Network for wildlife health surveillance in Europe

*EWDA Diagnosis Card*

# Avian Influenza

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**Etiology**

Caused by influenza A virus, family *Orthomyxoviridae*. Enveloped virus with single-strand negative RNA divided into 8 segments. Divided into subtypes according to antigenic variation in hemagglutinin (HA, 16 subtypes) and neuraminidase (NA, 9 subtypes) glycoproteins. Also divided in low (LPAIV) and high pathogenic avian influenza viruses (HPAIV) based on pathogenicity for chickens.

**Affected species** (wildlife, domestic animals, humans)

Natural reservoirs are wild waterbirds, especially Anseriformes and Charadriiformes, in which they are typically LPAIVs. LPAIVs also infect poultry. In terrestrial poultry, LPAIVs of H5 and H7 subtypes may mutate into HPAIVs. HPAIVs are typically restricted to poultry but may spill over to wild birds. HPAIV H5 of the Goose/Guangdong lineage (Gs/Gd) can infect and cause disease and death in a wide range of wild birds. LPAIV can also infect mammalian species, have caused disease in pilot whales (*Globicephala melas*), harbour seals (*Phoca vitulina*) and American mink (*Mustela vison*), and have established independent lineages in domestic horses, domestic pigs, domestic dogs and humans. Serological evidence of LPAIV infection exists in other mammalian species. Sporadic HPAIV infections in mammals. HPAIV H5N1 sporadically observed in a wider range of mammals, including domestic cats, domestic dogs, tigers (*Panthera tigris*), leopards (*Panthera pardus*), Owston's palm civets (*Chrotogale owstoni*), stone martens (*Martes foina*), American mink, raccoon dogs (*Nyctereutes procyonoides*), domestic pigs, donkeys and humans.

**Epidemiological characteristics and disease course**

LPAIV and HPAIV infections typically epidemic in birds and mammals. LPAIV prevalence in wild waterbirds generally peaks between late summer and early winter, depending on bird species and geographical regions. LPAIV prevalence varies greatly across geographical areas and among bird species. Outbreaks of HPAIV H5 Gs/Gd often associated with autumn migration. In recent years, some HPAIV genotypes belonging to H5 Gs/Gd lineage have been regularly circulating in the Eurasian continent in wild birds (in particular Anseriformes) where they have reassorted with LPAI strains. Prevalence of infection and mortality due to these viruses in wild bird populations varies from year to year based on the characteristics of the strain and to the susceptibility of involved species. No clear seasonal or geographical patterns for LPAIV or HPAIV H5N1 outbreaks in mammals. There is a chance for some susceptible species of predators, such as wild felids and mustelids living in an area with numerous cases of HPAI, to become infected after eating infected birds.

Course of LPAIV and HPAIV acute infection, ending with the mounting of a specific immune response or death (HPAIV). Infection usually lasts 4 to 8 days but may continue up to several weeks. LPAIV mostly infects the epithelium of the digestive tract and bursa of Fabricius in wild birds, and the epithelium of the respiratory tract in mammals. HPAIV H5 Gs/Gd infects respiratory epithelium and the parenchymal cells of internal organs, including pancreas, liver, kidney, adrenal glands, and brain in birds and mammals. Endothelium is rarely infected but has been reported in swans.

**Clinical signs**

*Birds*: LPAIV infection generally subclinical. HPAIV H5 Gs/Gd in wild birds sub-clinical to fatal. HPAIV H5 Gs/Gd infection in susceptible wild bird species causes prominent respiratory and neurological signs, including circling, ataxia and torticollis.

*Mammals*: LPAIV can cause respiratory disease (fever, weight loss, dry cough, labored breathing, and nasal discharge) that may be fatal. HPAIV H5N1 generally results in severe respiratory and

neurological disease, and death. Clinical signs include high fever, serosanguineous nasal discharge, respiratory distress, ataxia, circling and hind limb paralysis. Neurological signs (ataxia) were observed in American mink. Signs of severe illness including depression, ataxia and circling was observed in a HPAI H5N1 free-living infected stone marten.

**Gross lesions**

*Wild birds*: Gross lesions generally absent in LPAIV infection; variable in HPAIV H5 Gs/Gd infection: absent or in-keeping with known findings for HPAI in domestic species, i.e.: multifocal pancreatic and hepatic necrosis, haemorrhagic duodenitis, multifocal haemorrhages in the myocardium, air sac thickening pneumonia with haemorrhages and oedema, multifocal pulmonary consolidation.

