



**NATIONAL FLYWAY COUNCIL**

Pacific est. 1952 - Central est. 1948 - Mississippi est. 1952 - Atlantic est. 1952

# Early Detection and Monitoring for Avian Influenzas of Significance in Wild Birds

## A U.S. Interagency Strategic Plan



**June 2015**

*Table of Contents*

Executive Summary.....	3
Introduction .....	4
Goal of the Strategic Plan.....	7
A National Early Detection System for Avian Influenzas of Significance in Migratory Birds .....	8
Decentralized Planning and Execution.....	8
Centralized Coordination.....	9
Geographic Prioritization of Sampling Efforts .....	9
Sampling Strategies.....	10
Investigation of Morbidity/Mortality Events (Attachment 2): .....	10
Surveillance in Live Wild Birds (Attachment 3): .....	10
Surveillance in Hunter-Harvested Birds (Attachment 4) .....	11
Sentinel Species (Attachment 5): .....	11
Environmental Sampling (Attachment 6): .....	11
Sample Collection.....	11
Sample Size Determination .....	12
LaboratoryDiagnostics.....	12
Reporting and Data Management.....	13
Recommendations .....	13
ATTACHMENT 1: Migratory Bird Flyways in Asia and North America .....	15
ATTACHMENT 2: Investigation of Morbidity and Mortality Events in Wild Birds .....	16
ATTACHMENT 3: Surveillance for avian influenza viruses in Live Wild Birds.....	19
ATTACHMENT 4: Surveillance for Avian Influenza in Hunter-Harvested Waterfowl.....	22
ATTACHMENT 5: Sentinel Animal Methods .....	25
ATTACHMENT 6: Environmental Fecal Sampling .....	31
ATTACHMENT 7: Instructions for Collection and Shipment of Avian Carcasses for Diagnostic Evaluation ..	35
ATTACHMENT 8: Protocol for the Collection of Oropharyngeal/Cloacal Swab Samples.....	39
ATTACHMENT 9: Fecal Sampling and Shipping Protocol .....	41
ATTACHMENT 10: Charter of the Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds.....	44

## ***Executive Summary***

The purpose of this document is to detail the essential components of a unified national system for the early detection and monitoring for avian influenza viruses of significance in migratory birds. This monitoring includes but is not limited to highly pathogenic avian influenzas (e.g., Eurasian HPAI H5N8; Eurasian/North American HPAI H5N2; Eurasian HPAI H7N7), and low pathogenicity avian influenzas of public health significance (e.g., Eurasian LPAI H7N9, Eurasian LPAI H9N2). This plan is intended to guide regulators, animal industries, exhibitors, wildlife managers, and the public in making informed decisions about safety and biosecurity protocols when there is an imminent threat from an avian influenza virus of significance circulating locally in wild birds. This document provides guidance to Federal, State, university, and non-governmental organizations for conducting avian influenza monitoring and surveillance in migratory birds. It is expected that it will be used by agencies and organizations to develop regional and/or State-specific implementation plans for avian influenza surveillance.

Data collected in accordance with the guidelines presented in this document will be assimilated for use by all agencies, organizations, and policy makers. Furthermore, although the original purpose of this plan was to monitor migratory birds as a potential route of entry into the U.S., the standardized methodologies and procedures identified in this document are applicable to all wild birds, as well for endemic influenza viruses of concern. Agencies and organizations are encouraged to participate in this system by following the guidelines presented in this document when conducting avian influenza sampling in wild birds.

To provide a uniform structure for the development of local plans, this strategic plan recommends the consideration of five strategies for conducting monitoring and surveillance for avian influenza viruses in wild birds:

1. Investigation of Morbidity/Mortality Events
2. Surveillance in Live Wild Birds
3. Surveillance in Hunter-Harvested Birds
4. Sentinel Species
5. Environmental Sampling

Agencies and organizations are encouraged to use one or more of these strategies when designing surveys in wild birds.

The data collected for this national early detection and monitoring system will consist of samples submitted by many agencies and organizations. This will require coordination of animal and sample collection data through multiple routes, the ability to easily match, compare, and transfer laboratory data about these samples, and provide a platform in which information on avian influenza viruses of concern are made available to the public. The Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds will facilitate a coordinated and cooperative approach among federal and state agencies and other cooperators to the surveillance of wild birds for the presence of avian influenza viruses in the U.S.

## ***Introduction***

Avian influenza is a Type A influenza virus that is naturally found in certain species of waterfowl and shorebirds. Type A influenza viruses are classified on the basis of two viral surface proteins, hemagglutinin (H) and neuraminidase (N) into subtypes or strains. There are compositional differences between the various H and N proteins, so the protein subtype differences are uniquely numbered. For avian influenzas, there are 16 known H subtypes (H1-H16) and 9 known N subtypes (N1-N9) resulting in 144 possible combinations or subtypes based on this classification scheme. For example, an avian influenza virus might be designated as an H5N1, H7N7, H9N2, etc. We now also recognize two additional H and N subtypes that have thus far only been identified in bats, namely H17N10 and H18N11, but these are not considered avian influenza viruses.

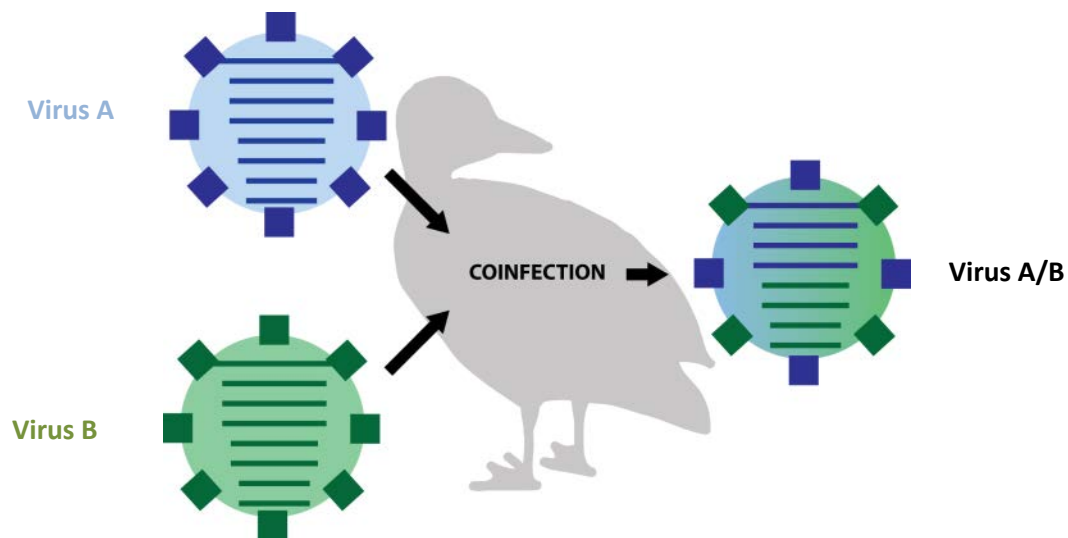
Avian influenzas are found globally in both wild and domestic avian species. Most avian influenza viruses are adapted to waterfowl and shorebirds, which are their natural hosts; however, some of these viruses can cause disease in these species. Whether a particular virus can cause disease in a specific bird depends on the species of bird, and the pathogenicity and virulence (disease causing potential and severity of infection in a specific host) of the virus. Avian influenza viruses move through migratory flyways along with their infected hosts. Large-scale movement is mainly confined to contiguous continental masses, but intercontinental migration of avian influenza host species does routinely occur. This means that viruses that evolved on one continent may be able to reach another continent and mix with the indigenous viruses to create reassortant avian influenza viruses.

Avian influenza largely circulates in waterfowl and shorebirds causing little to no disease. This allows the viruses to move efficiently along migratory flyways in these birds. Occasionally, such viruses (e.g., H4N6, H6N1) will spill over into domestic land-based poultry (e.g., chickens, turkeys, guinea fowl, etc.) where they may cause subclinical or mild disease, but generally do not result in significant disease issues. However, when poultry are infected with low pathogenic H5 and H7 viruses, these strains hold the potential to evolve into a serious disease-causing form of the virus termed highly pathogenic avian influenza (e.g., HPAI H5N1, HPAI H7N7). These highly pathogenic H5 and H7 viruses usually cause significant disease and mortality in domestic poultry and sometimes in wild birds. To date, all highly pathogenic avian influenza viruses are either of the H5 or H7 viruses, but not all H5 or H7 avian influenza subtypes are highly pathogenic. In fact, most avian influenza viruses do not cause disease and are found routinely in healthy wild waterfowl. It is thought that only when land-based poultry become involved in the viral interchange that an increase in pathogenicity/virulence may occur with these specific subtypes resulting in the creation of low pathogenic and highly pathogenic viruses in poultry.

Influenza viruses evolve by two different mechanisms that are influenced by intrinsic factors found in the virus and the host. The first mechanism is antigenic drift, where the virus mutates at a gradual rate due to inherent defects in the viral replication process. This antigenic drift introduces small mutations to the virus and allows a subtype to adapt to living in another species, usually a different avian species. Antigenic drift generally does not contribute to the pathogenicity or virulence of the virus. The second mechanism is antigenic shift. Antigenic shift can convey acute and extreme changes to host susceptibility as well as the pathogenicity and virulence of the virus. Antigenic shift can occur in several ways but the two most profound

mechanisms are direct species adaptation and genetic reassortment. In a direct species adaptation, the virus subtype infects a species that has not historically been known to harbor that subtype, and subsequently may cause disease. An example of this would be when equine (horse) influenza H3N8 jumped directly into dogs in the early 2000's to establish the widely circulating canine H3N8 virus, which causes respiratory disease in dogs.

The more common mechanism (and potentially more catastrophic) of antigenic shift is genetic reassortment. The genetic material (RNA) of the influenza virus is segmented into 8 individual and discrete units, meaning that the genes of the influenza virus are found on these 8 viral segments (see illustration below). When the cells of a waterfowl are co-infected with two different subtypes of influenza virus, for example LPAI H5N2 and LPAI H4N6, then the 8 discrete gene segments of each virus may “reassort” in the cell independently to create an entirely new virus containing a mixture of the viral gene segments from the two parent viruses, such as LPAI H5N6 (see illustration below):



Reassortment is a frequent event in waterfowl and may provide the virus with a rapid mechanism to adapt to a new species without having to wait on the progressive antigenic drift through gradual mutation. While reassortment would seemingly produce a number of random combinations, that is not the case because all the genes of the new virus must work in concert to make it efficient and successful, and some combinations are more viable and advantageous to the virus than others. The combination of gradual drifts and rapid shifts results in the production of a strain that may be better adapted to replicating in a different species (e.g., duck to a swan), but also may cause increased morbidity (i.e., the rate of disease induction) and mortality in susceptible hosts that are not well adapted to that virus (e.g., chickens).

Besides the normal movement of avian influenza viruses in wild migratory waterfowl, numerous potential routes for introduction of exotic viruses into the U.S. exist, including illegal movement of domestic or wild birds, contaminated products, or as a bioterrorism event. However, prior to 2005, it was widely believed that wild migratory birds could not harbor HPAI viruses and that there were only very rare instances of infection of wild birds by HPAI. This paradigm largely held true until the emergence of Eurasian HPAI H5N1 (EA HPAI H5N1) in 1996. EA HPAI H5N1 emerged in Asia in 1996 causing significant mortality in domestic poultry and associated illness and mortality in humans. The affected poultry were depopulated and it was believed that the virus had been eradicated. However, in 2003, the virus re-emerged in Asia again causing severe illness in both poultry and humans. Wild birds

associated with these poultry outbreaks were found ill or dead proximal to infected poultry farms. A major event occurred in 2005 on Qinghai Lake, China resulting in significant mortality of species infected with EA HPAI H5N1 such as bar-headed geese, brown-headed gulls, black-headed gulls, ruddy shelducks, and great cormorants. This seminal outbreak signaled the emergence of the pathogenic clades (strains) of EA HPAI H5N1 that would radiate out from Asia into Europe and Africa moving largely via infected poultry or their products, but also spread by migratory birds associated with outbreaks in domestic birds. Since 2003, EA HPAI H5N1 has become endemic to most of Southeast Asia, parts of greater Asia, Northern Africa in the Nile River delta, and perhaps parts of West Africa (FAO 2011). EA HPAI H5N1 continues to demonstrate significant zoonotic potential and continues to result in disease and death in humans throughout its range. However, despite the extensive range of this virus, it has not been detected in North America to date. This suggested that species capable of carrying EA HPAI H5N1 on a transcontinental migration to North America may have met with a range of fates including: the birds succumbed to the infection during the process; they cleared the infection prior to reaching North America; they succeeded in reaching North America but virus was not detected despite considerable efforts to find it in the flyways; or North America avoided infection through serendipity.

