

HIGHLY PATHOGENIC AVIAN INFLUENZA

Aetiology Epidemiology Diagnosis Prevention and Control References

AETIOLOGY

Classification of the causative agent

Virus family Orthomyxoviridae, genus *Influenzavirus A*. To date, all highly pathogenic isolates in birds have been influenza A viruses of subtypes H5 and H7.

Resistance to physical and chemical action

Temperature: Pasteurisation and cooking are effective means of inactivation. AIV is inactivated at 60°C for 188 seconds in whole eggs and 507 seconds in poultry meat. AIV is also inactivated in meat by cooking when reaching a core temperature of 70°C for 3.5 seconds. AIV can survive indefinitely when frozen.

pH: Inactivated by acid pH of ≤ 2 .

Chemicals: Inactivated by organic solvents and detergents (sodium desoxycholate, sodium dodecylsulphate). In presence of organic matter: aldehydes (formaldehyde, glutaraldehyde), β -propiolactone and binary ethyleneimine. After removal of organic matter: phenolics, quaternary ammonium compounds, oxidising agents (sodium hypochlorite, potassium peroxymonosulfate/sodium chloride), dilute acids (if pH ≤ 2), hydroxylamine, and lipid solvents.

Disinfectants: Clean surfaces in which organic material has been removed: sodium hypochlorite (5.25%), sodium hydroxide (2%), phenols, acidified ionophors, chlorine dioxide, strong oxidising agents, and sodium carbonate (4%)/sodium silicate (0.1%).

Survival: The tenacity of AIV in the environment is often underestimated. Especially cool and moist conditions and presence of organic material favour long survival. This is the case in many surface waters. Viable in liquid faeces for 30–35 days at 4°C and for 7 days at 20°C. Survived 4 days in chicken faeces held 25–32°C in the shade. LP H7N2 virus persisted for up to two weeks in feces and on cages. In water survived 26–30 days at 28°C, and 94–158 days at 17°C. Composting kills virus within poultry carcasses in <10 days.

EPIDEMIOLOGY

- Highly (Moderately) contagious
- Avian HPAI viruses have not established endemic infection status in domesticated poultry in developed nations.
- Reservoirs of LPAI virus occur worldwide in wild birds, especially waterfowl, sea birds and shore birds. Low pathogenic strains have been isolated from >90 species of 13 orders, but the reservoir is likely much greater. Most infections have not produced recognisable disease in wild birds. An outbreak of HPAI can occur in any country, due to the transmission of LPAI strains from reservoirs into poultry followed by mutation, presumably in gallinaceous poultry, to high pathogenicity. No evidence for HPAI generation in reservoir hosts so far.
- The current HPAI H5N1 outbreak began in poultry in Southeast Asia in 2003. From 2003 to 2007, 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 many countries have eradicated the virus from their domesticated poultry, this epidemic is ongoing and worldwide eradication is not expected in the short term.

Hosts

- Highly pathogenic avian influenza isolates have been obtained primarily from chickens and turkeys
- It is reasonable to assume all avian species are susceptible to infection
- Avian influenza viruses mainly infect birds, but some strains can also cause spill over into horses, mink, cats, dogs, ferrets, stone martens, palm civets, marine mammals and other species with clinical sequelae. Waterfowl and shore birds appear to be the natural

reservoirs for the influenza A viruses, and carry all of the known subtypes. In cage birds, most infections have been recorded in passerine birds. Psittacine birds are rarely affected.

