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Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Migratory Birds in the United States

July 1, 2016

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Introduction

This document describes a plan to conduct national level surveillance for highly pathogenic avian influenza virus (HPAI) in wild migratory birds. Collaborating entities include the USDA Animal and Plant Health Inspection Service (APHIS) Wildlife Services (WS) and Veterinary Services (VS); the United States Geological Survey (USGS); U.S. Fish and Wildlife Service (USFWS); and the National Flyway Council. This surveillance plan supplies detailed methods on the reasoning behind, and the development of, wild bird avian influenza surveillance. The Implementation Plan for Highly Pathogenic Avian Influenza (HPAI) Surveillance in the United States is a more concise supporting document that provides sample number targets for each state. Implementation of national level surveillance directly supports the U.S. Interagency Strategic Plan for the Monitoring of Avian Influenzas of Significance in Wild Birds (Interagency Strategic Plan).

Highly Pathogenic Avian Influenza in the United States

The first report of H5N1 HPAI occurred in Asia in 1996 and now a diverse viral gene pool exists in the world due to co-circulation of many avian influenza viruses (AIVs) in domestic and wild birds. The Asian H5N1 HPAI is the predecessor of multiple viral reassortants, including H5N2, H5N5, and H5N8.

In the fall of 2014, Eurasian HPAI H5N2 was identified in commercial poultry in the Fraser Valley region of southern British Columbia, Canada. Subsequent samples collected from wild birds in the United States, combined with mortality events associated with captive raptors, revealed a least three Eurasian H5 HPAIs in circulation. Between March and June of 2015, HPAI outbreaks in US domestic poultry operations resulted in the loss of nearly 50 million birds. In January of 2016, an HPAI event was identified in domestic turkeys in Indiana. Testing revealed a H7N8 virus of North American lineage and therefore different from the Eurasian HPAIs responsible for poultry outbreaks in 2014-2015. Surveillance associated with the 2015 Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Migratory Birds in the United States had identified a LPAI H7N8 in a wild bird from a neighboring state prior to the domestic turkey outbreak. Federal and state agencies continue to collect wild bird samples to define the extent of HPAI infection in specific avian species groups.

Specific Objectives

The purpose of this document is to detail the essential methods behind a national surveillance system for influenzas of interest in wild, migratory birds. This plan is intended to provide guidance for Federal agencies and other cooperators to conduct influenza surveillance in dabbling ducks, which are the avian group most likely to be infected with influenzas. This focus increases our chances of detecting AI viruses. Data generated through this surveillance efforts will provide information that can improve management actions that are taken to address the multitude of issues associated with HPAI. This risks include infections in commercial poultry, backyard poultry, game bird farms, wild birds, wild bird rehabilitation facilities, falconry birds and captive bird collections in zoos and aviaries. The first year of surveillance using the framework outlined in this document did not detect any confirmed HPAI positive wild birds; however, we did detect Eurasian H5 RNA (by PCR)

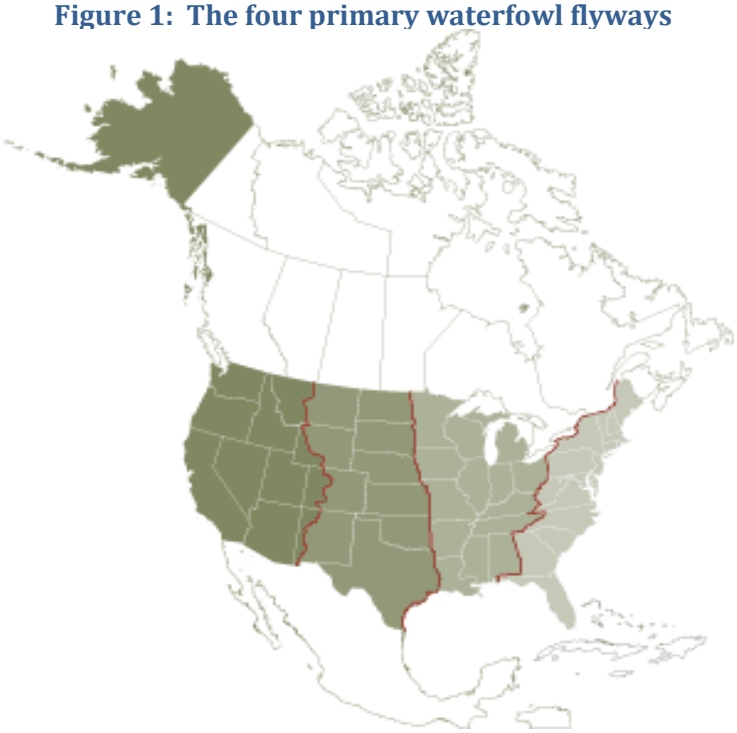
in wild birds as late as November 2015, suggesting that the viruses could still be present in the environment. There are also additional viruses in the same clade that are now circulating in Asia, demonstrating that introduction of novel viruses is always possible.

Objectives

1) Identify the distribution of HPAI influenzas of interest by U.S. flyways and through select, high priority watersheds
2) Detect spread of HPAI influenzas of interest to new areas of concern
3) Provide a flexible surveillance framework that can be modified to monitor wild waterfowl populations for re-assortments of influenzas, and introductions of new viruses,
4) To sequence influenza viruses from wild birds to better understand pathways that lead to outbreaks in commercial and backyard poultry

Migratory Waterfowl Movement

Figure 1: The four primary waterfowl flyways



Waterfowl and water 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). These flyways are broadly defined corridors where the migratory paths of many species of interest 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.

North American biological 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 the fall and spring. The Pacific Flyway is thought to be the most likely area of introduction for the HPAI viruses detected in Canada and the United States in December of 2014.

Species Identified for Collection

In 2006, the U.S. Departments of Agriculture and Interior, along with multiple State and tribal agencies, implemented a nationally coordinated avian influenza surveillance effort in wild birds. This large-scale surveillance system has provided an unprecedented amount of data on avian influenza viruses in U.S. wild bird populations and can inform current surveillance efforts. This dataset, along with many others, has identified dabbling ducks as the primary reservoir for avian influenzas, including H5s and H7s. Therefore, the primary focus of sampling will continue to be on these species (Genus *Anas*, *Aix*, *Cairina*, and *Dendrocygna*): American green-winged Teal, mallard, northern pintail, American black duck, wood duck, blue-winged teal, cinnamon teal, northern shoveler, gadwall, Muscovy duck, mottled duck, and American wigeon. The fulvous whistling duck is not taxonomically a dabbling duck but because of its foraging habits it is also included in the same functional group for urposes of this surveillance plan.