Evidence began to mount that EA HPAI H5N1 was becoming adapted to select species of waterfowl, specifically those dabbling ducks of the genus *Anas* such as mallards and their relatives. Many of the domestic duck species are derived from this genus, thus they play a role in the virus' maintenance and dissemination in countries where the virus is endemic. When land-based poultry become involved the ecology of the virus becomes complicated and difficult to predict.

EA HPAI H5N1 was not static and immediately began to diverge, such that there are now multiple clades (strains) of the virus present throughout its range. The virus demonstrates strain differences sufficiently diverse such that a vaccine made to one strain of EA HPAI H5N1 may not protect against infection by another H5N1 virus. Moreover, the virus began to reassort with endemic Eurasian LPAI viruses, generating a number of progeny including EA HPAI – H5N2, H5N5, H5N6, and H5N8, which have maintained their pathogenicity and virulence for poultry throughout this evolutionary process. Of these EA HPAI H5N1 progeny, EA HPAI H5N8 has proven to be an especially robust virus. EA HPAI H5N8 was first reported from China in 2010 and again in 2013 during which time it was undergoing continual evolution and reassortment with LPAI viruses. However, in January 2014, South Korea experienced catastrophic outbreaks in domestic poultry caused by an EA HPAI H5N8 virus and Japan had a focal outbreak in April 2014. While this virus killed both poultry and some wild birds, asymptomatic wild birds were found to also be infected in both South Korea and Japan. In early fall of 2014, outbreaks caused by EA HPAI H5N8 were reported in domestic poultry in Germany, Netherlands, and the United Kingdom. Wild bird surveillance in Germany found the virus in clinically normal migratory waterfowl. This strongly suggested that EA HPAI H5N8 was adapted to some wild waterfowl, allowing the long-range movement of the virus along migratory corridors.

In late November 2014, commercial turkey farms in southern British Columbia, Canada began to experience increased mortality in their flocks. Subsequent analysis revealed the presence of an HPAI H5N2 virus in the birds. Five of 8 genes segments including H5, were very closely related to EA HPAI H5N8, while 3 of the 8 gene segments including the N2 were of North American origin. This virus was subsequently labeled as Eurasian/North American HPAI H5N2 or EA/AM HPAI H5N2. Almost simultaneously, a significant wild bird die-off occurred

on a lake located in a northern border county in Washington State. Analysis of the mortalities found that the birds succumbed to a fungal infection (Aspergillosis). However, given the proximity of this mortality event to the commercial poultry outbreak in Canada, diagnostic laboratories tested for avian influenza and incidentally detected EA/AM HPAI H5N2 and EA HPAI H5N8. Since these first detections, the viruses have dispersed throughout the Pacific, Central and Mississippi flyways. Both of these viruses have been found in clinically normal migratory waterfowl.

Given this information, the likely scenario is that EA HPAI H5N8 reached North America via infected migratory waterfowl sometime in spring to early fall of 2014. This virus then likely reassorted with a North American H5N2 virus to generate novel EA/AM HPAI H5N2. Wild bird surveillance throughout the Pacific flyway has detected yet another reassortant with EA HPAI H5N8, namely EA/AM HPAI H5N1 of which 4 gene segments are derived from EA HPAI H5N8 and the remaining 4 derived from a North American low pathogenicity N1 virus. These findings may portend the discovery of yet other novel EA/AM reassortants as this H5 clade spreads through the wild bird population. Dogs in South Korea associated with large outbreaks in domestic poultry have been found to contain antibodies to EA HPAI H5N8 suggesting these animals may become asymptotically infected. Although these viruses are not known to have caused disease in humans, their appearance in North America might increase the likelihood of human infection in the U.S. (MMWR 2015). Consequently, personal safety precautions should be taken when handling sick or dead wild birds.

Whether these Eurasian origin H5 viruses and their progeny will persist within the North American wild bird population is unknown. Moreover, there is the potential for yet other Eurasian avian influenza H5 viruses to reach North America via the same pathway in which EA HPAI H5N8 arrived. Additionally, there are other threatening avian influenza viruses that may be introduced into the U.S. via transcontinental migration. Such viruses include Eurasian LPAI H7N9 which causes little to no disease in poultry, but has resulted in severe illness and death in humans. Also Eurasian LPAI H9N2 viruses have shown a significant potential to cause infections in people.

Given this palpable threat to both animal and human health, a sustained and extensive wild bird avian influenza surveillance plan is needed to provide early warning to animal stakeholders and the public which may be exposed to these infected birds. Therefore, in response to this threat personnel from the U.S. Departments of Agriculture and Interior re-convened the Interagency Wild Bird Avian Influenza Steering Committee in January of 2015. The purpose of this Steering Committee was to develop a comprehensive wild bird surveillance plan for avian influenzas that may pose a threat to public health or domestic poultry. This document is the result of those efforts.

## ***Goal of the Strategic Plan***

The goal of this plan is to describe the essential components of a unified national system for the early detection and monitoring for avian influenza viruses of significance in migratory birds. While the immediate concern is the recent introduction of highly pathogenic EA H5N8 avian influenza into the U.S., and its reassortants with North American strains, the development of a system that is capable of detecting all influenza viruses of concern in migratory birds significantly improves the biosecurity of the Nation. This document provides guidance to Federal, State, university, and non-governmental organizations for conducting avian influenza monitoring and surveillance in migratory birds. It is expected that this document will be used by

agencies and organizations to develop regional and/or State-specific implementation plans for avian influenza surveillance.

Data collected in accordance with the guidelines presented in this document will be assimilated for use by all agencies, organizations, and policy makers. Furthermore, although the original purpose of this plan was to monitor migratory birds as a potential route of entry into the U.S., the standardized methodologies and procedures identified in this document are applicable to other wild birds as well. Agencies and organizations conducting monitoring and surveillance in non-migratory birds are encouraged to follow these guidelines so that their data can be incorporated into this national strategy. This system will provide early warning for potentially catastrophic mortality events in North American wild birds and poultry, and minimize the potential for human exposures. Agencies and organizations are encouraged to participate in this system by following the guidelines presented in this document when conducting avian influenza sampling in wild birds.

While this plan focuses on detection of highly pathogenic avian influenza virus, it also fully supports efforts to characterize all influenza viruses in wild birds. Such information is critical to our understanding of the ecology of avian influenza viruses and their transmission among wildlife, livestock, and humans. Birds will be sampled in conjunction with existing studies when possible, and additional bird captures will be initiated as necessary to provide a broad species and geographic surveillance effort.

### ***A National Early Detection System for Avian Influenzas of Significance in Migratory Birds***

The ability to efficiently control the spread of a highly infectious, exotic disease such as highly pathogenic avian influenza virus is dependent upon the capacity to rapidly detect the pathogen if introduced. For this reason, a national early detection system for highly pathogenic and other avian influenza viruses of significance wild migratory birds is not only prudent, it is necessary. Effective implementation of this national detection system will require decentralized planning and execution at regional and State levels, combined with centralized coordination to ensure national level analysis of surveillance data for risk assessment. It also must involve a partnership between public and private interests and include efforts by Federal, State, and local governments as well as nongovernmental organizations, universities, and other interest groups. Lastly, it requires flexibility and commitment by all groups for successful implementation.

#### **Decentralized Planning and Execution**

Wild migratory birds, by their very nature, are not subject to disease containment controls as are domestic birds. While their movements are generally uncontrollable, these movements are largely predictable on both a daily and seasonal basis. Local movements within or between breeding, feeding, and roosting areas are frequently well known by State and local wildlife management authorities and others familiar with local bird populations. Long-range movements associated with seasonal migration are also well known for many species, especially those waterfowl and shorebird species of particular interest in highly pathogenic avian influenza detection and surveillance.

Coordinating groups such as the four Flyway Councils already exist to deal with issues related to migratory bird management on a broad geographic scale. These Councils include representation



from each of the States in their respective bird flyways as well as the U.S. Fish and Wildlife Service, and USDA Wildlife Services. Therefore, the planning and execution of local and regional avian influenza early detection efforts will best be accomplished by the States in collaboration with Federal agencies.

### **Centralized Coordination**

States and flyways are exposed to varying degrees of threat from avian influenza viruses of significance. Each has unique circumstances that will shape the direction and intensity of its early detection and monitoring efforts. Consequently, gaps among regional programs may emerge over time. Centralized coordination will evaluate the effectiveness of state and regional efforts, allowing for prioritization of available Federal resources.

Integration of this national early detection and monitoring system with similar influenza surveillance systems in other species (e.g., domestic, feral, captive and zoo) as well as humans will also require centralized coordination. Surveillance data from all of these systems will be incorporated into national risk assessments, and preparedness and response planning efforts.

### ***Geographic Prioritization of Sampling Efforts***

This Strategic Plan targets bird species in North America that have the highest risk of being exposed to or infected with avian influenza viruses of significance because of their migratory movement patterns. Currently, these include birds that migrate directly between North America and other continents, birds that may be in contact with species from areas in other continents with reported outbreaks, or birds that are known to be reservoirs of avian influenza.

In general, bird flyways represent migration corridors within continental landmasses. However, Alaska and areas in Eastern Siberia represent a unique situation where major flyway systems cross continental boundaries (Attachment 1, fig. 2-1). Two major Asian flyways (the East Asian-Australasian and East Asian) include both Southeast Asia and the Arctic regions of Siberia, the Russian Far East, and Alaska. The East Asian-Australasian Flyway, defined primarily in the context of shorebird use, extends across 20 countries from the Siberian and Alaskan Arctic through North and Southeast Asia including U.S. trust territories in the Pacific to Australia and New Zealand.

Similarly, in North America, the Pacific Flyway extends from Arctic Canada, Alaska, and Eastern Siberia through coastal and western regions of Canada, the United States and Mexico, and on to Central and South America (Attachment 1, Fig. 2-2). Many migratory species that nest in Arctic Siberia, Alaska, and Canada follow the Pacific Flyway to wintering areas. Although not considered a major pathway, birds from both Eastern Siberia and Alaska intermingle in both the Pacific and Central Flyways. Additionally, The Atlantic and Mississippi Flyways of North America overlap with the East Atlantic Flyway of Eurasia in northeastern Canada, Greenland, and Iceland. While intermingling of migratory birds occurs among these flyways, the degree to which it occurs is less than what is observed in Western North America and Eastern Asia. The overlap at the northern ends of these flyways and in Hawaii and Oceania establishes a potential path for disease transmission across continents and for mixing, re-assortment, and exchange of genetic material among avian influenza viruses from Eurasia and North America.

The above analysis of the major flyways suggests that the virus would most likely arrive first in the Pacific Flyway. Such a scenario has recently occurred with the introduction of HPAI EA

H5N8. Given the current knowledge on avian influenza distribution, the following prioritized sampling approach was developed based on the major North American flyways.

Biologically relevant sampling areas should be identified within Flyways should be identified. For example, watersheds can be used to identify key factors important to waterfowl and influenza biology and ecology. Such biologically relevant sampling units can be effectively used to establish the baseline distribution of influenzas across U.S. flyways and regions within flyways, and to detect early spread of influenza viruses of concern to new flyways and regions.

Agencies participating in the development of this plan are committed to efforts that ensure adequate sampling based on the biological units described above. However, experiences with previous introductions of exotic diseases into North America (e.g., West Nile Virus) have demonstrated that detection and surveillance systems must be adaptable to changes in pathogens and risk factors associated with their potential introduction. If changes in the relative risks of highly pathogenic avian influenza introduction into the US result in regional reprioritization, agencies must be prepared to redistribute resources accordingly.

## ***Sampling Strategies***

This strategic plan recommends decentralized planning and execution of avian influenza early detection efforts. To provide a uniform structure for the development of local plans, it recommends the consideration of five strategies for conducting monitoring and surveillance for avian influenza viruses in wild birds. Agencies and organizations are encouraged to use one or more of these strategies when designing surveys in wild birds. These strategies are:

### **Investigation of Morbidity/Mortality Events (Attachment 2):**

Highly pathogenic avian influenza viruses have been shown to cause morbidity and mortality in a wide variety of wild birds. The systematic investigation of morbidity and mortality events in wild birds to determine if highly pathogenic avian influenza is playing a role in causing illness and death offers the opportunity to detect the virus in new geographic locations. State natural resource agencies and Federal refuges and parks, primarily within the DOI's U.S. Fish and Wildlife Service National Wildlife Refuge System, are the principal authorities in a position to detect and respond to mortality events involving wild birds. Morbidity and mortality events involving wildlife are often detected by, or reported to, these agencies and entities. This strategy capitalizes on existing morbidity/mortality programs being conducted by DOI and its partners.