*Mammals*: Gross lesions of LPAIV and HPAIV H5N1 infection include multifocal pulmonary consolidation. In case of HPAIV H5N1 infection, lesions additionally include multifocal necrosis in one or more internal organs, notably the liver.

**Histological lesions**

*Wild birds*: Histological lesions of LPAIV infection generally absent, but mild epithelial degeneration of intestinal and bursal mucosa, as well as broncho-interstitial pneumonia observed in experimentally infected birds. Lesions of HPAIV H5 Gs/Gd infection absent or multifocal necrosis and inflammation in multiple organs, including pancreas, lung, air sac, brain, liver, heart, intestine, and adrenals. In swans (*Cygnus* spp.), hemorrhages in multiple organs, including heart and pancreas.

*Mammals*: Histological lesions of LPAIV infection include tracheobronchitis and necrotizing or hemorrhagic broncho-interstitial pneumonia. Lesions of HPAIV H5N1 infection include necrotizing or hemorrhagic broncho-interstitial pneumonia, and multifocal necrosis and inflammation in multiple organs, including brain, liver, heart, kidney, and adrenals.

**Differential diagnosis**

*HPAIV H5 Gs/Gd in birds*: Newcastle disease, botulism, avian cholera, chlamydiosis, duck virus enteritis (duck plague), West Nile virus infection, avian metapneumovirus infection, blue-green algal poisoning. *LPAIV and HPAIV H5N1 in mammals*: Adenovirus infection, morbillivirus infection, bacterial pneumonia, rabies, poisoning.

**Criteria for diagnosis**

Amplification of influenza A virus RNA; isolation of influenza A virus; detection of influenza A virus antigen in association with lesions.

**Recommended diagnostic method(s) and preferred samples** (incl. recommended amount and appropriate storage)

*LPAIV and HPAIV H5 Gs/Gd diagnosis in live birds or mammals*: Reverse-transcriptase polymerase chain reaction (RT-PCR)/ real time RT-PCR (rRT-PCR) with primer pairs based on conserved gene (e.g., matrix gene) for initial detection; rRT-PCR/RT-PCR on subtype specific genes (HA and NA) and/or virus isolation in specific pathogen free (SPF) embryonated chicken eggs or cell culture (less sensitive) on rRT-PCR/RT-PCR positive samples. Samples of choice: oro-pharyngeal and cloacal swabs in birds. Fresh faeces can be used as an alternative when it is not possible to collect swabs. Pharyngeal, nasal and rectal swabs in mammals. Placed in virus transport medium, either analyzed directly or stored at -70 °C (virus isolation) or -20 °C (RT-PCR) until analysis.

*LPAIV and HPAIV H5 Gs/Gd diagnosis in dead birds or mammals*: same as above. In addition, rRT-PCR/RT-PCR or virus isolation can be performed on fresh tissue samples, while immunohistochemistry on tissue samples fixed in 10% neutral-buffered formalin and embedded in paraffin. Tissue samples of choice: intestine, trachea, and lungs for LPAIV in birds; lungs, liver, brain for LPAIV in mammals; brain, trachea, lungs, pancreas, liver, kidneys for HPAIV H5 Gs/Gd in birds. Brain tissue should always be included in the set of samples collected when testing wild birds for HPAIV; lung, liver, heart, kidney, brain for HPAIV H5 in mammals.

**EWDA proposed protocol** (for harmonization at large scale)

Real time RT-PCR or RT-PCR for target genes (M, HA and NA) and virus isolation on rRT-PCR/RT-PCR positive samples. The above-mentioned samples must be transported to the laboratory refrigerated in virus transport medium with antibiotics and either analyzed directly or stored at -70 °C (virus isolation) or -20 °C (rRT-PCR/RT-PCR) until analysis.

**Laboratories that can be contacted for diagnostic support**

Influenza reference laboratories of the OIE, including EU reference laboratory (see: www.oie.int)
Department of Viroscience, Erasmus Medical Centre, Rotterdam, The Netherlands (http://virosciencelab.org/#home)

**Recommended literature**

1. AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS. 2006. Laboratory manual for the isolation and identification of avian pathogens. 5th Edition. American Association of Avian

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2. REPERANT, L.A., OSTERHAUS A.D.M.E., KUIKEN T. 2012. Avian influenza. In Infectious diseases of wild mammals and birds in Europe. D. Gavier-Widen, J. Duff, and A. Meredith (eds.). Wiley-Blackwell, London, UK< pp. 37-58.
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5. VANDEGRIFT K.J., SOKOLOW S.H., DASZAK P., KILPATRICK A.M. Ecology of avian influenza viruses in a changing world. Annals of the New York Academy of Sciences 1195: 113-128.