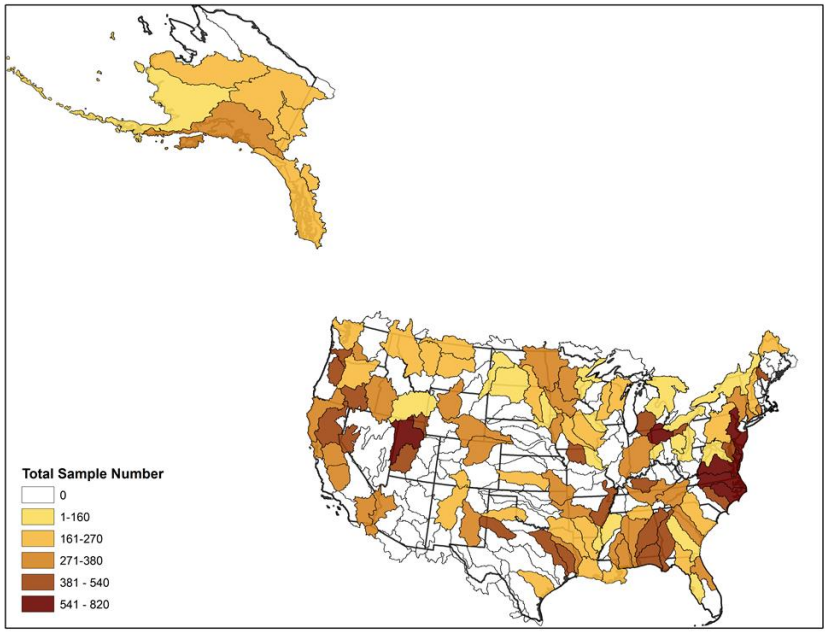
Identification of Priority Watersheds

With the intended goal of identification within a biologically relevant scale and not at the State level, watersheds were selected as the sampling unit for the surveillance design. Watersheds are used to identify key factors important to waterfowl and influenza biology and ecology. To identify the baseline HPAI distribution across U.S. flyways and constituent watersheds and to detect early spread of HPAI to new flyways and regions, sample units were defined geographically using the USGS 4-digit hydrologic unit code (HUC4). There are approximately 222 HUC4s in the United States and they represent an area drained by a river system, a reach of a river and its tributaries in that reach, a closed basin(s), or a group of streams forming a coastal drainage area [1].

Hawaii was not included in this analysis, because the data need to identify priority surveillance areas were not available. To identify which watersheds increase the likelihood of detection for influenzas of interest and are of agricultural importance, we used a combination of three measures and two constraints to capture the underlying biological aspects for influenza. The measures included: 1) watersheds with significant historic influenza clusters, 2) watersheds identified as having high inter- and intra-flyway mixing of dabbling ducks within the lower 48 States and 3), watersheds with high numbers of domestic chickens and turkey. The two constraints were based on median annual number of days above 0°C and below 0°C in a watershed. These were used in concert to develop targeted surveillance.

Figure 2. Targeted watersheds that ranked as a significant priority (colored) or low priority (gray/white) using network movements of dabbling ducks, influenza A clusters, and commercial poultry concentrations.

A) Targeted Watersheds were further prioritized by identifying watersheds in the upper 50th percentile of dabbling duck network mixing importance or with a historically significant influenza A cluster. Commercial poultry concentrations were also incorporated. Sampling would vary seasonally.



1) Historic influenza clusters

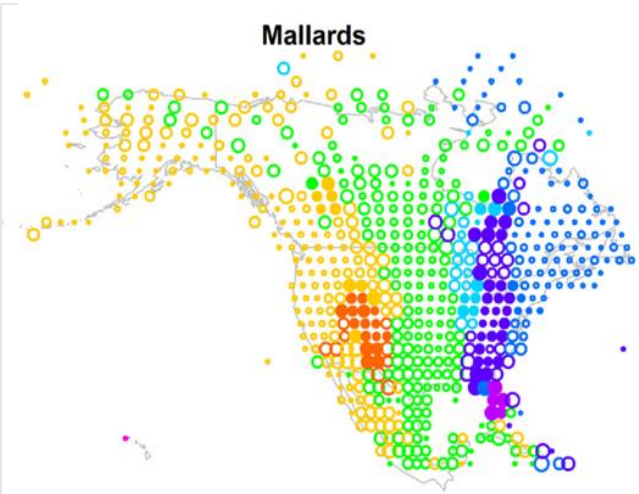
To identify watersheds with significant influenza clusters, we used LPAI data collected from dabbling ducks during the previous avian influenza surveillance effort to identify priority sample-collection areas. While using LPAI data is not ideal, the HPAI viruses circulating in the US during 2014/2015 appeared to behave similarly to LPAI in dabbling ducks. These data encompass known seasonal differences in influenza prevalence in waterfowl and important biological periods for waterfowl migration, both linked to influenza dynamics, the analysis considers three periods. These periods represent the summer breeding season (May-August), fall migratory season (August-December), and the over-wintering season (December-February). The spring northern migration was not included for three reasons. First, little is known about spring migration and data to estimate spring populations is not available. Second, data describing spring influenza is limited. Finally, logistical constraints on sampling during this time of year are significant.

All dabbling duck samples tested for the influenza A matrix gene were separated by three biologically relevant seasons and analyzed using the Getis-Ord Gi* spatial statistic to identify historic "hot spots" of influenza activity in dabbling ducks at a regional watershed scale. This resulted in identifying specific watersheds as having or not having significant influenza clusters (see Appendix 2). This analysis revealed geographic regions where influenza virus in sampled waterfowl was higher than expected [2]. A majority of these influenza clusters occur in more northern latitudes, a finding

supported by previous studies related to location of major waterfowl breeding grounds and additional research on the environmental persistence of influenzas at cold temperatures [3]. These results offer an important data stream to inform high priority areas for wild bird avian influenza surveillance.