### **Surveillance in Live Wild Birds (Attachment 3):**

This strategy incorporates sampling of live-captured, apparently healthy wild birds to detect the presence of highly pathogenic avian influenza virus. This effort will select bird species in North America that represent the highest risk of being exposed to, or infected with, highly pathogenic avian influenza virus because of their migratory movement patterns. This includes birds that migrate directly between other continents and North America, or birds that may be in contact with species from areas with reported outbreaks. Should highly pathogenic avian influenza virus be detected in domestic birds in the U.S., sampling of wild birds in the flyway in the affected area may become a high priority as well. Additional bird captures will be planned as necessary to provide a targeted species and geographic surveillance effort. This strategy capitalizes on research activities currently being conducted by DOI, USDA, State agencies, and their partners.

### Surveillance in Hunter-Harvested Birds (Attachment 4)

Perhaps the best opportunity to conduct surveillance for the presence of avian influenza viruses is from hunter-harvested wild birds. Similar to surveillance in live wild birds, sampling of hunter-harvested birds will focus on hunted species most likely to be exposed to avian influenza viruses. Collection of samples from these species will occur at hunter check stations and other hunter concentration areas during hunting seasons in areas where waterfowl stage during migration or over-winter.

### Sentinel Species (Attachment 5):

Waterfowl, exhibition gamefowl, and poultry flocks reared on backyard premises have been used as sentinels for active surveillance for avian diseases of interest to the commercial poultry industry and regulatory agencies. Placement of sentinel ducks has been used successfully for surveillance of diseases of importance to the poultry industry, including avian influenza viruses. Also, sentinel ducks in wild pelagic bird colonies improved virus detection rates fivefold, suggesting that this approach is advantageous in ecological studies. However, now that we have discovered HPAI in migratory waterfowl in the US, backyard flocks may serve as efficient local sentinels.

### Environmental Sampling (Attachment 6):

Avian influenza viruses are generally shed by waterfowl through the intestinal tract and viable virus can be detected in both feces and the water in which the birds swim, defecate, and feed. This is the principal means of virus spread to new avian hosts and potentially to poultry, and other susceptible species. Analysis of fecal material from waterfowl habitat can provide evidence of avian influenza viruses circulating in wild bird populations. Monitoring of fecal samples gathered from waterfowl habitat is a reasonably cost effective technologically. A surveillance system based on a validated water sampling method is not ready to implement at the present. However, if the validation of such a method becomes available, sampling of waterbodies could effectively identified areas where avian influenza virus is present.

## **Sample Collection**

Samples collected for avian influenza surveillance may include carcasses, oropharyngeal, and cloacal swabs, and feces. Prior to initiating a surveillance activity, it is important to identify the laboratory in which the samples will be submitted. Sample handling and transportation procedures may differ among laboratories. It is recommended that samples collected for inclusion into this national program be submitted to a laboratory that uses standardized procedures identified in the Laboratory Diagnosis section of this document or by using the attached detailed descriptions of sampling methodologies.

If birds are found morbid or dead, it is important to use proper personal protection techniques ([http://www.nwhc.usgs.gov/publications/wildlife\\_health\\_bulletins/WHB\\_05\\_03.jsp](http://www.nwhc.usgs.gov/publications/wildlife_health_bulletins/WHB_05_03.jsp)) and to submit the entire carcass to a veterinary diagnostic laboratory for necropsy (Attachment 7). Field biologists should contact the specific laboratory that they will be working with well in advance of any specimen collection and shipping to receive specific instructions for specimen submissions to that laboratory. Laboratories should always be notified ahead of time when a shipment is being

made to their facility.

When collecting samples from live or hunter-harvested birds, oropharyngeal and cloacal swabs are preferred. Most avian influenza strains tend to replicate more efficiently in the intestinal tract than in the respiratory tract of natural host species (i.e., waterfowl and shorebirds). However, recent isolations of highly pathogenic H5N1 avian influenza virus in wild birds have documented higher levels of virus in oropharyngeal and tracheal samples. Therefore, it is recommended that separate oropharyngeal and cloacal swabs be collected from individual birds and combined into a single media tube. Examples of oropharyngeal/cloacal swab collection protocols can be found in Attachment 8.

Monitoring of fecal samples gathered from waterfowl habitat is a reasonably cost effective, technologically achievable means to detect the presence of avian influenza viruses (Attachment 6). Fecal sampling is an established technique and is ready for use in surveillance with the establishment of sampling guidelines. The main advantages of this technique is that it can be more cost effective and logistically easier to implement than individual bird sampling. However, it requires fresh samples and is often difficult to identify species from which feces originated. It can also difficult to find specimens from target species (e.g., Anseriformes). Environmental fecal sampling could yield a spatial and habitat risk assessment for site contamination with avian influenza virus. The main considerations are where and when to get the samples, ensuring proper storage and transport, and the capacities and capabilities of the laboratories doing the analyses. Real-time reporting and the infrastructure to support such reporting is a serious constraint on any surveillance system. The ability to integrate, analyze, and responsibly disseminate these data is critical and needs to be addressed.

### ***Sample Size Determination***

Prior to initiating a surveillance program, it is important to determine the sample size necessary to make statistically valid inferences concerning the presence of avian influenza virus in a sample population. A biologically relevant sampling scheme should be developed to identify influenzas of concern within target populations. In the context of this strategic plan, the population of interest is not defined because this definition will vary by geographic location, time of year, species of interest, and sampling method employed. For example, sampling a breeding population versus a wintering population, for a single species, may result in very different interpretations of the geographic distribution of the population of interest. Therefore, it is crucial that prior starting the collections, statistically valid sample size estimations have to be incorporated into surveillance plans.

### ***Laboratory Diagnostics***

All samples collected for inclusion in this surveillance system should be analyzed in accordance with the standard procedures developed by the USDA National Veterinary Services Laboratories (NVSL). Samples will be analyzed as soon as possible after collection. Oropharyngeal/cloacal, and fecal swab samples will be analyzed using the Real-time Reverse Transcriptase-polymerase Chain Reaction (RT-PCR) Detection of Influenza A Virus and Avian Paramyxovirus Type-1 protocol (available from NVSL). Briefly, this protocol requires initial testing with the matrix gene RT-PCR assay, which is capable of detecting all 16 hemagglutinin and nine neuraminidase subtypes. Matrix gene RT-PCR-positive samples indicate the presence of avian influenza and they should be further characterized by the H5- and H7-specific RT-PCR assays.

Positive H5 and H7 RT-PCR tests would indicate the presence of avian influenza viruses with the potential of causing pathology in domestic poultry. Therefore, all samples positive for H5 and H7 by RT-PCR will be submitted to NVSL for confirmatory testing. Identification of a highly pathogenic H5 or H7 virus is a reportable disease and requires immediate notification to the agency submitting the sample, the state veterinarian, the USDA District Director and Assistant District Director, the State public health official, and the CDC/USDA Select Agent program. Samples will be immediately secured as required by the Select Agent Programs.

All positive H5 and H7 samples will also be sent to the USDA Agriculture Research Service Southeastern Poultry Research Laboratory in Athens, GA, for complete molecular sequencing. This will provide for complete typing of the virus and allow for phylogenetic analysis.

## ***Reporting and Data Management***

Highly pathogenic and zoonotic avian influenza viruses are reportable to the responsible State authority (State veterinarian, State public health authority, etc.). All laboratories must follow state regulations for reporting. To accomplish this, all laboratories (private, State, and university laboratories) that perform diagnostic procedures on wildlife should submit samples testing positive for H5 and H7 viruses, or those that are potentially zoonotic to the USDA National Veterinary Services Laboratory for confirmatory testing.

Real-time reporting and the infrastructure to support such reporting is a serious constraint on any surveillance system. The ability to integrate, analyze, and responsibly disseminate these data is critical. In addition, the data collected for this national early detection and monitoring system will consist of samples submitted by many agencies and organizations. This will require coordination of animal and sample collection data through multiple routes, the ability to easily match, compare, and transfer laboratory data about these samples, and provide a platform in which information on avian influenza viruses of concern are made available to the public. The Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds will facilitate a coordinated and cooperative approach among federal and state agencies and other cooperators to the surveillance of wild birds for the presence of avian influenza viruses in the U.S. (Attachment 10). All confirmed positive cases of HPAI and zoonotic avian influenza viruses will be posted on the USDA APHIS internet site.

## ***Recommendations***

Given the current state of knowledge of the epidemiology of avian influenza viruses it is recommended that a coordinated interagency/organization early detection and monitoring system be implemented in the U.S. An analysis of risk factors, including current worldwide distribution of avian influenza viruses and the migratory patterns of wild birds, indicated that this system should primarily focus sampling efforts in areas of historically high prevalence of these viruses. This includes areas where large concentrations of target species congregate, and where high concentrations of poultry production are located. Sampling should be conducted in breeding and wintering areas as well as during migration to detect and track movement of avian influenza viruses regionally and nationally.

State and Federal agencies should develop implementation plans based on the guidance provided in this Strategic Plan. Development of these plans should be conducted with the participation of all relevant management agencies and organizations such that sampling designs are produced that

allow for statistically sound inference regarding the presence or absence, and prevalence of avian influenza viruses in wild birds. Monitoring of avian influenza viruses should also be conducted to determine if new reassortments are evolving, which could adversely affect human or poultry health.

State and Federal agencies also should develop communication plans in the event that a HPAI is detected in wild birds. For example, the recent introduction of HPAI H5N8 from Asia required notification of the State Veterinarian, and the USDA District and Assistant District Director in the State. An introduction of a zoonotic HPAI, such as the EA HPAI H5N1, would also require notification of State Public Health Agency and the CDC/USDA Select Agent Programs. Additionally, HPAI detections require adherence to Select Agent guidelines.

Finally, it is recommended that the Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds, consisting of representatives from the DOI, USDA, HHS, and the National Flyway Council, continue to coordinate wild bird AI surveillance in the United States (Attachment 10). Specific roles of this Committee include:

- Facilitate communication between state and federal agencies, and organizations involved in avian influenza surveillance for wild birds.
- Coordinate implementation and data analysis of avian influenza surveillance programs nationally.
- Provide periodic summaries of avian influenza surveillance for wild birds in the United States.
- Provide periodic recommendations for avian influenza surveillance in wild birds based on previous sampling efforts and changes in virus epidemiology.
- Facilitate communication and coordination among State and Federal agencies for contingency planning and other preparations for the appearance of avian influenza viruses of concern in wild birds in North America.

Sampling strategies to detect avian influenza viruses in wild bird populations will change depending upon the risk assessment and management goals and prevailing status of the pathogen in North America. For early detection, efforts should focus on areas of high aggregations of waterfowl intersecting with areas of historically high prevalence of avian influenza viruses, and areas with large concentrations of poultry. The surveillance network should be placed along known waterfowl movement corridors. State-level sampling of wild birds and environmental sampling should target areas of strategic value (e.g., areas of high density of poultry production, human population centers). These areas would represent the highest level of risk to poultry, and in the case of zoonotic avian influenza viruses, to humans via contact with contaminated water and/or waterfowl. Sampling at ponds, lakes and waterfowl management areas around high density of poultry and humans could provide information used to assess and mitigate risk to these populations.

