RT-PCR

Highly Pathogenic Avian Influenza (HP AI) is clinically indistinguishable from virulent Newcastle Disease. Avian influenza can be diagnosed by variety of techniques including virus isolation. The virus can be recovered from oropharyngeal, tracheal and/or cloacal swabs in live birds. Feces can be substituted in small birds if cloacal samples are not practical (e.g., cannot be collected without harming the bird). Oropharyngeal, tracheal or cloacal swabs (or intestinal contents), and organ samples (trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart) are tested in dead birds. Virus isolation is performed in embryonated eggs; hemagglutinating activity indicates the presence of influenza virus. Because of the broad spectrum of signs and lesions a definitive diagnosis must be made by virology and serology. The identity of the virus can be confirmed with agar gel immunodiffusion (AGID) or ELISAs. Avian influenza viruses are subtyped with specific antisera in AGID or hemagglutination and neuraminidase inhibition tests. Virulence tests in susceptible birds, together with genetic tests to identify characteristic patterns in the hemagglutinin, are used to differentiate LPAI from HPAI viruses. [Photo from National Institute of Animal Health]

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Diagnosis, cont'd

- RT-PCR
  - Clinical samples
  - Can distinguish different subtypes
- Viral antigen detection
  - Best for flocks, not individuals
- Serology
  - AGID
  - Hemagglutination inhibition
  - ELISA

RT-PCR assays can identify avian influenza viruses in clinical samples, and can replace virus isolation in some cases. These tests can also distinguish some subtypes. Real-time RT-PCR is the method of choice for diagnosis in many laboratories. Viral antigens can be detected with ELISAs including rapid tests. As of 2008, the World Organization for Animal Health (OIE) recommended that antigen detection tests be used to identify avian influenza only in flocks and not in individual birds. Serological tests including agar gel immunodiffusion, hemagglutination, hemagglutination inhibition and ELISAs are useful as supplemental tests. Although most gallinaceous birds and other susceptible birds die before developing antibodies, serology can be valuable for surveillance and to demonstrate freedom from infection. AGID tests can recognize all avian influenza subtypes in poultry, but hemagglutination inhibition tests are subtype specific and may miss some infections. AGID tests are not reliable for detecting avian influenza in ducks or geese. In wild birds, some serological tests may underestimate the prevalence of H5N1 infections.

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Treatment

- No specific treatment
- HPAI in poultry usually not treated
- Outbreaks controlled by eradication
- Antivirals (amantadine) effective in reducing mortality
  - Not approved in food animals
  - Results in resistant viruses

No practical, specific treatment exists for avian influenza virus infections in commercial poultry. In most countries including the U.S. and Canada, high pathogenicity avian influenza in poultry is not treated; outbreaks are controlled by eradication. Amantadine has been shown experimentally to be effective in reducing mortality but the drug is not approved for food animals and quickly results in amantadine resistant viruses. Photo: USDA.

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DISEASE IN HUMANS

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Incubation in Humans

- Difficult to determine
  - 2-17 days possible
- Symptoms usually appear in 2-5 days

World Health Organization
Recommends using incubation period of seven days for field investigations and monitoring patient contact

The incubation period for avian influenza is difficult to determine in humans. Human influenza viruses usually cause disease in one to four days. Limited data from Asian lineage H5N1 infections suggest that the incubation period for this virus may range from two to eight days and could be as long as 17 days. In most cases, the first symptoms appear in two to five days. The World Health Organization (WHO) currently suggests using an incubation period of seven days for field investigations and monitoring patient contacts.

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H5N1 Infections in Humans

- Cause severe disease
  - High fever
  - Upper respiratory symptoms
  - Mucosal bleeding
  - Gastrointestinal symptoms
- Patients may deteriorate rapidly
- Late symptoms
  - Organ failure, DIC

The Asian lineage H5N1 HPAI viruses appear to cause more severe disease than other HPAI viruses or LPAI viruses. High fever and upper respiratory symptoms resembling human seasonal influenza tend to be the initial signs. In some patients, there may also be mucosal bleeding, or gastrointestinal symptoms such as diarrhea, vomiting and abdominal pain. Respiratory signs are not always present at diagnosis, but may include chest pain, dyspnea, tachypnea, hoarseness of the voice and crackles during inspiration. Most patients deteriorate rapidly. Heart failure, kidney disease, encephalitis and multiorgan dysfunction are common in the later stages, and disseminated intravascular coagulation can occur. Milder cases have been reported occasionally, particularly among children. Asymptomatic infections with Asian lineage H5N1 viruses seem to be rare.

