

Subject: Summary Report of 2006 Highly Pathogenic Avian Influenza Surveillance in the Atlantic Flyway

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Prepared by: USDA/APHIS Wildlife Services Wildlife Disease Surveillance and Emergency Response Program, Kerri Pedersen and Seth Swafford

Introduction

Waterfowl (Order Anseriformes), shorebirds (Order Charadriiformes), and several other species of wild birds are considered natural reservoirs for avian influenza (AI) viruses. Type A influenza viruses are known to infect many different species of wild birds as well as domestic poultry. AI viruses in general are classified by a combination of two groups of proteins: the hemagglutinin or H proteins of which there are 16 (H1-H16) and neuraminidase or N proteins, of which there are 9 (N1-N9). AI viruses can also be classified based on their ability to cause disease: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). Pathogenicity is determined by genetic comparison with known isolates and/or the chicken pathogenicity test which involves the inoculation of 4 to 8 week old disease-free chickens with observations over a 10 day period.

All 16 H proteins and 9 N proteins have been isolated from wild birds, especially waterfowl and shorebirds. The combination of these proteins creates subtypes (i.e. H₅N₁, H₃N₂, etc) which are adapted to survive in certain species of wild birds without causing disease. However, gradual genetic drift (i.e., mutation) can occur and a particular subtype can become adapted to infect other species of wild birds and domestic birds. Although this slight genetic change in the virus allows it to infect new species, it usually does not cause disease in the new host. The virus can also change if a host is simultaneously infected with another type A influenza virus. In these situations, mixing of the genetic material from the two virus strains (genetic shift) can occur, resulting in the formation of a new strain. The combination of gradual drifts and rapid shifts results in the production of a strain that may cause morbidity and mortality in susceptible hosts. If the morbidity and mortality is significant, the virus may be classified as HPAI.

Due to concerns that HPAI would be introduced into the United States, *An Early Detection System for Asian H₅N₁ Highly Pathogenic Avian Influenza in Wild Migratory Birds, US Interagency Strategic Plan* was developed. The plan standardized sample collection and diagnostic methods for conducting surveillance to detect HPAI in wild, migratory birds. It also identified species of wild birds that were thought to be important due to their specific migratory patterns. Development of the plan involved cooperation and collaboration between the U. S. Department of Agriculture (USDA), Department of the Interior (DOI), the Association of Fish and Wildlife Agencies (AFWA), and many others to formulate an approach for surveillance. The *US Interagency Strategic Plan* was adopted by the USDA and DOI in early March 2006.

The *US Interagency Strategic Plan* was then stepped down to individual flyway plans by Flyway Council technical sections and study committees. *Surveillance for Early Detection of Highly Pathogenic Avian Influenza Asian H5N1 in Wild Migratory Birds, A Strategy for the Atlantic Flyway* was developed to address surveillance in the Atlantic

Flyway. This important phase of implementation allowed for more direct input from state wildlife agencies and set species priorities and sampling locations. Many state wildlife agencies furthered the process by developing state level plans and surveillance approaches by working collaboratively with agricultural, natural resource and public health officials to develop specific approaches to meet the goals and objectives set forth by all involved parties. USDA established sample size numbers for states in the Atlantic flyway based on a priority rating system that included input from the AFWA and the Atlantic Flyway Council technical section. Overall, cooperative efforts in the Atlantic flyway were designed to account for sampling approximately 23,000 wild birds. In addition, USDA planned to collect approximately 17,000 fecal samples from migratory birds.

Strong interagency relationships were developed as a result of the cooperation and collaboration required to collect the large number of samples in each state. Everyone's dedication and commitment yielded an unprecedented wildlife disease surveillance effort that resulted in a highly successful sampling season.

