APHIS Factsheet

Veterinary Services

May 2013

Avian Influenza Diagnostics and Testing

Avian influenza (AI), commonly known as "bird flu," is caused by an influenza type A virus which can infect poultry (such as chickens, turkeys, pheasants, quail, domestic ducks, geese, and guinea fowl) and some wild bird species (such as wild ducks and swans). AI viruses are classified by a combination of two groups of proteins: hemagglutinin or "H" proteins, of which there are 17 (H1–H17), and neuraminidase or "N" proteins, of which there are 10 (N1–N10). AI viruses are further classified by their pathogenicity—the ability of a particular virus strain to produce disease in domestic chickens.

Highly pathogenic avian influenza (HPAI) virus strains are extremely infectious, often fatal to domestic poultry, and can spread rapidly from flock-to-flock. Low pathogenicity avian influenza (LPAI) virus strains occur naturally in wild migratory waterfowl and shorebirds without causing illness. However, LPAI H5 and H7 viruses are capable of evolving into HPAI viruses in poultry. Because of this potential, infection of commercial poultry by LPAI H5 and H7 viruses—and certainly HPAI H5 or H7 viruses—is reportable to State, Federal, and international animal health authorities per World Organization for Animal Health (OIE) standards.

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) works closely with States and the poultry industry to prevent AI from becoming established in the U.S. poultry population.

Sample Collection

Commercial poultry, live bird markets, and avian importation quarantine facilities are systematically sampled and tested to monitor for AI viruses. Wild birds are monitored for AI viruses through investigation of large-scale avian mortality events and opportunistic sampling of live birds in conjunction with other mission projects. Samples are taken from live birds, dead birds, or the environment that they inhabit. In live domestic birds, samples are taken by swabbing the throat. Vent and throat swabs may be taken from wild birds, as well as environmental samples. Samples are placed into sealed tubes and taken to USDA-approved laboratories for AI screening, or sent directly to USDA's National Veterinary Services Laboratories (NVSL) for screening and confirmatory tests.

Testing Facilities

Many of the AI screening tests, including those done on samples from wild birds and live bird markets, are conducted by one of more than 50 USDA-approved laboratories that are part of the National Animal Health Laboratory Network (NAHLN). The NAHLN is a cooperative effort between APHIS, USDA's National Institute of Food and Agriculture (NIFA), and the American Association of Veterinary Laboratory Diagnosticians (AAVLD). NAHLN laboratories conduct nationwide surveillance testing for early detection of animal diseases and have the resources to test large numbers of samples during a disease outbreak.

Screening tests performed at these NAHLN laboratories determine whether AI virus is present in the sample and whether the virus is an H5 or H7 subtype. All positive samples originating from commercial poultry are forwarded to the NVSL in Ames, IA, for confirmation and additional testing. All wild bird samples testing positive for AI at a NAHLN laboratory are further analyzed to determine if the virus is an H7 or H7 subtype. Samples originating from wild birds containing H5 or H7 subtypes are forwarded to NVSL.

National Poultry Improvement Plan H5/H7 LPAI Monitored Program

The National Poultry Improvement Plan (NPIP) is a voluntary program administered cooperatively by USDA, States, and the poultry industry. The program's H5/H7 LPAI Monitored Program requires the testing of meat-type chickens (broilers, roasters, cornish, and fryers), commercial table-egg layers, meat-type turkeys, meat-type waterfowl and game birds, and raised-for-release waterfowl and game birds. Screening tests are performed in 129 NPIP-authorized laboratories throughout the country.

APHIS operates secure data reporting systems that incorporate NPIP testing data in national AI surveillance. This information helps to assure international partners that the U.S. AI surveillance system is working to verify the safety of our poultry exports.