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Communicability

- Rare cases of person-to-person transmission
- NO cases of sustained transmission
- Fecal shedding and transplacental transmission may occur

Rare cases of probable person-to-person transmission, and no cases of sustained transmission, have been reported in humans infected with avian influenza viruses. Fecal shedding of an Asian lineage H5N1 virus has been documented in a child with diarrhea. Transplacental transmission of this virus may be possible.

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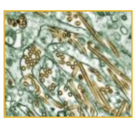
Diagnosis in Humans

- RT-PCR
  - Primary test to identify H5N1
- Antigen detection
- Virus isolation
  - WHO Reference Laboratories
- Serology
  - Microneutralization



Avian influenza viruses can be identified by reverse transcription polymerase chain reaction (RT-PCR) tests, antigen detection or virus isolation. RT-PCR is usually the primary test for infection with Asian lineage H5N1 viruses. Virus isolation is done at World Health Organization (WHO) H5 Reference Laboratories. In the U.S., samples that test positive by RT-PCR or antigen tests are confirmed by the Centers for Disease Control and Prevention (CDC). RT-PCR and antigen testing of avian influenza viruses must be carried out in Biosafety Level (BSL) 2 laboratory conditions. Enhanced BSL 3+ laboratory conditions are required for the isolation of H5N1 HPAI viruses. Serology has been used for surveillance. The microneutralization assay is the most reliable test for detecting antibodies to avian influenza viruses. Photo: Colorized transmission electron micrograph of Avian influenza A H5N1 viruses (seen in gold) grown in MDCK cells (seen in green). CDC PHIL.

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Treatment in Humans

- Antiviral drugs
  - Amantadine
  - Rimantadine
  - Zanamivir
  - Oseltamivir
- Currently circulating H5N1 viruses may be resistant to amantadine, rimantadine

Four antiviral drugs -- amantadine, rimantadine, zanamivir, and oseltamivir - are active against human influenza viruses. Studies suggest that these drugs may also be helpful in avian influenza infections in humans. Oseltamivir appears to increase the chance of survival in patients infected with Asian lineage H5N1 viruses, particularly if it is given early. Further testing, particularly on the optimum dose and duration of treatment, is still needed. The H5N1 viruses currently circulating in poultry are resistant to amantadine and rimantadine. Although resistance to zanamivir and oseltamivir has been reported in these viruses, it is uncommon.

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PREVENTION AND CONTROL

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Recommended Actions

- Notification of Authorities
  - Federal:
    Area Veterinarian in Charge (AVIC)
    - www.aphis.usda.gov/vs/area_offices.htm
  - State veterinarian
    - www.aphis.usda.gov/vs/sregs/official.htm
- Quarantine

Due to the economically devastating nature of this disease, authorities should be notified immediately of any suspicious cases of highly pathogenic avian influenza. While waiting for the authorities or a confirmed diagnosis, all suspect animals should be quarantined.

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Prevention in Humans

- People working with infected poultry
  - Follow good hygiene practices
  - Wear protective clothing (gloves, masks)
  - Consider antiviral prophylaxis
  - Be vaccinated against human influenza
  - Do not have contact with sick birds if experiencing symptoms of influenza

Controlling epidemics in poultry decreases the risk of exposure for humans. People working with infected birds should follow good hygiene practices and wear appropriate protective clothing such as boots (or shoe covers), coveralls, gloves, respirators and goggles. The specific recommendations may vary with the virus. In addition, WHO recommends prophylaxis with antiviral drugs in people who cull birds infected with H5N1 HPAI viruses. To prevent reassortment between human and avian influenza viruses, people in contact with infected birds should be vaccinated against human influenza. They are also discouraged from having contact with sick birds while suffering flu symptoms.

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Prevention in Humans

- If Asian lineage H5N1 is present, avoid contact with:
  - Domesticated poultry
  - Poultry farms
  - Live bird markets
- Prepare food properly
  - Practice good hygiene
  - Cook chicken and eggs thoroughly

In areas where Asian lineage H5N1 viruses could be present in domesticated poultry, poultry farms and live bird markets should be avoided. Precautions should also be taken when handling raw meat and eggs, which can contain virus. Sanitary precautions and cooking methods recommended to destroy *Salmonella* and other poultry pathogens are sufficient to kill avian influenza viruses. The hands should be washed thoroughly with soap and warm water after handling meat or eggs. Cutting boards and utensils should be washed with soap and hot water. Poultry should be cooked to a temperature of at least 74ºC (165ºF). Eggs should be cooked until the whites and yolks are both firm. Photo: CDC PHIL.

