Avian Influenza

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Agent

- Avian influenza can occur in two forms: highly pathogenic avian influenza (HPAI), also known as fowl plague, and low-pathogenic avian influenza (LPAI). Both forms are caused by influenza type A virus. HPAI spreads rapidly among flocks and often is highly fatal. Conversely, LPAI generally does not cause severe disease and is more commonly seen in US poultry flocks

Agent

- **Viral classification and genetic composition**
  Family: Orthomyxoviridae
  Genus: Influenza
- Virions 80 to 120 nm in diameter
- May be filamentous
- Eight different segments of negative-stranded RNA; allows for genetic reassortments in single cells infected with more than one virus and may result in multiple strains that are different from the initial ones.

Types: A, B, and C

- Type designation is based on the antigenic character of the M protein located in the virus envelope and the nucleoprotein within the virus particle

Influenza A virus causes human, swine, equine, avian, and marine mammal influenza and is the type associated with pandemic disease in humans.

Influenza B virus causes disease in humans only.

Influenza C virus causes a relatively mild illness in both humans and swine and occurs uncommonly.

HPAI and LPAI are caused by influenza A viruses.

The virus envelope glycoproteins have hemagglutinin (HA) and neuraminidase (NA) activity; these characteristics are used to subtype the A, B, and C viruses.

For influenza A viruses, there are 15 different HA antigens (H1 to H15) and nine different NA antigens (N1 to N9).

Only subtypes H5 and H7 of influenza A virus have caused HPAI.

The H5 and H7 strains also are identifiable according to a nucleic acid sequence at the hemagglutinin cleavage site.

Environmental Survival of HPAI Virus

- **Influenza A virus remains viable at moderate temperatures for long periods in the environment and can survive indefinitely in frozen material. It can survive for 4 days in water at 22°C and for over 30 days at 0°C .**

Inactivation of the virus occurs under the following conditions:

- Temperatures of 56°C for 3 hours or 60°C or more for 30 minutes
- Acidic pH conditions
- Presence of oxidizing agents such as sodium dodecyl sulfate, lipid solvents, and B-propiolactone
- Exposure to disinfectants: formalin, iodine compounds

Hosts

- It is likely that all birds are susceptible to HPAI

Species that have been shown to be susceptible either experimentally or naturally include:

- **Chickens Turkeys Ducks**
- **Quail**
- **Pigeons Partridges Pheasants**
- **Geese Guinea Fowl**
- **Ostriches and other ratites**

Migratory waterfowl, sea birds, shore birds, and imported pet birds have been found to be carriers of highly-pathogenic influenza A viruses without clinical signs.

As noted above, influenza A viruses also cause illness in humans and other animal hosts (pigs, horses, sea mammals, and mustelids).

Epidemiology

a) Transmission

- **Routes of infection include:**
  - **Oral**
  - **Conjunctival**
  - **Respiratory**

- **Vertical transmission is not known to occur**

Common modes of infection include:

- **Direct transmission through secretions (feces, respiratory secretions) of infected birds.**
- **Broken contaminated eggs in incubators infecting healthy chicks.**
- **Movement of infected birds between flocks**

Fomites such as contaminated equipment, egg flats, feed trucks, and clothing and shoes of employees and service crews

Contact with wild birds and waterfowl, which appear to be natural reservoirs for the virus

Fecal contamination of drinking water

Garbage flies (suspected of transmitting the virus during the 1983-1984 epidemic in Pennsylvania)

Airborne transmission if birds are in close proximity

- Do not take live infected chickens out of the pen to euthanize them

The disease is highly contagious. One gram of contaminated manure can contain enough HPAI virus to infect 1 million birds.

As stated above, waterfowl, ostriches and other ratites, shore birds, sea birds, and other wild birds can act as carrier of the virus.

These birds (and quail)may not show clinical signs but shed the virus.

Occurrence of HPAI in Avian Populations

- **Because HPAI usually arises from an LPAI virus that has mutated at its hemagglutinin surface proteins, it is difficult to define specific areas where the disease is endemic.**
- **HPAI types of virus have been isolated from free-living birds in Europe and other regions; however, there is no recognized wild bird reservoir for the HPAI subtypes of the virus.**

Apathogenic and mildly pathogenic influenza A viruses occur worldwide.