2) Inter and intra-flyway mixing of dabbling ducks

Figure 3. Example map showing nodes that depict the spatial distribution of mallards. Color represents flyway membership and the size of the node is scale to the CF metric, representing regions important to flyway structure and thus potential areas of increased mixing. Filled circles are statistically significant CF nodes at α=0.05.



To identify watersheds important for mixing of waterfowl populations, we used the consolidation factor (CF), a recently developed metric that pinpoints geographic areas of high mixing for multiple dabbling duck species [4] . Specifically, this metric is a measure of within- and between-flyway movement processes important for influenza, and is based on historic U.S. Fish and Wildlife Service banding data. The metric was used to identify watersheds within each flyway that account for the majority of within-flyway mixing of waterfowl and to identify watersheds important for linking watersheds (identifying watersheds where mixing of birds from multiple flyways occurs.) See Appendix 3 for detailed methods.

3) Commercial poultry production

To ensure that surveillance encompassed areas with substantial domestic poultry production, watersheds were identified that contained an estimated annual average inventory greater than 10 million birds. These data were generated by the Farm Location and Animal Population Simulator (FLAPs) [5] and include all chicken and turkey production (http://flaps.biology.colostate.edu). The FLAPs data are generated using a probabilistic model that represents the likelihood of farm presence based on 2012 National Agricultural Statistics Service data. Watersheds with more than 10 million birds were used in concert with historic avian influenza clusters and with areas with high inter and intra-flyway mixing of dabbling ducks to identify which watersheds to target for avian influenza surveillance in wild birds.

4) Temperature constraints

These watersheds were then constrained to address biological processes for both the host and pathogen. Temperature is associated with waterfowl migration and influences avian influenza persistence in the environment. There is a well-documented pattern of reduced avian influenza prevalence in waterfowl at southern latitudes, especially during the breeding season [3]. This is largely attributed to viral degradation at higher temperatures [6]. To address this within the framework of a targeted surveillance effort, we incorporated the documented decay of virus at increasing temperatures above 0˚C to identify watersheds during the breeding period in which AI was less likely to persist. Because water salinity and pH also influence avian influenza, we used a conservative approach—removing watersheds with a 30-year median annual number of days above 0˚C of 365. See Appendix 4 for detailed methods.

Ambient temperature and number of consecutive days below 0˚C have direct consequences for waterfowl [7,8], and can affect their energy requirements. These cumulative effects also influence water temperature and ice formation. Increasing ice coverage can decrease availability of wetland foods, reducing nutrient acquisition by wetland-obligate waterfowl (e.g., gadwall, northern shoveler) and resulting in migration. We addressed this by removing watersheds from surveillance during the overwintering period in which waterfowl were unlikely to be present. Because migration can be influenced by a myriad of other factors such as snow and ice cover precluding foraging for field-feeding waterfowl (e.g., mallard), we removed watersheds with an estimated 30-year median number of days below 0˚C of at least 180 from the winter surveillance effort.

Sample Size Estimation

1) Waterfowl Population Estimates

Currently, seasonal population estimates for waterfowl at a watershed or regional level are not available for the entire United States. To mitigate this, we developed a Bayesian Gamma-Poisson Mixture model to estimate a 15-year (1998-2013) median seasonal population within each watershed. The approach integrated bird band recovery data and the estimated annual continental dabbling duck population available from the U.S. Fish and Wildlife Service. The result was an estimate of the median population of ducks (with 95 percent credible intervals) that might be expected in the watershed during the fall and winter. We used an alternative method to derive breeding season (May-August) population estimates because bird banding data at the continental scale is not available for the spring or summer periods. To estimate summer populations, we used the breeding bird survey data, one of the only large-scale datasets available, to develop an aggregate estimate of relative abundance at the watershed scale [9]. We did not estimate spring populations because band recovery data or other survey data are not available. Our approach for developing coarse population estimates ensures that the influenza surveillance effort accounts for the movement of migratory dabbling duck species across space and time. While these approaches have limits, they provide a relative understanding of the distribution and potential populations

across the United States. Appendix 4 describes the method for estimating populations and the corresponding assumptions in greater detail.

2) Avian Influenza Occurrence in U.S. Waterfowl Populations

To estimate true seasonal expected influenza A prevalence within watersheds, we used prior dabbling duck surveillance data from 2006 to 2011. A Bayesian Beta-Binomial model was used to estimate the true seasonal and monthly prevalence. The Bayesian model accounted for uncertainties in the diagnostic test process (sensitivity and specificity), differences in seasonal and monthly sampling efforts, and observed variability from year to year in influenza A prevalence. This method resulted in estimates for each watershed for the seasonal median prevalence (with 95 percent credible intervals) expected during the fall, winter, and breeding periods, should influenza be introduced. Appendix 4 describes specific details about the model.

3) Estimation of Watershed Sample Sizes

Sample sizes required to identify influenzas of interest were developed for each season within each priority watershed. Using the seasonally expected dabbling duck population (part 1) and the expected influenza prevalence (part 2) given introduction, we estimated the number of samples required to detect influenzas of interest for each season. We assumed that the influenza of interest represented 20 percent of the expected influenzas in a watershed, which is within the range of previously reported H5 prevalence values in dabbling ducks. This was reinforced during surveillance from 2015-2016, where mallards were the most commonly sampled dabbing duck species by far, and their Influenza A prevalence was 19.85%. This provided the data needed to compute detection prevalence thresholds.