- Several clades of HPAI H5N1 viruses are currently circulating in poultry. These viruses can infect and cause disease in numerous species of birds in addition to poultry. Unusually, the H5N1 viruses have caused severe disease and deaths in some species of wild waterfowl, which usually carry avian influenza viruses subclinically. Most HPAI H5N1 viruses of the current lineages have been isolated from birds in the order Anseriformes, particularly the families Anatidae (ducks, swans and geese) and Charadriiformes (shore birds, gulls and terns). Clinical infections have also been reported in pheasants, partridges, quail, guinea fowl and peafowl (order Galliformes); egrets, storks and herons (order Ciconiiformes); pigeons (order Columbiformes); eagles, falcons and buzzards (order Falconiformes); owls (order Strigiformes); cranes, moorhens and sultans (order Gruiformes); cormorants (order Pelecaniformes), emus (order Struthioniformes), grebes (order Podicipediformes), budgerigars (order Psittaciformes) and flamingos (order Phoenicopteriformes). Natural clinical or experimental infections have also been reported in passeriform birds including zebra finches, house finches, house sparrows, Eurasian tree sparrows, mynahs, crows, magpie robins, munias, orioles and magpies.
- Several sublineages/strains of HPAIV H5N1 cause productive but subclinical infections in domestic ducks. These hosts serve as motors for the perpetuation of the virus in poultry populations.
- A few fatal H5N1 infections have been reported in zoo tigers, zoo leopards, housecats, captive palm civets, a dog and a stone marten. Experimental infections have been established in housecats, dogs, pigs, ferrets, rodents, macaques and rabbits. Miniature pigs were resistant to infection in one study. Ferrets are susceptible experimentally.
- Human beings can be infected although there seems to be a high, species-specific transmission barrier. Established infections, however, frequently take a fatal course.

Transmission

- Clinically normal waterfowl (domesticated or wild) and sea birds may introduce the virus into flocks, and people and their equipment readily transport the virus. Secondary dissemination is by fomites, movement of infected poultry, and possibly airborne.
- Direct contact between infected and susceptible birds: sharing of faeces and secretions. Airborne transmission may occur if birds are in close proximity and with appropriate air movement. Faeces are significant because of the large volume excreted and ability to contaminate fomites.
- Important fomites: people (shoes and clothing), feed, water, egg crates and equipment.
- Broken contaminated eggs may infect chicks in the incubator. HPAI virus has been recovered from the eggshell and internal egg contents, but proof of vertical transmission is lacking because most virus strains are embryolethal and eggs do not hatch.

Sources of virus

- Faeces, saliva, nasal and respiratory secretions. Faeces contain large amounts of virus, and faecal-oral transmission is usually the predominant means of spread in wild bird reservoirs. However, some recent isolates of HPAI H5N1 have been found in higher quantities in tracheal samples than faeces, suggesting that the primary means of transmission for HPAI H5N1 virus may no longer be the fecal-oral route in some species.
- With HPAI viruses high levels occur in tissues of infected birds.
- HPAI viral titres in meat vary with virus strain, bird species and clinical stage of infection.

Occurrence

- Apathogenic and mildly pathogenic influenza A viruses occur worldwide.
- Wild birds have the potential to introduce these viruses into domestic poultry anywhere in the world, with potential mutation to a highly pathogenic form for representatives of the H5 and H7 subtypes.
- Highly pathogenic avian influenza A (HPAI) viruses of the H5 and H7 HA subtypes have been isolated occasionally from free-living birds in Europe and elsewhere, with the potential for introduction to domestic poultry. It is currently not clear whether occurrence

of HPAIV in wild birds requires previous spill-over infection from infected poultry or whether HPAIV can establish endemic status in wild bird populations.

- There have been 26 documented epidemics of HPAI through 2007 since the discovery of AIV in 1955, including the current H5N1 outbreak still in progress.

Currently, the world is experiencing the most extensive HPAI outbreak ever recorded. The outbreaks feature a zoonothroponotic strain, and worldwide eradication is not currently feasible. Since 1997, HPAI viruses of the H5N1 subtype appeared in 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 H5N1 viruses reached other parts of Asia, Europe, Africa and the Middle East. The H5N1 strains responsible for this epidemic appear to be unusually virulent. From 2003 through August 2009, these viruses have caused over 440 human infections, generally as the result of close contact with poultry; 262 cases were fatal (www.who.int).

For more recent, detailed information on the occurrence of this disease worldwide, see the OIE World Animal Health Information Database (WAHID) Interface [<http://www.oie.int/wahis/public.php?page=home>] or refer to the latest issues of *the World Animal Health* and the *OIE Bulletin*.

DIAGNOSIS

Incubation period in individual birds usually is short (2–5 days), but the rate of infection in a flock can be highly variable (days to weeks) depending on the environmental conditions (cages versus free-range etc.).

