National H5/H7 Avian Influenza Surveillance Plan

United States Department of Agriculture
Animal Plant Health Inspection Service
Veterinary Services
October 2013

Table of Contents

EXECUTIVE SUMMARY

The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) national H5/H7 avian influenza (AI) surveillance plan has five objectives:

- Rapidly detect H5/H7 AI in domestic poultry populations
- Assure that low pathogenicity H5/H7 avian influenza (LPAI) strains are not circulating in poultry populations where they may spread and mutate into highly pathogenic avian influenza (HPAI)
- Provide consistency with international surveillance guidelines for trade purposes
- Protect public health through early detection and control of H5/H7 AI viruses
- Demonstrate to trading partners and consumers that U.S. poultry is free of potentially dangerous influenza viruses

This national H5/H7 AI surveillance plan was developed to provide surveillance recommendations for the different components of the National Avian Influenza Surveillance System (NAISS). The NAISS components are actively undertaken by USDA-APHIS-VS in partnership with other Federal and State agencies and the commercial poultry industry. The NAISS collectively provides the information necessary to safeguard the health of U.S. poultry and promote the marketability of U.S. poultry and poultry products. Certain elements of the plan will require that USDA-APHIS-VS and individual States work together to implement statistically representative sampling strategies as outlined in the Sampling Methods section of this document.

USDA-APHIS-VS currently conducts or supports domestic poultry surveillance for H5/H7 AI in commercial poultry through the National Poultry Improvement Plan (NPIP), the Live-Bird Marketing System (LBMS), and backyard flocks. It is assumed that participants in the NPIP, LBMS, or other State-sponsored programs will continue their current level of vigilance and participation, which provides additional confidence in the Nation's disease-free status. **The recommendations outlined in this surveillance plan do not change the regulations or standards for the NPIP, LBMS, or other State-sponsored programs.**

The surveillance recommendations provided in this plan provide the **minimum** level of testing required for a State to achieve 95 percent confidence of disease freedom and detection of avian influenza if the prevalence among premises within a State is 1 percent and the prevalence within an infected premises is 25 percent. Three cost-efficiency strategies are incorporated in the sampling plans: 1) targeting sick birds to greatly increase the probability of finding infection if present, 2) using likelihood ratios to reduce sampling in flocks that are less likely to be exposed, and 3) focusing surveillance in areas likely to experience the highest consequences from an outbreak.

The National AI Surveillance Plan divides the domestic poultry population in the United States into the following categories:

- Large-volume commercial poultry including:
    - Commercial meat-type chickens

    - Commercial table-egg layers
    - Commercial meat-type turkeys
    - Breeding meat-type chickens
    - Breeding egg-type chickens
    - Breeding meat-type turkeys
- Small-volume but high-value commercial poultry
    - Hobbyist and exhibition waterfowl
    - Exhibition poultry
    - Game birds breeding flocks
    - Raised-for-release upland game birds and waterfowl;
- Live-bird marketing system
    - Production, market, and distribution birds
- Backyard poultry flocks

These categories are based primarily on risk of disease introduction and the level of management practices as well as commercial characteristics.

Three methods of surveillance are conducted in domestic poultry, with oversight provided by official State agencies or the commercial poultry industry: passive surveillance, active observational surveillance, and active laboratory surveillance. Each method is specifically designed for detecting H5/H7 AI as defined by the World Organization for Animal Health (OIE) within the various subpopulations.

Passive surveillance is a process involving individual poultry growers and flock service personnel who notice atypical disease signs and report them to private veterinarians or directly to diagnostic laboratories. This reporting ultimately results in sample submission to diagnostic laboratories. Active observational surveillance is the flock monitoring process conducted by contract growers and flock service personnel who actively and frequently observe the birds for clinical disease signs and mortality and record these observations as part of their routine management practices. Serologic surveillance involves collection of blood samples to check for antibodies that represent recent infections in apparently healthy poultry. However, detectable levels of antibodies can take a week to 10 days to develop after exposure. Antigen detection techniques in apparently healthy poultry are also used, but can only detect the avian influenza virus while it is shed – usually within the most recent 7 to 14 days (Lu and Castro 2004). Active laboratory surveillance (serologic and antigen) is conducted through State NPIP programs for large-volume commercial poultry, through the LBMS program for H5/H7 LPAI, and through cooperative agreement funding for smaller poultry operations and backyard birds. Each sampling strategy has different utility in the various domestic poultry populations and for the purposes identified within this surveillance plan. The combination of all the State-level AI sampling strategies then supports the national H5/H7 AI surveillance objectives of rapidly detecting H5/H7 in domestic poultry to ensure marketability, support trade, protect public health, and meet international surveillance guidelines.

INTRODUCTORY INFORMATION

1. DISEASE DESCRIPTION

ETIOLOGIC AGENT

The agent responsible for avian influenza, an orthomyxovirus, has been described extensively (Kalthoff *et al.*, 2010; Alexander, 2000; Webster *et al.* 1992). Influenza viruses are classified by examining nuclear and matrix proteins that divide them into three groups: influenza types A, B, and C. All influenza viruses from birds and most from mammals are type A. Type A influenza viruses are further classified into various virus subtypes through testing of two surface proteins, hemagglutinin (HA) and neuraminidase (NA). Historically, subtypes including H5 and H7 are associated with disease in poultry.

For disease surveillance purposes related to trade in commercial poultry products, avian influenza reportable to the OIE are those infections of poultry caused by any influenza A virus of the H5 or H7 subtypes or other subtypes meeting specific requirements for high virulence[1] (OIE 2013). Reportable AI viruses can be divided into highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) H5 or H7.

The rationale for focusing surveillance on H5 and H7 subtypes of AI is that H5/H7 LPAI subtypes circulating within poultry over a period of time may mutate into highly pathogenic forms and cause significant losses to the commercial poultry industry (Veits *et. al.* 2012). For official control purposes, designation as LPAI or HPAI is based on pathogenicity according to *in vivo* tests or molecular determinants (severity of disease defined by pathogenicity index or amino acid sequence in the HA receptor protein).

CLINICAL SIGNS

AI virus infections in domestic poultry may be clinically inapparent or result in disease that ranges from mild transient syndromes to 100 percent morbidity and/or mortality, depending on virus pathogenicity types (Swayne and Suarez 2000). In addition to pathogenicity, other factors such as genetics, nutrition, and co-infection with other pathogens affect clinical outcome. When seen, clinical signs may be evident as respiratory, enteric, cardiovascular, or reproductive. Low pathogenicity strains typically cause either no disease signs or result in mild cases, but may cause increased mortality, decreased feed consumption, respiratory signs (e.g., nasal discharge, coughing, sneezing), and decreased egg production (Dunn *et al.* 2003). Infection with LPAI virus only sporadically leads to appreciable virus shedding in the gastrointestinal tract. Therefore, subclinical cases may shed low amounts of virus and have inconsistent or incomplete seroconversion (antibody production) on a flock basis, causing a concern for detection and control of this form of AI. In contrast, birds infected with HPAI generally have a greater level of

[1] A full reading of the OIE definition of avian influenza can be found in Chapter 10.4 of the 2013 OIE Terrestrial Animal Health Code.

illness and could exhibit one or more of the aforementioned clinical signs and any of the following: sudden death, lack of energy and appetite, soft-shelled or misshapen eggs, swelling and purple discoloration of the combs or wattles, hemorrhages on the unfeathered parts of legs and feet, lack of coordination, and diarrhea (Elbers *et al.* 2005). It is possible that some H5 and H7 AI virus strains are genetically classified and reported as HPAI, but present clinically the same as non-pathogenic or low-pathogenic viruses, as found during the last HPAI outbreak in the United States (Pelzel *et al.* 2006).

EPIDEMIOLOGY

Disease Transmission

AI is spread by direct contact between healthy and infected birds and indirect contact with contaminated equipment and materials. The virus is primarily excreted through the feces of infected birds, as well as secretions from the nose, mouth, and eyes.

HPAI viruses cause higher levels of viral shedding than H5/H7 LPAI viruses, with a related increase in infectiousness. Although HPAI may cause rapid death within 4 to 10 days, the infectious period[2] induced by HPAI virus *is not* reduced, unless birds die acutely, and is actually longer for birds infected with HPAI compared with LPAI virus (van der Goot *et al.* 2003). Transmission of HPAI virus *is* strongly reduced in a population where all animals previously went through an infection with H5/H7 LPAI virus (van der Goot *et al.* 2003).

Waterfowl and shorebirds are considered natural reservoirs of LPAI viruses. Wild waterfowl are generally asymptomatic, may excrete virus in feces for long periods, may 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 (Halvorson D.A. 2002). Influenza A viruses generally remain in evolutionary stasis within wild birds and usually do not cause wild bird morbidity or mortality (Webster *et al.* 2006).

Human AI infections are relatively rare. Most human AI infections have resulted from direct exposure to infected domestic poultry or from visiting an AI-contaminated environment, such as a live bird market; sustained human-to-human AI transmission has not occurred (Kaye and Pringle 2005; To *et al.* 2012; Van Kerkhove *et al.* 2011). Direct exposure routes leading to human infection have included caring for poultry, culling infected flocks, slaughtering, and food preparation activities (Belser *et al.* 2009; Van Kerkhove *et al.* 2011).

Though human AI infections are relatively rare, two current zoonotic AI outbreaks necessitate careful monitoring of disease transmission. In 2013, a novel LPAI H7N9 from an unknown origin was detected among people in China. Surveillance sampling of domestic poultry, wild birds, and other animals in China

[2] Length of virus shedding measured from time of first detection until virus is no longer detected.

have resulted in relatively few culture-positive detections (0.07 percent) in chickens, ducks, pigeons, and environmental samples (CDC 2013).

The origins of the HPAI H5N1 virus responsible for the current epizootic in Asia, Europe, and Africa can be traced to an outbreak in domestic geese in southern China in 1996 (Sims *et al.* 2005). Expansion of the host range from geese to ducks was probably a key event in the genesis of the epizootic in 2004. Epidemiologic studies suggest that domestic ducks played a key role in the spread of these viruses to terrestrial poultry through widespread seeding of the virus on farms and rice paddies. During 2005-2006, the H5N1 virus began circulating widely in the southern Asian, Middle East, European, and African wild bird populations (Le *et al.* 2011). Multiple genotypes of the Asian HPAI H5N1 continue to evolve (Kim *et al.* 2012; Capua and Alexander 2006).

2. Purpose and Rationale of the National Avian Influenza Surveillance System (NAISS)

The OIE requires reporting of all H5/H7 AI detections in domestic poultry. Although H5/H7 LPAI infections usually do not result in the same mortality as HPAI in domestic poultry, the economic impacts to international trade losses are substantial, and there is potential for them to mutate into an HPAI form. The length of time that H5/H7 LPAI virus has circulated in poultry before becoming highly pathogenic has varied from 11 days to more than 2 years (Senne *et al*. 2006). Due to increased international concerns over the possibility of mutation, trading partners have used this concern to restrict U.S. poultry exports after detection of H5 or H7 LPAI in U.S. LBMS or backyard flocks (Hall 2004).

Economic Impact of H5/H7 AI Detection

The U.S. poultry industry is the second most valuable livestock industry at the farm level, second only to cattle and calf production. It accounted for $38 billion in farm cash receipts in 2012 (NASS Poultry-Production and Value 2012; NASS 2007 Census of Agriculture). The industry is primarily composed of large-volume commercial poultry including meat-type chickens, meat-type turkeys, and table egg-layers (described in Appendix A). Total farm cash receipts for specialty chicken production amounted to $79 million in 2012 (NASS Poultry-Production and Value 2012) and, therefore, the size of theses sub-industries has minimal influence on this economic discussion. Other poultry-related industries exist in the United States such as duck, goose, and game birds; however, USDA does not routinely follow the markets for these production types.

The meat-type chicken (broiler) industry is the largest and most valuable of the U.S. poultry sub-industries. In 2012, farm cash receipts for broiler production were $24.8 billion (NASS Poultry-Production and Value 2012). The retail value of the U.S. broiler industry was $48.7 billion (ERS Meat Price Spreads, ERS Livestock and Meat Domestic Data). Eighteen percent of broiler production was exported at a value of $4.4 billion (NCC U.S. Broiler Exports, Global Trade Information Services).