Some H5 and H7 viruses are of low pathogenicity.

Because of a lack of appropriate laboratory facilities in many parts of the world, it is difficult to accurately determine the actual incidence of HPAI in the world's poultry flocks

Outbreaks of HPAI have occurred throughout the world:

Where does it come from?

AI in East Asia

- 1996 - Guangdong Province, CHINA
- ?
- 1997 Hong Kong
- 2002 Hong Kong
- ?
- Still in China?
- How spread to rest of Asia?

Outbreaks of HPAI have occurred throughout the world:

- **An H5 strain affected Pennsylvania in 1983 and caused severe clinical disease and high mortality rates in chickens, turkeys, and guinea fowl**
- **A serologically identical but apparently mild virus had been circulating in poultry in the area for 6 months**
- **Did it mutate?**
- **A total of 17 million birds were culled. Retail egg prices increased by 30 cents**

Outbreaks of HPAI have occurred throughout the world:

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- **A total of 17 million birds were culled. Retail egg prices increased by 30 cents**

Outbreaks of HPAI have occurred throughout the world:

- **An H5N1 strain of AI was responsible for a significant outbreak of influenza in domestic poultry in 1997 in Hong Kong.**
- **Prior to this outbreak, H5N1 was not known to infect humans.**
- **Six human deaths were attributed to this strain.**
- **The virus was isolated from chickens, and mortality rates were high.**
- **A total of 1.5 million birds were culled in 3 days.**
- **A monitoring system was instituted for birds in live markets.**

An H7N1 strain occurred in the Veneto region of Italy in December 1999.

A total of 30,000 turkeys died or were destroyed to contain the outbreak.

In 1998, H5N2 strains of the virus were responsible for at least eight outbreaks on Italian farms

Other countries that have had outbreaks include Australia (H7), England (H7), South Africa (H5), Scotland (H5), Ireland (H5), Mexico (H5), and Pakistan (H7).

An outbreak of HPAI occurred in the Netherlands in 2003. Over 28 million birds out of a total 100 million birds in the country were killed.
There were reports of over 80 human cases, and one veterinarian died. The disease spread to Belgium but was quite rapidly contained.

An outbreak of H5N1 in poultry in Asia started in late 2003 and continues to spread at a rate that is historically unprecedented. counting those culled in China.

- **So far, it involves South Korea, Vietnam, Japan, Thailand, Cambodia, Indonesia, Laos, and China (plus low-pathogenic avian influenza [H5N2] in Taiwan and an H7 HPAI strain in Pakistan).**
- **Of special concern is that fact that in Vietnam and Thailand, the avian strain (H5N1) has been confirmed in numerous cases of fatal human respiratory disease.**
- **A total of about 80 million birds have been sacrificed.**

Infections in cats (leopards, tigers and domestic cats) fed raw infected poultry carcasses have also been reported.

As of February 27, there have been a total of 33 laboratory confirmed human cases, of which 22 have died.

Cases of H5N1 were also reported in other avian species such as ducks, falcons and cranes.

Historically, H5N1 is the only Avian Influenza virus strain that has been able to cause severe human illness (Hong Kong 1997).

H7N7 and H9N2 strains have also been reported to cause mild illness and one death.

Infection reappeared in Asia in July,2004.

An outbreak of avian flu was reported in Delaware in early February 2004, followed by outbreaks at farms in New Jersey and Pennsylvania. The strains involved (H2N2 and H7N2) are not related to those circulating in Asia and do not infect humans.