2006 Number of Samples and Collection Locations

Surveillance for HPAI was conducted in all four North American Flyways with corresponding activities in Canada and Mexico to ensure the goal of early detection in wild, migratory birds. Upon completion of the *US Interagency Strategic Plan*, sampling was initiated first in Alaska in early April 2006. Sampling in the Atlantic Flyway began shortly thereafter and continued through March 31, 2007. In less than a year, 85,424 cloacal samples were collected from wild birds nationwide. This total includes the combined efforts of state wildlife agencies in all 50 states and various tribes that participated in surveillance under the direction and funding provided by USDA's Animal and Plant Health Inspection Service, Wildlife Services (WS). The following numbers do not include samples collected by DOI, states under contract with DOI, research activities, or other projects.

Of the total number of wild bird samples, 24,852 were collected in the Atlantic Flyway, 23,597 in the Mississippi Flyway, 18,462 in the Central Flyway, 17,993 in the Pacific Flyway, and 520 in Hawaii and the Pacific Islands. The Atlantic Flyway accounted for approximately 29% of the overall wild bird sampling effort. Wild, migratory bird collection locations including sample collections in Puerto Rico and Guantanamo Bay, Cuba are depicted in Figure 1.

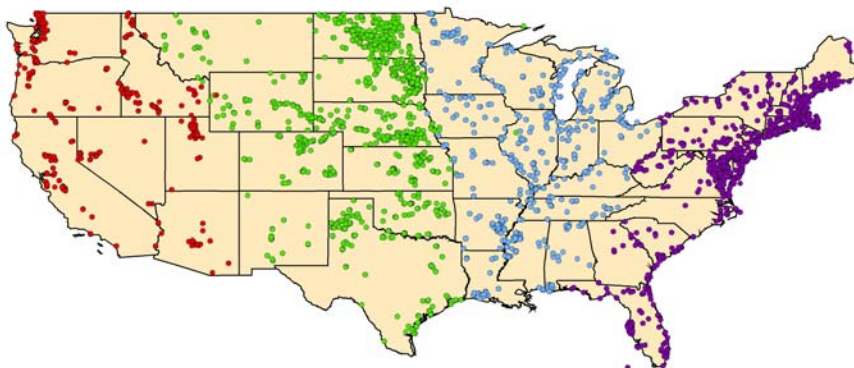


Figure 1. Collection Sites of Wild Bird Samples in 2006

Sample locations in the Atlantic Flyway were distributed geographically as depicted in Figure 2. The Chesapeake Bay area and coastal areas along the New England States provided appropriate locations to intercept migratory birds that were identified in *A Strategy for the Atlantic Flyway*. Surveillance in Florida and other coastal States along the Gulf of Mexico reinforced the efforts of Northern States as well as provided adequate coverage if migratory birds moved HPAI viruses north from South or Central America. Even though HPAI has not been detected in the Western Hemisphere, 2006 sample locations provided excellent geographic distribution throughout the entire Atlantic Flyway.