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Prevention in Humans

- Avoid wild bird contact
- Report dead or diseased wildlife
- Do not handle or eat sick game
- Wear gloves while handling or cleaning wild birds
- Wash hands
- Cook game thoroughly

Avian influenza viruses can be carried in wild birds, and these birds could be the initial source of infection in an area. Wild birds should be observed from a distance; close contact is discouraged. If birds or potentially contaminated surfaces are touched, the hands should be washed with soap and water before eating, drinking, smoking, or rubbing the eyes. Dead or diseased wildlife should be reported to state, tribal or federal natural resource agencies. Hunters should not handle or eat sick game, and should always wear rubber or latex gloves while handling and cleaning wild birds. The hands, as well as equipment and surfaces, should be thoroughly washed after dressing the carcass. All game should be cooked thoroughly.

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Pandemic Precautions

- If a pandemic occurs:
  - Avoid crowded conditions and close contact with other people
  - Consider wearing respirators or other protective equipment
  - Follow good hygiene measures
  - Practice social distancing
  - Quarantine ill individuals
  - Vaccination

If a human avian influenza pandemic occurs, additional precautions will be necessary. During a pandemic, crowded conditions and close contact with other people should be avoided. Respirators and other protective equipment may be advisable during close contact with an infected individual. In addition, infection control measures such as good hygiene, cancellation of social events and voluntary quarantine of infected individuals can limit the spread of disease. H5N1 vaccines have also been developed. In the U.S., these vaccines are stockpiled by the government and will be distributed by public health officials if they are needed. Avian influenza vaccines for humans are not commercially available in the U.S.

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Prevention in Birds

- All-in/all-out flock management
- Prevent contact with wild birds or their water sources
- Do not allow birds to return to the farm from live markets
- Practice strict hygiene and biosecurity measures

Poultry can be infected by contact with newly introduced birds or fomites, as well as by contact with wild birds, particularly waterfowl. The risk of infection can be decreased by all-in/all-out flock management, and by preventing any contact with wild birds or their water sources. Birds should not be returned to the farm from live bird markets or other slaughter channels. In addition, strict hygiene and biosecurity measures are necessary to prevent virus transmission on fomites.

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Prevention in Birds

- Depopulation of infected flocks
- Proper carcass disposal
  - Burying
  - Composting
  - Rendering
- Strict biosecurity measures
- Quarantine
- Cleaning and disinfection

Outbreaks can be controlled by rapid depopulation of infected and exposed flocks, proper disposal of carcasses and contaminated materials, and strict biosecurity measures. Farms should be quarantined, and movement controls and surveillance should be established. Infected premises must be thoroughly cleaned and disinfected. Insects and mice on the premises should be eliminated, then the flock depopulated and the carcasses destroyed by burying, composting or rendering. Once the birds have been killed, the manure and feed should be removed down to a bare concrete floor. If the floor is earthen, one inch or more of soil should be removed. The manure can be buried at least five feet deep. It may also be composted for 90 days or longer, depending on the environmental conditions. The compost should be covered tightly with black polyethylene sheets to prevent the entry of birds, insects and rodents. Feathers can be burned or composted; alternatively, they may be removed and the area wet down with disinfectant. High–pressure spray equipment should be used to clean all equipment and building surfaces. Once all surfaces are clean and free of all organic material, the entire premises should be sprayed with an approved residual disinfectant. Photo: Danelle Bickett-Weddle, CFSPH.

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Prevention in Birds

- Vaccination
  - Not used routinely in the U.S.
  - Usually autogenous
  - Requires approval of State veterinarian and USDA (H5, H7 vaccines)
  - May not prevent virus shedding
  - Differentiating Infected from Vaccinated Animals (DIVA) strategy used to recognize field viruses in vaccinated flocks

HPAI vaccines are not used routinely in the U.S. or most other countries; however, nations may consider vaccination as a preventative or adjunct control measure during an outbreak. Avian vaccines are usually autogenous or from viruses of the same subtype or hemagglutinin type. Currently licensed vaccines in the U.S. include inactivated whole virus and recombinant fowlpox- H5 vaccines. The use of these vaccines requires the approval of the state veterinarian and, in the case of H5 and H7 vaccines, USDA approval. Because vaccines can allow birds to shed virus while remaining asymptomatic, good surveillance and movement controls are critical in a vaccination campaign. Methods used to recognize infections with field viruses in vaccinated flocks include a “DIVA” (differentiating vaccinated from infected animals) strategy, and the use of sentinel birds. Vaccination may place selection pressures on avian influenza viruses, and might eventually result in the evolution of vaccine-resistant isolates.