Clinical diagnosis

- Clinical signs vary from sudden death to highly variable clinical presentations: based on species, age, type of bird, viral strain, concurrent infections, and environment.
- Respiratory signs: ocular and nasal discharges, coughing, snicking, dyspnoea, swelling of the sinuses and/or head
- Severe depression, reduced vocalisation, marked reduction in feed and water intake
- Cyanosis of unfeathered skin, wattles and comb
- Incoordination and nervous signs
- Diarrhoea
- Drastic decline in egg production and increased poor quality eggs
- Sudden deaths (mortality is high and can reach 100%)
- In domestic ducks, most HPAI viruses produce few clinical signs.
- Virus detection needed for definitive diagnosis

Lesions

Gallinaceous poultry Variety of oedematous, haemorrhagic and necrotic lesions in visceral organs and the skin.

- Lesions may be absent in cases of sudden death
- Swelling of head, face, upper neck and feet from subcutaneous oedema
- Severe congestion of the musculature
- Dehydration
- Nasal and oral cavity discharge
- Severe congestion of conjunctivae, sometimes with petechiae
- Excessive mucous exudate in the lumen of the trachea, or severe haemorrhagic tracheitis
- Petechiae on the inside of the sternum, on the serosa and abdominal fat, serosal surfaces and in the body cavity
- Severe kidney congestion, sometimes with urate deposits in the tubules
- Haemorrhages and degeneration of the ovary
- Haemorrhages on the mucosal surface of the proventriculus, particularly at the juncture with the gizzard
- Haemorrhages and erosions of the gizzard lining
- Haemorrhagic foci on the lymphoid tissues in the intestinal mucosa
- Necrotic foci common in pancreas, spleen and heart

Ducks infected with HPAI and excreting the virus, may not show any clinical signs or lesions. Geese more likely develop central nervous disorders.

Differential diagnosis

- Acute fowl cholera
- Velogenic Newcastle disease
- Respiratory diseases, especially infectious laryngotracheitis
- Heat exhaustion, water deprivation, and some toxins

Laboratory diagnosis

Procedures

Samples

Identification of the agent

- Oropharyngeal and cloacal swabs (or fresh faeces) from live birds.
- Intestinal contents (faeces) or cloacal swabs and oropharyngeal swabs from dead birds. Samples from trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart should also be collected and processed either separately or as a pool.
- Swabs from up to five birds can be pooled in the same tube of broth – but do not mix swabs from different sites or tissues.

Serological tests

- Clotted blood samples or serum from several birds in standard serum tubes.

Identification of the agent

All virulent strains isolated to date have been either of the H5 or H7 subtype, although most H5 and H7 isolates have been of low virulence.

Characterisation of suspected virulent strains of the virus should be conducted in a virus-secure laboratory at biocontainment level 3 or greater. If HPNAI is used in challenge studies, the facility should meet the OIE requirements for Containment Group 4 pathogens.

*Virus isolation with pathogenicity testing (the prescribed test in the OIE *Terrestrial Manual*)*

- Virus isolation: inoculation of 9-11-day-old embryonated chicken eggs followed by:
 - Demonstration of haemagglutination in the allantoic fluid suggests presence of virus.
 - Immunodiffusion test confirms the presence of influenza A virus.
 - Subtype is determined by using monospecific antisera prepared against the 16 haemagglutinin (H1–H16) and 9 neuraminidase (N1–N9) subtypes of influenza A viruses or by haemagglutination and neuraminidase inhibition tests against a battery of polyclonal antisera to a wide range of strains covering all the subtypes.
- Strain virulence is evaluated by inoculation of eight or more 4–8-week-old chickens: strains are highly pathogenic if they have greater than 1.2 IVPI in 6-week-old chickens, or, as an alternative, cause at least 75% mortality in 4–8 week old chickens infected intravenously. H5 and H7 viruses which do not meet these criteria should be sequenced to determine whether multiple basic amino acids are present at the HA0 cleavage site of the haemagglutinin molecule. If the amino acid motif is similar to other virulent viruses, the strain should be considered as HPNAI.
- Isolation in embryos has recently been replaced, under certain circumstances, by real-time reverse-transcription polymerase chain reaction (RT-PCR) which offers advantages regarding economy of time until diagnosis, high-throughput possibilities and protection of laboratory staff.