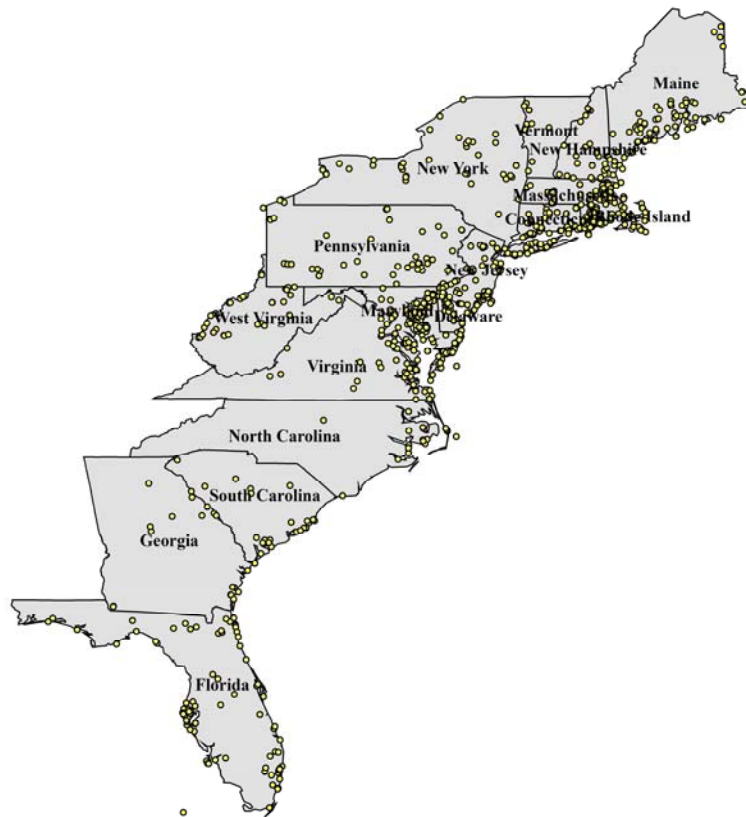


Figure 2. Collection Sites of Wild Bird Samples in the Atlantic Flyway

In addition to collecting samples directly from wild, migratory birds, environmental sampling for detecting HPAI was also implemented throughout the Atlantic Flyway. Environmental sampling consisted of the collection of fresh fecal material deposited by migratory birds. Specific collection sites included locations representative of habitat used by large concentrations of priority species (e.g., migratory waterfowl and/or shorebirds). Number of sites per state varied depending on amount of suitable habitat and migratory bird presence. Fecal samples were collected in all 50 states by WS and as well as state wildlife agencies in Alaska, Washington, Oregon and California. A total of 50,184 samples were collected in 2006, with the Atlantic Flyway accounting for 34% (16,929) of the fecal sampling effort.

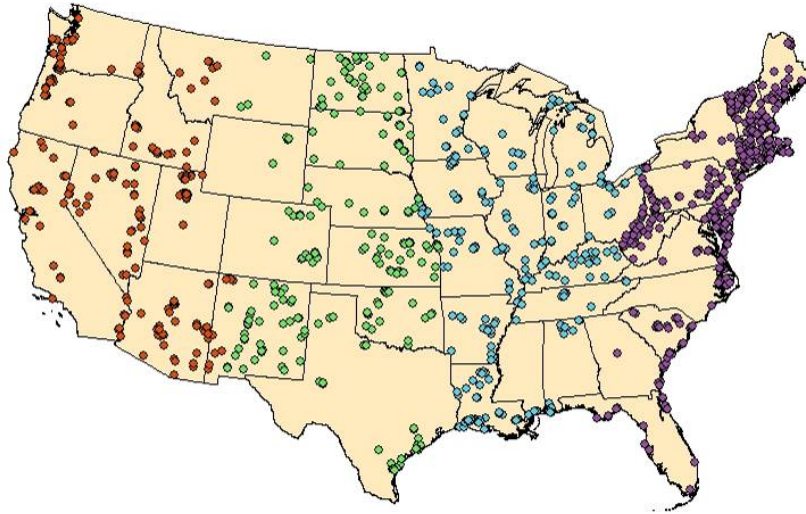


Figure 3. Fecal Sample Collection Sites in 2006

Diagnostic Testing of Wild Bird Samples

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 US by positioning the National Veterinary Services Laboratory (NVSL) as the lead US 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 μ 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 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.

Functional Groups and Collection Strategies

Grouping wild birds by related species relies on the fact that birds with similar behavior and feeding habits have comparable probability of contracting AI viruses. It facilitates examination of results and trends at a larger scale rather than examining each species individually. Percentages from 2006 sampling of functional groups are depicted in Figure 4. Dabbling ducks (Genus *Anas* and *Aix sponsa*) accounted for 55% of the overall effort, followed by geese & swans (Subfamily Anserinae) at 16%; and shorebirds (Order Charadriiformes, except Families Laridae and Alcidae) at 13%. The reason for the apparently large percentage of dabbling ducks is due to the relative facility in collecting samples from hunter check stations in comparison to shorebirds and other species where live trapping was the only active surveillance option available. Historical banding operations of dabbling ducks and population size also factored in to the large percentage represented in Figure 4. Functional groups of diving ducks (Subfamily Anatinae, except Genus *Anas* and *Aix sponsa*), gulls, terns, and Alcids (Families Laridae and Alcidae) and other (all birds not represented in another functional group) accounted for 7%, 5%, and 4% of the overall approach, respectively.