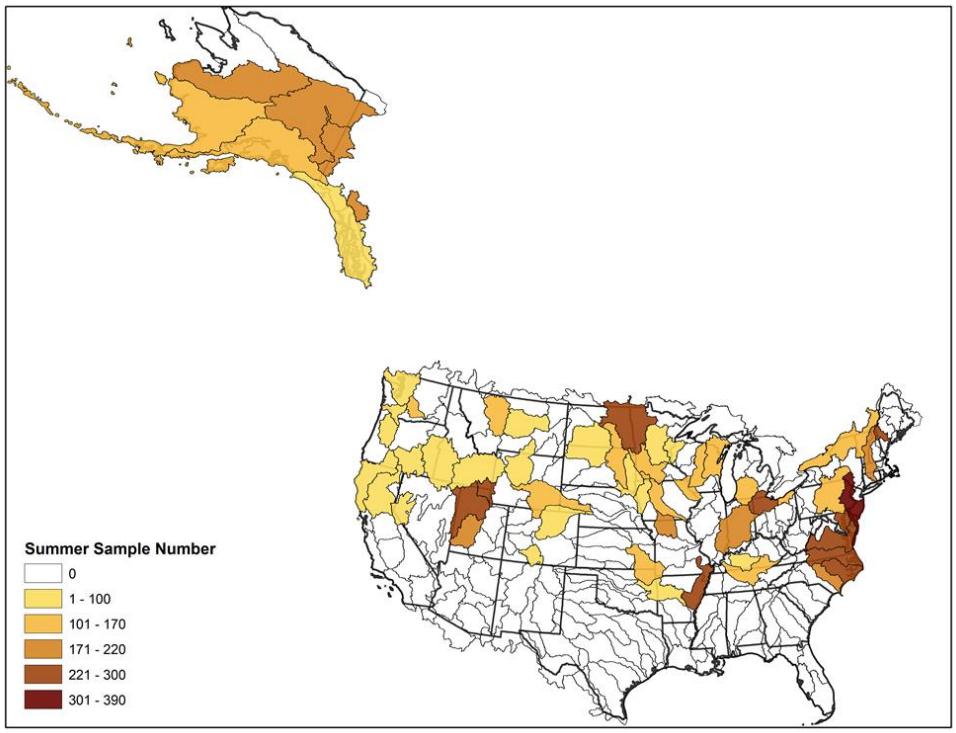
The numbers in Tables 1 represents the total annual number of samples [10] needed across watersheds to detect the presence of influenza A of interest at a 95 percent confidence level within a given watershed if the prevalence meets or exceeds the detection prevalence threshold. Detection prevalence thresholds for influenzas of interest vary among and within each sampled watershed by season, but have a minimum level of detection of 1%. The sample sizes also assume 86.3 percent diagnostic sensitivity for the matrix rRT-PCR (Janice Pedersen, personal communication; Mia Torchetti, personal communication). Sampling all watersheds requires extremely large sample numbers. In contrast, targeting sampling of high priority watersheds (Figure 4) lowers the sample numbers required while still allowing us to infer distribution across the United States for influenzas of interest, because the targeting criteria preferentially selects watersheds with greatest connectivity and potential for spread (i.e., those most at risk for introduction of influenzas of interest).

Table 1. Estimated seasonal sample sizes needed by watershed ranking

Targeted Priority Watersheds	No. of Watersheds	Sample Sizes[1]	Percent
Summer	55	**8430**	**27%**
Fall	92	**11300**	**36%**
Winter	67	**11770**	**37%**
All Seasons	**105**	**31500**	**100%**

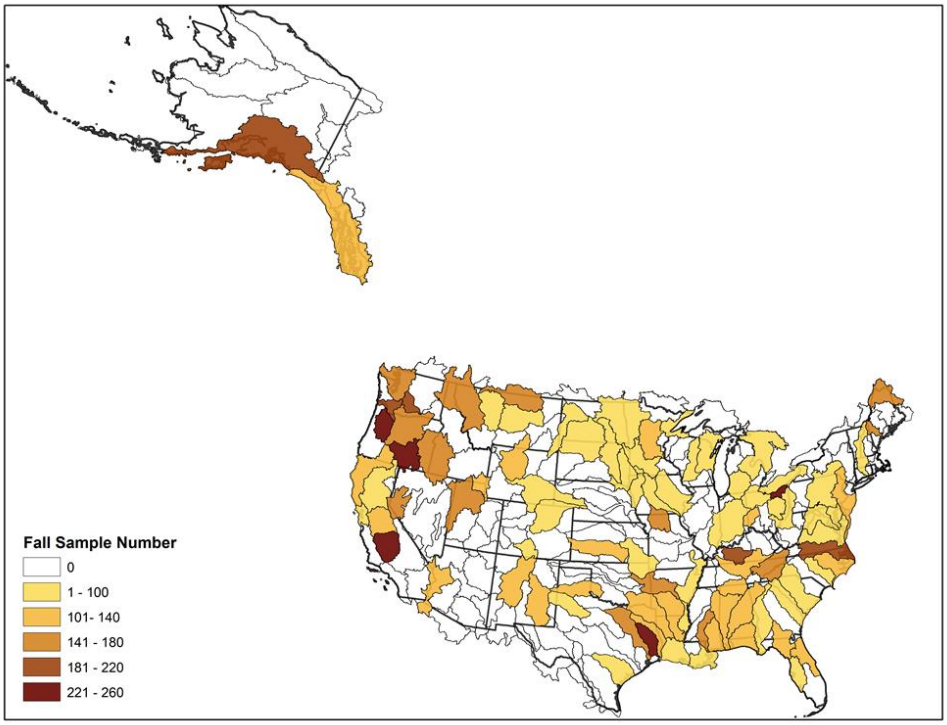
Figure 4. Number of samples to collect annually by watershed and season for surveillance based on targeted watersheds