## ATTACHMENT 1

### Migratory Bird Flyways in Asia and North America

Figure 2-1: Asian Migratory Bird Flyways

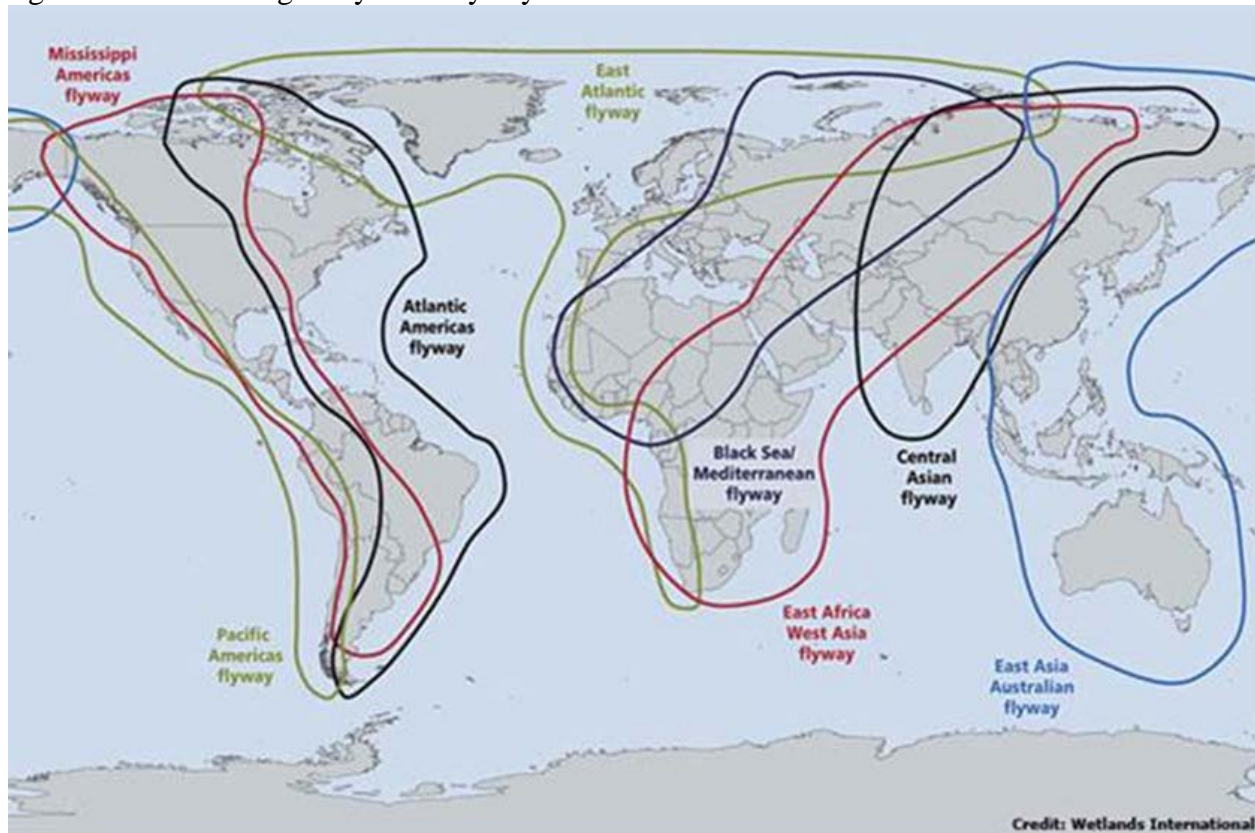
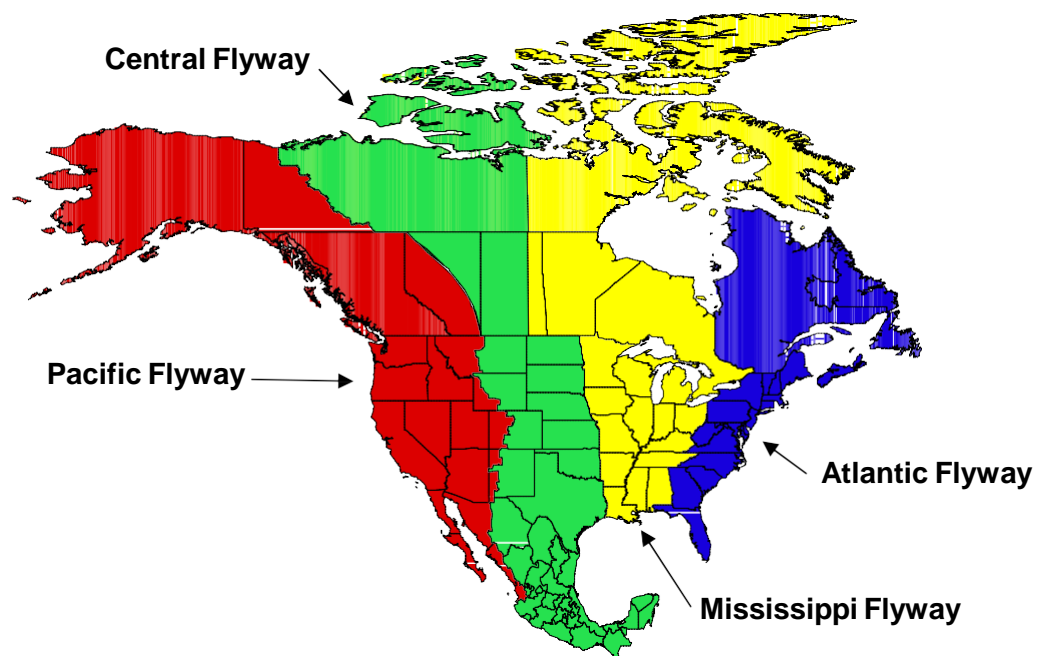


Figure 2-2. North American Migratory Bird Flyways



## ATTACHMENT 2

### Investigation of Morbidity and Mortality Events in Wild Birds

#### *Overview*

The systematic investigation of morbidity and mortality events in wild birds provides an opportunity to detect avian diseases in new geographic areas. As an example, investigation of an avian mortality event led to the first detection of West Nile virus in North America. Because a few highly pathogenic avian influenza viruses have demonstrated the ability to cause mortality in several wild bird families, mortality event investigations are included as a detection method in this national avian influenza early detection and monitoring system.

#### Methodology

##### Wild bird morbidity and mortality events:

1. **Early detection** of wild bird morbidity and mortality
  - a. State, Federal, and tribal resource personnel are encouraged to increase vigilance and to establish routine and systematic monitoring of wild bird populations for morbidity and mortality.
2. Rapid **reporting and submission** of appropriate biological specimens to qualified diagnostic laboratories
  - a. A uniform protocol for reporting mortality events to identified diagnostic facilities will be developed. Field and response personnel will be trained in the safe handling and shipment of specimens. Reporting of mortality events will be through appropriate channels within each State, Federal, or tribal entity to the U.S. Geological Survey (USGS) National Wildlife Health Center (NWHC).
  - b. Representative and suitable carcasses and other biological samples and specimens will be submitted to one or more identified diagnostic facilities capable of conducting immediate necropsy and laboratory analyses. Guidelines will be developed to assure that the appropriate number and types of samples are collected to ensure that there is a statistically-based confidence in the sample size analyzed in response to a mortality event.
3. Immediate **assessment** of the field event (descriptive epidemiology)
  - a. Field personnel or teams designated by respective land management agencies will respond to mortality events by conducting field investigations to determine onset, course, duration, distribution, species, and other environmental conditions associated with mortality events. The NWHC and U.S. Department of Agriculture (USDA) Wildlife Services (WS) will assist in developing guidelines and training. In certain circumstances, NWHC and USDA-WS personnel will conduct field investigations or assist other agencies.
4. Rapid, accurate, and consistent **diagnosis** and confirmation of cause of death
  - a. Necropsies, histology, and laboratory investigations will be utilized to substantiate a diagnosis of highly pathogenic avian influenza virus. Virus isolation, hemagglutination inhibition tests, and molecular testing specifically for H5 and H7 types will be performed.



5. Immediate **reporting** of diagnostic results once they are confirmed
  - a. Reporting of results to submitters will be done as early as possible. Final results of avian influenza testing will be reported immediately to the submitter - public release of information will occur after that.
6. Pre-planned contingency and **response** training
  - a. Wildlife disease contingency plans will be established at an appropriate landscape scale to enable rapid deployment of personnel and resources to take action. Disease contingency plans can be developed for general response to a mortality event, with special reference and consideration for highly pathogenic avian influenza viruses. The NWHC and USDA will assist in providing guidelines and training in the establishment of contingency plans.

Rehabilitated wild birds, falconry birds, captive raptors, and pen-reared gallinaceous birds and waterfowl:

These species are particularly susceptible to clinical disease associated with highly pathogenic avian influenza viruses and serve as good sentinels (or indicators) for circulation of the virus in a particular geographic region. Morbidity and mortality events in rehabilitated wild birds, falconry birds, captive raptors, and pen-reared gallinaceous birds and waterfowl should be immediately reported through appropriate channels within each state, Federal, or tribal entity.

**Call or email the laboratory before submitting any carcasses or samples.**

Marine mammal morbidity and mortality events:

Because marine mammals are also susceptible to infection and clinical disease associated with influenza viruses, unusual mortality events in dolphins and pinnepeds in areas experiencing concurrent avian mortality events should also be reported through the appropriate channels.

Wildlife professionals employed by state natural resource agencies and by the U.S. Fish and Wildlife Service are the principal authorities positioned to detect and respond to morbidity and mortality events involving wild birds. The DOI Bureau of Land Management, Tribal Nations, and several other state, Federal, and local agencies (including the U.S. Department of Defense) also have authority over lands that they administer and manage. Morbidity and mortality events involving wildlife are often detected by, or reported to these agencies and entities.

Investigations into the causes of wildlife mortality events are dependent on the perceived significance of the event and on the knowledge or availability of disease diagnostic facilities capable of providing assistance. The USGS - NWHC, located in Madison, Wisconsin, is a full-service wildlife diagnostic and research laboratory that assists Federal, state, and tribal agencies in responding to wildlife disease outbreaks. Numerous state natural resource agencies also have established wildlife disease laboratories and programs with staff that respond to wildlife disease outbreaks in their respective states. USDA, state and university diagnostic laboratories, and regional entities such as the Southeastern Cooperative Wildlife Disease Study (SCWDS) are also involved in wildlife disease investigations. The NWHC maintains an extensive database on wildlife mortality events across the United States and Canada to which Federal, state, provincial, and tribal agencies contribute.

## Discussion

The primary strength of the strategy of targeted investigations of avian mortality events is based

upon the observation that highly pathogenic avian influenza viruses kill some species of wild birds. As such, a wild bird die-off serves as a “trigger event” that immediately focuses the investigation to a given area and species. Recovering carcasses and samples from wild bird die-offs affords a timely opportunity to detect avian influenza.

## Recommendations

Because the primary goal of the process outlined in this plan is the earliest possible detection of avian influenzas of concern in wild birds, investigation of avian mortality events is important, however it should be noted that highly pathogenic avian influenza will not be the cause of most of the mortality events investigated through a targeted surveillance strategy. Rather, other bacterial and viral diseases that are either zoonotic or important to agriculture may be detected through these surveillance programs. Mortality event investigation provides the opportunity to obtain the greatest amount of information about health and disease in wild birds without an a priori bias. Supplemental wildlife disease information will be prioritized and gathered as funding and personnel allow. Surveillance for highly pathogenic avian influenza will remain the top priority.

## Appendix

*DOI Updated Employee Health and Safety Guidance for Avian Influenza Surveillance and Control Activities in Wild Bird Populations, 2014*

<http://www.doi.gov/emergency/employeeemergency/upload/Updated-Employee-Health-and-Safety-Guidance-for-AI-Surveillance-and-Control-Activities-in-Wild-Bird-Pops-2014-FINAL-w-SIGNED-memo-JULY2014.pdf>

## ATTACHMENT 3

### Surveillance for avian influenza viruses in Live Wild Birds

#### **Overview**

This surveillance strategy incorporates sampling of live-captured, apparently healthy migratory birds to detect the presence of avian influenza virus. Virus isolation from oropharyngeal and cloacal samples is a common method for detecting avian influenza. This effort focuses on bird species in North America that represent the highest risk of being exposed to, or infected with, avian influenza viruses based on previous surveillance programs and because of their migratory movement patterns.

In general, bird flyways represent migration corridors within continental land masses. However, Alaska and corresponding areas in the Russian Far East represent a unique case where major flyway systems cross continental boundaries. Many migratory species that nest in Arctic Siberia, Alaska, and Canada follow the Pacific Flyway to wintering areas. Although not considered a major pathway, birds from both Eastern Siberia and Alaska intermingle in both the Pacific and Central Flyways. The overlap at the northern end of these flyways establishes a path for potential disease transmission across continents and for mixing, re-assortment, and exchange of genetic material among avian influenza viruses from Eurasia and North America. Similarly, the convergence and overlap of the North American flyways in the prairie pothole region, including the Dakotas, Minnesota, and the south central Canadian provinces, provide opportunities for transmission and movement of any avian influenza virus into other flyways. Recent surveillance of wild birds and outbreaks in domestic poultry in western North America demonstrate that a highly pathogenic H5N8 virus from Asia has entered the continent.

There is less concern about the spread of avian influenza viruses of concern westward from Asia to Europe, then into North America. There is comparatively little movement of wild birds between Europe and North America, however, studies in Iceland and Greenland suggest that entry by this route cannot be discounted (Hjulsager et al, 2010; Hall et al, 2014).

#### **Methodology**

##### **Identification of Priority Species**

Birds should be sampled in conjunction with existing studies when possible (e.g., banding), and additional bird captures should be initiated as necessary to provide a targeted species, geographic and temporal surveillance effort. As with other surveillance methods, sampling should be directed at functional groups rather than particular species. Initial efforts should focus on dabbling ducks. As a functional group, dabbling ducks accounted for 91.5% of the H5 matrix positive samples and 89.7% of the H7 matrix positives in the prior surveillance program which operated from 2006-2010 (Bevins et al. 2014). Diving ducks, shorebirds, gulls

geese, and swans are lower priorities but may be sampled in some cases. These species have also been shown to serve as reservoirs for some avian influenza viruses.