- A complete diagnosis and characterisation of HPAIV can be accomplished by molecular means.

Serological tests

- Agar gel immunodiffusion (alternative test in the OIE *Terrestrial Manual*): detects antibodies to antigens common to all influenza A viruses. Sera from infected chickens usually produce AGID positive antibody tests as early as 3–4 days after appearance of clinical signs. AGID testing is best used for Low Pathogenic AI surveillance and requires use of H5 and H7 specific HI tests to identify Notifiable AI virus infections. AGID is not a reliable test for detecting AI virus infections in ducks or geese.
- ELISA: detects antibodies to antigens common to all influenza A viruses. Subtype-specific ELISA (e.g. H5, H7) are increasingly made available commercially.
- *Haemagglutination* or Haemagglutination inhibition tests (HI is alternative test in the OIE *Terrestrial Manual*): determines the haemagglutinin subtype. Use of a homologous or closely related antigen is pivotal to ensure suitable sensitivity of HI when examining an outbreak.

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapter 2.3.4 Avian influenza in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Diagnostic Techniques”.

PREVENTION AND CONTROL

No treatment

Sanitary prophylaxis

- Avoidance of contact between poultry and wild birds or their fomites (including surface waters!), in particular waterfowl
- Avoidance of the introduction of birds of unknown disease status into flock
- Birds should not be returned to the farm from live bird markets or other slaughter channels.
- Control of human traffic: strict hygiene and biosecurity measures to prevent fomite transmission.
- One age/species group per farm ('all in-all out') breeding is recommended

In outbreaks

- Farms must be quarantined, and movement controls and surveillance be established.
- Flock to be depopulated and the carcasses rendered innocuous. Staff must observe personal protection rules to avoid human infections (HPAIV but also Chlamydiae etc.)
- Disposal of carcasses and all animal products by burying, composting or rendering.
- 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 tightly covered with black polyethylene sheets to prevent entry of birds, insects and rodents. Feathers can be burned or composted; alternatively, they may be removed and the area wet down with disinfectant.
- Thorough cleaning first, then disinfection. High-pressure spray equipment should be used to clean all equipment and building surfaces (beware of aerosols, personal protection equipment required). Once all surfaces are clean and free of all organic material, the entire premises should be sprayed with an approved residual disinfectant.
- Allow at least 21 days before restocking.
- Cats and dogs should not be fed poultry or other birds that may be infected with H5N1 avian influenza viruses. During outbreaks, they should be kept indoors.

Medical prophylaxis

- 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. Because vaccines may allow birds to shed virus while remaining subclinical, good surveillance and movement controls are critical in a vaccination campaign. Methods used to recognise infections with field viruses in vaccinated flocks include a “DIVA” (differentiating infected from vaccinated 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. Limited evidence suggests that some newer strains of H5N1 viruses, which were isolated in China in 2006, and in Egypt since 2007, may be resistant to the currently available vaccines.

- Inactivated oil-emulsion vaccines, although fairly expensive, have been demonstrated to be effective in preventing clinical signs and death, increasing resistance to infection, reducing infections, reducing shedding of AI virus from respiratory and intestinal tracts, and preventing contact transmission in a variety of birds. In addition, recombinant fowl poxvirus with H5 AI hemagglutinin gene insert and recombinant Newcastle Disease vaccine strains have shown similar protection in chickens. However, no AI vaccine will provide absolute prevention of infections and environmental contamination: biosecurity, surveillance and other management practices must be practiced concurrently for AI control to be successful.
- Anti-influenza drugs should not be used in poultry.

For more detailed information regarding vaccines, please refer to Chapter 2.3.4 Avian influenza in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Requirements for Vaccines and Diagnostic Biologicals”.

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the OIE *Terrestrial Animal Health Code*.

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department (scientific.dept@oie.int). Last updated October 2009.