AI was also identified in Texas and in Canada

HPAI As a Biological Weapon

- **HPAI is considered a potential biological weapon because of the following factors:**
- **High mortality rate**
- **Severe economic consequences of an outbreak:**
- **Large numbers of birds are destroyed or die.**
- **Control measures disrupt trade of poultry products from affected areas.**
- **Prices of retail poultry products may increase significantly**

species

The Hong Kong epidemic of 1997 and the associated human cases demonstrate the ability of the virus to affect humans and birds

Clinical Features

- **The clinical signs of HPAI are severe and result in high mortality rates in many species of birds, especially domestic fowl.**
- **As mentioned above, waterfowl, ratites, and other birds may not be as susceptible to clinical signs, but can act as carriers for the virus.**

Pathogenic Avian Influenza in Animals
Feature Characteristics

- **CHICKENS**
- **Incubation Period - 3-7 days**
  Clinical signs
- **—Sudden death**
  —Severe depression with ruffled feathers
  —Inappetence
  —Drastic decline in egg production

Lesions

- **Edema of head and neck**
- **—Swollen and cyanotic combs and wattles**
- **—Petechial hemorrhages on internal membrane surfaces**
- **—Excessive thirst**
  —Watery diarrhea that begins as bright green and progresses to white
  —Swollen and congested conjunctiva with occasional hemorrhage
  —Diffuse hemorrhage between hocks and feet

Respiratory signs are dependent on tracheal involvement

- —Nasal and ocular discharge
- —Mucus accumulation (varies)
- —Lack of energy
- —Coughing/sneezing

Nervous signs

- —Incoordination
- —Nervous system signs such as paralysis

Complications

- —Cessation of egg production, and eggs laid immediately prior to infection often soft-shelled and misshapen
- —Surviving birds are in poor condition and resume laying only after a period of several weeks

Case-fatality rate

- —Can be as high as 100%
- —Death may occur prior to any symptoms or as late as a week after symptoms, though it is frequently within 48 hr

TURKEYS

- **Incubation period**
  - **3-7 days**
  - **Clinical signs**
    - **—Sudden death**
    - **—Severe depression with ruffled feathers**
    - **—Inappetence**
    - **—Drastic decline in egg production**
    - **—Edema of the head and neck**
    - **—Swollen and cyanotic combs and wattles**
    - **—Petechial hemorrhages on internal membrane surfaces**
    - **—Excessive thirst and evidence of dehydration**
    - **—Watery diarrhea that begins as bright green and progresses to white**
    - **—Swollen and congested conjunctivae with occasional hemorrhage**
    - **—Diffuse hemorrhage between hocks and feet**
    - **—Respiratory signs are dependent on tracheal involvement**
    - **—Nasal and ocular discharge**
    - **—Mucus accumulation (varies)**
    - **—Lack of energy**
    - **—Coughing/sneezing**
    - **—Incoordination**
    - **—Nervous system signs such as paralysis**
    - **—Sinusitis**
    - **—Dehydration**
  - **Complications**
    - **—Decrease in egg production**
    - **—Sudden death**
    - **—Surviving birds are in poor condition and resume laying only after a period of several weeks**
  - **Case-fatality rate**
    - **—Can be as high as 100%**
    - **—Most turkeys die within 3 to 10 days**

DOMESTIC DUCKS AND GEESE

- **Incubation period**
  3-7 days
  Clinical signs
- **—Signs of depression, inappetence, and diarrhea similar to those seen in layers**
  —Swollen sinuses
  —Neurologic signs in younger birds
  —Sinusitis
- **Complications**
  —Decrease in egg production
  —Sudden death
  —Surviving birds are in poor condition and resume laying only after a period of several weeks
- **Case-fatality rate**
  As high as 100%

Chickens

Necropsy Lesions

HPAI can be recognized by the high mortality rate in affected flocks as well as by the clinical signs. Characteristic necropsy lesions, listed in the table below, also can help make the diagnosis.

Lesions Associated With Highly Pathogenic Avian Influenza in Animals*

- **Type of Bird**
- **Characteristics**

Chickens

—Lesions may be absent in young birds and birds that die from peracute disease

—Severe congestion of musculature

—Severe congestion of conjunctivae, sometimes with petechia

—Excessive mucous exudates in lumen of trachea

—Severe hemorrhagic tracheitis

—Petechiae on inside of sternum

—Petechiae on serosal and abdominal fat and in body cavity

—Severe kidney congestion, sometimes with urate deposits in tubules

—Hemorrhages on mucosal surface of proventriculus, especially at juncture with gizzard

—Hemorrhages and erosions of gizzard lining

—Hemorrhagic foci on lymphoid tissues in intestinal mucosa

—Ovary may be hemorrhagic or degenerated with darkened areas of necrosis

—Peritoneal cavity often filled with yolk from ruptured ova

Turkeys

- Lesions similar to those in chickens but may not be as severe

Domestic ducks

- **Lesions may be similar to those seen in chickens though not as marked, or they may be absent altogether**

Quail

- More susceptible than chickens
- May not show clinical signs
- But may shed virus.