Since the majority of positives have been detected in dabbling ducks the primary focus of sampling will continue to be on these species (American Black Duck, American Green-winged Teal, American Wigeon, Blue-winged Teal, Cinnamon Teal, Gadwall, Mallard, Mottled Duck, Northern Pintail, Northern Shoveler, and Wood Duck). If it is not feasible to collect the specified number of samples from the dabbling duck functional group, the secondary focus should be on collecting samples from the diving duck or geese/swan functional groups as specified by the regional surveillance plan. A tertiary focus of sampling shorebirds, gulls, terns, skimmers, alcids and other birds (Appendix 4) can be considered for sampling if indicated in the surveillance plan and State wildlife agencies or other cooperators have current projects or interest. Close coordination should occur at the local level to ensure complementary and additive surveillance and sampling approaches. Coordinating sampling with planned banding activities, waterfowl hunts, or other similar projects would provide an opportunity to increase efficiency and provide valuable cost-saving measures. Note that collecting swab samples from live-captured birds must be in compliance with the U.S. Fish and Wildlife Service collection permits.

## ***Sample Collection***

When sampling for highly pathogenic avian influenza virus it is critical that an appropriate sample size for each species or functional group in each designated sample population is obtained. Detailed surveillance plans should provide sample sizes and sample locations. As prevalence decreases the likelihood of detecting the disease in an individual bird also decreases due to the low probability of detection and practical limitations on laboratory processing capability.

Oropharyngeal and cloacal swabs should be collected from individual birds and combined into a single tube with transport media (Attachment 8). Swabs from different birds should not be combined.

## ***Discussion***

Wild birds, particularly waterfowl and other water birds, are natural hosts of avian influenza viruses and are believed to play an important role in the epizootiology of these viruses. Detection of various highly pathogenic avian influenza subtype combinations in waterfowl in the Pacific flyway in late 2014-early 2015, demonstrate that dabbling ducks can be infected without displaying any clinical signs of illness. In this respect, the newly found highly pathogenic strains are behaving similar to the native North American strains found previously in waterfowl. Most hemagglutinin and neuraminidase subtypes have been found in waterfowl and shorebirds (Webster et al., 1992; Krauss et al., 2004; Widjaja et al., 2004). This proposed sampling effort provides the best opportunity for detection of highly pathogenic avian influenza viruses in the natural reservoir species.

## ***Recommendations***

Sampling live birds will allow for early detection of avian influenza viruses of concern in

specific regions of the U.S., evolution of new viruses, and for estimating the risk of transmission to poultry and humans. When collecting samples from live birds, combined oropharyngeal and cloacal swabs are preferred. Specific implementation plans should be developed for each zone/State/flyway. It is strongly advised that agencies and organizations coordinate their sampling efforts to assure that adequate sample sizes are obtained from each functional group. Coordination should be achieved through the existing Interagency Wild Bird Avian Influenza Steering Committee (Attachment 10).

It is also strongly recommended that wild bird surveillance activities on the breeding grounds and migratory staging sites in Canada are supported however possible. Surveillance in Canada may be critical to provide early warning for the US regarding the circulation of avian influenza viruses, their pathogenicity, presence of new reassortments, which species are involved, and the possible path by which these viruses may move during migrations.

### ***Literature Cited***

- Bevins, S.N., K. Pedersen, M.W. Lutman, J.A. Baroch, B.S. Schmit, D. Kohler, T.Gidlewski, D.L. Nolte, S.R. Swafford, T.J. DeLiberto. 2014. Large-Scale Avian Influenza Surveillance in Wild Birds throughout the United States. *PLoS ONE* 9(8): e104360. doi:10.1371/journal.pone.0104360.
- Hall, J.S., G.T. Hallgrimsson, K. Suwannarn, S. Sreevatsen, H.S. Ip, E. Magnusdottir, J. L. Teslaa, S.W. Nashold, and R.J. Dusek. 2014. Avian influenza virus ecology in Iceland shorebirds: intercontinental reassortment and movement. *Infection, Genetics and Evolution* (28): 130-136.
- Hjulsager, C.K., S.Ø. Breum, R. Trebbien, K. J. Handberg, O.R. Therkildsen, J.J. Madsen, K. Thorup, J.A. Baroch, T.J. DeLiberto, L.E. Larsen, and P.H. Jørgensen. 2010. Surveillance for avian influenza viruses in wild birds in Denmark and Greenland, 2007–10. *Avian Diseases*, 56: 992-998.
- Krauss, S., D. Walker, S.P. Pryor, L. Niles, L. Chenghong, V.S. Hinshaw, and R.G. Webster. 2004. Influenza A viruses of migrating wild aquatic birds in North America. *Vector-Borne and Zoonotic Diseases* 4:177-189.
- Krauss, S., S.P. Pryor, G. Raven, A. Danner, G. Kayali, R.J. Webby, and R.G. Webster. 2012. Respiratory tract versus cloacal sampling of migratory ducks for influenza A viruses: are both ends relevant? *Influenza Journal*. doi:10.1111/j. 1750-2659.2012.00359.
- Widjaja, L., S.L. Krauss, R.J. webby, T. Xie, and R.G. Webster. 2004. Matrix gene of influenza A viruses isolated from wild aquatic birds: ecology and emergence of influenza A viruses. *Journal of Virology* 78:8771-8779.
- Webster, R.G., W.J. Bean, O.T. Gorman, T.M. Chambers, and Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. *Microbiological Reviews* 56:152-179.

## **ATTACHMENT 4**

### **Surveillance for Avian Influenza in Hunter-Harvested Waterfowl**

#### ***Overview***

Waterfowl habitat, and associated waterfowl hunting activity, occurs across the landscape based on available and suitable wetlands that maintain densities of waterfowl. This distribution often concentrates waterfowl hunters at limited public access areas associated with the marshes, lakes and rivers on public hunting areas or private waterfowl clubs properties. Many public hunting areas have check stations operated by State and Federal natural resource agencies on State hunting units or National Wildlife Refuges providing agency managers the opportunity to collect information on the local waterfowl harvest. Additionally, check stations, boat ramps, hunting clubs and other hunter constrictions provide an opportunity to also collect samples to monitor for the presence of avian influenza viruses.

Hunter-harvested bird sampling supplements live bird surveillance (Attachment 4) by increasing the number of selected species, geographic locations, and time periods represented in surveillance efforts. Sampling should concentrate on dabbling duck species. Previous sampling and research has shown dabbling ducks have higher prevalence of virus than diving or sea ducks and geese and dabbling ducks make up the majority of the duck harvest ( $\approx 85\%$  in the United States; Bevins et al. 2014). Sampling of hunter-harvested dabbling ducks in the Pacific flyway in late 2014 and early 2015 detected HPAI H5 virus subtypes in all species of dabbling ducks present, except for blue-winged and cinnamon teal, however, these species are relatively rare in the harvest and fewer were sampled.

From research, we also anticipate persistence of the Eurasian origin HPAI H5 viruses as influenza viruses are known to remain viable for months in colder wetland environments. This surveillance strategy is focused on monitoring current HPAI occurrence in the Pacific flyway and early detection and movement of the circulating HPAI viruses to eastern flyways. The complete design, implementation and development of an operational plan and the funding necessary for this strategy require close coordination with States through the Interagency Wild Bird Avian Influenza Steering Committee (Attachment 10) and the Flyway Councils..

## ***Methodology***

**Sampling Areas** - Some States routinely check hunter- harvested waterfowl. The present agency infrastructure for hunter field checks provides the most efficient sampling strategy of HPAI surveillance. In most circumstances, collection of HPAI samples will occur at check stations and public access sites. Additional samples may be collected at private waterfowl hunting clubs requiring landowner cooperation. States should develop implementation plans for these sampling strategies in consultation with their respective flyway council using guidance provided in this strategic plan.

**Target Species** - Primary target species and populations identified for highly pathogenic avian influenza virus sampling include all species of dabbling ducks commonly encountered in North America including mallard, American black duck, mottled duck, gadwall, American wigeon, green-winged teal, blue-winged teal, cinnamon teal, northern shoveler, northern pintail, and wood duck. Given the ephemeral nature of birds at specific sites along their migration routes, sampling efforts should be concentrated on the wintering grounds. Specific sampling sites (i.e., check stations or other areas where hunter-harvested birds are sampled) will be determined by state and federal agencies, and organizations participating in the national surveillance effort.

## ***Discussion***

Sampling of hunter-harvested birds will supplement targeted surveillance in live wild birds (Attachment 3) and other strategies identified in this strategic plan. The advantages of hunter-harvested bird sampling is that it is efficient and cost-effective because 1) most of the avian influenzas of concern are commonly found in harvested waterfowl, and 2) hunter check stations exist in most wintering areas, and 3) statistically sufficient numbers of birds can be acquired from hunter-harvested birds on many wintering wetland habitats. Due to the numerous sampling sites throughout the U.S., training of sampling personnel will be required to ensure samples are properly acquired, preserved, and shipped to the laboratory for virus evaluation and isolation. Public perception of sampling hunter-harvested birds provides good outreach opportunities between managers and hunters and hunters may appreciate the wildlife health surveillance effort. Conversely, if accurate information about the low zoonotic risks of the circulating highly pathogenic avian influenza viruses is not relayed, hunters may become unnecessarily alarmed about exposure, especially if agency samplers are wearing protective equipment.

## ***Recommendation***

Sampling hunter-harvested birds supplements other surveillance approaches in an efficient and cost-effective manner and provides determination of virus presence and relative rates of infection by species. Federal and State partners should work collaboratively on funding, sampling strategies, and specific state implementation plans.

Unlike other surveillance approaches, the use of hunter-harvested birds has a high public profile. Therefore, the implementation of this action should be discussed with all agencies and the Association of Fish and Wildlife Agencies and organizations such as Ducks Unlimited regarding the public relations to ensure development of an appropriate and consistent message to hunters.

## ***References***

- Fox, A.D., Christian Glahder, Carl R. Mitchell, David A. Stroud, Hugh Boyd, and John Frikke. 1996. North American Canada geese (*Branta canadensis*) in west Greenland. *Auk* 113(1): 231-233.
- Bevins, S.N., K. Pedersen, M.W. Lutman, J.A. Baroch, B.S. Schmit, D. Kohler, T. Gidlewski, D.L. Nolte, S.R. Swafford, T.J. DeLiberto. 2014. Large-Scale Avian Influenza Surveillance in Wild Birds throughout the United States. *PloS ONE* 9(8): e104360. doi:10.1371/journal.pone.0104360.



## ATTACHMENT 5

### Sentinel Animal Methods

#### **Overview**

This methods section reviews two sentinel animal methods that have been used in avian disease surveillance programs and that may be used for the early detection of avian influenza virus infection along migratory flyways in the U.S.

#### **Backyard poultry flocks, raptors, and wild gallinaceous birds**

Backyard poultry are defined as domesticated fowl, including chickens, turkeys, waterfowl, and game birds (except doves and pigeons) maintained for hobby or noncommercial egg and meat production (NAHRS FAQ 2005). Backyard poultry are typically allowed to forage freely or may be confined in partially enclosed fenced areas. The evaluation of poultry flocks reared on backyard premises for diseases of interest to the commercial poultry industry has been used as a surveillance method to estimate seroprevalence of selected disease agents as part of health surveys in backyard flocks adjacent to commercial operations. (McBride; Hird; Carpenter; Snipes; Danaye-Elmi, and Utterback 1991; Johnson; Colby; Tablante; Hegngi; Salem; Gedamu, and Pope C. 2004).

Since the discovery of high pathogenic avian influenza in wild birds in Washington, infected backyard poultry flocks have been identified. These operations often keep various domestic galliform and anseriform species. There is often either a history of, or actual exposure to, wild waterfowl. These operations are abundant and provide excellent opportunities for surveillance. Producer education and cooperation are the keys to maximizing this surveillance stream. The experience in the States of Washington and Oregon, as well as British Columbia suggests that the arrival of highly pathogenic avian influenza in wild birds is heralded by poultry mortality.

In addition to the infected backyard poultry operations, numerous raptors, both wild and captive, have died from highly pathogenic avian influenza. There are numerous raptor rehabilitator organizations throughout the U.S. Based on observations in captive poultry, it is reasonable to assume that many of our wild gallinaceous birds such as turkeys, pheasants and grouse may very well be susceptible to avian influenza and suffer notable mortality.