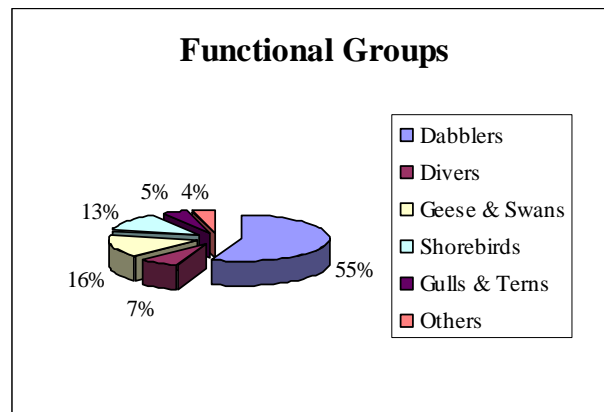


Figure 4. Functional groups of samples collected for AI surveillance in 2006.

There were essentially 5 different collection strategies described in the *US Interagency Strategic Plan* that could be utilized in collecting samples from wild birds (morbidity/mortality, live wild bird, hunter harvest, sentinel species, and environmental). Birds lethally collected by state wildlife agencies and WS are represented in the hunter harvest strategy. Decisions on which collection strategies to use were made through flyway plans and at the state level.

Although morbidity/mortality events made up a very small fraction (1%) of the total percentage of samples collected (Figure 5), the strategy is still a very important method for detecting HPAI in wild birds. Recent literature has shown that many species

of wild birds will likely succumb to infection of HPAI, and natural immunity and pre-exposure to LPAI viruses might afford some level of protection. Wild birds that do not act as natural reservoirs of LPAI viruses are more likely to die from infection of the virus, thus making collection of samples at morbidity/mortality events most important.

As seen in Figure 5, hunter harvest and environmental sampling accounted for 35% and 38% of the total number of samples collected, respectively. Note: Operational control (birds lethally collected by state wildlife agencies and WS) accounted for 5% of the total samples and could be included within the hunter harvest category. These three collection strategies are perhaps the easiest and most predictable methods of collecting samples for HPAI surveillance. Live wild birds (21%) and sentinel flocks (1%) also played important roles in the overall strategy of early detection of HPAI in wild birds.

Within the Atlantic Flyway, 12,555 live wild birds were captured for AI surveillance. Of these, 7,788 live wild birds captured by Wildlife Services employees resulted in only 37 mortalities (0.5%). Accidental deaths were attributed to various causes such as mist net or cannon net injuries but none of these were from species listed as birds of conservation concern.

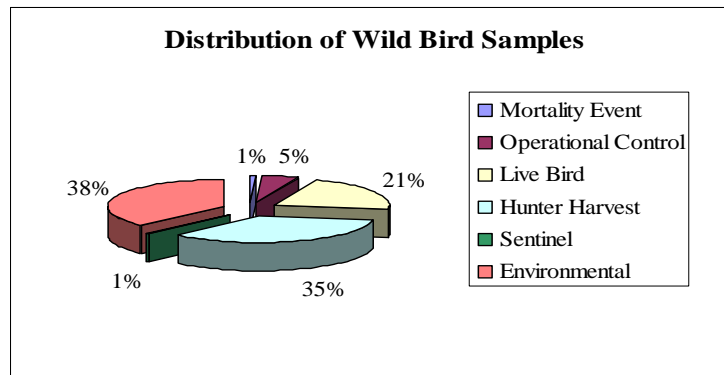


Figure 5. Collection strategies for samples collected for AI surveillance in 2006