The United States is the world's largest producer and consumer of turkey meat. Farm-level value of the U.S. turkey industry in 2012 was $5.4 billion and the retail value was $8.1 billion (ERS Meat Price Spreads, ERS Livestock and Meat Domestic Data; NASS Poultry-Production and Value 2012). Turkey meat exports were valued at $585 million (Global Trade Information Services).

At the farm level, the value of U.S. egg production – which includes table eggs and hatching eggs – was $7.8 billion in 2012 (NASS Poultry-Production and Value 2012). Table eggs accounted for 69 percent of total egg production in 2012. The retail value of the U.S. table egg industry was $12 billion (ERS Meat Price Spreads, Livestock Market Information Center).Value of exports for eggs and egg products in 2012 was $341 million (American Egg Board, Global Trade Information Services).

Primary breeders are the foundation of the $30-plus billion poultry industry in the United States. Exports of poultry products account for over $5 billion annually. The exports accounted for by the primary breeder companies in the United States are estimated at approximately $250 million with total value (including the U.S. market) 2 to 3 times that of exports. The United States primary breeders account for over 95 percent of the world's broiler production. Estimates indicate that the genetics originated in the United States account for at least 60 percent of that amount (D.L. Brinson, personal communication, 2013).

IMPORTANCE OF SURVEILLANCE IN ALL POULTRY TYPES

The U.S. poultry sectors outside of the intensely managed large-volume commercial operations present a higher risk for AI introduction due to their generally lower emphasis on biosecurity practices. Live-bird markets have been implicated as potential reservoirs for AI viruses and may serve as an amplifier and reservoir of infection (Bulaga *et al.* 2003a, Bulaga *et al.* 2003b, Mullaney 2003, Nguyen *et al.* 2005, Trock *et al.* 2003, Webster and Hulse 2005). These markets house birds from many different sources and species, including waterfowl; they continuously maintain live birds on the premises and, in some cases, may practice suboptimal sanitation. Since 1996, five outbreaks of low pathogenicity H7N2 in commercial poultry have been linked to the LBMS in the Northeastern United States (Senne *et al.* 2003). Of four H5/H7 LPAI outbreaks in Pennsylvania since 1983, two were traced to connections with live-bird markets (Dunn *et al.* 2003).

Backyard flocks present a risk to the commercial poultry industry due to varying biosecurity practices by flock owners and their proximity to commercial poultry operations (National Animal Health Monitoring System 2004). Hence, it is not surprising that index cases of AI have been identified in backyard flocks prior to the onset of AI outbreaks in commercial flocks (Kinde *et al.* 2003). Game birds raised under semi-wild conditions for eventual release on shooting preserves have become infected with LPAI strains found previously in wild waterfowl (Groocock 1994). Along with the risk from these populations to the commercial poultry industry, an added risk is that human contact is minimally restricted in the live-bird markets and backyards. Viruses in these birds with the potential to infect people pose increased public health risks and therefore provide further rationale for surveillance of these poultry populations.

PUBLIC HEALTH CONSIDERATION

Some subtypes of H5/H7 AI, particularly China LPAI H7N9 and Asian HPAI H5N1, are zoonotic and have recently presented substantial risk to the health and well-being of the public. It is extremely important to detect these and other zoonotic H5/H7 AI subtypes as rapidly as possible should they occur in the United States.

The China LPAI H7N9 subtype was first reported in March 2013 and by the end of August, there were at least 135 known human infections and 44 deaths; all infections occurred in China and Taiwan (WHO 2013). Since 2003, Asian HPAI H5N1 has infected domestic poultry and wild birds in Asia, Europe, and Africa, causing 637 known human infections and 378 deaths (WHO August 29, 2013). Other AI subtypes have sporadically infected humans causing less severe disease. From 1996 to 2009, two people in the United States were detected with infection of an avian H7 subtype and both infections resulted in respiratory illness (Belser *et al.* 2009). Other zoonotic H5/H7 AI subtypes have primarily caused mild influenza-like illness and/or conjunctivitis in humans (Belser *et al.* 2009; Kaye and Pringle 2005).

Direct exposure to infected poultry has been the primary route of human infection, with no sustained human-to-human transmission. However, a major concern is the potential for these zoonotic AI viruses to mutate or change into a subtype that could spread from person to person in pandemic proportions. As a result, prevention, rapid detection, and control of an outbreak in poultry are essential to minimize the potential public health hazards and subsequent economic consequences.

3. SURVEILLANCE OBJECTIVES

The primary objectives of the national H5/H7 AI surveillance system are to:

- Detect the presence of H5/H7 AI in domestic poultry populations if the prevalence among premises within a State is 1 percent and the prevalence within an infected premises is 25 percent.
- Assure that H5/H7 LPAI strains are not circulating in poultry populations where they may spread and mutate into HPAI
- Provide consistency with international surveillance guidelines for trade purposes
- Protect public health through early detection and control of H5/H7 AI viruses and
- Demonstrate to trading partners and consumers that U.S. poultry is free of H5/H7 AI viruses.

This plan outlines recommended national surveillance activities to support the national H5/H7 AI surveillance goals. USDA-APHIS-VS currently conducts or supports domestic poultry surveillance for H5/H7 AI in three major areas: commercial poultry, coordinated by the NPIP; the LBMS; and backyard flocks. It is assumed that current participants in the NPIP, LBMS, or other State-sponsored programs will continue their current levels of vigilance and participation, which provides additional confidence in the

Nation's disease-free status. **The recommendations outlined in this surveillance plan do not change or supersede the regulations and standards for the NPIP and LBMS respectively, or other State-sponsored programs.**

4. Expected Outcomes: Products, Decisions, and Actions

Expected outcomes include:

- A systematic mechanism to gather surveillance data and document the H5/H7 AI status of U.S. poultry
- Early detection of H5/H7 AI in poultry, triggering response plans to control and eliminate H5/H7 AI in a timely manner
- Quarterly and annual NAISS reports demonstrating the level of surveillance within all poultry types among States with avian health cooperative agreements
- Quarterly posting of summary level National Chicken Council surveillance data to the National Animal Health Surveillance System (NAHSS) Web site (http://www.aphis.usda.gov/vs/nahss/poultry/index.htm)
- Analysis of surveillance implementation efforts and data to determine whether surveillance goals are being achieved
- Decision-making and policy development regarding design and implementation of future H5/H7 AI surveillance programs and control efforts
- Reassurance to consumers and international trading partners regarding our ability to detect H5/H7 AI.

5. Stakeholders and Responsible Parties

Stakeholders in H5/H7 AI surveillance include producers, industry representatives, and individuals responsible for designing, implementing, managing, and/or disseminating information. Stakeholders may use the surveillance information to formulate policy, negotiate trade, and, if necessary, take additional security measures. Table 1 summarizes the specific parties that may have an interest in this surveillance plan.

Table 1. Stakeholders and their AI surveillance responsibilities and interests.

Stakeholder	Interest/Responsibility
USDA-APHIS-VS	Cooperative Data Sharing
Surveillance, Preparedness, and Response Services (SPRS)	• Field implementation NAI surveillance and reporting • Coordination of disease response • Policy and budget
Science, Technology, and Analysis Services (STAS)	• Development, evaluation, and revision of surveillance plan; data analysis • Risk-based analysis • Diagnostic laboratory support; reference laboratory services; sample testing and data reporting
National Import Export Services (NIES)	• Import, export, and international health status management
National Animal Health Laboratory Network (NAHLN)	• Sample testing and data reporting
Approved NPIP laboratories	• Sample testing
State Veterinarians and field staff	• Jointly responsible with VS District Directors and Assistant District Directors for field implementation; data reporting; coordination of disease response
Veterinarians, industry field representatives, and individual producers	• Animal health and production issues; disease detection and reporting; sample collection and submission; biosecurity plans
NPIP[3], LBMS stakeholders, and other stakeholders[4]	• Financial interest; disease detection and prevention; flock health status; surveillance

[3] NPIP stakeholders: commercial table-egg producers, meat-type chicken and turkey producers and processors, and their parent hatching egg production flocks, exhibition poultry, upland gallinaceous gamebirds, and domesticated waterfowl (small-volume high-value commercial breeders).

[4] Other stakeholders: auctions, small sales, flea markets, swap meets, farmers markets, production facilities, backyard or hobby flock owners, distributors (dealers, haulers, wholesalers), botanicas, custom exempt poultry facilities, feed stores, and other programs (fairs, poultry shows, exhibitions, interstate movement).

	data
Industry producer groups	• Industry policy, scientific issues, surveillance data
Fancy (show) bird groups, 4-H groups	• Information users
U.S. Poultry and Egg Association, USA Poultry and Egg Export Council, National Turkey Federation, National Chicken Council, United Egg Producers; USDA APHIS International Services and Foreign Agricultural Service	• Trade issues
Trading partners	• Trade issues
Commercial diagnostic and reagent companies	• Manufacture and sales of commercial reagents and assays

POPULATION DESCRIPTION AND SAMPLING METHODS

6. POPULATION DESCRIPTION

The domestic poultry population in the national AI surveillance plan is divided into the following categories for the purpose of designing an avian influenza surveillance plan: the large-volume commercial poultry industry (both commercial and breeding meat-type and egg-type chickens and meat-type turkeys), the small-volume but high-value commercial poultry industry, the LBMS (raised-for-release upland game birds and waterfowl, producers, markets and distributors), and backyard poultry flocks. The categories are primarily based on risk of disease introduction and management practices. Appendix A provides a complete description of the poultry industry.

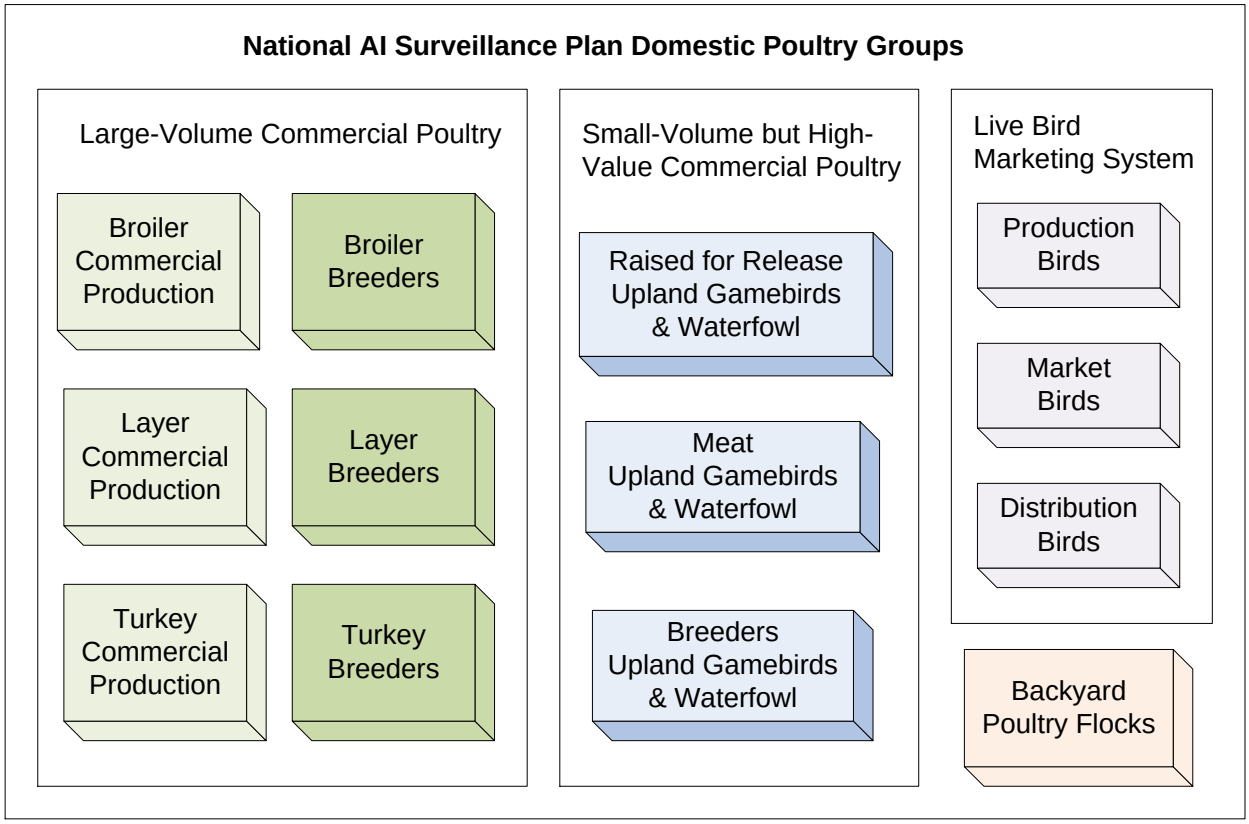

Figure 1. Division of poultry types for the National H5/H7 AI surveillance system

7. Case Definition

A comprehensive case definition for H5/H7 AI surveillance in the United States includes clinical and laboratory diagnostic criteria for both active and passive surveillance. Recognition of clinical sign combinations and gross lesions is an essential component of passive and active observational surveillance. Recognition triggers the reporting of suspicious cases for further investigation and enables appropriate control measures to be taken rapidly and efficiently (Kradel *et al.* 1986, Weaver *et al.* 2006). Active laboratory surveillance is necessary for detecting H5/H7 AI infections that do not cause noticeable clinical signs. Laboratory confirmation is necessary for index cases.

