

## USDA AND STATE/TRIBAL COOPERATOR SUMMARY FOR 2006/2007 NATIONAL EARLY DETECTION SYSTEM FOR HPAI IN WILD MIGRATORY BIRDS: ACCOMPLISHMENTS, FINDINGS, AND FUTURE DIRECTIONS

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**Abstract:** In late 2005, at the request of the Homeland Security Council's Policy Coordinating Committee for Pandemic Influenza Preparedness, the United States Departments of Agriculture (USDA) and Interior began developing a United States Interagency Strategic Plan for the early detection of highly pathogenic avian influenza (HPAI) introduction into North America by wild migratory birds. This plan was stepped down by various regional, state, and tribal entities and was implemented nationwide in mid-2006. USDA funded Federal, State, and Tribal cooperators began with a goal of collecting over 75,000 surveillance samples from wild migratory birds during the 2006/2007 surveillance period. The 2006 accomplishments, findings, and AI surveillance results are reported. The USDA's HPAI surveillance strategies and goals are discussed for the 2007 sampling season.

**Key words:** avian influenza, HPAI, interagency strategic plan, LPAI, surveillance, USDA,

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### INTRODUCTION

Avian influenza (AI) is the common reference for type A influenza viruses that are found naturally in certain species of waterfowl and shorebirds (Alexander 2000). The recent emergence and subsequent spread of the highly pathogenic avian influenza (HPAI) H5N1 subtype in Eurasia has raised concerns about the potential impacts to wild birds, domestic poultry, and human health. The HPAI H5N1 virus could enter the United States via several routes, including illegal movement of domestic or

wild birds, contaminated products, infected travelers, bioterrorism, and migrations of infected wild birds (Kilpatrick et al. 2006). A National Strategy for Pandemic Influenza was implemented in November 2005 (Homeland Security Council 2005). The *National Strategy for Pandemic Influenza* guides the United States preparedness and response to an influenza pandemic. At the request of the Homeland Security Council's Policy Coordinating Committee for Pandemic Influenza Preparedness, the United States Departments of Agriculture

(USDA) and Interior were asked to develop a coordinated United States Interagency Strategic Plan (USISP) for early detection of HPAI introduction into North America by wild migratory birds. The USISP was created and implemented in March 2006 (USDA 2006). This plan focuses primarily on the detection of a potential introduction of HPAI by wild migratory birds.

Bird migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas. There are four major

flyways in North America (Figure 1) that are broadly defined corridors where the migratory paths of many birds tend to converge. They are associated with major topographical features in North America, which also tend to be aligned along a north-south axis. The four flyways, Atlantic, Mississippi, Central, and Pacific, have areas of overlap and convergence, particularly at the north and south ends. The flyway boundaries are defined administratively, and are not biologically fixed or sharply defined.



**Figure 1. Four major flyways for migratory birds in North America.**

North American Flyways represent the predominant pathways of migratory bird movements within broad geographic areas. Many migratory bird species use specific flyways; however, many species migrate across flyways during fall and spring. Alaska and the Pacific Flyway are thought to be the most likely points of introduction of infected birds because of their proximity

to Siberia and the East Asian flyway. There are other potential routes of introduction that may occur via migratory birds including the Atlantic, Central, and Mississippi Flyways (Kilpatrick et al. 2006, Shoham and Rogers 2006).

## METHODS

The goal of the 2006 USDA funded surveillance program was to collect a minimum of 75,000 wild bird samples and 50,000 fecal samples through the efforts of Wildlife Services (WS), state wildlife agencies, and tribal cooperators.

Five collection strategies are identified in the USISP: live wild bird, sentinel, hunter-harvest, morbidity/mortality investigation, and environmental sampling. All five strategies were employed nationwide during the 2006 sampling season. The USISP identifies lists of migratory bird species that potentially have been exposed to highly pathogenic H5N1 avian influenza, establishes diagnostic procedures, and provides guidelines for other aspects of the surveillance effort. The four Flyway Councils also developed regional plans which list priority species for sampling. In most cases, individual states further stepped down the Strategic and Flyway level plans to identify those migratory bird species that were most abundant in their states and target numbers were set up for each species.

WS and its cooperators collected one cloacal swab from each bird sampled by hunter-harvest, sentinel, and live wild bird collection strategies. Separate cloacal and tracheal swabs were taken during morbidity/mortality investigations. Environmental samples consisted of a single swab taken from fresh bird feces.