#### **Sentinel Duck Flocks**

This is the placement of sentinel duck flocks in wetland environments where they are potentially exposed to and share infections as they commingle with wild birds. Sentinel flocks of domestic ducks has been used to successfully identify avian influenza viruses and detect influenza epizootics in pelagic bird colonies, and yielded much higher isolation rates

compared to isolations from wild birds (Turek; Gresikova, and Tumova 1984; Sinnecker; Sinnecker; Zilske, and Koehler 1982; Sinnecker; Sinnecker, and Zilske 1982). The timing of avian influenza infections in sentinel ducks has been found to be associated with the arrival of wild migratory waterfowl in wetland habitats when such studies were conducted in areas adjacent to market turkey production flocks (Halvorson; Karunakaran; Senne; Kelleher; Bailey; Abraham; Hinshaw, and Newman 1983; Halvorson; Kelleher, and Senne 1985; Kelleher; Halvorson; Newman, and Senne 1985).

Ideally, surveillance activities should occur at a time when migratory birds are actively nesting and at locations where they marshal and intermingle with other migratory birds transiting the area prior to winter migration. Avian influenza infection in sentinel ducks has been shown to occur in late July and early August in summer breeding areas (infection of range reared turkey flocks was shown to occur about 6 to 8 weeks later) (Halvorson et al. 1985). Avian influenza virus prevalence estimates from published waterfowl surveys indicate that virus can first be detected in naïve juvenile birds in summer breeding areas in July or August (prevalence ranged from 11% to 61% in published surveys). It is at that time that the juveniles emerge from hiding and intermingle with other broods. There is a subsequent high rate of re-infection as birds marshal for winter migration in October (Hanson et al. 2003; Hinshaw et al. 1985). Avian influenza virus prevalence generally decreases during late fall and winter and may reach a level of 1% or less in over-wintering areas. (Stallknecht; Webster; Bean; Gorman; Chambers, and Kawaoka 1992) However, virus was isolated from 11% of teals and from 15% of northern pintails in one recent survey of wintering ducks in Texas, suggesting that the avian influenza season may not be a fall season event (Hanson 2003). As a result of early migration, blue winged teal are thought to serve as an immunologically naïve host in wintering areas.

Most virus isolations have occurred in mallards and other species of dabbling ducks, but less commonly in wood ducks and similar species (Stallknecht 2003). Mallards are commonly associated with habitats located near man, livestock, and poultry and would be more likely to interact with backyard poultry flocks compared with other waterfowl species (Stallknecht and Shane 1988). Although H5, H7, and H9 subtypes have been poorly represented in most waterfowl surveys (H3, H4, or H6 subtypes have been isolated most frequently), pintails and mallards have been shown to be significant reservoirs in one recent survey where H5, H7, and H9 virus subtypes were isolated 21.5% of the time in Minnesota (Hanson and others 2003). The prevalence of AI isolated from blue winged teal on wintering grounds in February in Texas was found to be 22% in 2001 and 15% in 2002 (Hanson 2003). Migration of blue-winged teal occurs in late summer and early fall (typically September), prior to the highest period of avian influenza prevalence. Early migration of this species is thought to play a role in maintenance of avian influenza infection on wintering grounds by providing a susceptible population with little or no prior exposure or immunity.

The approach to the design of a targeted surveillance method for the detection of avian influenza should incorporate what is presently known about the ecology and natural history of avian influenza infection in wild waterfowl reservoir species. Sentinel ducks are most likely to become infected with avian influenza if exposed to reservoirs in nature during periods of highest viral shedding. As described above, prevalence of infection as measured by virus isolations in published waterfowl surveys has been shown to vary temporally by location, age, season, and species. A targeted approach to sentinel duck surveillance should be designed to:

- Target specific locations where avian influenza has been isolated from wild waterfowl historically;
- Target locations where known primary reservoir species (mallards, blue winged teal, ruddy turnstones) congregate for breeding (resulting in higher concentrations of juveniles susceptible to infection) or wintering (higher concentrations of species with little or no previous exposure) resulting in a higher prevalence of infection;
- Be timed to coincide with periods (seasons) of highest prevalence in the reservoir species, in particular migratory species that originate from an area having high incidence of avian influenza (Southeast Asia).

## ***Methods***

### **Backyard Poultry Surveillance Method**

- Morbidity and mortality events in backyard poultry can be early warning signs of potential highly pathogenic avian influenza introduction
- Many of these facilities contain various species and are more accurately classified as hobby farms rather than poultry producers
- Highest risk for avian influenza infection are flocks where birds have an opportunity to directly intermingle with waterfowl (especially mallards) at or near the common watershed via open range or open enclosure or by sharing a common source of water.
- Closely monitor farms adjacent to wetlands or containing water features especially where avian influenza virus has been isolated from waterfowl
- State and federal veterinary authorities conduct the investigations, diagnostics and determine the final disposition of the flock

### **Raptor Surveillance Method**

- Raptor rehabilitator facilities routinely encounter sick and dying birds
- The state and federal wildlife agencies in conjunction with the USGS National Wildlife Health Center determine the appropriate diagnostic protocol to follow
- Captive raptor mortalities are often handled by the State Diagnostic Laboratory

## ***Discussion***

### **Major advantages of the use of sentinel birds to detect avian influenza:**

- Once highly pathogenic avian influenza is introduced into a country by wild waterfowl, monitoring the health of backyard poultry operations is a very efficient way to measure spread and pathogenicity
- Backyard facilities often maintain minimal biosecurity, contain multiple galliform and anseriform species, and are often associated with a riparian areas or bodies of water

- Raptors are highly charismatic wildlife with a rehabilitation network in place throughout most of the United States
- Serves as an early warning to commercial poultry

### Major disadvantages of the use of sentinel birds:

- Traditional use of duck sentinels is expensive and logistically complicated
- Duck sentinels provided little added value to the original H5N1 surveillance program
- By the time the disease is diagnosed in backyard poultry the disease has probably already been widely spread by wild birds
- Sentinel poultry or raptors would be a poor way to monitor the geographic extent of infection and the evolution of strains

### **Recommendations**

Since the discovery of highly pathogenic avian influenza in wild waterfowl in the United States, it has become obvious that backyard operations are susceptible. Multiple species, lack of biosecurity and the presence of bodies of water accessible by wild waterfowl are risk factors for infection. Raptors appear very likely to succumb to the disease, presumably by ingesting infected waterfowl.

### **References**

NAHMS Poultry '04 Part I; 2004.

NAHRS FAQ [Web Page]. Accessed 2005 Nov 14. Available at:

[http://www.aphis.usda.gov/vs/ceah/ncahs/nahrs/NAHRS\\_faq.htm](http://www.aphis.usda.gov/vs/ceah/ncahs/nahrs/NAHRS_faq.htm).

Notes: The National Animal Health Reporting System (NAHRS) is a cooperative effort between the American Association of Veterinary Laboratory Diagnosticians (AAVLD), the U.S. Animal Health Association (USAHA) and USDA's Animal and Plant Health Inspection Service (APHIS).

Alfonso, C. P.; Cowen, B. S., and van Campen, H. Influenza A viruses isolated from waterfowl in two wildlife management areas of Pennsylvania. *J Wildl Dis.* 1995 Apr; 31(2):179-85.

Bevins SN, Pedersen K, Lutman MW, Baroch JA, Schmit BS, et al. (2014) Large-Scale Avian Influenza Surveillance in Wild Birds throughout the United States. *PLoS ONE* 9(8):

Cannon, R. M. and Roe, R. T. *Livestock Disease Surveys A Field Manual for Veterinarians* Department of Primary Industry Bureau of Rural Science. Canberra: Australian Government Publishing Service; 1982.

Halvorson, D.; Karunakaran, D.; Senne, D.; Kelleher, C.; Bailey, C.; Abraham, A.; Hinshaw,

- V., and Newman, J. Epizootiology of avian influenza--simultaneous monitoring of sentinel ducks and turkeys in Minnesota. *Avian Dis.* 1983 Jan-1983 Mar 31; 27(1):77-85.
- Halvorson, D. A.; Kelleher, C. J., and Senne, D. A. Epizootiology of avian influenza: effect of season on incidence in sentinel ducks and domestic turkeys in Minnesota. *Appl Environ Microbiol.* 1985 Apr; 49(4):914-9.
- Hanson, B. A. Temporal, spatial and species patterns of avian influenza viruses among wild birds. Athens, Georgia: University of Georgia; 2003.
- Hanson, B. A.; Stallknecht, D. E.; Swayne, D. E.; Lewis, L. A., and Senne, D. A. Avian influenza viruses in Minnesota ducks during 1998-2000. *Avian Dis.* 2003; 47(3 Suppl):867-71.
- Hinshaw, V. S.; Nettles, V. F.; Schorr, L. F.; Wood, J. M., and Webster, R. G. Influenza virus surveillance in waterfowl in Pennsylvania after the H5N2 avian outbreak. *Avian Dis.* 1986 Jan-1986 Mar 31; 30(1):207-12.
- Hinshaw, V. S.; Wood, J. M.; Webster, R. G.; Deibel, R., and Turner, B. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. *Bull World Health Organ.* 1985; 63(4):711-9.
- Johnson, Y. J.; Colby, M. M.; Tablante, N. L.; Hegngi, F. N.; Salem, M.; Gedamu, N., and Pope C. Application of commercial and backyard poultry geographic information system databases for the identification of risk factors for clinical infectious laryngotracheitis in a cluster of cases on the delmarva peninsula. *International Journal of Poultry Science.* 2004; 3(3):201-205.
- Kawaoka, Y.; Chambers, T. M.; Sladen, W. L., and Webster, R. G. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? *Virology.* 1988 Mar; 163(1):247-50.
- Kelleher, C. J.; Halvorson, D. A.; Newman, J. A., and Senne, D. A. Isolation of avian paramyxoviruses from sentinel ducks and turkeys in Minnesota. *Avian Dis.* 1985 Apr-1985 Jun 30; 29(2):400-7.
- McBride, M. D.; Hird, D. W.; Carpenter, T. E.; Snipes, K. P.; Danaye-Elmi, C., and Utterback, W. W. Health survey of backyard poultry and other avian species located within one mile of commercial California meat-turkey flocks. *Avian Dis.* 1991 Apr-1991 Jun 30; 35(2):403-7.
- McCluskey, Brian J. Epidemiology of Vesicular Stomatitis Viruses in the Southwestern United States: Colorado State University; c2003.
- Sinnecker, H.; Sinnecker, R., and Zilske, E. Detection of influenza A viruses by sentinel domestic ducks in an ecological survey. *Acta Virol.* 1982 Jan; 26(1-2):102-4.
- Sinnecker, H.; Sinnecker, R.; Zilske, E., and Koehler, D. Detection of influenza A viruses and influenza epidemics in wild pelagic birds by sentinels and population studies. *Zentralbl Bakteriol Mikrobiol Hyg [A].* 1982 Dec; 253(3):297-304.
- Slemons, R. D.; Hansen, W. R.; Converse, K. A., and Senne, D. A. Type A influenza virus

surveillance in free-flying, nonmigratory ducks residing on the eastern shore of Maryland. *Avian Dis.* 2003; 47(3 Suppl):1107-10.

Slemons, R. D.; Johnson, D. C.; Osborn, J. S., and Hayes, F. Type-A influenza viruses isolated from wild free-flying ducks in California. *Avian Dis.* 1974 Jan-1974 Mar 31; 18(1):119-24.

Slemons, R. D.; Shieldcastle, M. C.; Heyman, L. D.; Bednarik, K. E., and Senne, D. A. Type A influenza viruses in waterfowl in Ohio and implications for domestic turkeys. *Avian Dis.* 1991 Jan-1991 Mar 31; 35(1):165-73.

Stallknecht, D. E. Ecology and epidemiology of avian influenza viruses in wild bird populations: waterfowl, shorebirds, pelicans, cormorants, etc. *Proceedings of the Fourth International Symposium on Avian Influenza; Athens, GA. U.S. Animal Health Association.* 2003.

Stallknecht, D. E. and Shane, S. M. Host range of avian influenza virus in free-living birds. *Vet Res Commun.* 1988; 12(2-3):125-41.

Stallknecht, D. E.; Shane, S. M.; Zwank, P. J.; Senne, D. A., and Kearney, M. T. Avian influenza viruses from migratory and resident ducks of coastal Louisiana. *Avian Dis.* 1990 Apr-1990 Jun 30; 34(2):398-405.

Turek, R.; Gresikova, M., and Tumova, B. Isolation of influenza A virus and paramyxoviruses from sentinel domestic ducks. *Acta Virol.* 1984 Mar; 28(2):156-8.

Webster, R. G.; Bean, W. J.; Gorman, O. T.; Chambers, T. M., and Kawaoka, Y. Evolution and ecology of influenza A viruses. *Microbiol Rev.* 1992 Mar; 56(1):152-79.