Summer

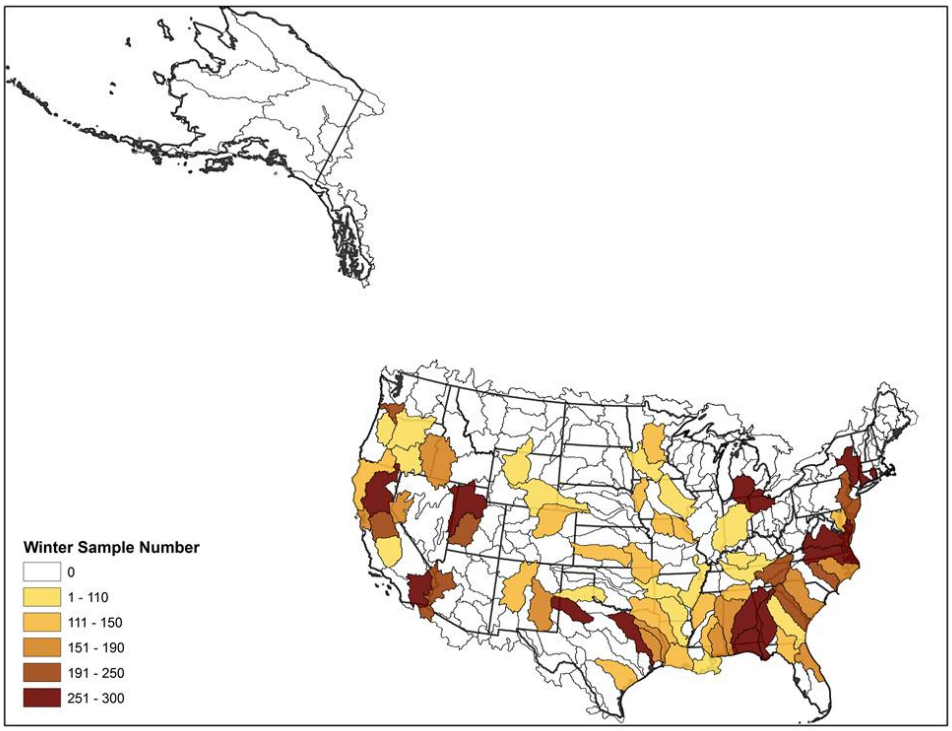


[1] Sample sizes are computed at 95% confidence assuming a diagnostic test sensitivity of 86.3% and are a function of detection threshold with a minimum of 1% and estimated population sizes per watershed in each season. The range of detection level is based on the observed influenza A in the watersheds and assuming influenzas of concern account for 20% of all influenzas.

Fall


Fall Sample Number
0
1 - 100
101- 140
141 - 180
181 - 220
221 - 260

Winter


Winter Sample Number
0
1 - 110
111 - 150
151 - 190
191 - 250
251 - 300

Sample Collection

National-level surveillance for HPAI in wild, migratory birds will follow a biological year beginning May 1, 2016, through February 28, 2017. The sampling season dates defined in this document (summer, fall, winter) are purposely broad so that they can apply to all states, regardless of geographic location. Biologists can determine what time period within any given season is most appropriate for the waterfowl in their state. Summer samples however will ideally be focused on hatch-year-birds, since they are the population that is most likely to be infected. Samples should be collected when dabbling ducks or other species of interest are migrating into or through a specific state. Timing of seasonal migratory movements can vary widely depending on species, region, and current weather patterns. Sample collection efforts should be coordinated within each state and include efforts by federal, state, local, university, and non-governmental participants. Coordination will allow for efficient and cost-effective collection of wild bird samples. Local expertise should be utilized to attempt to collect informative samples from areas within targeted watersheds and species of high importance.

Multiple collection strategies are identified in the Interagency Strategic Plan: live wild bird, hunter harvest, morbidity/mortality investigation, and environmental sampling. This surveillance plan supports using a combination of strategies to increase the probability of detecting HPAI. Sampling morbidity and mortality events may be informative, but to date, the AIV viruses circulating in wild birds in the United States cause limited morbidity and mortality in most dabbling duck species. There is evidence that multiple raptor species and owl species may die from HPAI infections (see Appendix 5). States should remain flexible to incorporate opportunistic sampling of morbidity/mortality events in highly susceptible species as needed. We recommend contacting the appropriate personnel from raptor rehabilitation or related outreach programs when signs of infection are detected in raptors and briefing them on appropriate communication, sampling, and biosecurity responses.

Opportunistic sampling of any morbidity or mortality event in these species could provide highly relevant information. In the case of large numbers of sick or dying birds, a subset of wild birds should be sampled depending on the circumstances surrounding the morbidity/mortality event (including the number of wild birds involved and potential causes of death.) Investigations related to morbidity/mortality events should be conducted regardless of time of year, number of species involved, or number of samples already collected in the state. In addition, as more data become available on how these novel influenzas impact avian species, managers and Federal agencies may be interested in sampling areas that intersect with species of conservation concern (see Appendix 6). A similar approach can be used in regions of poultry production. Targeted priority watersheds identified in this surveillance effort inherently include areas of poultry production. However, in specific regions of concern, we recommend sampling that also informs poultry producers of local influenza risk so they can increase biosecurity measures to help prevent direct or indirect introduction of HPAIV from wild birds (see Appendix 7).

Spreading the sampling activities over multiple time periods during migration and in multiple locations will increase the temporal and spatial coverage of sampling efforts and provide more independent data.

Recognizing the need for flexibility - because each state will have specific logistical and resource constraints - the Surveillance Plan does not mandate how and when sampling should be done. There is also value in distributing sampling among multiple species particularly if samples are being collected from only a few locations and during a few sampling collection periods. Spreading the effort over multiple species should increase the probability of detecting AI under the assumption that various species will have varying exposure histories. This should also lead to greater independence of collected samples. However, as noted above, to provide states with the maximum flexibility to achieve sampling goals (based on the species available within their local watersheds), the Surveillance Plan does not provide specific mandates for sampling.

WS and its cooperators will continue to collect one cloacal and one oropharyngeal swab from each wild bird sampled by hunter harvest, sentinel, and live wild bird collection strategies. Cloacal and oropharyngeal swabs will be combined in the same tube of media per the AI Procedures Manual. Sampling during morbidity/mortality events will follow the same procedures as previous years (i.e., one tracheal and one cloacal sample will be collected from specimens and placed in different tubes.) The AI Procedures Manual provides details on sample collection, packaging, and shipping. All samples will be submitted to an approved National Animal Health Laboratory Network (NAHLN) laboratory. The NAHLN laboratory will screen samples to determine if type A influenza virus is present; if the test is positive, the sample will be further analyzed by H5 and H7 specific assays. Samples testing H5 or H7 positive at a NAHLN laboratory will be sent to the National Veterinary Services Laboratory (NVSL) for confirmatory testing and final diagnosis.

Appendix 1. Wild Bird Species Confirmed Positive for HPAI in the United States (December 2014-February 2016)

1. American green-winged teal
2. American wigeon
3. Gadwall
4. Mallard
5. Northern pintail
6. Northern shoveler
7. Ring-necked duck
8. Wood duck
9. Canada goose
10. Lesser snow goose
11. Bald eagle
12. Cooper's hawk
13. Peregrine falcon
14. Red-tailed hawk
15. Snowy owl

Appendix 2. Methods for Historical AI Clusters in Wild Birds

Dabbler matrix infection data were stratified by date collected (Fall=August-December, Winter=December-February, Breeding=May-August; time periods overlap because of variability across migrations) and related spatially on USGS 4-digit hydrologic unit code (HUC4). Clusters of matrix positive wild birds were identified using the Getis-Ord Gi* spatial statistic in ArcGIS. The analysis examines wild bird data (the number of samples and the AI status of each sample) that has been aggregated at the HUC4 watershed scale. Avian influenza activity within a watershed is analyzed in the context of neighboring watersheds. To be considered a statistically significant hotspot, the watershed will have a higher value and the surrounding watersheds will also have a higher value than would be expected due to chance.