Differential Diagnosis

- **Other diseases to consider when examining birds suspected of having HPAI include:**
- **Velogenic (exotic) Newcastle disease**
  Infectious laryngotracheitis
  Acute Escherichia coli infections
  Acute fowl cholera (Pasteurella multocida)
  Bacterial sinusitis (ducks)

Laboratory Diagnosis

- Sample Collection
  All specimens sent to the laboratory for testing should come with a history of the clinical and gross lesions observed.

- Multiple specimens from the same farm should be sent because tests on a single specimen may fail to yield the virus.

Samples need to be kept frozen during shipping

Dead Birds

Lungs
Air sacs
Trachea
Brain
Pancreas
Spleen
Serum (ducks and geese

Live birds:

- **Tracheal and cloacal swabs or feces**
- **Swabs should be transferred to brain and heart infusion broth or other cell culture maintenance medium containing high levels of antibiotics**
- **Cloacal swabs from up to five birds can be pooled in the same tube of broth**
- **Clotted blood or serum from several live birds.**

Identification of the Agent

- **The agent is identified by inoculating 9- to 11-day-old embryonated chicken eggs with swabs or tissue specimens, followed by:**
- **Demonstration of hemagglutination**
- **Immunodiffusion test to confirm the presence of avian influenza A virus**

Subtype determination with monospecific antisera

Strain virulence can be assessed by evaluation of the intravenous

pathogenicity index (IVPI) in 4- to 8-week-old chickens.

Serologic Tests

- **Because sera from infected chickens can yield positive antibody tests as early as 3 to 4 days after the first signs of disease appear, serologic tests can be useful to diagnose the disease**

Specific hemagglutination inhibition tests are the basis for determining the antibodies associated with the influenza viral strain

Agar gel immunodiffusion

Commercial enzyme-linked immunosorbent assay (ELISA) test kits

All positive readings are confirmed with agar gel precipitation tests

Treatment

- There is no effective treatment for HPAI in poultry.

Prevention

A) Biosecurity

- **The best way to prevent HPAI from spreading is to prevent exposure of flocks to the influenza virus. This can be done in several ways:**

1. Avoid contact between domestic poultry and wild birds, especially waterfowl.

Open-range operations have a greater risk of acquiring influenza virus in regions where migratory waterfowl, sea birds, and shore birds are found.

2. Avoid the introduction of birds of unknown disease status into a flock.

3. Control human traffic.

4. Provide clean clothing and disinfection facilities for employees.

5. Permit only essential workers and vehicles to enter the farm.

Follow proper cleaning and disinfection procedures.

6. Use an all-in/all-out production system.

7. Keep poultry away from any water source that may have been contaminated by migratory waterfowl, sea birds, or shore birds.

Chlorinate drinking water from ponds.

8. Thoroughly clean and disinfect equipment and vehicles entering and leaving the farm; the tires and undercarriage of vehicles should be included in the process.

9. Do not loan or borrow equipment or vehicles from other farms.

10. Avoid visiting other poultry farms.
If unavoidable or if visiting a live-bird market, change footwear and clothing before working with your own flock

11. Do not bring birds from slaughter channels, especially live-bird markets, back to the farm.

12. Thoroughly disinfect crates before returning them to the farm.

Live Market Practices

- **The 1997 HPAI outbreak in Hong Kong demonstrated the difficulties of preventing spread of influenza virus in live markets.**
- **Once the virus is established in such a market, it can easily spread via the movement of birds, crates, or trucks to other farms and/or markets.**
- **t is important to follow biosecurity protocols at live-bird markets as well as on the farm.**
- **Use plastic instead of wooden crates for easier cleaning.**
- **Keep scales and floors clean of manure, feathers, and other debris.**
- **Clean and disinfect all equipment, crates, and vehicles before returning them to the farm.**
- **Keep incoming poultry separate from unsold birds, especially if birds are from different lots.**
- **Clean and disinfect the marketplace after every day of sale.**
- **Do not return unsold birds to the farm.**