Clinical Description

Clinical signs noted earlier in this document (see introductory information) provide the trigger for sampling and laboratory testing to determine if one of the more virulent strains of the AI virus is causing the illness or mortality. The clinical manifestations and mortality from other H5/H7 AI infections can vary considerably depending on species, age, sex, concurrent infections, virus strain, and environmental conditions. The digestive, respiratory, nervous, reproductive, or circulatory systems may be affected. The clinical definition below describes several of the clinical manifestations of AI viruses that may characterize an outbreak, although some strains of HPAI and many strains of LPAI may not show overt

disease manifestations that would be detected by direct observation of clinical signs. Due to this characteristic, passive and active observational surveillance are supplemented by active serologic and antigen detection surveillance, such as the NPIP certification program.

CLINICAL CRITERIA

AI virus can infect almost all species of birds. Domestic poultry identified as having illness compatible with OIE-reportable AI infection (HPAI and H5/N7 LPAI) are those with one or more of the following clinical signs and gross lesions: reduction in normal vocalization; listlessness; conjunctivitis; drops in egg production sometimes with pale, misshapen or thin-shelled eggs; respiratory signs such as rales, snicking, and dyspnea; neurological signs such as incoordination or torticollis; a drop in feed and/or water consumption; swollen or necrotic combs and wattles; swollen head and legs; lungs filled with fluid and blood; tracheitis and airsacculitis; hemorrhages on the unfeathered parts of legs and feet; petechial hemorrhages on internal organs (Easterday *et al.* 1997); **OR***,* flocks that experience mortality as listed for each compartment as follows (S. Malladi and E. Gingerich, personal communications, 2013):

- Commercial broilers: mortality exceeding 3.5 birds/1,000 per day
- Commercial layers: mortality exceeding 3 times the normal daily mortality per day (normal: 0.13 birds/1,000 per day for layers from 2 to 50 weeks, and 0.43 birds/1,000 per day for layers over 50 weeks); OR 5 percent drop in egg production for 3 consecutive days
- Commercial turkeys: mortality exceeding 2 birds/1,000 per day
- Broiler breeders: mortality exceeding 2 birds/1,000 per day
- Layer breeders: mortality exceeding 3 times the normal daily mortality per day (normal: 0.2 birds/1,000 per day prior up to 50 weeks, and 0.37 birds/1,000 per day after 50 weeks)
- Turkey breeders: mortality exceeding 2 birds/1,000 per day; OR a decrease in egg production of 15 percent occurring over a 2-day period
- Small-volume high-value commercial poultry and backyard flocks: any sudden and significant mortality event or sudden drop in egg production should be investigated

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 H5/H7 LPAI defined by one or more of the following diagnostic strategies:

Virus isolation and identification: preferred specimens for virus isolation include tracheal/oropharyngeal and cloacal swabs, fresh feces from live birds, samples from pools of organs (trachea, lungs, air sacs, intestine, spleen, kidney, liver) and feces from dead birds. A preparation of the specimen is inoculated into the allantoic cavity of susceptible embryonated chicken eggs. The eggs are incubated at 37 °C for 4 to 5 days. The amniotic-allantoic fluid is harvested from inoculated embryos and tested for presence of virus by the following methods:

- Demonstration of hemagglutination **AND**
- Confirmed presence of influenza A virus by PCR, antigen capture, agar gel immunodiffusion (AGID) **AND**

- Subtype (HA and NA) determination by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests.

Strain virulence evaluation:

- Classification of the isolate as HPAI by having an intravenous pathogenicity index greater than 1.2 or by causing at least 75 percent mortality within 10 days in 4- to 8-week-old chickens infected intravenously; **OR**, if no mortality occurs:
- Determination of the amino acid sequence at the hemagglutinin cleavage site (of H5 and H7 viruses) to identify viruses that have the capacity to become highly pathogenic.
- If H5 or H7 subtypes do not meet the criteria for HPAI, they are classified as H5/H7 LPAI

Antigen capture and molecular techniques: typically testing swab material, refer to virus isolation for other sample types

- *Antigen detection* – enzyme immunoassays (*swab samples only, for flock level testing*)
- *Direct RNA detection* – Reverse transcriptase polymerase chain reaction (RT-PCR) using nucleoprotein specific or matrix-specific conserved primers and subtype determination using H5- or H7-specific primers

Serological tests:

- Hemagglutination-inhibition (for H1-H16) and neuraminidase-inhibition (for N1-N9)
- Agar gel immunodiffusion (AGID)
- Enzyme linked immunosorbent assay (ELISA)

2.2. *Assumptions:* Influenza virus may be detected 48 hours post-infection (HPAI within 24 hours post-infection) by virus isolation or real-time reverse transcriptase polymerase chain reaction (rRT-PCR) (E. Spackman, personal communication, 2006) and 1-5 days post-infection by antigen capture enzyme immunoassay, when virus is shed at moderate to high levels (Gelb and Ladman 2006). Orpharyngeal/tracheal specimens are preferred for poultry because there generally are fewer inhibitors and therefore higher test sensitivity, especially during the early phase of infection. While oropharyngeal/tracheal swabs are preferred for detection of AI in poultry, cloacal swabs are preferred in wild birds. Presence of blood or fecal material in swab specimens (i.e., cloacal swabs) can result in lower test sensitivity on the rRT-PCR assay due to the presence of non-specific inhibitors, and should be processed appropriately.

CASE CLASSIFICATION FOR H5/H7 AI

Suspect Case: Domestic poultry having illness compatible with H5/H7 AI infection **OR** positive AGID or ELISA samples taken during routine surveillance with or without the presence of compatible illness.

Presumptive Positive Case: A suspect case with one of the following criteria:

- Detection of antibodies to influenza A in sera as determined by AGID serological test that cannot be explained by vaccination (USDA permission required for use in the United States) and subsequent subtyping by HI and NI as H5 or H7 with any NA subtype **OR**

- Detection of influenza A antigen using a commercially available influenza A antigen detection kit approved by the NPIP administrator **OR**
- Identification of influenza A RNA by rRT-PCR

Confirmed Index Case: Requires antigen detection (virologic or molecular detection methods) **AND** the confirmation of the H5 or H7 subtype **WITH** subsequent determination of *pathogenicity* as described in Section 2.2 of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (HPAI or H5/H7 LPAI) by USDA's National Veterinary Services Laboratories (NVSL).

Required Reporting

Suspected cases of HPAI in domestic poultry should be reported in accordance with **VS Guidance 12001.1**, "Policy for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI)." Suspected cases or laboratory cases consistent with H5/H7 LPAI in domestic poultry should be reported in accordance with **VS Guidance 8602.1** "Response, Communications, and Investigation of Notifiable Avian Influenza (NAI) in Domestic Poultry" and **VS Guidance 8604.1,** "Reporting Confirmed Findings of Low Pathogenic Notifiable Avian Influenza (LPNAI) (H5 and H7 Subtypes) to the World Organization for Animal Health (OIE) and to Trading Partners." In addition, State animal health officials should report the presence or absence of H5/H7 AI in commercial poultry to APHIS through the National Animal Health Reporting System (NAHRS) following NAHRS reporting guidelines.

8. Data Sources and Sampling Methods

Data Sources

APHIS relies on a variety of voluntary State and commercial programs to monitor and test domestic poultry. The surveillance sampling streams include:

- The NPIP administered by official State agencies
- Live-bird marketing system surveillance program
- National Chicken Council (NCC) avian influenza monitoring program
- Foreign animal disease investigations
- Slaughter inspection
- Targeted surveillance of backyard poultry at shows, fairs, exhibits, and flocks located in high-risk areas
- Passive surveillance activities

Information from all of these streams generates useful surveillance data for determining the status of AI virus in the United States as well as compliance with international standards, whether the data result from structured population-based surveys or from non-random data sources. Although each sampling stream listed above provides information about the status of avian influenza in the United States, this surveillance plan focuses primarily on the NPIP and the live-bird market surveillance programs.

States and producers receiving avian health cooperative agreement funding for NPIP and LBMS H5/H7 AI surveillance submit quarterly reports to APHIS-VS regional offices. Data submitted in the updates include, at a minimum, the number of birds sampled and tested, the number and type of diagnostic tests performed, and summarized flock testing data (i.e., number of participating table-egg layer flocks; meat-type chicken and turkey slaughter plants; egg- and meat-type chicken breeding flocks; turkey breeding flocks; raised-for-release waterfowl and upland game birds; meat upland game birds and waterfowl; LBMS production birds, market birds, and distribution birds; and backyard flocks).

VS also collaborates with the NCC, which represents the U.S. broiler industry and conducts rigorous testing for AI virus. VS and the NCC cooperate to maintain a secure data reporting system that allows NCC testing data to be used in national avian influenza surveillance. The NCC Avian Influenza Monitoring Plan focuses on extensive private laboratory testing in which every participating company tests all broiler flocks before slaughter; this testing is part of the NPIP program and exceeds the minimum national standards established by USDA for AI surveillance. NCC participating companies represent 98 percent of the U.S. broiler production. Summary surveillance information regarding NCC avian influenza testing in meat-type chickens is provided on the NAHSS Web site (http://www.aphis.usda.gov/vs/nahss/poultry/ai/index.htm).

Sampling Methods

In the United States, three methods of surveillance enable detection of H5/H7 subtype avian influenza virus. Each has benefits and limitations for use different production types as described below.

Active observational surveillance is the active effort to detect evidence of disease through routine observation rather than laboratory sampling. Growers whose commercial interests are directly tied to disease prevention and biosecurity practices actively observe production flocks on a scheduled basis to detect and report certain disease syndromes to flock service personnel or industry veterinarians. Active observational surveillance is similar to active laboratory surveillance in that it is ongoing and follows a pre-planned schedule. Its advantage over laboratory types of surveillance is that the screening "test" is the observation of clinical signs and is done frequently and routinely—generally once or twice daily in large-volume commercial poultry operations—and recording the observations is part of the operation's routine management practices. Its utility is highest for diseases that show overt clinical signs such as HPAI, and it is used as a trigger for further investigation and laboratory sampling. Virtually all large-volume commercial operations use active observational surveillance to detect a multitude of diseases, including diseases that have signs compatible with AI viruses. Many of the other industry sectors, such as small-volume high-value flocks and backyard flocks, also use active observational surveillance, but there is no industry standard and documentation of management practices is not readily available.

Passive surveillance is used by all industry sectors. This type of surveillance involves reporting suspicious mortality or clinical signs by concerned individuals to their attending veterinarian, the local animal health official, or State animal health official. It differs from active observational surveillance in that it is not an ongoing, scheduled practice. The likelihood of voluntary reporting varies with flock owners, disease awareness, laboratory cost, and rate of mortality within the flock. Nonetheless, it adds value to the overall AI surveillance system.

To encourage passive surveillance and reporting of clinical signs in backyard birds, APHIS supports education and outreach activities such as the ongoing biosecurity information campaign called "Biosecurity for Birds," which can be accessed at http://www.aphis.usda.gov/animal_health/birdbiosecurity/. The educational program reaches out to backyard poultry producers and pet-bird owners to educate them about the signs of infectious poultry diseases, the need to practice biosecurity, and the importance of reporting sick or dead birds. By 2007, the campaign had distributed nearly 1 million copies of materials to all 50 States and more than 50 countries, and placed bilingual biosecurity information on more than 1.7 million poultry feed sacks. The campaign has placed radio ads on national and regional agricultural radio networks reaching an estimated 23 million listeners in 29 States, and has advertised in newspapers and magazines reaching nearly 30 million readers. APHIS also held a number of stakeholder briefings on avian influenza and partnered with FFA and 4-H to distribute materials at county and State fairs.