We ran the analysis for both number of positive birds aggregated at the HUC4 watershed scale (count data) and on matrix prevalence aggregated at the HUC4 scales (proportion data). Watersheds identified as having a significant cluster of AI matrix positive dabblers or watersheds with a significant cluster of high matrix positive prevalence values were identified as high priority sample targets. Analyses used a previously calculated distance band of 100,000 meters [2].

Appendix 3. Development of Watershed Level Consolidation Factor (Waterfowl Mixing) Metrics

Buhnerkempe *et al.* (2016) developed network metrics for network regions at a resolution of 200km [4]. To assign these metrics to watersheds, a method for disaggregation and assignment of the measures to watersheds was developed. The network nodes represent band recoveries for distinct square regions in North America extending 100km out from a central reference location. In order to disaggregate and assign measures to watersheds, the network measure of interest for each HUC4 watershed, CF_i^{HUC4}, were estimated using the method described in Equation 1 and Equation 2.

The network measure for three dabbling duck species (northern pintail [NOPI], American green-winged teal [AGWT], and mallards [MALL]) is $\boldsymbol{W_i^{HUC4}}$ for each HUC4 watershed. This is the weighted sum of the network metric M_{jk} for HUC8 watershed *j* (within 100 km of the central reference location for network node *k*.) P_{jk}^{HUC8} is a weighting factor representing the 20-year mean proportion of dabbling duck band recoveries, *njk*, in HUC8 watershed *j* within the neighborhood of network node *k*. The CF was first assigned to the HUC8 watershed because the course spatial resolution of the HUC4 watersheds contained multiple network nodes. The HUC8 watersheds provided a resolution that allowed representation of the neighborhoods of each network node (Figure 5).

Equation 1. Assignment of network measures to watersheds

$$W_i^{HUC4} = \sum_{i=1}^{n} M_{jk}\, P_{jk}^{HUC8}$$

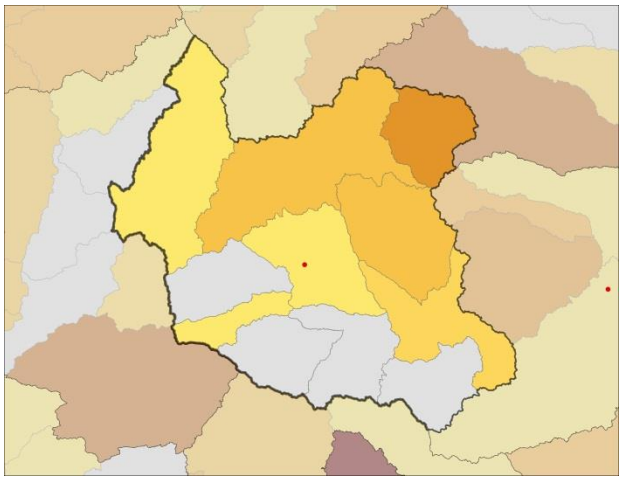
$$\text{Where, } P_{jk}^{HUC8} = \frac{n_{jk}}{\sum_{k=i,j=1}^{J} n_{jk}}$$

To estimate the consolidation factor for dabbling ducks, we used an aggregate measure for mallards, American green-winged teal, and northern pintails. These three species are assumed to represent the majority of mixing and connectivity within North American waterfowl; thus, this aggregate metric can be considered a proxy for dabbling ducks. The aggregate measure assumed a linear relationship across the three species and was calculated as:

Equation 2. Aggregate measure for consolidation factor.

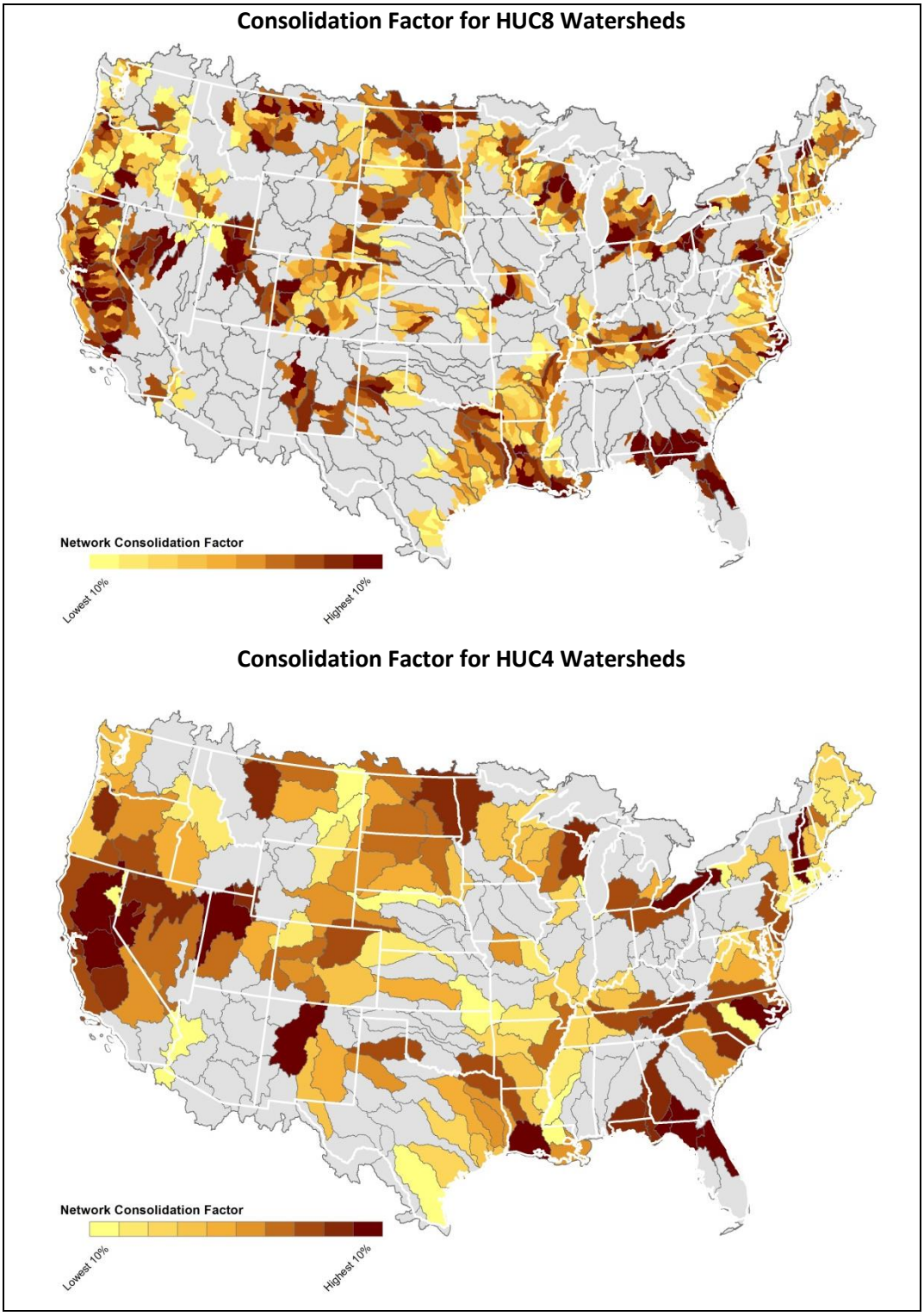
$$CF_i^{HUC4} = \sum_{i=1}^{n} W_{jk}^{MALL} + W_{jk}^{AGWT} + W_{jk}^{NOPI}$$