Results

Of the 85,424 samples submitted to NAHLN laboratories for screening, 1414 individual samples from 186 locations in 43 states were sent to NVSL for confirmation. Not all of the 1414 samples were screened as positive, but were part of a pool which had at least 1 sample that tested positive by the rRT-PCR H5 assay. Of the 1414 samples, NVSL confirmed that 254 samples collected from 138 locations in 40 states were LPAI H5 positive. As depicted in Figure 6, these were collected from American Black Duck, American Green-winged Teal, American Wigeon, Blue Winged Teal, Cackling Geese, Canada Geese, Common Goldeneye, Domestic Ducks, Gadwall, Lesser Snow Goose, Muscovy Duck, Mute Swan, Mallard, Northern Pintail, Northern Shoveler, Ring-necked Duck, and a Tundra Swan. Figure 6 also depicts the temporal distribution of all LPAI H5 positive samples collected during the 2006 surveillance effort. None of the wild bird samples tested positive for HPAI.

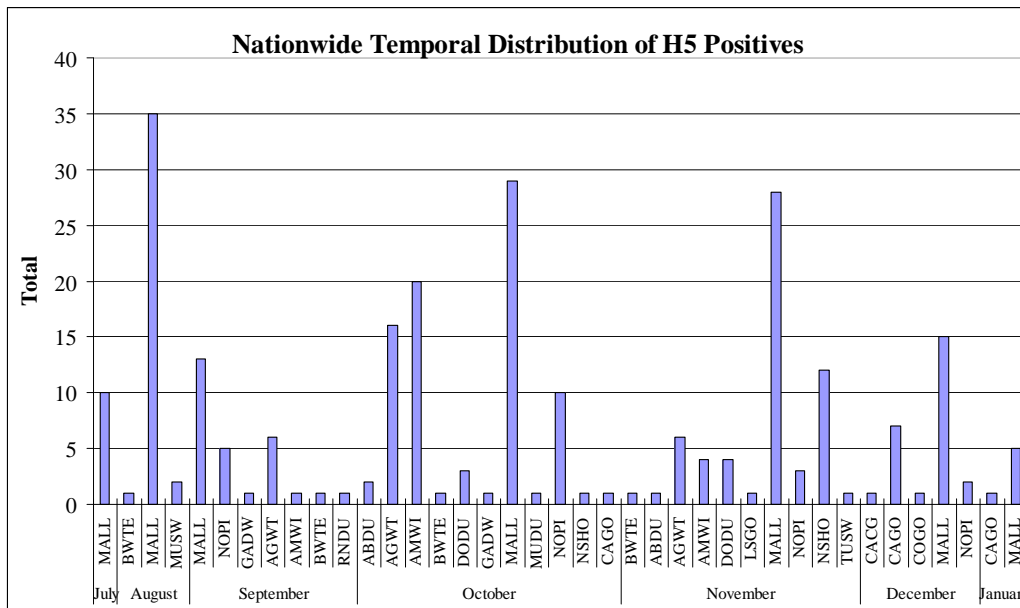


Figure 6. Nationwide Distribution of H5 Positives by Species

Of the 50,184 fecal samples collected during 2006, there were 103 matrix positive pools. Of the matrix positive pools, 24 pools from 16 states screened H5 positive and were sent to NVSL for confirmation. Three of these pools were confirmed positive. Within the Atlantic Flyway there were 4 H5 positive pools from 2 states (2 from Pennsylvania and 2 from Georgia) sent to NVSL for confirmation. One of these from Georgia was confirmed H5 positive. None of the fecal samples tested positive for HPAI.

Within the Atlantic Flyway, 12 NAHLN labs were responsible for testing the wild bird samples. Through the screening process, 240 samples from 13 states collected at 31 locations screened H5 positive as part of larger pools at a NAHLN laboratory. These samples were sent to NVSL for confirmation. Forty-one samples from 20 locations were confirmed LPAI H5 positive by NVSL (Figure 7). These were collected from American Black Duck, American Green-winged teal, Canada Goose, Common Goldeneye, Domestic Duck, Muscovy Duck, Mallard, and Northern Pintail. LPAI H7 and HPAI was not detected in any of the samples submitted for AI testing.