Active laboratory surveillance for H5/H7 AI involves periodic sampling of flocks and/or their environment (premises) to detect the presence of antibodies or antigen. Active laboratory surveillance is used to detect circulating H5/H7 AI virus in poultry in the absence of high mortality. The objective of active laboratory surveillance in commercial poultry is to detect circulating H5/H7 LPAI and to prevent its persistence within poultry flocks, thereby eliminating opportunities for these viruses to mutate into HPAI.

Tests that detect antibodies (serology) are sensitive during the period that antibodies are circulating in the birds. AGID and ELISA are recommended serological screening tests for H5/H7 AI surveillance. The antibody titer rises to detectable levels in 7-10 days after infection and declines after several months. These assays are valuable because they can detect evidence of disease for a period spanning several months; however, if there are indications of several infections, they cannot determine the most recent infection, nor can they indicate if virus remains in the flock. A very useful application of serology testing is to prove disease freedom in stable longer-lived flocks over a period of time (e.g., layers, breeders).

Real-time RT-PCR and ACIA tests for antigen (virus protein) and virus isolation (VI) are sensitive as long as circulating virus is present. They are valuable tools for detecting active infection in flocks prior to movement or where tracing the source of virus is a likely outcome. Since AI virus is generally only shed for about 10 days by infected poultry, its utility is high for current infections but declines as virus circulation decreases in the flock. ACIA tests are most effective when used as pen-side tests of sick and dead birds during an acute outbreak (day 3-5 post infection) when there is a moderate to high viral load present (Synbiotics 2005). ACIA will not detect AI infections that do not result in moderate to high viral loads.

Surveillance methods vary depending on the bird populations described in the NAISS components. Active laboratory surveillance (i.e., serologic or antigen detection surveillance) is conducted in commercial poultry flocks and the LBMS using different types of diagnostic tests, depending on surveillance objectives. Serology is the most common test method used to identify evidence of infection

in long-lived commercial flocks. In the LBMS, production flocks are evaluated using serology; short-lived flocks of birds for sale in markets are tested for the presence of viral antigen; and environmental swabs of cages that housed birds in market premises are tested for persistent viable virus. See Table 2 for recommended diagnostic tests per poultry type.

Serum samples are initially screened using an ELISA antibody detection assay or the AGID assay at approved/authorized NPIP laboratories or the National Animal Health Laboratory Network (NAHLN); samples found positive by the ELISA assay are confirmed by the AGID assay. Samples found positive by either antibody assay lead to follow-up investigations, which may include sampling from the originating flock for virus isolation and sample submission to USDA's NVSL for subtype and pathogenicity determination. AI antigen rRT-PCR assays to detect H5 and H7 subtypes are conducted by NAHLN laboratories. All H5 or H7 subtypes identified are further analyzed to determine pathogenicity.

Table 2. Screening diagnostic test recommendations per poultry type for the NAISS. Other tests not listed may be permitted.

Poultry Type	AGID	ELISA	rRT-PCR assays/ACIA	VI	Notes
Large-Volume Commercial Breeder					
Broiler	Yes	Yes	Yes – prior to movement	No	
Table-Egg Layer	Yes	Yes	Yes – prior to movement	No	
Turkey	Yes	Yes	Yes – prior to movement*	No	*used also in vaccinated flocks
Large-Volume Commercial Production					
Broiler	Yes	Yes	Yes – prior to movement	No	
Table-Egg Layer	Yes	Yes	Yes – prior to movement	No	
Turkey	Yes	Yes	Yes – prior to movement*	No	*used also in vaccinated flocks
Small-Volume, High-Value Commercial Poultry					
Upland Gallinaceous Gamebirds breeding/raised for release	No (see note)	No (see note)	Yes	No	Serology okay on eggs in breeding groups
Waterfowl breeding/meat/ raised for release	No	No	Yes	Yes	
LBMS					
Production	Yes	Yes	Yes – prior to movement	No	

Market	No	No	Yes – gallinaceous, waterfowl	Yes - waterfowl, environment	
Distribution: auction/small sales/whole sales/feed stores	No	No	Yes – gallinaceous birds, waterfowl	Yes – waterfowl, environment	Do not sample if birds held for less than 72 hours
Backyard/Fairs/Shows	No	No	Yes	No (Yes – see note)	VI used for exotic species with no validated PCR and for States that do not have PCR available for waterfowl

Sampling for active laboratory surveillance is performed by the poultry industry and through cooperation among commercial producers and Federal and State government. The following factors complicate the description of the sampling methods:

- Differences exist in the level of participation. For example, because not all States have live-bird markets, not all States maintain LBMS programs. In addition, not all producers participate in NPIP programs.

- National surveillance depends upon varying individual State plans, which must consider the economics and availability of individual screening assays (not every participating laboratory is approved to perform all of the assays). This national avian influenza surveillance plan provides recommendations regarding appropriate screening diagnostic assays and associated targeted sampling numbers per poultry type, but each individual State decides which screening test to use and the number of samples to collect.

Active Surveillance Sampling Strategy Recommendations

Three sampling strategies will be used to achieve cost-efficiency within the NAISS. Since influenza viruses often weaken or kill birds, the first strategy is to target sick or dead birds to reduce sample numbers and increase surveillance effectiveness. A second sampling strategy uses likelihood ratios to quantify the risk associated with different types of poultry and production management; this focuses resources where they are most needed. The third sampling strategy is to emphasize surveillance in poultry types where consequences of H5/H7 AI introduction are greatest to the poultry industry. For a detailed description of likelihood ratios and the incorporation of consequence based sampling, see Appendix B. The output from the three sampling strategies is encompassed in the sampling protocol below.

1. Each State determines the number of premises that are present per poultry type.
2. Each State determines the number of premises per poultry type to sample using table 3 below and the following guidelines:
    a. District Directors, State veterinarians, or their designees randomly select premises for testing.

b. Premises for testing should be selected to represent the population and avoid all known sources of bias. All phases of the poultry type must be represented in the sample pool (i.e., layers should not only be tested at the end of their production cycle).
c. The recommended minimum sampling frequency for premises will be every 6 months.

3. Once the number of premises to sample for each poultry type is determined, the number of birds to sample on each premises must be calculated.
4. Each barn/house on a premises should be sampled. Use the sampling guidelines below for number of birds to sample by diagnostic test.
5. First select sick and dead birds for sampling. If there are not enough sick and dead birds in a barn/house, use the alternate sample size numbers for the number of apparently healthy birds to sample.
6. Birds should be sampled during a time period when they are susceptible to avian influenza virus (i.e., do not test broilers when they are covered by maternal immunity. There is evidence that maternal immunity starts to decrease significantly in 10-day-old chicks [Abdelwhab et. al., 2012] if not earlier [Lebdah and Shahin, 2010]).[5]
7. All testing must follow guidelines established by the test manufacturers and the NVSL.

- If using RRT-PCR:
  - Swab 15 sick or dead birds from each barn/house on the premises (3 pools of 5 swabs or up to 11 swabs per pool[6]).
  - If fewer than 15 sick or dead birds per barn/house, sample all sick and dead, then sample healthy birds to obtain a total of 15 samples. Evenly distribute samples among 3 pools of up to 5 swabs or up to 11 swabs per pool[6].
  - If no sick or dead birds are present per barn/house, select 30 healthy birds from the house to sample (6 pools of up to 5 or 11 swabs per pool[6]).
- If using AGID:
  - Select 18 sick birds to sample from each barn/house on the premises.
  - If fewer than 18 sick per barn/house, sample all the sick present in the barn/house, then sample healthy birds to obtain a total of 18 samples.
  - If no sick birds are present in a barn/house, select 30 healthy birds to sample from the barn/house.
  - If sampling eggs from upland gallinaceous game bird breeding groups, select 30 eggs to sample from the barn/house.

[5] In studies using chicks produced by vaccinated hens, by day 14 the mean haemagglutination inhibition antibody titer in sera of 14-day-old progeny chickens was approximately eight-fold lower than the mean titer in sera of vaccinated hens (Maas *et. al.*, 2011), while some amount of maternally derived antibodies have been found to be present in chicks up to 3 weeks of age (De Vriese *et. al*., 2010).

[6] If pooling more than 5 swabs, additional broth must be used.

- If using ACIA:
    - Select 17 sick or dead birds to sample from each barn/house on the premises.
    - If fewer than 17 sick or dead birds per barn/house, sample all the sick or dead birds present in the barn/house, then sample healthy birds to obtain a total of 17 samples.
    - Not recommended for use if there are no sick or dead birds.
- If using ELISA:
    - Select 14 sick birds to sample from each barn/house on the premises.
    - If fewer than 14 per barn/house, sample all sick birds present in the barn/house, then sample healthy birds to obtain a total of 14 samples.
    - If no sick birds are present in a barn/house, select 30 healthy birds to sample from the barn/house.
    - If sampling eggs from upland gallinaceous gamebird breeding groups, select 30 eggs to sample from the barn/house.
- If using VI
    - Select 11 sick or dead birds to sample from each barn/house on the premises.
    - If fewer than 11 per barn/house, sample all sick and dead present in the house, then sample healthy birds to obtain a total of 11 samples.
    - If no sick and dead birds are present in a barn/house, select 30 healthy birds to sample from the barn/house.
    - Take environmental samples only from cages that housed birds.
- If premises includes a combination of upland gallinaceaus gamebirds, waterfowl, and/or environmental samples:
    - Select 15 sick or dead birds to sample from each barn/house on the premises (3 pools of 5 swabs or up to 11 swabs per pool for rRT-PCR[7]).
    - If fewer than 15 sick or dead birds per barn/house, sample all sick and dead, then sample healthy birds to obtain a total of 15 samples. Evenly distribute samples among 3 pools of 5 or up to 11 swabs if rRT-PCR.
    - If no sick or dead birds are present per barn/house, select 30 healthy birds to sample from the barn/house (3 pools of 5 or up to 11 swabs per pool for rRT-PCR[7]).

Table 3. Sample size (number of premises) required for a State to attain 95% confidence in detecting disease at 1% prevalence level for premises of various sizes using a test with perfect specificity and a within-premises sensitivity of 95%, assuming a simple random sample. Examples: If there are 172 production layer premises in a State, sample 151 premises. If 23 production broiler premises in a State, sample 4 premises.

[7] If pooling more than 5 swabs, additional broth must be used.

Number of premises to sample by bird type			
Number of premises by bird type in State	**Commercial table-egg layer, commercial meat-type turkey, meat-type chicken breeder, egg-type chicken breeder, meat-type turkey breeder, LBMS, backyard**	**Commercial meat-type chicken**	**Upland gamebirds and waterfowl**
0-140	All	(No. of premises*__0.12__)+1	(No. of premises*__0.19__)+1
141-150	136	17	27
151-175	151	19	30
176-200	163	21	32
201-250	183	23	36
251-300	199	25	39
301-400	221	28	43
401-500	236	29	46
501-600	247	31	48
601-700	256	32	50
701-800	262	32	51
801-900	267	33	52
901-1,000	271	34	52
1,001-1,500	285	35	55
1,501-2,000	292	36	56
2,001+	300	37	58

*0.12 and 0.19 are likelihood ratios derived from methodology described in Appendix B: Likelihood ratios and consequence-based sampling

Active Observational Surveillance Recommendations

- Producers should maintain written records of observational efforts. Written records should include times, locations (e.g., barns/house/pen observed), any type of observation efforts (mortality levels, feed intake, etc.), any events that trigger veterinary investigation, and results of follow-up tests.
- Each facility should have a written biosecurity plan to minimize disease introduction risks. This should be reviewed or revised annually in consultation with an accredited veterinarian.