Figure 5. Example of the neighborhood for one network node (red dot and black line) using HUC8 watersheds. Colors represent the number of band recoveries in each watershed. Gray indicates no band recoveries.



This method of disaggregation allowed the identification and weighting of watersheds by their contribution to the CF. Figure 5 illustrates this point and represents the neighborhood of watersheds and the band recoveries that would have contributed to the network metric. There is obvious heterogeneity across the neighborhood from watershed to watershed. Figure 6 illustrates the HUC8 level aggregate CF and the HUC4 aggregated CF. As presented in Figure 6, for some HUC4 watersheds only a small fraction of sub-watersheds (HUC8) were important for mixing. For this reason we only considered HUC4 watersheds that had a minimum proportion of 0.3 represented by HUC8 watersheds important for mixing in the ranking of watersheds for surveillance. We acknowledge that the minimum proportion of 0.3 is somewhat arbitrary, but it allows the identification of priority HUC4 watersheds. It also serves to address logistical considerations of allocating sampling to a HUC4 watershed with only a small proportion important for waterfowl mixing.

Figure 6. Aggregate consolidation factor for HUC8 and HUC4 watersheds. Note some HUC8 watersheds with CF values only represent a small proportion of HUC4 watersheds.



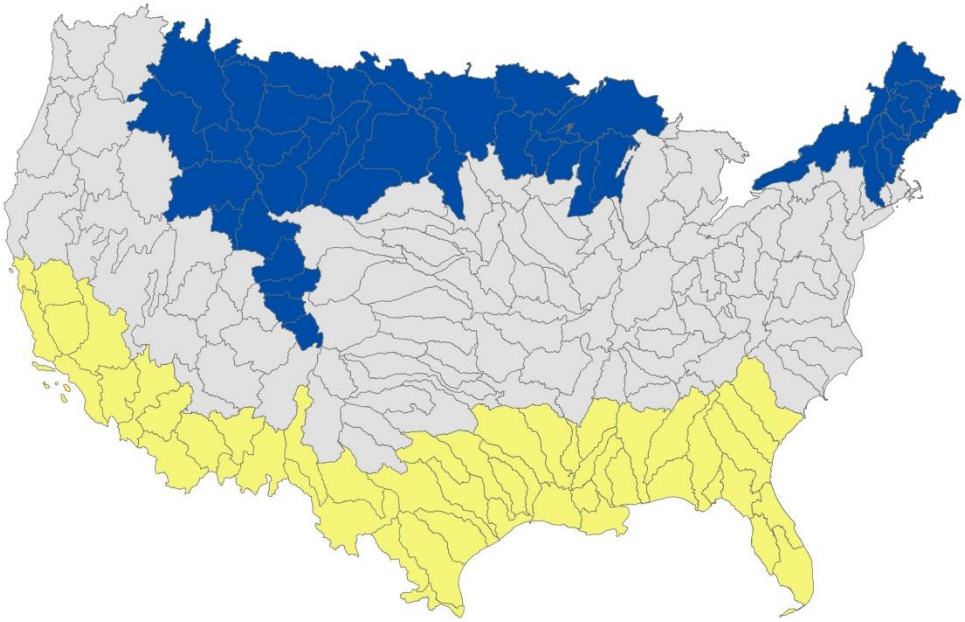
Appendix 4. Dabbling Duck Population Estimation and Temperature Constraints

Temperature constraints, the cumulative number of days above 0˚C and below 0˚C, were derived for each watershed in a given year from the National Oceanic and Atmospheric Administration (NOAA) weather station data. We identified weather stations within 250 km of each watershed centroid (up to 10 closest stations), then calculated the number of days each station had an observed maximum temperature above 0˚C and the number of days with an observed minimum temperature below 0˚C. We then adjusted for the difference in elevation between each weather station and the watershed centroid using the average adiabatic lapse rate temperature correction formula [11]:

$$\Delta T = 6.49^{\circ}C/1000\ m$$

where ΔT represents a change in temperature of 6.49˚C for every 1,000 meters of elevation gained or lost between the weather station location and the watershed centroid. We averaged across the selected weather stations and over 30 years of observations, or all years in a 30-year time period for which data were available. The results of the temperature constraint are presented in
Figure 7.

Figure 7. Watersheds identified with a 30-year median number of 365 days above 0˚C (yellow) and 180 days below 0˚C (blue).



