

## Case Definition

### Avian Influenza (AI) (Notifiable)

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#### 1. Disease Information

**1.1 General Disease and Pathogen Information:** Avian influenza (AI) is a viral infection of birds including chickens, turkeys, guinea fowl, and other avian species. The agent responsible for avian influenza is an orthomyxovirus. Influenza viruses are classified by examining nuclear and matrix proteins. All influenza viruses from birds and many from mammals are type A. Type A influenza viruses are further classified into subtypes based on antigenic differences of two surface proteins, hemagglutinin (HA) and neuraminidase (NA). There are at least 16 HA and 9 NA subtypes recognized to be of avian origin. Subtypes H5 and H7 low pathogenic avian influenza (LPAI) have been demonstrated to have the potential to mutate to highly pathogenic avian influenza (HPAI) after replication in non-host species such as gallinaceous birds; however, these highly pathogenic viruses typically do not persist in wild birds. There is currently one exception: the H5 HPAI goose/Guangdong-lineage (gs/GD) viruses which emerged in Hong Kong live bird markets in the mid 1990's and continue to circulate and evolve in both poultry and wild birds. Recently the World Organisation of Animal Health (OIE) updated the HPAI definition to an infection of poultry by any influenza A virus that has been determined to be high pathogenicity in accordance with the Terrestrial Manual.

Additionally, some avian-origin strains can cause zoonotic disease, with most of the human cases occurring after direct contact with infected poultry. The incubation period is variable (1-7 days), and dependent upon the AI virus strain, dose, route of exposure, and species of bird. The OIE recognizes a 14-day incubation period at the flock level for high pathogenicity avian influenza for virus spread in a bird population.

AI is spread by direct contact between healthy and infected birds and by indirect contact with contaminated equipment and materials. The virus is excreted through the feces of infected birds and secretions from the upper respiratory tract and eyes and may be dependent upon the course of infection as well as the strain itself. Waterfowl and shorebirds are considered natural reservoirs of influenza A viruses. Wild waterfowl generally show no signs of illness due to AI virus infection but may excrete virus in feces for long periods. However, morbidity and mortality have been documented across multiple wild bird species infected with H5 HPAI goose/Guangdong-lineage (gs/GD) viruses. They may also be simultaneously infected with multiple subtypes and often do not develop detectable levels of antibody. Seasonal infection with AI virus occurs in conjunction with hatching, brooding, and fledging of susceptible juveniles. Influenza A viruses evolve and reassert at an expected rate in natural hosts and typically do not cause mortality.

**1.2 Clinical Signs:** AI virus infections in domestic poultry may be clinically inapparent or result in disease that ranges from mild transient clinical signs to 100 percent morbidity



and/or mortality, depending on strain virulence. Factors such as genetics, nutrition, and co-infection with other pathogens can also affect clinical outcome. When seen, clinical signs can include respiratory, enteric, cardiovascular, or reproductive (e.g., decreased egg production).

**1.2.1** LPAI H5/H7: Often cause either mild or no disease signs, but may cause increased mortality, decreased feed consumption, respiratory signs (e.g., nasal discharge, sneezing), and decreased egg production.

**1.2.2** HPAI: Usually associated with sudden and increased morbidity and mortality along with significant drops in feed and water intake. Swelling and purple discoloration of the combs or wattles, and hemorrhages on the unfeathered parts of legs and feet, as well as neurologic signs may be seen.

## 2. Laboratory Criteria

Subclinical infections identified through active laboratory surveillance or clinical cases with compatible clinical signs and pathologic lesions in a susceptible species are evaluated using laboratory criteria for HPAI and LPAI H5/H7 defined by one or more of the following diagnostic strategies:

**2.1 Agent Isolation and Identification:** Detect presence of influenza A virus by:

**2.1.1** Antigen capture immunoassays (ACIA): collect tracheal/oropharyngeal and/or cloacal swab samples from clinically ill or dead birds. ACIA (test kits approved by APHIS) are for flock level testing; the ability to detect low levels of infection is enhanced by testing multiple samples. Molecular confirmation of positive results is required; negative results with clinical signs require confirmatory diagnostics as indicated in VS Guidance 12001, "Policy for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI)." Samples will be forwarded to USDA's National Veterinary Services Laboratories (NVSL) to determine subtype and pathotype.

**2.1.2** Direct RNA detection: Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) using NVSL-approved molecular assays for influenza A and H5/H7 subtypes, WITH molecular determination of subtype and pathotype direct from swab sample by Sanger sequence methods, OR virus isolation with antigenic and/or molecular characterization.

**2.1.3** Virus isolation and identification: Preferred specimens for virus isolation include tracheal/oropharyngeal and cloacal swabs, or fresh feces from live or dead birds, or samples from organs pooled by system (e.g., respiratory-trachea, lungs, air sacs; enteric-intestine, spleen, kidney, liver; reproductive) from dead birds. Isolated virus is characterized by sequencing methods and in vivo testing where indicated.

**2.1.4** Assumptions: Influenza virus may be detected within 48 hours post-infection (HPAI within 24 hours post-infection) by rRT-PCR or by virus isolation, and 1-5 days post-infection by antigen capture enzyme immunoassay, when virus is shed at moderate to high rates. Oropharyngeal/tracheal specimens are generally



preferred for poultry because virus is typically shed at higher titers via the respiratory route, especially during the early phase of infection<sup>1</sup>. For poultry, all sudden and unexplained spikes in mortality should be investigated.

## **2.2 Agent Characterization:** Strain virulence evaluation:

**2.2.1** Determination of the amino acid sequence at the hemagglutinin cleavage site to identify viruses that have the capacity to become highly pathogenic with or without elevated mortality in *in vivo* assays (see 2.2.2).

**2.2.2** Viruses with an intravenous pathogenicity index (IVPI) greater than 1.2, or that cause at least 75 percent mortality within 10 days in 4- to 8-week-old chickens infected intravenously, are classified as HPAI.

**2.2.3** If H5 or H7 subtypes do not meet the criteria for HPAI, they are classified as H5/H7 LPAI.

## **2.3 Serology:** Demonstration of influenza A antibody by:

**2.3.1** Agar gel immunodiffusion (AGID) or USDA-licensed influenza A enzyme-linked immunosorbent assay (ELISA); and

**2.3.2** Confirmation of antibody to H5 or H7 by hemagglutination inhibition (HI).

## **3. Case Definition and Reporting Criteria**

### **3.1 Suspect Case:**

**3.1.1** Illness compatible with H5/H7 AI infection; **OR**

**3.1.2** Detection of antibodies to influenza A that cannot be explained by vaccination<sup>2</sup>, as determined by AGID or ELISA serological test with or without the presence of compatible illness; **OR**

**3.1.3** Detection of influenza A antigen using a commercially available influenza A antigen test kit (ACIA, approved by USDA) with the presence of compatible illness.

### **3.2 Presumptive Positive Case:**

**3.2.1** A suspect positive case as defined above with detection of H5 or H7 antibodies by HI subtyping; **OR**

**3.2.2** Poultry with identification of influenza A H5/H7 RNA by rRT-PCR with or without the presence of compatible illness.

### **3.3 Confirmed Positive Case:**

**3.3.1** Poultry with influenza A antigen detection (virologic or molecular detection methods) **AND**

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<sup>1</sup> [https://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/downloads/WIAV0020.pdf](https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/WIAV0020.pdf)

<sup>2</sup> USDA permission is required for H5 and H7 vaccine use.



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- 3.3.2** The confirmation of the H5/H7 subtype WITH determination of pathogenicity by NVSL as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (HPAI or H5/H7 LPAI).