Analysis Reporting and Presentation

9. Data Analysis and Interpretation

State personnel currently record avian influenza surveillance data on State avian health cooperative agreement spreadsheet templates and forward to the VS Surveillance, Preparedness, and Response Services (SPRS) poultry epidemiologists as part of their cooperative agreement quarterly reporting. The VS SPRS poultry epidemiologists then send the spreadsheets to VS Center for Epidemiology and Animal Health (CEAH) Surveillance Design and Analysis (SDA) for compilation and analysis. The methodology is viewed as a temporary data management solution until APHIS establishes a permanent data collection and management system that meets data and security requirements.

CEAH-SDA is responsible for avian influenza surveillance data analysis, working in collaboration with all stakeholders. Analysis will be provided to VS management and all pertinent VS units. Information and selected samples will also be shared with industry and other stakeholders as appropriate. Data release will meet all Federal privacy law requirements and appropriate VS policy statements.

VS data analysis is currently limited to surveillance data supplied through testing funded by VS cooperative agreements. Testing supported by States or producers is not reported to VS and is therefore not included in the data analysis. This gap in surveillance knowledge limits the interpretations that can be made regarding H5/H7 avian influenza surveillance activities in the United States.

Confirmed stakeholder and management-approved summary surveillance data may be posted on the NAHSS Web site (http://www.aphis.usda.gov/vs/nahss/poultry/index.htm) or other USDA Web sites for sharing with the public.

10. Presentation and Reporting

- Quarterly State and national level surveillance reports will be generated to inform stakeholders (e.g., VS SPRS, STAS, NIES, NCC) of the avian influenza surveillance activities supported by VS through cooperative agreements.

- Summary level NCC surveillance data will be posted quarterly on the NAHSS Web site (http://www.aphis.usda.gov/vs/nahss/poultry/index.htm).

- Annual summary reports of the surveillance data supported by cooperative agreements will be generated during normal reporting cycles. Annual reports will minimally include:
    - Number of surveillance samples collected and tested versus the expected numbers
    - Analysis of problems or issues within the sampling stream
    - A summary of individual State submission data
    - Review of compiled summary data reported *and*
    - Evaluation of *s*ample stream efficacy and identification of needed changes.

Implementation, Budget, and Evaluation

11. Surveillance System Implementation: Priorities, timelines, Internal Communication

This national H5/H7 AI surveillance plan was developed to provide surveillance recommendations for the different components of the NAISS. The NAISS collectively provides the information necessary to safeguard the health of U.S. poultry and promote the marketability of U.S. poultry and poultry products through existing and ongoing activities performed as part of the NPIP and LBMS programs. Activities in these programs to meet requirements described in regulations (NPIP) or standards (LBMS) will not change. These recommendations are intended to assist national and State planners assess existing activities and gauge whether surveillance is adequate to meet national NAISS goals. To that order, VS will continue to work with States to plan statistically valid sampling methods.

The avian health commodity team and CEAH-SDA staff will meet and discuss the use of the surveillance plan regularly to continuously refine surveillance strategies.

12. Resources and Budget

The NAISS is funded under an avian commodity-specific line item. Avian health cooperative agreement funding is allocated to the States and other cooperators each year to carry out H5/H7 AI surveillance. Human resources essential for the success of the NAISS include the VS avian health commodity team, VS district staff, State animal health personnel, industry personnel, and STAS personnel (NVSL and CEAH), and the approved NAHLN laboratories.

13. Surveillance Plan Performance Metrics

The primary performance metric of this surveillance plan is the success of attaining State-level 95 percent confidence to detect H5/H7 AI virus if the prevalence among premises in the State is 1 percent and the prevalence within an infected flock is 25 percent. This ensures adequate surveillance on the national level. Because requirements to meet NPIP certification or LBMS requirements may exceed the minimum recommended in this plan, the plan is not intended to measure success of each State meeting its own NPIP and LBMS objectives.

14. Surveillance System Evaluation

The national reportable avian influenza surveillance system should be evaluated regularly to determine how well the system fulfills its stated objectives and meets the performance metrics identified above. CEAH-SDA personnel, in collaboration with the avian health commodity team, will annually assess implementation progress, actual obtained sample numbers, applicability of performance metrics, and attainment of stakeholder goals. Modifications to the plan will be made as necessary in consultations with stakeholders of the H5/H7 AI surveillance plan.

APPENDIX A. U.S. POULTRY POPULATION DESCRIPTION, CHARACTERISTICS, AND MANAGEMENT PRACTICES

The domestic poultry population in the National AI Surveillance Plan is divided into four categories: the large-volume commercial poultry industry, the small-volume but high-value commercial poultry industry, the Live-Bird Marketing System, and backyard poultry flocks (Figure A-1).

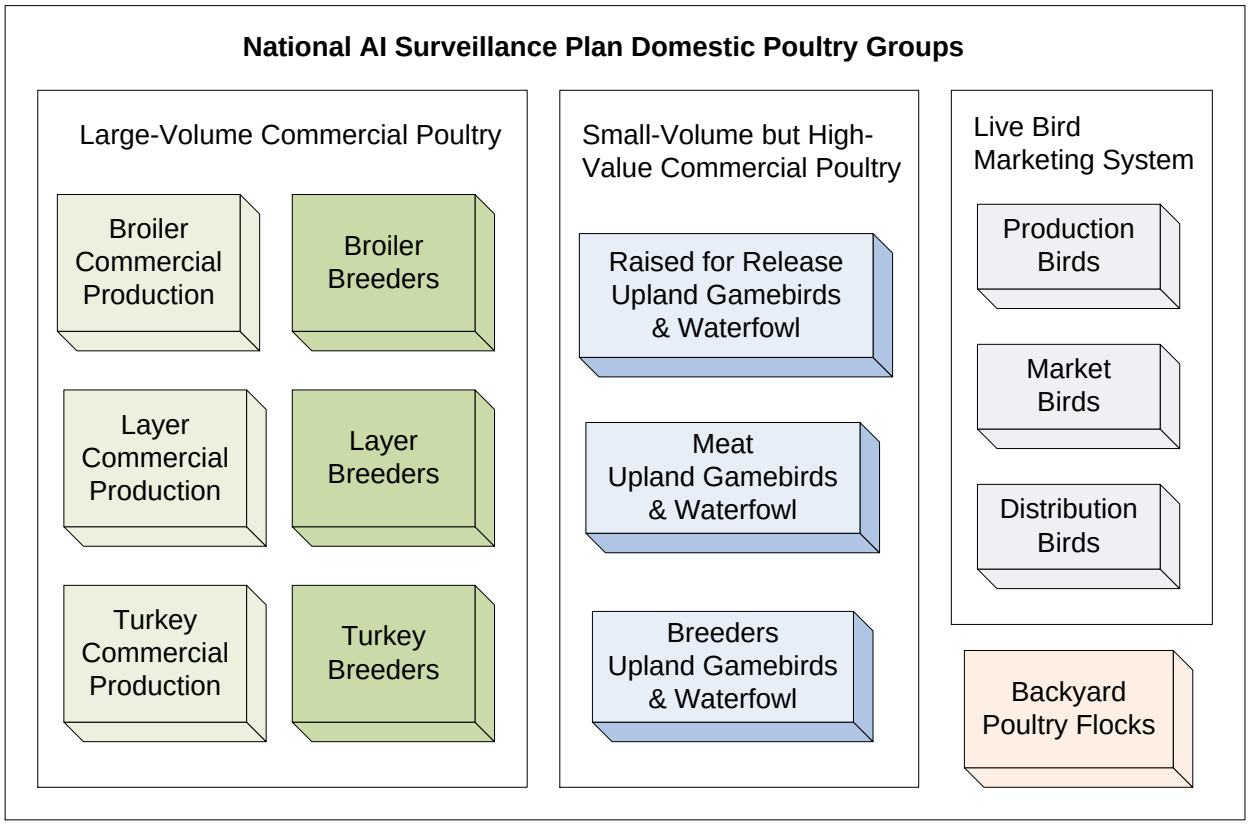

Figure A-1. The domestic poultry population groups in the H5/H7 AI surveillance plan

The categories are primarily based on risk of disease introduction and management practices. Most surveillance in domestic commercial poultry populations occurs through the NPIP, a joint industry-State-Federal program administered through official State agencies in cooperation with USDA-APHIS-VS.

This plan does not include wild birds and pet bird populations. Currently, measures are in place to restrict or prohibit the importation of avian commodities (including pet birds) from HPAI H5N1-affected countries and/or regions. Domestic pet birds are housed indoors; they are generally not exposed to waterfowl or their habitat and are considered to be at negligible risk for AI.

Population Group I: Large-Volume Commercial Poultry

Large-volume commercial poultry production is the largest segment of the U.S. poultry industry and includes commercial meat-type chickens, commercial table-egg layers, and commercial meat-type turkey production. While the product, location, and integrator vary, this industry segment has the most standardized production practices of all U.S. livestock industries. High levels of biosecurity, daily monitoring, and restricted access to the poultry are important characteristics of this segment.

Broiler, Layer, and Turkey Production

The commercial poultry industry includes three main components: commercial meat-type chickens (broiler production), commercial table-egg production, and commercial meat-type turkey production.

Indoor housing is the norm for commercial broiler, layer, and turkey operations, especially breeders, reducing the risk presented by wildlife and migratory wild birds. The level of biosecurity, monitoring, and management practices is very high. In an outbreak of H5/H7 LPAI H7N2 in commercial farms in Virginia, however, raccoons possibly acting as mechanical vectors were found to be associated with the outbreak (McQuiston *et al.* 2005). Risk factors for introduction of virus to flocks include service personnel, catching crews, vaccination crews, employees (especially if they own birds), rendering facilities, feed trucks, egg pickup and processing (racks and crates going to different farms), shared equipment, and bird placements (spiking males, flock additions).

Commercial Production Broiler Flocks

Meat-type chickens include all domesticated chickens grown primarily for producing meat, including but not limited to broilers, roasters, fryers, and Cornish game hens. A total of 8.6 billion broilers were produced in the United States in 2011 (NASS Poultry-Production and Value 2011).

Meat-type chicks are placed in the grower house at 1 day of age and are raised on the floor on litter until market age (5–8 weeks). Caked litter is removed between flocks, and litter is replaced every 1-3 years. The grower house environment, including temperature, ventilation, and light, is frequently computer-controlled, and birds are housed in total confinement. Farms are operated in all-in, all-out management systems. Feed is withdrawn 8 hours prior to processing. Although mechanical catching is becoming more common, broilers are mostly caught and loaded into coops or cages by hand. The typical operation experiences five to six turns per year depending on economic conditions.

Commercial Production Table Egg Layer Flocks

Table-egg layers are domesticated chickens grown primarily to produce eggs for human consumption. On average in 2011, there were 277 million table-egg layers on hand (NASS Chickens and Eggs 2011).

According to a 1999 NAHMS study of the layer industry, AI risk factors include the opportunity for disease transmission between flocks from racks and flats via the processor. Biosecurity practices

employed by U.S. egg producers include: prohibiting non-business visitors (68.1 percent), prohibiting employees from owning poultry (75.7 percent), fencing (26.7 percent), and employee footbaths (24.5 percent). Only 3.9 percent of U.S. egg producers provide workplace shower facilities for employees and/or visitors.

While there are many independent small farms, they account for a small percentage of production; the majority of egg production occurs through vertically integrated commercial operations with more than 30,000 layers. The average size of these large layer farms is 163,000 layers; over half (56 percent) of the farm sites have more than 70,000 layers, and over a third (36.5 percent) have more than 100,000 layers. Two-thirds (63.9 percent) of farm sites have one flock, and one-third have two or more concurrent flocks. The average flock size is 63,000 birds (National Animal Health Monitoring System 1999).

According to a 1999 NAHMS national study, pullets are raised on pullet farms, where approximately three-fourths are cage-reared and one-fourth floor-reared. Layers are placed in layer houses at 18-20 weeks. Layers are nearly always housed in cages; non-caged layers accounted for less than 1 percent of layer houses. On the majority of farm sites, eggs were gathered by egg belts. Eggs were gathered by hand on about 30 percent of farm sites, accounting for 10.6 percent of eggs gathered. This practice was most common in the Western United States. Eggs were processed on-farm at 19 percent of farm sites, and 81 percent of farms had their eggs processed off-farm. Egg pickup occurred every 1-2 days for 48 percent of farms and every 3-5 days for 45 percent of farms. Eggs were transferred to the processor in crates or flats on racks (National Animal Health Monitoring System 1999).