Vaccination

- Vaccination can be an option and has
- been used to control outbreaks in Pakistan and Mexico. However, it can cause additional long-term damage because some vaccinated birds may still become infected and shed the virus
- There is no cross-protection between the 15 different known hemagglutinin subtypes of influenza A virus, so specific subtype vaccines are necessary to gain protection

Several different types of vaccines are available:

- **Inactivated oil-emulsion vaccines**
- **These vaccines are more expensive.**
- **They have been demonstrated to be effective in reducing mortality rate and preventing disease in chickens and turkeys**

vaccines

- Vaccines using naturally avirulent or attenuated strains

  These types are more economical.
  They may create a genetic reassortment of different influenza viruses with unpredictable characteristics

- Vaccine must be multivalent or else the vaccination must be postponed until the prevalent disease-causing subtype in the area is identified
  Recombinant fowlpox virus vaccine contains the gene encoding production of the H5 antigen

Vaccines

- **Experiments have been done using infectious laryngotracheitis virus as a vector for expression of the H5 antigen**
- **Results have been promising for preventing disease and reducing shedding of a challenge virus.**
- **The vaccine would allow rapid generation of emergency vaccines against influenza epidemic strains.**

Outbreak Control

- **Several steps must be taken to control an outbreak of HPAI:**
- **Slaughter of all birds suspected of being infected (and compost in the shed)**
- **Proper disposal of carcasses and all animal products in contact with the infected flock**
- **Stringent cleaning and disinfection of the facilities and equipment**
- **No new birds allowed in facilities for at least 21 days after depopulation and disinfection**

Personal Protective Equipment

- **Prior to commencing the Avian Influenza decontamination programme all personnel must be provided with and wear personal protective equipment comprising of:**

  respirator face mask
  eye protection
  coveralls
  heavy duty rubber gloves
  rubber or polyurethane boots

First Disinfection

- **Once all birds/carcasses have been removed from the f**
  1.Thoroughly disinfect all bird carcasses and remove for burning or burial
  2.Spray disinfectant on all areas where there has been direct or indirect contact with the birds (buildings, cages, runs, etc).
- **3.Thoroughly soak all nesting, litter and faecal material with disinfectant.**
- **4.Ensure disinfectant is sprayed on all interior walls, into roof spaces and voids.**
  5.External walls need only be disinfected where they face onto an area where infected birds have passed through or been kept (external surfaces and roofs are not required to be disinfected).
  6.Disinfectant must be left to soak into all surfaces, bedding etc for 24 hours.
  7.Disinfect farm roads and footpaths thoroughly ensuring that any drains are sealed so as not to contaminate watercourses.

Secondary Disinfection

- **Do not undertake secondary disinfection until at least 24 hours after the first disinfection has been completed.**

1.Remove all litter and dirt from the buildings to a manure heap, spraying the outside of the heap thoroughly with disinfectant.

2. Leave the manure heap for at least 42 days prior to spreading or burn it off in small quantities.

3. Dry clean and then disinfect ventilation and extractor systems by wiping with a cloth soaked with disinfectant. If the systems cannot be cleaned in situ, remove them, clean and disinfect them, and then put them back into place.

4. Using Antec Biosolve heavy duty cleaner in conjunction with a pressure washer (on a low pressure setting), thoroughly soak the floors, walls, ceilings, roof spaces, nest boxes, feeders etc of all buildings that have been in direct contact with birds.

Remove and cobwebs and dust by either spraying or using an industrial vacuum cleaner

5 . Upon completion of point 4, all equipment should be degreased using Biosolve (apply using a foaming lance). Allow at least 10 minutes for the detergent to penetrate and loosen the dirt from all surfaces before rinsing at high pressure with clean water.

Ensure that all equipment is then thoroughly disinfected.

6. The farm buildings, loading bays, paths etc should then be treated with disinfectant leaving it to soak for at least 30 minutes before rinsing with clean water.