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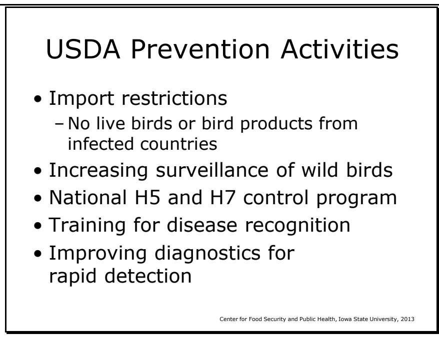

Many prevention and control activities are occurring in the US. The USDA has import restrictions on birds and bird products. No live birds or bird products are imported from countries with HPAI. Humans that have traveled to areas where H5N1 infections are occurring are not allowed on a US poultry farm for at least 7 days after return to this country. Introduction of H5N1 HPAI by migratory wild birds or intentional introduction will not be stopped by the border controls and effort is therefore focused on rapid detection. USDA along with the Department of the Interior have increased surveillance of the migratory wild bird population. The USDA has also increased surveillance at live bird markets. There is a National Poultry Improvement Plan (NPIP) AI Surveillance program for layer and breeder flocks of poultry in the US. Under this plan layer and breeder bird flocks are routinely tested for AI. Many broiler flocks are also under surveillance for AI although the current NPIP AI survellance plan does not include broilers. There is a NPIP AI surveillance plan for broiler flocks in the final stages of approval. This will increase the likelihood of early detection of an AI virus in the US poultry. Other prevention and control activities include training for veterinarians, laboratorians and poultry workers to recognize the disease and improved rapid diagnostics. The veterinary laboratories have formed the National Animal Health Laboratory Network to quickly share information and reagents. USDA is also supporting collaboration and influenza research to advance our understanding of the virus.

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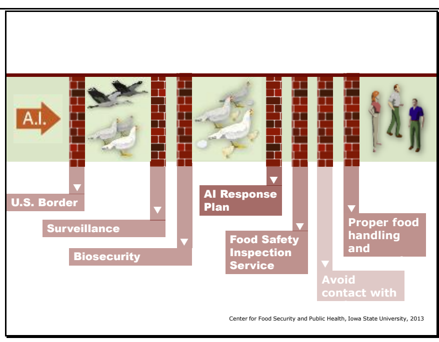

This diagram summarizes the many ways humans in the US are protected from infection by avian H5N1 influenza. The USDA is active in other countries trying to help control the H5N1 outbreak in birds so there is activity even before the US border. At the border, the USDA has import restrictions on birds and bird products. No live birds or bird products are imported from countries with HPAI. USDA has increased surveillance in the live bird markets and, together with the Department of the Interior, has increased surveillance of the migratory wild bird population. Biosecurity procedures in poultry operations is of the greatest importance. These practices include control of human traffic, shower in and shower out facility, truck washing, cleaning and disinfection, careful introduction of new birds, and keeping wild birds and poultry separate. If AI would get through those prevention practices there is a robust AI response plan that has been practiced. The virus would be quickly identified and eliminated. Only the necessary workers would have contact with infected birds and they would be wearing PPE. The infected birds would be humanely euthanized. If for some reason an infected bird would be sent to slaughter, the Food Safety Inspection Service (FSIS) inspects the birds at slaughter. Any sign of disease would prevent those birds from entering the food supply. Other barriers for protecting humans from infection include avoiding contact with live poultry or poultry feces or respiratory secretion. In the unlikely event that poultry an avian influenza infected bird entered the food supply, proper handling and cooking would easily kill the virus. [Graphic by Clint May, CFSPH, ISU, CVM]

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Prevention in Mammals

- Do not feed infected poultry or birds to mammals
- Prevent contact with potentially infected flocks and wild birds
- Keep cats and dogs indoors during avian influenza outbreaks

Mammals should not be fed poultry or other birds that may be infected with Asian lineage H5N1 viruses or other HPAI avian influenza viruses. They should also be kept from contact with potentially infected flocks and wild birds. During outbreaks, cats and dogs should be kept indoors whenever possible.

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Disinfection

- Sodium hypochlorite
- 70% ethanol
- Oxidizing agents
- Quaternary ammonium compounds
- Aldehydes
- Phenols
- Acids
- Povidone-iodine

The influenza viruses are susceptible to a wide variety of disinfectants. Avian influenza viruses seem to be more resistant to high temperatures and low pH than mammalian influenza viruses.

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Disinfection, cont'd

- Heat
  - 56°C (133°F) for a minimum of 60 minutes
- Ionizing radiation
- Low pH (pH 2)

Influenza viruses can also be killed by heat, radiation, and low pH conditions.

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Additional Resources

- Center for Food Security and Public Health
  - www.cfsph.iastate.edu
- Centers for Disease Control and Prevention (CDC)
  - www.cdc.gov
- World Organization for Animal Health (OIE)
  - www.oie.int
- World Health Organization (WHO)
  - www.who.int

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Acknowledgments

Development of this presentation was made possible through grants provided to the Center for Food Security and Public Health at Iowa State University, College of Veterinary Medicine from the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the Iowa Homeland Security and Emergency Management Division, and the Multi-State Partnership for Security in Agriculture.

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