The NAHMS study determined that egg production peaked at 27-29 weeks with a peak hen-day egg production of 90 percent. Approximately three-fourths of flocks were molted when production dropped (at approximately 60 weeks) and a second laying cycle occurred. Molting was most common in the Southeast and least common in the Central United States. Molted flocks ended production at an average of 111 weeks and unmolted flocks at 74 weeks. Most) spent hens (86.1 percent) went to processing while 2.6 percent of spent hens (from 10.8 percent of farm sites) went to live-bird markets. The average down time between flocks was 17 days.

Commercial Production Turkey Flocks

Meat-type turkeys are domesticated turkeys grown primarily for producing meat. In 2011, a total of 248.5 million turkeys were raised in the United States (NASS Poultry-Production and Value 2011). Housing in the turkey industry has moved mostly indoors. Poults (young turkeys) are now brooded to 6 to 8 weeks in one operation and then moved to one or more grow-out operations. Turkeys are separate-sex reared and a typical operation experiences three to four turns per year.

Broiler, Layer, and Turkey Breeders

Primary breeder flocks for the large-volume commercial industry have the highest levels of biosecurity measures. These measures include daily monitoring; showers and designated clothing for employees;

visitor restrictions; vehicle sprays; and locating parking areas away from bird housing. Birds are housed indoors and rarely have contact with wild birds.

The National Poultry Improvement Plan (NPIP) is an industry-State-Federal cooperative program that awards "avian influenza clean" status to poultry breeders (http://www.aphis.usda.gov/newsroom/content/2009/03/aphisnpid.shtml). All breeder farms included in a 2010 population estimate study participated in the NPIP avian influenza program (USDA 2011).

Eggs for hatching are incubated at hatcheries, and usually day-old chicks and poults are placed at grower farms for meat production or pullet farms for table-egg layers. Layer pullets are usually placed in layer production houses at 16–18 weeks.

Broiler Breeders

Within the 12-month period between July 2010 and June 2011, a total of 6,471 broiler breeder flocks were monitored as part of the NPIP avian influenza surveillance program (C.S. Roney, personal communication, 2011).

Broiler breeders are placed in breeder houses at 20-22 weeks, with 8-10 males per 100 hens. Hens are provided with nest boxes, as floor eggs are undesirable. Egg production peaks at 30-40 weeks and flocks generally will be in lay until 60-65 weeks. Uncompetitive males are culled, and new young males are introduced into the flock (spiking males). Eggs are removed from the hen house at least daily and stored up to 7 days. Eggs are transported to the hatchery for incubation and hatching.

Layer Breeders

Within the 12-month period between July 2010 and June 2011, a total of 331 layer breeder flocks were monitored as part of the NPIP avian influenza surveillance program (C.S. Roney, personal communication, 2011).

The majority of egg-type primary breeder stock is controlled by a few companies, who maintain ownership of the multiplier breeder stock, with approximately 20 percent of day-old multiplier breeding stock sold to large integrators to maintain their own multiplier breeding stock. Pullets for breeding are raised by pullet growers on litter floor to 18 weeks, at which time they move to contract layer houses on slats. Production ends at 70 weeks of age and hens are not molted. Males and females are kept together throughout the process; males make up approximately 8-10 percent of breeder inventory. Eggs are sent to company-owned hatcheries. Day-old chicks are then sold to commercial producers for table-egg production.

Turkey Breeders

Within the 12-month period between July 2010 and June 2011, a total of 634 turkey breeder flocks were monitored as part of the NPIP avian influenza surveillance program (C.S. Roney, personal communication, 2011).

Because turkeys are artificially inseminated, turkey toms and hens are raised separately. Hens and toms are selected at 16 weeks and moved to a dark-out house, where they are gradually exposed to increasing light. At 30 weeks they are moved to laying/stud facilities, and production begins at about 32 weeks. Hens are inseminated every 1-2 weeks and will have a lay cycle of 25 weeks. Farm personnel at the stud farms collect semen manually, and different personnel at the laying farms do the insemination. The addition of extenders has allowed storage of semen, and thus semen can be delivered to longer distances. Hens lay eggs in nests, and eggs are collected by hand several times per day (University of Minnesota 2006)

Population Group II: Small Commercial and Other Industries

The remaining 10 percent of the production value for poultry and eggs occurs in what the reportable AI surveillance system describes as small-volume and high-value production sectors. Production characteristics for many of these producers are undocumented and production practices are diverse, including outdoor and free-range flocks. This industry segment produces poultry and eggs for commercial sales, although not through the same channels as described for large-volume commercial operations.

Upland Gallinaceous Game birds and Raised-for-Release Waterfowl

Upland game birds include domesticated fowl such as pheasants, partridge, quail, grouse, and guineas, but not doves and pigeons. The industry is guided by the North American Game Bird Association and its membership base. In 2007, there were a total of 65 million game birds and waterfowl included in inventory (as of December 2007) and sold (during 2007) in the United States (NASS 2007 Census of Agriculture). This category includes breeding stock and raised-for-release birds.

A total of 8 million pounds (live weight) of poultry other than chickens, turkeys, or ducks were slaughtered in Food Safety and Inspection Service (FSIS) inspected plants, accounting for about 0.01 percent of total poultry slaughtered in 2005 (NASS 2006a), though the proportion that is game birds is unknown. The proportion of upland game birds and waterfowl slaughtered on farm or sold for custom slaughter in smaller facilities, and not at federally inspected plants, also is unknown.

Although much of the upland game bird industry focus appears to be on stocking hunting preserves and wildlife restocking, some producers sell meat and eggs of upland game birds, mainly in specialized gourmet markets. Some upland game birds are also raised for exhibition and some farms sell day-old chicks (Iowa State University Agricultural Marketing Resource Center 2006). Inherently, this industry has less strict biosecurity measures, because these birds are released into environments for contact with wild birds. Primary AI risk factors include movement of birds off property and exposure to wild birds.

Game fowl are breeds of chickens intended primarily for exhibition/competition and bred for visual characteristics, strength, health, vitality, and longevity. Nearly 9,000 game fowl breeders in 34 States

belong to the United Game Fowl Breeders Association (UGBA) or a State association not affiliated with UGBA. Game fowl breeding is a diverse industry with a wide array of practices. In general, birds used for experimental purposes or to develop blood lines are penned with one rooster and one or two hens. Pullets, young hens intended for egg laying, are raised free-range until ready for production. Hens are allowed to forage on a free range in the winter when they are not producing. Spent hens—mature hens that have reached the end of their productive lives—are often sold at live-bird markets; younger hens that are no longer needed may be sold to another producer as brood hens (L. Mathews, personal communications, 2006).

Commercial Waterfowl

Commercial waterfowl are defined as domesticated ducks or geese grown under confinement primarily for producing meat for human consumption, but they are also raised for breeding and release. In 2007, there were 31.6 million ducks and geese included in inventory and sold in the United States (NASS 2007 Census of Agriculture)

In 2007, 27 million ducks were sold in the United States (NASS 2007 Census of Agriculture).
The U.S. duck industry is widely dispersed throughout the country, but FSIS slaughter data reports indicate that most commercial ducks are raised in Wisconsin and Indiana.

According to the Cornell University Duck Research Lab (Cornell University 2006), commercial duck housing is either total or semi-confinement. Properly designed confinement housing will restrict contact with wild birds. Under a semi-confinement housing plan, ducks more than 2 to 3 weeks old are allowed outside during the day, and ducks over 4 weeks spend most of their time outdoors. Ponds are not required for commercial waterfowl production as long as the birds are provided ample clean, fresh drinking water and access to shade, if kept outdoors.

In 2007, 161,133 geese were sold in the United States (NASS 2007 Census of Agriculture). Geese in commercial production are raised under cover until approximately 6 weeks of age. Brooding is done in a temperature-controlled environment. After this period, geese are kept on range, where they graze and are fed some supplemental grain for another 14 to 20 weeks, until slaughter.

Risk factors for exposure to AI viruses are the same as for other poultry with similar management practices. An additional consideration for this poultry sector is that many AI viruses pathogenic to other poultry show few, if any, clinical signs in ducks and geese (Swayne and Suarez 2000). The only exceptions have been the recent Asian HPAI reportable AI H5N1 virus in a variety of wild bird species (Webster *et al.* 2006) and the African H5N3 that led to one mortality event in terns (Becker 1966). Because waterfowl may not show clinical signs of infection, they serve as an H5/H7 AI transmission risk when collocated with other poultry species. Other poultry in contact with subclinically infected waterfowl can develop clinical illness upon infection with avian influenza virus.

PASTURED, FREE-RANGE, AND ORGANIC POULTRY

The total number of farms that raise poultry as pastured, organic, and free-range is unknown. Some of their population numbers are included in the other domestic poultry categories. In 2008, a total of 5.5 million layers, 9 million broilers, and nearly 400,000 turkeys were certified organic (ERS Organic Production 2008).

Pastured poultry is a production system that involves raising chickens directly on pasture using moveable shelters. Birds receive up to 20 percent of their feed intake from pasture forage and are moved regularly to fresh pasture. Processing is often done on the farm, although larger producers transport birds to slaughter facilities.

In order to receive USDA "Free-Range" certification, producers must demonstrate to USDA that chickens raised for meat have daily access to the outdoors, although there are no industry guidelines for how long birds must remain outdoors (FSIS Fact Sheet, 2007).

"Certified organic" production means that the production methods meet the national standards established by USDA's Agricultural Marketing Service, as certified by accredited State, private, or foreign organizations or other approved certifying agents. The national standard requires that animals for slaughter must be raised under organic management from the last third of gestation, or no later than the second day of life for poultry. Producers are required to feed livestock agricultural feed products that are 100 percent organic, but may also provide allowed vitamin and mineral supplements. Organically raised animals may not be given hormones to promote growth or antibiotics for any reason. Preventive management practices, including the use of vaccines, will be used to keep animals healthy. Producers are prohibited from withholding treatment from a sick or injured animal; however, animals treated with a prohibited medication may not be sold as organic. All organically raised animals must have access to the outdoors. They may be temporarily confined only for reasons of health, safety, the animal's stage of production, or to protect soil or water quality (ERS Organic Production 2008).

Population Group III: Live-Bird Marketing System

Live-bird markets are part of a complex marketing system that provides a source of fresh poultry meat often preferred by ethnic populations (Senne *et al.* 2003). Customers select live birds that they wish to purchase and the birds are then individually slaughtered and prepared according to the customer's specifications. In 2012, the total number of LBMS-associated premises in the United States was estimated at 5,253; including 522 production premises, 283 markets, and 4,448 distribution sites (A. Pelzel, personal communication, 2012).

Market characteristics and practices vary according to region. The NAHMS Poultry 2004 study found that markets were larger in the Northeast region (New Jersey, New York, Massachusetts, Maine, Vermont, New Hampshire, Connecticut, Rhode Island, and Pennsylvania) compared to the Southwest region (California, Florida, and Texas). In the North region, over two-thirds of markets sold 1,000 or more birds per week. In the South region, over half of markets sold less than 500 birds per week. Nearly

all markets in the North region always slaughtered birds on site, whereas birds left the market alive in over half of markets in the South region (mostly Florida botanicas[8]). A higher percentage of markets in the North region sold spent laying hens, turkeys, ducks, and guinea fowl, while geese and pigeons were sold by a higher percentage of markets in the South region (National Animal Health Monitoring System 2004).

The LBMS includes the live-bird markets and their production and distribution systems. Birds entering the LBMS come from a variety of sources, including farms that raise birds specifically for live-bird markets, backyard flocks, and spent hens from smaller layer farms. While some markets receive birds directly from farm deliveries, most receive birds from distributors or wholesalers who collect birds at the farm and deliver them either directly to markets or to distribution centers, where the shipments are mixed to fill orders.

Population Group IV: Backyard Flocks

For the reportable AI surveillance system, a backyard flock is defined as a premises having fewer than 1,000 birds, other than pet birds (NAHMS Poultry 2004). Exact estimates for the number of backyard flocks are unavailable.

According to a 2004 NAHMS study, the average backyard flock size is 35 birds, with more than half of flocks numbering fewer than 20 birds. Some common types of birds are table-egg laying chickens, game fowl, ducks, meat-type chickens, guinea fowl, and game birds. Approximately 20 percent of backyard poultry flocks include ducks. A total of 8.7 percent of backyard flocks report having waterfowl other than ducks. While nearly one in four backyard flocks have game fowl, they account for only 10 percent of backyard birds. This indicates that game fowl flocks tend to be smaller than the average flock. Table A-1 lists the common types of birds in backyard flocks.