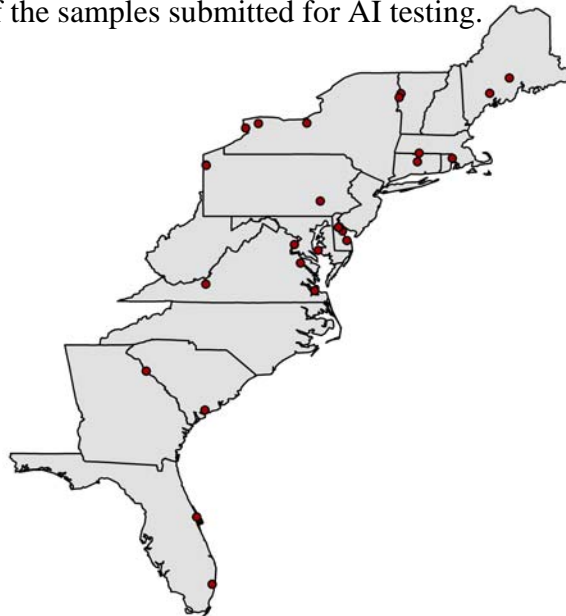


Figure 7. NVSL confirmed LPAI H5 Positive Locations in the Atlantic Flyway

Changes in HPAI surveillance for 2007

As presented at the late winter/spring Atlantic Flyway Council Technical Section, several changes are being implemented due to 2006 findings and input from state wildlife agencies, Flyway Councils, and research studies. Analysis of data from the 2006 HPAI surveillance effort has yielded the need for adapting surveillance to a targeted approach that will be implemented over a long-term maintenance phase. The targeted approach includes shifting toward increasing sampling of dabbling ducks, while not eliminating other species of wild, migratory birds or deviating from priority species listed in the *US Interagency Strategic Plan* or *A Strategy for the Atlantic Flyway*. This shift was based on 2006 findings presented above as well as the analysis of other surveillance data. Functional group approaches are also being enacted to allow greater flexibility to achieve significant sample sizes from hard to capture species (i.e. shorebirds).

Another significant change for 2007 includes collecting an oropharyngeal swab (different than tracheal) in addition to the cloacal swab. Sample collection methods are clearly described in the *Wildlife Services & State/Tribal Cooperator Avian Influenza Surveillance Procedures Manual*. These samples should be labeled with one barcode number and will be the main sample type for every collection strategy except morbidity/mortality events. For morbidity/mortality events, no changes have been enacted for 2007. One cloacal and one tracheal swab will again be collected from each bird and placed in different tubes with different barcode numbers.

Due to the repetitive paperwork required in filling out both a field datasheet and lab submission form, a universal datasheet was created to reduce the paperwork burden. The new datasheet can be found in the *Wildlife Services & State/Tribal Cooperator Avian Influenza Surveillance Procedures Manual*. Following the instructions of simply removing GPS coordinates and collection site name on the datasheet allows sample submitters to use the universal datasheet as the laboratory submission form.

Pooling samples has been eliminated for sample collection in 2007. This step will more easily allow for the analysis of LPAI viruses, which is an added value of HPAI surveillance in wild, migratory birds. The elimination of pooling allowed for more samples to be recorded on an individual datasheet, which also reduced the paperwork burden. This step greatly improves the efficiency of NAHLN laboratories by allowing for the analysis of individual samples, rather than pools.

New procedures for shipping samples went into effect on January 1, 2007. Samples submitted for surveillance purposes are considered exempt animal specimens. Any labels on shipping boxes marked UN3373 should either be removed or covered with an exempt animal specimen label. These new procedures can be reviewed in the *Wildlife Services & State/Tribal Cooperator Avian Influenza Surveillance Procedures Manual*.