Webster, R. G.; Morita, M.; Pridgen, C., and Tumova, B. Ortho- and paramyxoviruses from migrating feral ducks: characterization of a new group of influenza A viruses. *J Gen Vir*

## ATTACHMENT 6

### Environmental Fecal Sampling

#### ***Introduction***

Avian influenza viruses are released by waterfowl through the intestinal tract and viable virus can be detected in both feces and the water in which the birds swim, defecate and feed. This is the principle means of virus spread to new avian hosts and potentially to poultry and other susceptible livestock. Analysis of fecal material from waterfowl habitat can provide evidence of avian influenza viruses circulating in wild bird populations, identify the specific viruses, and levels of pathogenicity.

#### **Technical Aspects of Sampling Feces**

Fecal sampling has been used extensively in monitoring studies for avian influenza in wild bird populations. The principal advantages of this method are that the costs and effort of capturing birds are avoided and large sample numbers can be quickly and easily obtained (Table 7-1). It also is a good method to determine the presence or absence of virus in bird populations present at a specific location. The disadvantages are that source species identification is not always possible, fresh feces are required, determination of prevalence is complicated by the possibility of repeated sampling of individuals, and the sensitivity of the method is lower than for oropharyngeal and cloacal swabs.

Fresh fecal samples that are either processed quickly or frozen until processing provide the most reliable test results. Thus this method of sampling, while providing good information, is best applied while birds are present at a location such that the samples are as fresh as possible. By restricting fecal collection to fresh samples, it allows for population census data to be collected and, by inference, estimates of the species sources of the contamination. Species and individual identification through genetic typing of feces would allow estimates of prevalence.

Accredited laboratories have the capacity and infrastructure to analyze a limited number of samples for avian influenza. The anticipated sampling effort for this surveillance study will require an investment in equipment and staff to provide results in a timely fashion. Equipment needs include real-time PCR thermocyclers, RNA extraction capabilities, DNA sequencing capabilities, tissue culture and egg culture facilities, ultracold freezers, centrifuges and vacuum pumps.

#### ***Methodology***

Sampling for avian influenza from environmental deposition of virus by waterfowl should be accomplished by collecting and analyzing feces (Attachment 9). from areas of known use by high

risk species (e.g., dabbling ducks, transcontinental migrants, etc). The general challenges faced include determining locations used by high risk species, and design of a sampling system using composite samples for analysis.

Table 7-1. Qualitative comparison of environmental fecal sampling methods.

Fecal Sampling	
Pros	Cons
Technically easy sample acquisition. Sampling represents non technical approach and would not require extensive training or experience by field personnel.	Viable virus restricted to fresh samples (1-4 days)
Generate large sample numbers quickly.	Large sample numbers can swamp lab systems (applies to all methods)
Does not require handling or capturing animals	Difficult to obtain fecal samples from reservoir species
Low cost, well established technique amenable for high through-put screening (modified APHIS RT-PCR method). Sample analysis is transferable across labs.	Lower sensitivity and specificity due to environmental contaminants.
Capable of identifying HPAI contaminated sites/locations/regions. Prevalence would be estimated on a site basis. Information from the field could be used to generate an environmental risk map related to specific areas (habitats) associated with potential AIV transmission.	Identity of species and individuals unlikely, estimates of prevalence not possible. Species identification possible through molecular fingerprinting, but at additional cost.
BSL-2 laboratory conditions sufficient for initial diagnostic screening.	Requires Biosafety level 3 capabilities for virus isolation
<b>Summary:</b> An approach based on fecal sampling could be immediately implemented and may represent the only reasonable approach in areas where bird capture is not practical.	

For logistically practical and economic reasons sample analysis should focus on composite samples on a per site basis; this minimizes effort in both data collection and analysis, while greatly increasing the probability of detection. Table 7-2 provides a hypothetical, but plausible, example of the expected number of tests per composite fecal sample necessary to detect avian influenza virus. When prevalence is very low (e.g.,  $10^{-7}$ ) almost all composites will test negative and on average only a single test will be needed to determine the absence of avian influenza virus in that composite sample.

The approximate sample sizes necessary for assuring a high probability of detecting avian influenza virus depends on its prevalence in the population. However, a preliminary estimator is:

$$p^* = 1 - (1-r/m)^{1/n} \quad (\text{eq. 1})$$

where  $p^*$  is the proportion of infected individual samples across all composite samples,  $r$  is the number of composite samples that test positive for the presence of avian influenza virus,  $m$  is the total number of composite samples tested, and  $n$  is the number of individual samples in each composite sample (e.g., fecal count or volume). Rearranging eq. 1 provides an estimate of the number of individual fecal samples needed to detect highly pathogenic H5N1 avian influenza virus, for a given population level prevalence;

$$n = \ln(1-r/m) / \ln(1-p^*) \quad (\text{eq. 2})$$



7-2. Expected number of tests needed for a single positive reaction for each composite sample containing 100 individual fecal samples,  $n$ , as a function of expected prevalence of avian influenza,  $p$ . Calculation is based on the binomial probability model describing the average number of tests needed as  $(n+1) - n(1-p)^n$

Prevalence in Waterfowl ( $p^*$ )	Individual fecal samples/composite ( $n$ )	Mean # composite samples to test
$10^{-3}$	100	10.5
$10^{-4}$	100	2.0
$10^{-5}$	100	1.1
$10^{-6}$	100	1.0
$10^{-7}$	100	1.0

The results for various hypothetical values of  $r$ ,  $m$ ,  $n$ , and  $p^*$  are shown in Table 7-2. Thus, if avian influenza virus prevalence is  $10^{-6}$  and 10,000 independent fecal samples are collected, analysis of 100 composite samples would result in detecting the presence of avian influenza virus in one composite. These two equations allow us to initially estimate the number of fecal samples to be collected and to estimate prevalence of avian influenza virus in the population.

Table 7-3. Number of individual fecal samples  $n$ , for a fixed prevalence  $p^*$ , needed to detect the presence of HPAI in 1 out of 100 composite samples. Calculation is based on the probability model given by eq. 2.

Prevalence in Waterfowl ( $p^*$ )	Number of positive composites ( $r$ )	Number of composites ( $m$ )	Number of individual samples ( $n$ )
$10^{-3}$	1	100	10
$10^{-4}$	1	100	100
$10^{-5}$	1	100	1005
$10^{-6}$	1	100	1050
$10^{-7}$	1	100	1050

## Safety

Given the potential for exposure to zoonotic avian influenzas, precautions should be taken by workers conducting environmental sampling and laboratory testing. In the lab, standard BSL-3 precautions are required for virus isolation, and BSL-2 precautions for molecular diagnostics. In the field, workers should wear disposable gloves and garments. Gloves should be decontaminated with 70% ethanol frequently, or changed often as necessary. Mucous membranes (eyes, nose, throat) should be protected from

splashes and aerosols. This may require covering with protective equipment such as goggles and N95 or higher rated masks in some cases. Field workers should avoid direct contact with animals after handling environmental samples until decontamination procedures are completed (e.g. changing garments and gloves). Untrained workers (such as the general public) should be discouraged from collecting and submitting environmental samples for testing.

## ***Summary***

Monitoring of fecal samples gathered from waterfowl habitat is a reasonably cost effective, technologically achievable means to assess the presence of avian influenza viruses in waterfowl. Fecal sampling is an established technique and is ready for use in surveillance with the establishment of sampling guidelines. This approach, like all methodologies has advantages and disadvantages, which should be considered before environmental fecal sampling is implemented.

## ATTACHMENT 7

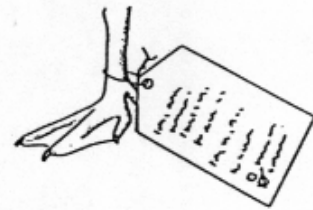
# Instructions for Collection and Shipment of Avian Carcasses for Diagnostic Evaluation

The following are general guidelines for collecting and shipping wildlife carcasses to veterinary diagnostic labs to insure adequate and well preserved specimens. Field biologists should contact the specific laboratory that they will be working with well in advance of any specimen collection and shipping to receive specific instruction for specimen submissions to that lab. Labs should always be notified ahead of time when a shipment is being made to their facility. Once you have determined what equipment and supplies will be needed for specimen shipping, keep adequate numbers of shipping containers, frozen ice packs, shipping labels and packing materials available at all times. If you plan to collect animals while in the field, take along a cooler with ice packs to chill the carcasses.

1. More than one disease may be affecting the population simultaneously. Different species may have varying susceptibility to disease agents. Therefore, collect and ship specimens representative of all species and geographic areas affected.

Obtain good specimens for necropsy. Carcasses that are decomposed or scavenged are unacceptable. If the carcass has an odor, is soft and mushy, has skin discoloration, feathers or skin that easily rubs off, or has maggots present, it is too decomposed for testing.

2. Collect animals under the assumption that an infectious disease or toxic substance is involved and other animals or humans may be at risk. Remember to protect yourself as some of these diseases and toxins are hazardous to humans. Guidelines for personal protection against disease exposure for individuals working with sick or dead wild animals can be obtained from the USGS National Wildlife Health Center, the Centers for Disease Control and Prevention, and OSHA websites.



Always wear latex or nitrile gloves when picking up sick or dead animals. If you are dealing with a significant number of dead animals, or you suspect the presence of a zoonotic disease agent, additional protective equipment including coveralls, eye protection and N95 respiratory protection should be used.

Attach a leg tag to each animal with the following information in pencil/waterproof ink:

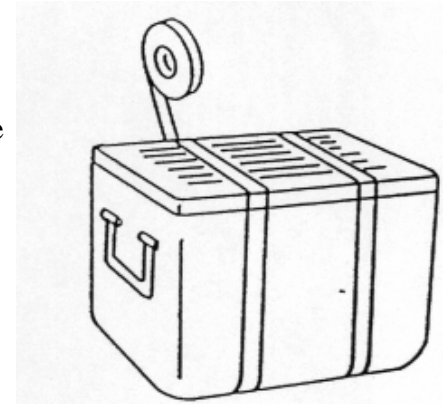
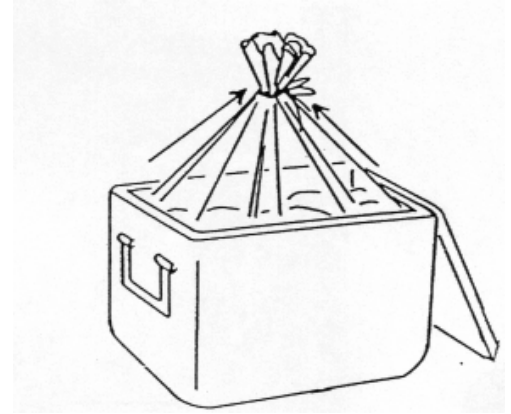
- species
- date collected
- location (state, county, location name, and latitude/longitude if available)
- found dead or euthanized
- collector (name/address/phone)
- additional history or comments on back of tag

Place each animal in a plastic bag, tie shut, then place inside a second bag and tie shut. This system of double bagging prevents cross-contamination of individual specimens and leaking shipping containers that can contaminate vehicle surfaces and handlers during transportation. Contact the diagnostic lab for guidance in assistance with collecting samples from animals that are too large to ship.

3. Ship animals in a sturdy hard sided plastic cooler. These coolers can be disinfected and returned to you if a pre-paid shipping label or commercial shipping company account number is provided to the diagnostic lab. Be sure to provide a street address for return of the cooler.

Line the shipping cooler with a large plastic bag and pack the individually bagged animal(s) in the cooler with enough blue ice to keep carcasses cold. Disperse blue ice packs among the carcasses so that all carcasses are kept chilled. If you are shipping blood tubes, culture tubes, or other specimen

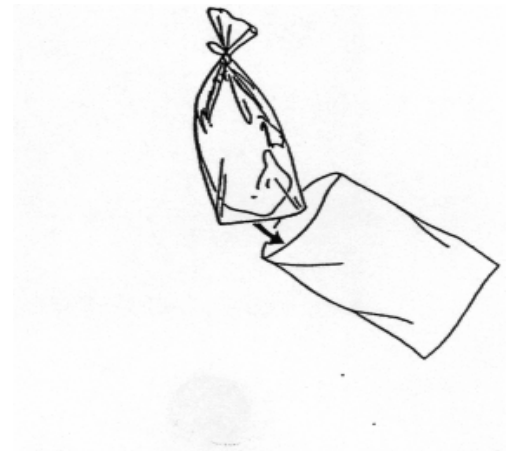
containers along with the carcasses, these specimens should be placed within a sturdy cardboard or plastic box or screw cap container with padding material to prevent breakage. That container should be placed next to blue ice packs within the large cooler. Do not use bagged wet ice for shipments in order to avoid fluid leakage during shipment. Do not use dry ice unless instructed to do so by the diagnostic lab. Place crumpled newspaper or similar absorbent material in the cooler with the bagged carcasses to fill unused space, hold the ice in contact with carcasses, provide insulation, and absorb any liquids. Tape the cooler shut with sturdy strapping tape.