7. After 7 days have passed, repeat the complete decontamination programme and procedures once again.

Important Notes

- 1. If a farm building has an earth, clay or chalk floor, it must be first scraped clean then the surface should be broken up and thoroughly soaked with disinfectant.
- Cobble or hardcore floors should be soaked with disinfectant and then rinsed.
- Any material scraped from the floors should either be burnt or removed to the manure heap.

- 2. Wooden floors must be taken up and cleaned & disinfected if they are to be reused. The underlying soil should be broken up and soaked with disinfectant.

- 3. Any timber or scrap wood such as pallets, old fence posts and sleepers not of good enough quality to be satisfactorily disinfected must be burned

Important notes, Cont'd

- **4. When disinfecting out side pay particular attention to:**
  Roadways
  Paths
  Gates
  Gateways
  Runs
- **For environmental awareness take care to avoid any solutions entering surface water drains or water courses.**

- **5. Pay particular attention to the cleansing and disinfection of any hired utensils, machinery (Antec Vehicle Disinfection Programmes) and equipment, which may have been contaminated.**
- **6. Straw, baled hay, wood chippings and any paper should be sprayed with disinfectant. Any loose material lying around should be sprayed and then removed to the manure heap or burnt.**

Public Health Issues

- **In the past several years, it has become clear that HPAI viruses can infect humans.**
- **Examples that have been identified include the following:**

During the outbreak of H5N1 avian influenza in Hong Kong in 1997, 18 human cases were recognized, with six fatalities .

Fortunately, sustained person-to-person transmission did not occur and the outbreak stopped when all birds in the Hong Kong commercial poultry industry (approximately 1.4 million) were slaughtered.

Two additional cases of H5N1 influenza were reported in humans in the spring of 2003

In March 1999, H9N2 avian influenza occurred in two children (ages 4 years and 13 months) Both had been hospitalized with influenza-like illness and both recovered uneventfully

No additional cases of person-to-person transmission occurred. Further investigation demonstrated that H9N2 strains were circulating in poultry in Hong Kong and China, although the viruses were not highly pathogenic for birds

During the outbreak of H7N7 HPAI in the Netherlands in 2003, the H7N7 strain spread to poultry workers and their families in the area. Over 80 cases in humans occurred; most had conjunctivitis and several complained of influenza-like illness

One patient (a 57-year-old veterinarian) died of acute respiratory distress syndrome.

Human cases have occurred with the current outbreak of H5N1 in Asia..

Despite the lack of serious public health consequences in most of the examples above, the greatest public health concern with HPAI continues to be the potential emergence of a viral strain in the avian population that could ultimately lead to a global influenza pandemic in humans.

For a pandemic to occur, the novel strain would need to have the following features:

Highly pathogenic for humans
Easily transmitted between humans
Genetically unique (ie, lack of pre-existing immunity in the human population)

Past influenza pandemics occurring during the 20th century apparently all arose from the Eurasian avian lineage of viruses.

These strains underwent genetic reassortment, most likely in pigs, before spreading widely among humans.

It is unclear whether reassortment in another animal host is necessary or whether an avian strain could directly cause a global pandemic in humans

Background on Pandemics

- **An influenza pandemic is a global outbreak of influenza and occurs when a new influenza virus emerges, spreads, and causes disease worldwide. Past influenza pandemics have led to high levels of illness, death, social disruption and economic loss.**

There were 3 pandemics in the 20th century. All of them spread worldwide within 1 year of being detected. They are:

1918-19, "Spanish flu," [A (H1N1)], caused the highest number of known flu deaths: more than 500,000 people died in the United States, and 20 million to 50 million people may have died worldwide. Many people died within the first few days after infection and others died of complications soon after. Nearly half of those who died were young, healthy adults.

1957-58, "Asian flu," [A (H2N2)], caused about 70,000 deaths in the United States. First identified in China in late February 1957, the Asian flu spread to the United States by June 1957.

1968-69, "Hong Kong flu," [A (H3N2)], caused approximately 34,000 deaths in the United States. This virus was first detected in Hong Kong in early 1968 and spread to the United States later that year. Type A (H3N2) viruses still circulate today.