Virus Detection

Screening tests conducted by the NVSL and NAHLN laboratories include polymerase chain reaction

(PCR) assay and virus isolation. PCR testing detects the presence of the virus' genetic material in the sample. The PCR assays performed at these laboratories include assays targeting the matrix gene, which screens for type A AI viruses, as well as subtype-specific assays for H5 and H7 viruses. Neuraminidase-specific assays are also performed at NVSL for some subtypes in order to detect the specific subtype for the "N" component (e.g., N1). PCR testing can determine H5, H7, and available N subtypes within 24 hours; however, differentiating between HPAI and LPAI viruses requires additional testing.

The NVSL conducts all confirmatory testing, which include virus isolation, H/N subtyping, genetic sequencing, and determination of chicken pathogenicity. Results of confirmatory tests are generally available within 14 days after sample receipt.

Virus isolation is the gold standard test used to diagnose AI virus infections. The virus is isolated in embryos inside chicken eggs to specifically identify H and N subtypes of the AI virus. Positive virus isolation is an accurate method of confirming the presence of a viable virus (i.e., a virus that could infect other birds).

Serology

Serology may be used to detect the presence of antibodies to AI viruses in a bird's serum, or blood plasma. Antibodies are generated in response to exposure or infection with AI virus. While the presence of antibodies indicates previous infection, the bird may no longer be infected with that virus. Antibody detection (positive serology) for AI in commercial poultry requires confirmation at NVSL. Where H5 or H7 antibodies are found, field investigation and additional sampling are required per OIE-recommended standards, as serology results are not sufficient to confirm a "notifiable" AI infection.

Pathogenicity

Additional work at NVSL is conducted to confirm the pathogenicity of an AI virus. This includes genetic sequencing to determine the genetic profile of the hemagglutinin (H) part of the virus. HPAI viruses have a very specific hemagglutinin genetic signature that differs from LPAI viruses. Determination of chicken pathogenicity is performed per OIE standards; OIE defines HPAI as any AI virus that is lethal for 6 or more of the 8 chickens, or a 75 percent or greater mortality rate.

Response to AI Detection

Response actions depend on the subtype and pathogenicity of the AI virus and what animal compartment (poultry versus wild bird) the virus originated from. APHIS works cooperatively with States and the poultry industry to ensure proper disease response, which helps protect public health and food safety and minimize economic hardships for producers. The National HPAI Response Plan and individual LPAI Initial State Response and Containment Plans (part of the NPIP LPAI H5/H7 Monitored Program) outline specific actions.

If an HPAI infection is found in a commercial poultry flock, all surviving birds are depopulated to prevent the spread of the virus. If laboratory testing detects LPAI H5 or H7 viruses, the infection may be controlled through depopulation or controlled marketing, depending on the Initial State Response and Containment Plan. Controlled marketing allows poultry that are infected with or exposed to H5/H7 LPAI to move to market on a limited basis, according to the process laid out by each State's response and containment plan. Under controlled marketing, infected or exposed poultry may not be transported for 21 days after the acute phase of the infection, and poultry must test negative for H5/H7 LPAI viruses within 7 days prior to slaughter. Testing reduces the risk of spreading H5 or H7 LPAI strains and maintains the safety of the controlled marketing process, while allowing producers to recover value from infected poultry. However, if animal health officials determine that an LPAI H5 or H7 virus poses a significant threat to avian and/or public health, it is likely that that flock(s) would be depopulated.

APHIS cooperates with State partners to conduct surveillance in the area surrounding AI-infected birds and enforces movement controls to reduce the risk of spreading the virus. APHIS reports all findings of HPAI, as well as findings of H5 or H7 AI viruses in commercial poultry, to the OIE.

For More Information

USDA efforts to protect against and respond to bird flu: www.usda.gov/birdflu

Report Sick Farm Birds: If your farm birds are sick or dying, call USDA's Veterinary Services toll free at 1-866-536-7593 or your State veterinarian or local extension agent.

Report Dead Wild Birds: Dead wild birds can be reported to State or Federal wildlife agencies. Information on how to make contact with wildlife officials in your State is available at www.usda.gov/birdflu

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