Table A-1. Common Types of Birds in Backyard Flocks (NAHMS 2004)

Bird Type	% Flocks	% Birds
Table-egg chickens	63.2	37.5
Game fowl	23.2	10.2
Ducks	20.6	6.4
Other waterfowl	8.7	1.3
Meat-type chickens	17.2	11.5
Guinea fowl	11.8	4.7
Game birds	4.4	17.8

[8] Botanicas are markets that sell birds for slaughter offsite, primarily for ritual slaughter.

Backyard flock owners rarely use the services of a veterinarian (2.9 percent). Primary risk factors include: exposure to wild birds, birds leaving the property, ponds that attract wild waterfowl, trading birds between premises, frequent flock additions, and minimal biosecurity practices.

The NAHMS Poultry 2004 study determined that on average, there are 1.9 backyard flocks located within a 1-mile radius of commercial poultry operations. In 47.1 percent of the flocks, birds are housed in a manner that allows them to leave the property. Two-thirds of flocks have contact with wild birds. Footwear precautions are rarely used (11.4 percent). On sites with backyard flocks, 38.4 percent have ponds on the property (most common in Midwest) and 40 percent have wild-bird feeders. Although biosecurity practices are minimal, bird movement and interaction are also uncommon. Only one-third of backyard operations reported new flock additions in the year, most commonly from a private individual and generally from the same county. Only 17.8 percent of operations sell or give away live birds. Movement to fairs, shows, and other events where other birds are present is extremely rare (3.6 percent of flocks), and these events are mostly within the same county or within the State (NAHMS Poultry 2004).

APPENDIX B. LIKELIHOOD RATIOS AND CONSEQUENCE-BASED SAMPLING

Likelihood ratios (LRs) are epidemiologic measures of association, similar in function to relative risks or odds ratios. LRs measure the predictive strength of a particular risk characteristic or demographic. When incorporated as variables in the equations of Bayes theorem, they quantify the predictive strength of the characteristics or context of the population to be sampled and adjust the sample size accordingly. For example, if LRs suggest that a population is protected from disease by biosecurity practices at the facility, the calculations define the number of samples required. LRs are calculated as the prevalence of a risk characteristic among an infected group divided by the prevalence of the same risk characteristic among an uninfected group.

Poultry production types are expected to vary in disease risk by differing biosecurity practices, where stronger biosecurity should equate to reduced likelihood of disease introduction. Production types are also expected to vary by differences in susceptibility to disease and longevity of production cycles, where greater durations should mean greater opportunities for virus to circulate and for viral re-assortment or mutation. Additionally, production types are stratified by consequences of disease introduction. By capturing this risk differential, surveillance can be focused on the highest likelihood of introduction and highest consequence production types, and reduce demands for surveillance elsewhere.

In this plan, LRs were constructed for each bird type. Disease occurrence data by bird type were obtained from VS regional records. Denominator data estimates for occurrence of each production type were based on NASS Census figures, (NASS 2007 Census of Agriculture). Preliminary likelihood ratios were then reviewed and modified by experts from the VS regions (operational epidemiology) and surveillance unit (surveillance epidemiology planning and analysis).

From those results, bird types were ranked according to likelihood of infection as follows. Where LRs are equal to or greater than 1, the production types need target levels of surveillance sampling to demonstrate freedom. Where LRs are less than 1, the sampling can be reduced to (LR*target level) and still achieve full confidence in disease freedom (Gustafson et al., 2010). One is then added to the product [(LR*target level) +1] to ensure the estimate is conservative. The LBMS and backyard LRs were not calculated because they were both known to have higher likelihood of exposure and disease. The expert opinion shows that the 'other commercial' sector has a high likelihood of becoming infected but a low consequence of disease introduction (i.e., infection in this sector is unlikely to spread to commercial production facilities). Therefore, the experts agreed upon the low calculated LR generated from the outbreak data as shown in Table B-1 below.

Table B-1. Data-derived and expert-accepted likelihood ratios per bird type. A likelihood ratio less than 1 indicates the bird type requires less sampling.

	Bird Type	Likelihood Ratio
Breeders		
	Meat-type chicken	2.5
	Egg-type chicken	12
	Meat-type turkey	12.5
Production		
	Commercial meat-type chicken	0.12
	Commercial table-egg layer	1
	Commercial meat-type turkey	4.4
Other Commercial (upland gamebirds, waterfowl)		0.19

Each State's targeted sample size was calculated per bird type. Confidence in disease freedom for a given premises was computed using the appropriate diagnostic test sensitivity for each test[9] and specificity of 100 percent, and among birds, a pre-set prevalence detection threshold of 25 percent. Maximum sample size was determined using the methodology as described in Cannon, 2001. Confidence in disease freedom for a State was computed using a hypergeometric approximation (Cannon, 2001) with the within-premises sensitivity derived from the average confidence achieved in sampled sites, a within-premises specificity of 100 percent, and among premises, a pre-set prevalence detection threshold of 1 percent.

For commercial meat-type chickens and 'other commercial' bird types (upland game birds and waterfowl), the LR calculated above was then applied to the targeted sample size to decrease the level of sampling in those bird types. Commercial meat-type chickens are only alive for 5-8 weeks, and the majority of that time they are covered by maternal immunity. Also, the 'other commercial' category has

[9] Diagnostic test sensitivities: rRT-PCR Matrix 85% (J. Pedersen, personal communication, 2012), AGID 60%, ELISA 76% (Brown 2009), VI 95% (D. Swayne, personnel communication 2012), ACIA 65.9% (Elvinger 2007)

a low consequence of disease introduction. Thus, sampling in these bird types may be substantially reduced while still achieving confidence in the disease free status. The avian health commodity team may determine that funding levels allow for more sampling to occur within the cooperative agreement surveillance stream than is necessary to meet the minimum surveillance requirements outlined in this plan.

REFERENCE LIST

Abdelwhaba E.M., Grund C., Aly M.M., Beer M., Harder T.C, and Hafez H.M. (2012). Influence of maternal immunity on vaccine efficacy and susceptibility of one day old chicks against Egyptian highly pathogenic avian influenza H5N1, *Vet Microbiology*, 155(1), 13-20.

Alexander, D.J. (2000). A review of avian influenza in different bird species, *Vet Microbiology* 74, 3-13.

American Egg Board, http://www.aeb.org/egg-industry/industry-facts/shell-egg-distribution, Accessed 8/8/2013.

Becker, W.B. (1966). The isolation and classification of tern virus: influenza virus A/tern/South Africa/1961. *Journal of Hygiene*, *64*(03), 309-320.

Belser, J. A., Bridges, C. B., Katz, J. M., & Tumpey, T. M. (2009). Past, present, and possible future human infection with influenza virus A subtype H7. *Emerging infectious diseases*, *15*(6), 859.

Brown, J.D., Stallknecht, D.E., Berghaus, R.D., Luttrell, M.P., Velek, K., Kistler, W., Costa, T., Yabsley, M.J., Swayne, D. (2009). Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. *Clinical and vaccine immunology* 16(6), 824-829.

Bulaga, L. L., Garber, L., Senne, D., Myers, T. J., Good, R., Wainwright, S., and Suarez, D. L. (2003a). Descriptive and surveillance studies of suppliers to New York and New Jersey retail live-bird markets. *Avian Dis* 47, 1169-76.

Bulaga, L. L., Garber, L., Senne, D. A., Myers, T. J., Good, R., Wainwright, S., Trock, S., and Suarez, D. L. (2003b). Epidemiologic and surveillance studies on avian influenza in live-bird markets in New York and New Jersey, 2001. *Avian Dis* 47, 996-1001.

Capua, I. and Alexander, D. J. (2006). The challenge of avian influenza to the veterinary community. *Avian Pathol* 35, 189-205.

Centers for Disease Control and Prevention (CDC). (2013). Emergence of avian influenza A(H7N9) virus causing severe human illness – China, February-April 2013. Morbidity and Mortality Weekly Reports (MMWR). 62(18);366-371.

Cornell University. Duck Research Laboratory; www.duckhealth.com/ducklab.html. (2006). Accessed on September 18, 2006.

De Vriese J., Steensels M., Palya V., Gardin Y., Dorsey K.M., Lambrecht B., Van Borm S., and van den Berg T. (2010). Passive protection afforded by maternally derived antibodies in chickens and the antibodies' interference with the protection elicited by avian influenza-inactivated vaccines in progeny, *Avian Dis.*, 54, 246–252.

Dunn, P. A., Wallner-Pendleton, E. A., Lu, H., Shaw, D. P., Kradel, D., Henzler, D. J., Miller, P., Key, D. W., Ruano, M., and Davison, S. (2003). Summary of the 2001-02 Pennsylvania H7N2 low pathogenicity avian influenza outbreak in meat type chickens. *Avian Dis* 47, 812-6.

Easterday, B. C., Hinshaw, V. S., and Halvorson, D. A. (1997). Influenza. pp. 583-605. (Iowa State University Press: Ames IA).

Economic Research Service (ERS), Livestock and Meat Domestic Data, WADSE reports, http://www.ers.usda.gov/data-products/livestock-meat-domestic-data.aspx#26126, USDA. Accessed 8/8/2013.

Economic Research Service (ERS), Meat Price Spreads, Retail prices for beef, pork, poultry cuts, eggs, and dairy products, http://www.ers.usda.gov/data-products/meat-price-spreads.aspx, USDA. Accessed 8/8/2013.

Economic Research Service (ERS). Organic Production in 2008. http://www.ers.usda.gov/Data/Organic/, USDA. Accessed 07/2012.

Elbers, A. R., Koch, G., and Bouma, A. (2005). Performance of clinical signs in poultry for the detection of outbreaks during the avian influenza A (H7N7) epidemic in The Netherlands in 2003. *Avian Pathol* 34, 181-7.

Elvinger, F., Akey, B. L., Senne, D. A., Pierson, F. W., Porter-Spalding, B. A., Spackman, E., and Suarez, D. L. (2007). Characteristics of diagnostic tests used in the 2002 low-pathogenicity avian influenza H7N2 outbreak in Virginia. *Journal of veterinary diagnostic investigation*, *19*(4), 341-348.

Food Safety and Inspection Service (2007). Meat and Poultry Labeling Terms Fact Sheet, http://www.fsis.usda.gov/Fact_Sheets/Meat_&_Poultry_Labeling_Terms/index.asp. Accessed 3/23/2007.

Gelb, J., and Ladman, B. (2006). Appropriate Applications of Agent and Antibody Detection in AI Surveillance. High Path Avian Influenza Workshop, April 27, 2006.

Global Trade Information Services, Global Trade Atlas – 2013, Accessed 8/8/2013.

Groocock, C. Avian Influenza in gamebirds in Maryland. Foreign Animal Disease Report. (Summer 1994).

Hall, C. (2004). Impact of avian influenza on U.S. poultry trade relations-2002: H5 or H7 low pathogenic avian influenza. *Ann N Y Acad Sci* 1026, 47-53.

Halvorson D.A. (2002). 25 years of avian influenza in Minnesota. Proceedings of the 53rd North Central avian disease conference. 65-69.

Iowa State University Agricultural Marketing Resource Center. (2006). Gamebird Profile. Sept. 18, 2006.

Kalthoff, D., Globig, A., and Beer, M. (2010) (Highly pathogenic) avian influenza as a zoonotic agent, *Vet Microbiology* 140, 237-245.

Kaye, D. and Pringle, C. R. (2005). Avian influenza viruses and their implication for human health. *Clin Infect Dis* 40, 108-12.

Kim, H-R., Lee, Y-J., Park, C-K., Oem, J-K., Lee, O-S, Kang, H-M., Choi, J-G, and Bae, Y-C. (2012) Highly pathogenic avian influenza (H5N1) outbreaks in wild birds and poultry, South Korea. *Emerging Infectious Diseases* 18(3), 480-483.

Kinde, H., Read, D. H., Daft, B. M., Hammarlund, M., Moore, J., Uzal, F., Mukai, J., and Woolcock, P. (2003). The occurrence of avian influenza A subtype H6N2 in commercial layer flocks in Southern California (2000-02): clinicopathologic findings. *Avian Dis* 47, 1214-8.