Place a detailed history of the animal and circumstances associated with the mortality event in a paper envelope or a plastic sleeve and tape it to the outside of the cooler. A copy of this history should be faxed or e-mailed to the diagnostic lab at the time of shipment. A standard wildlife specimen history form can be found on the last page of these instructions.

4. Prior to shipping contact the diagnostic lab to inform them of the type and number of specimens being shipped. Ship specimens for next day delivery (overnight service) from Monday through Wednesday to guarantee arrival at the diagnostic lab before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday contact the diagnostic lab to make special arrangements for receipt of specimens.

Freezing and thawing can make isolation of some pathogens difficult and damage tissues needed for microscopic examination. Diagnostic labs prefer unfrozen specimens if



they can be sent within 24 – 48 hours of collection or death. The diagnostic lab can provide guidance on when or if to freeze samples on a case-by-case basis. If you are in the field and cannot call or ship within 24-48 hours, freeze the animal(s).

5. Prior to shipping contact the commercial shipping company to obtain guidelines for shipping diagnostic or biological specimens. Label coolers with clear, legible labels including the diagnostic lab name, street address, and telephone number. In addition to the mailing address, attach a label reading **“DIAGNOSTIC SPECIMENS –WILDLIFE”** to the side of the cooler. If dry ice was used in the shipment a standard dry ice warning label will be required. These can be obtained from the shipping company. Please make note of the tracking number in case packages are delayed.



## ATTACHMENT 8

### Protocol for the Collection of Oropharyngeal/Cloacal Swab Samples

1. Contact Laboratory to determine specific protocol to use. Laboratories may request samples be placed in tubes containing Viral Transport Medium (VTM) or brain-heart infusion broth (BHI).
2. Thaw appropriate number of pre-labeled tubes of Viral Transport Medium (VTM) or brain-heart infusion broth (BHI) at refrigerator temperature (4 °C) overnight and keep chilled with wet/blue ice packs in a cooler during the day of collection.
3. Unwrap a Dacron swab from the stem-end of the packaging.
4. Remove swab and insert the entire head of the swab into the trachea or cloaca. Use gentle pressure and in a circular motion, swab the inside circumference of the trachea/cloaca two or three times.
5. For Cloacal swabs, shake off large pieces of feces.
6. Inserting the swab into the tube containing VTM or BHI broth. With the swab in the media, swirl the stem end of the swab between fingers vigorously. Lift the swab approximately ¼” from the bottom of the vial and bend the stem over the edge of the vial to break off the stem so that the swab remains in the vial and the cap can be screwed tight.  
The entire swab end and a portion of the stem will be left in the tube.  
If the stems are unable to be broken (some small swabs will have metal stems) then they can be cut with scissors. Scissors should be wiped with 70% alcohol each time they are used to cut a stem.
7. Record sample tube number on banding sheet or the Sample History Sheet along with date, species, age, sex, and location data (GIS coordinates if possible)..
8. Replace tube into cooler for transport back to the base camp. Samples should be kept cold (<4 °C, frozen if possible) and out of direct sunlight.
9. At camp, transfer tubes into liquid nitrogen shippers or into a freezer as soon as possible. Note any exceptions to the collection or storage conditions in field sheets and note such information on the "Sample History and Packing List Form".
10. Place tubes into a hard plastic shipping container with enough frozen gel packs to keep samples cold for at least two days.
11. Notify laboratory that samples are being shipped, the method of shipment (FEDEX is preferred), and the expect date of arrival. Packages should only be shipped on Monday, Tuesday, or Wednesday.



### Sample History and Packing List H5N1 Avian Influenza Project

Submitter \_\_\_\_\_  
 Address \_\_\_\_\_  
 \_\_\_\_\_  
 Phone \_\_\_\_\_  
 E mail \_\_\_\_\_  
 \_\_\_\_\_

Sample ID	Bird Species	Age	Sex	Band Number	Location	Comments or Sampling problems



## ATTACHMENT 9

# Fecal Sampling and Shipping Protocol

### ***Fecal Sampling***

#### Purpose

The purpose of this standard operating procedure is to describe the essential elements of proper handling and collection of field fecal samples for surveillance of avian influenza.

#### Identification of Sampling Sites

- Environmental samples should be collected near bodies of water where waterfowl are currently active. Also, environmental sampling may be useful for passively sampling waterfowl around poultry operations.

#### Identification and Sampling of Feces

- Wear gloves while handling samples to protect the sterility of the sample as well as for personal hygiene purposes. Change gloves if they become soiled or contaminated. When finished collecting, wash your hands with antibacterial soap or antibacterial waterless hand sanitizer.
- Feces must be less than 24 hours old; obtaining the freshest sample possible is critical for virus detection.



- A sample of feces is collected using a sterile swab. Swirl the swab through the feces until the head of the swab is well coated with feces.
- Place the swab (tip first) into a cryovial containing transport media. Point the swab, liquid and vial away from your face (in case splashing occurs), lift swab just off the bottom of vial, and break the swab handle at the lip of the vial. Fasten the vial cap,

making sure that it is sealed tightly.



- Label the cryovial and data sheet with corresponding barcode. The barcode label is placed lengthwise on the vial. Ensure that the barcode label on the vial is the same as the barcode label next to the corresponding information on the data sheet.
- Store samples in a chilled field cooler.
- Fill out data sheets with the necessary information for the collection site, including site name, county, state, GPS coordinates (Datum: WGS84; units: decimal degree), species present (see example data sheet), and date of collection.

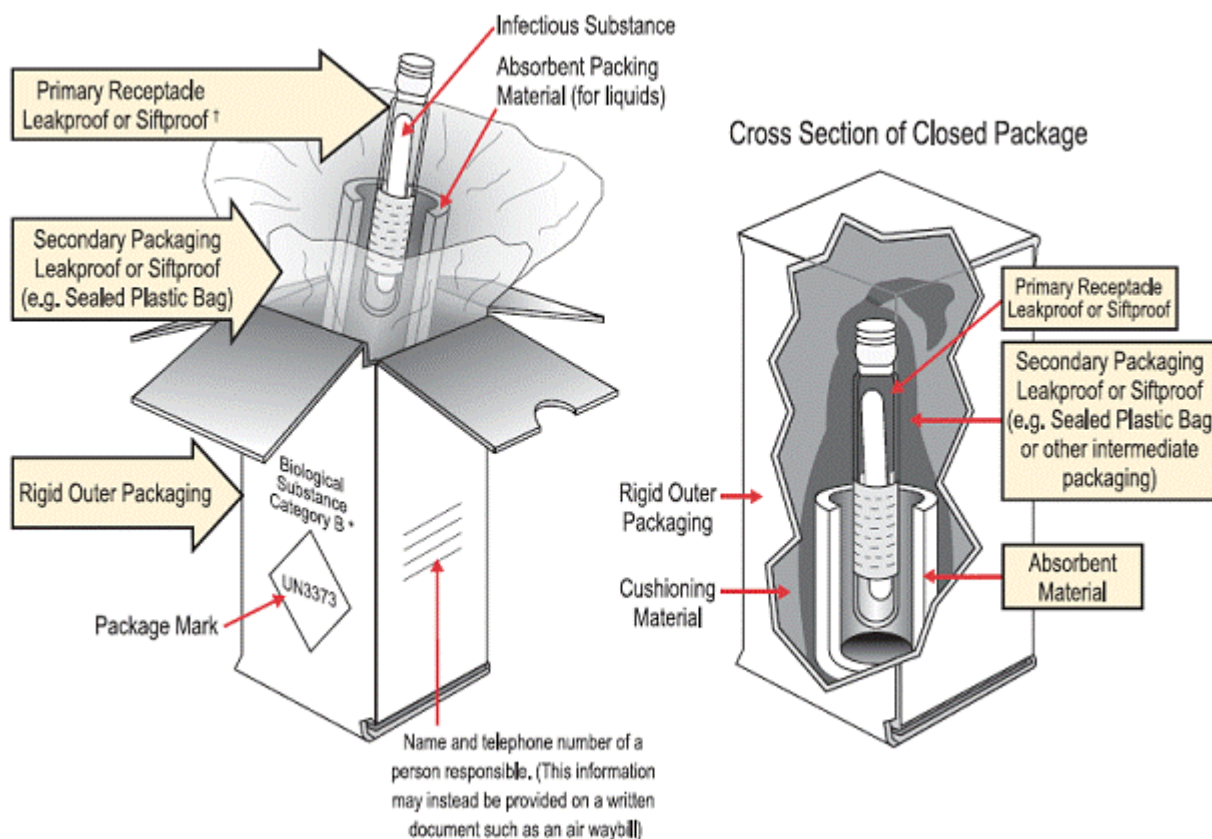
## Shipping

It is imperative that shippers ensure their diagnostic specimens are shipped safely and in compliance with governing regulations and requirements. Diagnostic specimens include biological and environmental samples that are being transported to undergo routine screening tests or for the purpose of initial diagnosis and are considered Category B Infectious Substances and must comply with the International Air Transportation Association (IATA) Dangerous Goods Regulations. The shipper must also ensure that shipments are prepared in such a manner that they comply with the individual carriers (e.g., Federal Express [FedEx] or United Parcel Service [UPS]) requirements and that they arrive at their destination in good condition and present no hazard to persons or animals during shipment.

- United Parcel Service is the preferred carrier for the USDA/NWRC.
- Category B materials may be packaged and shipped by personnel who have been trained and read and understand the following instructions and diagram (see Figure 1).
- The primary receptacle (e.g., vial, test tube, jar, etc.) may be glass, metal or plastic and must have a positive means of ensuring a leak-proof seal (e.g., metal crimp seal or screw cap sealed with adhesive tape). For liquid specimens, the primary receptacle must be leak-proof and must not contain more than 1 liter of liquid. For solid specimens, the primary receptacle must not contain more than 4 kg of material.

- The primary receptacle must be placed inside a leak-proof secondary receptacle (e.g., sealed plastic bag, jar, can, etc.) with sufficient absorbent material to absorb the entire liquid contents of the primary receptacle.
- The outer packaging must be of a sturdy and rigid material (e.g., cardboard box or plastic cooler), and must be at least 7" x 4" x 2". The outer package must be capable of withstanding a 4-foot drop without leakage of the primary receptacle or significant damage to the outer packaging. Packages containing liquids should also have orientation markings (e.g., up arrows).
- The inner receptacles should be marked with appropriate identification and biohazard symbol, and an itemized list or Material Safety Data Sheet (MSDS) of the contents should be enclosed outside of the secondary packaging.
- The outer package must have a diamond-shaped label "UN3373" (at least 2" x 2" in size), with the text "**Biological Substance Category B**" (at least ¼" high font) adjacent to the shipping label. **The name, address and phone number of the person responsible for shipping the package must also be marked on the outer package.**

**Figure 1: Biological Substance Category B packaging.**



## **ATTACHMENT 10**

### **Charter of the Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds**

#### Purpose

To facilitate a coordinated and cooperative approach among federal and state agencies and other cooperators to the surveillance of wild birds for the presence of highly pathogenic avian influenza virus in the U.S.

#### Organization

The Interagency Steering Committee (ISC) will be comprised of the following members:

- i) a representative of the USDA Animal and Plant Health Inspection Service;
- ii) a representative of the DOI U.S. Geological Survey;
- iii) a representative of the DOI U.S. Fish and Wildlife Service;
- iv) a representative of the DHHS Centers for Disease Control and Prevention;
- v) a representative of the Association of Fish and Wildlife Agencies;
- vi) a representative of the National Flyway Council;
- vii) representatives of other agencies or organizations, as mutually determined by the membership.

Ad hoc subcommittees and working groups may be formed under the guidance of the members. Membership in such a group does not constitute membership in the ISC.

#### Functions

The Interagency Steering Committee will perform the following functions:

- i) facilitate interagency coordination and cooperation in field implementation of wild bird surveillance for highly pathogenic avian influenza (HPAI);
- ii) cooperate in developing management systems and communication messages relating to surveillance and response for HPAI in wild birds;
- iii) coordinate interactions with other cooperators in wild bird surveillance efforts for HPAI;
- iv) facilitate coordination of agency response efforts in the event of an occurrence of HPAI in wild birds in the U.S.;
- v) resolve differences in the implementation of wild bird surveillance for HPAI by the member agencies and organizations that impede interagency coordination and cooperation; and

- vi) any other functions that the members may agree upon.

#### Operations

The Steering Committee will select a chair from among their membership, who will be responsible for scheduling periodic meetings or conference calls, drafting meeting/conference call agendas, and managing discussions at scheduled calls or meetings. The position of committee chair will come up for renewal semi-annually.

Decisions will be made through a consensus of the Committee members.

Activities undertaken by this Interagency Steering Committee will be subject to authorization and policies of the member agencies or organizations.