All wild bird samples were sent for testing to one of 42 diagnostic laboratories that are part of the National Animal Health Laboratory Network (NAHLN). The NAHLN is part of a national strategy to coordinate animal health diagnostic services in the United States by positioning the National Veterinary Services Laboratories (NVSL) as the lead United States animal health laboratory and allowing select laboratories operated by State, Federal and

university officials to cooperate in foreign animal disease surveillance and related services. NVSL serves as the national reference laboratory by providing other diagnostic laboratories with animal disease information and technical guidance as well as confirmatory testing of foreign animal diseases.

Wild bird samples were pooled within species, location, and date in the laboratories by taking an aliquot of 60  $\mu$ L from each of the samples. No more than 5 samples constituted a pool. Each pool of samples was initially screened for type A influenza with the matrix H5 bead real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay. If the matrix assay was positive for type A influenza, subtyping was conducted with the H5 and H7 specific rRT-PCR assays. Testing at the NAHLN laboratories was completed within 48 hours of receipt of samples at the laboratory. Individual samples comprising H5 or H7 positive pools were then forwarded to NVSL for confirmation. NVSL conducted rRT-PCR on each individual sample comprising the pool by testing for H5, H7 and N1. Virus isolation was conducted on each sample sent to NVSL for confirmation by inoculating a suspension of each specimen into the embryos of chicken eggs to replicate the virus in order to determine the H and N subtypes of the virus. Genetic sequencing of H5 and N1 positive isolates was used to determine the pathogenicity of the virus. Additionally, 4 to 8-week old disease-free chickens were inoculated with all H5 isolates to determine pathogenicity. All H5N1 detections in 2006 were considered low path avian influenza (LPAI) because the AI virus was never lethal for 6 or more of the 8 chickens that were inoculated, and all H5 isolates were genetically related to North American strains of known LPAI viruses.

All environmental samples were submitted to WS National Wildlife Research Center where diagnostic testing was conducted. Fecal samples were pooled in the laboratory with up to 5 samples and screened for the presence of AI viruses using rRT-PCR matrix assays. Positive pools were then screened for H5 and H7 and positive pools were sent to NVSL for confirmation.

All cooperator sample data and results were stored in a single database managed by National Biological Information Infrastructure. This database is the Highly Pathogenic Avian Influenza Early Detection Data System (HEDDS) and it can be found at <http://wildlifedisease.nbii.gov/ai/>.

## RESULTS

In the 2006 wild bird surveillance program, the WS and its state wildlife and tribal agency partners collected more than 85,500 samples from wild birds in all 50 United States states, trusts, and territories (Figure 2). In this unprecedented effort, all samples tested negative for HPAI H5N1 virus. Additionally, WS and 4 State wildlife agencies collected and evaluated 50,184 environmental samples from wild birds. All environmental samples (feces) tested negative for HPAI H5N1. Nineteen bird species were found to be infected with H5 LPAI viruses in the 2006 sampling season (Table 1).