Kradel, D. C., Miller, W. L., Braune, M. O., and Rothenbacher, H. R. (1986). Avian influenza H5N2 - Necropsy and laboratory test observations during the 1986 outbreak. Proceedings of the American Association of Veterinary Laboratory Diagnosticians. 29.

Lebdah, M.A. and Shahin, A.M. (2010) Evaluation of Avian Vaccines used in Broiler Flocks in Egypt, *J of Am Science*, 6(12), 918-926.

Li Y., Liu L., Zhang, Y., Duan Z., Tian, G., Zeng, X., Shi, J., Zhang, L, and Chen, H., (2011) New avian influenza virus (H5N1) in wild birds, Qinghai, China, *Emerging Infectious Diseases* 17(2), 265-267.

Livestock Market Information Center, http://lmic.info/tac/spreadsheets/spreadsheets.html, Denver, CO, Accessed 8/8/2013.

Lu, H. and Castro, A. E. (2004). Evaluation of the infectivity, length of infection, and immune response of a low-pathogenicity H7N2 avian influenza virus in specific-pathogen-free chickens. *Avian Dis* 48, 263-70.

Maas R., Rosema S., van Zoelen D., Venema S. (2011) Maternal immunity against avian influenza H5N1 in chickens: limited protection and interference with vaccine efficacy, *Avian Pathol.*, 40, 87–92.

McQuiston, J. H., Garber, L. P., Porter-Spalding, B. A., Hahn, J. W., Pierson, F. W., Wainwright, S. H., Senne, D. A., Brignole, T. J., Akey, B. L., and Holt, T. J. (2005). Evaluation of risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms. *J Am Vet Med Assoc* 226, 767-72.

Mullaney, R. (2003). Live bird market closure activities in the northeastern United States. *Avian Dis* 47, 1096-8.

National Agricultural Statistics Service (NASS) 2007 Census of Agriculture – United States Data. USDA, http://quickstats.nass.usda.gov/ July 2012.

National Agricultural Statistics Service (NASS) Chickens and Eggs 2011 Summary, USDA, February 2012.

National Agricultural Statistics Service (NASS). Poultry-Production and Value 2012 Summary, USDA, April 2013

National Agricultural Statistics Service (NASS). Poultry Slaughter 2005 Annual Summary. USDA, February 2006.

National Animal Health Monitoring System. NAHMS Layers 1999. October 1999.

National Animal Health Monitoring System. NAHMS Poultry '04 Part I. 2004.

National Association of State Departments of Agriculture Research Foundation. Animal Health Safeguarding Review. 2001. Accessed August 23, 2006.

National Chicken Council (NCC). How Broilers are Marketed; http://www.nationalchickencouncil.com/statistics/stat_detail.cfm?id=7 . Accessed 4/28/2005.

National Chicken Council (NCC), U.S. Broiler Exports Quantity and Share of Production, http://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-exports-quantity-and-share-of-production/, Accessed 8/3/2012.

Nguyen, D. C., Uyeki, T. M., Jadhao, S., Maines, T., Shaw, M., Matsuoka, Y., Smith, C., Rowe, T. , Lu, X., Hall, H., Xu, X., Balish, A., Klimov, A., Tumpey, T. M., Swayne, D. E., Huynh, L. P., Nghiem, H. K., Nguyen, H. H., Hoang, L. T., Cox, N. J., and Katz, J. M. (2005). Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. *J Virol* 79, 4201-12.

OIE (World Organization for Animal Health). (2013). Avian Influenza. Terrestrial Animal Health Code. Chapter 10.4; http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm

OIE (World Organization for Animal Health). (2012). Avian Influenza. Terrestrial Animal Health Manual. Chapter 2.3.4; http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.04_AI.pdf

Pelzel, A. M., McCluskey, B. J., and Scott, A. E. (2006). Review of the highly pathogenic avian influenza outbreak in Texas, 2004. *Journal of the American Veterinary Medical Association*, *228*(12), 1869-1875.

Roney, C. Stephen (2011) NPIP 2011 Annual Report. Senne, D. A., Pedersen, J. C., and Panigrahy, B. (2003). Live bird markets in the Northeastern United States: a source of avian influenza in commercial poultry. Schrijver R.S., G. Koch. Proceedings of the Frontis workshop on Avian Influenza: Prevention and Control.

Senne, D. A., Suarez, D. L., Stallnecht, D. E., Pedersen, J. C., and Panigrahy, B. (2006). Ecology and epidemiology of avian influenza in North and South America. *Dev Biol (Basel)* 124, 37-44.

Songserm, T., Jam-on, R., Sae-Heng, N., Meemak, N., Hulse-Post, D. J., Sturm-Ramirez, K. M., and Webster, R. G. (2006). Domestic ducks and H5N1 influenza epidemic, Thailand. *Emerg Infect Dis* 12, 575-81.

Swayne, D. E. and Suarez, D. L. (2000). Highly pathogenic avian influenza. *Rev Sci Tech* 19, 463-82.

Synbiotics Corporation. (2005). Flu Detect™ avian influenza type A Antigen test kit for the surveillance of avian influenza synopsis of validation studies. Available at: http://www.synbiotics.com/Products/ProductionAnimals/Avian/AvianInfluenzaDiagnosticKits/FluDETECT-AI-TypeAAntigenTestKit/96-6800-SynopsisofValidationStudies.pdf

To, K. K., Ng, K. H., Que, T. L., Chan, J. M., Tsang, K. Y., Tsang, A. K., ... & Yuen, K. Y. (2012). Avian influenza A H5N1 virus: a continuous threat to humans. *Emerging Microbes & Infections*, *1*(9), e25.

Trock, S. C., Senne, D. A., Gaeta, M., Gonzalez, A., and Lucio, B. (2003). Low-pathogenicity avian influenza virus in live bird markets--what about the livestock area? *Avian Dis* 47, 1111-3.

U.S. Poultry and Egg Association. Economic Information; http://www.poultryegg.org/EconomicInfo/index.html. Accessed on September 25, 2006.

University of Minnesota. Turkey Management; http://www.ansci.umn.edu/poultry/resources/turkeymgmt.htm#status. Accessed 9/21/2006.

USDA. The National Poultry Improvement Plan. September 8, 2006.

USDA Animal and Plant Health Inspection Service. (2006). 2005 United States Animal Health Report.

USDA, APHIS, VS. VS Guidance 12001.1, Policy for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI). 10/25/2012.

USDA, APHIS, VS. VS Guidance 8602.1, Response, Communications, and Investigation of Reportable Avian Influenza (REPORTABLE AI) in Domestic Poultry. 07/11/2012.

USDA, APHIS, VS. VS Guidance 8604.1, Reporting Confirmed Findings of Low Pathogenic Reportable Avian Influenza (H5/H7 LPAI) (H5 and H7 Subtypes) to the World Organization for Animal Health (OIE) and to trading Partners. 07/25/2012.

USDA, APHIS, VS, CEAH National Surveillance Unit. Reportable Avian Influenza Case Definition. USDA/VS/CEAH/NCAHS.

USDA (2011) Poultry 2010. "Reference of Health and Management Practices on Breeder Chicken Farms in the United States, 2010. USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO #585.1111

van der Goot, J. A., de Jong, M. C., Koch, G., and Van Boven, M. (2003). Comparison of the transmission characteristics of low and high pathogenicity avian influenza A virus (H5N2). *Epidemiol Infect* 131, 1003-13.

Van Kerkhove, M. D., Mumford, E., Mounts, A. W., Bresee, J., Ly, S., Bridges, C. B., & Otte, J. (2011). Highly Pathogenic Avian Influenza (H5N1): Pathways of Exposure at the Animal-Human Interface, a Systematic Review. *PLoS One*, *6*(1), e14582.

Veits, J., Weber, S., Stech, O., Breithaupt, A., Graber, M., Gohrbandt, S, Bogs, J., Hundt, J., Teifke, J.P., Mettenleiter, T.C., and Stech, J. (2012). Avian influenza virus hemagglutinins H2, H4, H8, and H14 support a highly pathogenic phenotype. Proc Natl Acad Sci USA 109(7), 2579-2584.

Weaver, J. T., Grogan, K.B., Trampel, D., McDonald, R., Tilley, B., McCarter, S., Brennan, P., Coats, M., Senne, D., Klein, P., Pelzel, A., Garber, L., Hall, C., and Myers, T. J. (2006). Surveillance Working Group. (2006). High Pathogenicity Avian Influenza Workshop for Poultry Industry, State, and Federal Government Stakeholders. April 27, 2006. Webster, R. and Hulse, D. (2005). Controlling avian flu at the source. *Nature* 435, 415-6.

Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M., and Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. Microbiol Rev 56, 152-79.

Webster, R. G., Peiris, M., Chen, H., and Guan, Y. (2006). H5N1 outbreaks and enzootic influenza. Emerg Infect Dis 12, 3-8.

World Health Organization (WHO). Cumulative number of confirmed cases of avian influenza A(H5N1) reported to WHO. http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html Accessed 5/9/2013.

DEFINITIONS OF TERMS/ACRONYMS USED IN THIS DOCUMENT

AGID - Agar-gel immunodiffusion assay; one of several screening assays used to detect antibodies against avian influenza

AOS - Active observational surveillance

ALS - Active laboratory surveillance

AI - Avian influenza; Infection of birds by any orthomyxovirus of the influenza A genus

AVHS - Avian Health Surveillance database, housed and maintained by the USDA

BHI - Blood-heart infusion media

Botanica - Retail live-bird markets where live birds are sold for off-site slaughter or for offsite ritual use

Commercial meat-type flock - At the discretion of the official State agency, any group of poultry segregated from another group in a manner sufficient to prevent the transmission of H5/H7 LPAI and has been so segregated for a period of at least 21 days may be considered as a separate flock

Contract grower - Poultry producers who contract with integrators (companies) to grow poultry under very specific management programs

DOI - Department of Interior

ELISA - Enzyme-linked immunosorbent assay. Commercially available test kits used to screen for antibodies against or antigens of influenza A viruses in domestic poultry

Exhibition poultry - Domesticated fowl that are bred for the combined purposes of meat or egg production and competitive showing

Flock - A group of birds of similar age considered as a production unit

Functional group - Groups of wild migratory birds (e.g., dabbling ducks, light geese, dark geese, and swans) that share similar characteristics including, but not limited to, behavior, habitat use, geographic distribution, migration patterns, and host pathogen dynamics.

Game birds - Domesticated gallinaceous birds such as pheasants, partridge, quail, grouse and guineas

Game fowl - Breeds of chickens, such as Kelso, Hatch, Claret, and Roundhead, intended primarily for exhibition/competition and bred for beauty, strength, health, vitality, and longevity

Highly pathogenic avian influenza (HPAI)- AI viruses that have been shown to fulfill virulence criteria established by OIE

Live-Bird Market (LBM) - Any facility that gathers live poultry to be slaughtered and sold on site

Live-Bird Marketing System (LBMS) - The Live-Bird Marketing System includes live-bird markets and their production and distribution systems

Low pathogenicity avian influenza (LPAI) - All AI viruses that are not REPORTABLE AI viruses

Meat-type chicken - A domesticated chicken grown for the primary purpose of producing meat, including but not limited to broilers, roasters, fryers, and Cornish

Meat-type chicken slaughter plant - A federally inspected meat-type chicken slaughter plant

Meat-type turkey - A domesticated turkey grown for the primary purpose of producing meat

NPIP - National Poultry Improvement Plan

NSU - USDA-APHIS Veterinary Services National Surveillance Unit

OIE - Office International des Epizooties; currently known as World Organization for Animal Health

Raised-for-Release - Upland game birds or waterfowl that are raised for eventual release in game preserves and are not breeding stock

RRT-PCR - Real-time reverse transcriptase polymerase chain reaction. Screening assays used to detect genetic material (RNA) of avian influenza viruses.

Table-egg layer- A domesticated chicken grown for the primary purpose of producing eggs for human consumption

Table-egg layer flock -All of the birds in one barn or house

Table-egg layer operation-All of the flocks under common ownership on one premises

Upland game birds - Domesticated fowl such as pheasants, partridge, quail, grouse, but not doves and pigeons.

VI - Virus isolation

Waterfowl - Domesticated fowl that normally swim, such as ducks and geese