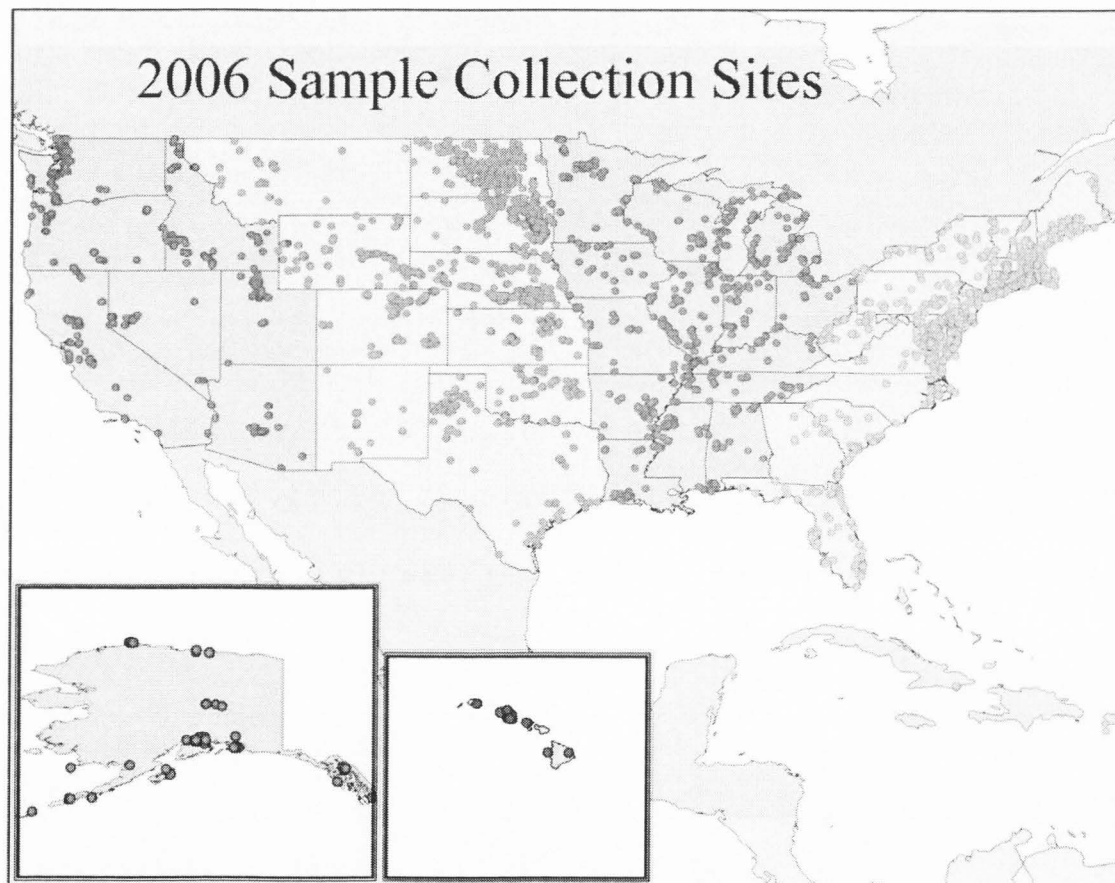


Figure 2. 2006 USDA AI Sample Collection Sites.

**Table 1. 2006 LPAI H5+ species from which samples were collected that contained low path avian influenza, H5 virus.**

<b>H5 Positive Species</b>		
Mallard	American Green-winged Teal	Canada Goose
Northern Shoveler	Blue-winged Teal	Ruddy Duck
Northern Pintail	American Wigeon	Mute Swan
Gadwall	Greater White-fronted Goose	Muscovy Duck
Cackling Goose	American Black Duck	Common Goldeneye
Ring-necked Duck	Lesser Snow Goose	Tundra Swan
Domestic Duck		

**DISCUSSION**

HPAI H5N1 continues to spread in Asia, Europe, and Africa. Wild migratory birds likely play a role in the spread of the virus (Kilpatrick et al. 2006) and serve as an important component in overall surveillance. Because wild migratory birds are a potential pathway that cannot be ignored, surveillance will continue in 2007.

In 2006, implementation of the early detection system was based on the best available knowledge of the HPAI H5N1 circulating overseas and LPAI research findings. Over the last year, additional research findings and cooperative surveillance efforts have provided valuable information on LPAI viruses circulating in the US. Findings from 2006 have guided the targeted surveillance for 2007 with the main objective remaining the early detection of HPAI in wild migratory birds.

Given the tremendous success of the 2006 program, the 2007 program is very similar, with several key changes that will improve efficiency and effectiveness of the early detection system. USDA will continue to offer financial support to state wildlife agencies, tribes, and diagnostic laboratory partners participating in the collection and testing of samples to support the early detection of HPAI viruses in wild migratory birds. For initial screening, all wild bird

samples collected in support of this partnership will continue to be shipped to NAHLN facilities approved for avian influenza testing. Finally, all confirmatory testing for HPAI will be conducted at NVSL.

WS again considers morbidity and mortality investigations in wild birds highly important for potential detections of HPAI, and will continue to support the need for sampling birds associated with those events. Additionally, surveillance will focus sampling of apparently healthy birds (i.e., hunter/agency harvest, live bird sampling) on those species most likely to be carriers of HPAI, by encouraging the sampling of the 19 bird species that were infected with H5 LPAI viruses in 2006 (e.g., mallards, American green-winged teal, Northern shovelers, Northern pintails, etc.). Other species listed in the US Strategic Plan and regional Flyway Plans remain valid and worthy of sampling.

In 2007, approximately 50,000 samples from wild migratory birds will be submitted for surveillance. Environmental sampling focuses on areas most likely to receive birds from Asia, Europe, and South and Central America. WS personnel will collect 25,000 fecal samples in 2007 to support the early detection system.

Considerable debate has occurred over the relative value of tracheal, oropharyngeal, and cloacal swabs. Published (Brown et al. 2006) and unpublished data suggest that there is utility in swabbing the oropharyngeal cavity when looking for HPAI viruses. For samples collected in association with a morbidity/mortality event, surveillance design includes collecting both tracheal and cloacal swabs from each bird. Swabs will be placed into separate tubes and tested individually. For apparently healthy birds, such as those collected by hunter-harvest, agency collection, sentinel and live bird sampling, two swabs will be collected per bird. An oropharyngeal and a cloacal swab will be collected and placed into the same tube. Diagnostic testing will analyze the swabs from apparently healthy wild birds as a single sample. Pooling of samples has been discontinued for the 2007 surveillance effort.

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