Expected Dabbling Duck Populations

To estimate seasonal expected population that might be present in a watershed, we used two available datasets: bird band and recovery data and the estimated annual continental dabbling duck population available from the U.S. Fish and Wildlife Service from 1998 to 2012. These data were used to estimate

the expected proportion of the continental dabbling duck population present within each watershed in each season. This proportion was then used in concert with the estimated annual continental dabbling duck population to estimate the median population within each watershed. We used a Poisson-Gamma mixture model to model the median population in each watershed. The model structure for the population of dabbling ducks in HUC4 watershed *j* in time *t* and year *k* is estimated using the hierarchical model in Equation 3. The watershed population is modeled using the deterministic model in Equation 4.

Equation 3.

$$[\lambda_{jtk}, \sigma_k^2 | b_{jtk}, t_{tk}, \theta_k] \propto Poisson(\lambda_{jtk}) Gamma\left(\lambda_{jtk} | \frac{g(b,t,\theta)}{\sigma_k^2}, \frac{g(b,t,\theta)}{\sigma_k^2}\right) InverseGamma(\sigma_k^2 | .001, .001)$$

Where $g(b, t, \theta)$ is the expected proportion of the population in watershed *j* in time *t* and year *k.* This is estimated using the band recoveries for watershed *j* in month *t* and year *k* and the total recoveries for North America in month *t* and year *k,* $\left(\frac{b_{jtk}}{t_{tk}}\right)$. This proportion is multiplied by the estimated annual population of dabbling ducks θ_k.

Equation 4.

$$g(b,t,\theta) = \left(\frac{b_{jtk}}{t_{tk}}\right)\theta_k$$

Aggregating the data by month, year, and watershed allowed for including multiple forms of uncertainty in the estimates. These include but are not limited to variation in hunter effort, band reporting, recovery, and banding effort. While we did not model these explicitly, our intent is to provide an estimate of the expected population that may be present during the fall and winter periods. Our assumption is that these uncertainties do not greatly influence the median estimates for the population that might be present. However, this assumption might be violated in regions with low hunter effort or small populations. Our method also assumes that the band recoveries are proportional to the continental population at a given time and represent the spatial distribution of the continental population. This assumption may be violated early in the fall during the teal seasons, before the primary waterfowl seasons have opened. An example is if migration begins early and species such as mallards have begun to move prior to the season opening, which is typically in October. We have attempted to address this variation by including multiple years of data.

Expected Influenza A Prevalence

To estimate seasonal true expected prevalence within watersheds, we used prior dabbling duck surveillance data from 2006 to 2011. The data was aggregated by month and year for each watershed, resulting in the count of sampled birds and the diagnostic test results. Aggregating the data by month, year, and watershed allowed for the inclusion of uncertainty in the estimates from differences in sampling effort and annual variation in prevalence. Our model structure used a binomial sampling distribution that accounted for uncertainty in the diagnostic test process. The expected seasonal prevalence was estimated using Equation 5.

Equation 5.

$$\left[\pi_{jtk}, \mathrm{Se}, \mathrm{Sp}, \theta \,\middle|\, y_{jtk}\right] \propto Binomial(n, g(\pi_{jtk}|\mathrm{Se}, \mathrm{Sp}, \theta))\mathrm{Beta}(Se|\alpha, \beta)\mathrm{Beta}(Sp|\alpha, \beta)Beta(\theta|\alpha, \beta)$$

Equation 6.

$$g(Se, Sp, \theta) = \theta\mathrm{Se} + (1 - \theta)(1 - \mathrm{Sp})$$

Where n is the number of birds tested and $g(Se, Sp, \theta)$ is the estimated true probability of an individual bird is infected with AI. θ is the unknown prevalence of AI in the watershed in the month and year. Se and Sp are the sensitivity and specificity, respectively, of the diagnostic test. We used an uninformative Beta prior for the unknown prevalence:

$$\theta \sim Beta(\alpha = 1, \beta = 1)$$

Uncertainty of sensitivity (Se) and specificity (Sp) was modeled using independent informative beta prior distributions [12] using known estimates for the diagnostic test Se (86.3%) and Sp (99.99%) (Janice Pedersen, personal communication; Mia Torchetti, personal communication). Specifically these priors were:

$$Se \sim Beta(\alpha = 20.833, \beta = 4.148)$$

$$Sp \sim Beta(\alpha = 8.403, \beta = 1.001)$$

Model Fitting and Evaluation

The models for expected population and prevalence were fit for each watershed and biological season using Markov chain Monte Carlo (MCMC) techniques and implemented in WinBUGS software [13]. Posterior inferences were based on 100,000 iterations with a sampling lag of 5, after a burn-in of 20,000 iterations was discarded. We assessed convergence by running five chains from dispersed starting values, observing autocorrelation among samples and investigating the Brooks-Gelman-Rubin convergence statistic [14]. We used the median of the posterior distributions as an estimate for the parameters of interest, and the 2.5 and 97.5 percent points as estimates of the 95 percent credible intervals.

Appendix 5. Morbidity and Mortality Surveillance

The technical guidance in this plan has focused on active surveillance of live or hunter-harvested waterfowl species to achieve the stated surveillance objectives. However, passive surveillance through investigation of morbidity and mortality events in wild birds can provide an ancillary source of information or surveillance stream for detection of influenzas of interest. Within a given HUC, a sample collected from surveillance of morbidity and mortality events should provide at least as much information regarding the detection of novel influenzas of interest as a sample collected from hunter harvested or live-bird surveillance. Thus, samples collected during passive surveillance can potentially be used to achieve active surveillance sample size goals. Additionally, if individuals involved in a morbidity or mortality event have a higher probability of being infected with influenzas of interest, including samples from these events may increase the overall probability of detection of the surveillance program. These types of differential probabilities of infection between apparently healthy and sick individuals have been exploited for other wildlife diseases to maximize the efficiency of detection surveillance efforts [15]. However, passive surveillance is only useful when the goal of a surveillance program is detection of a pathogen. It should not be used when intensity metrics (e.g., prevalence) are the focus.

Including morbidity and mortality surveillance directly in the statistical design of this technical plan is not feasible due to the highly stochastic nature in the occurrence and location of these events and the resulting lack of a useful underlying probability model. Therefore, we recommend investigating all significant morbidity and mortality events, particularly if they occur in regions important to species conservation or domestic poultry production. These passive surveillance efforts should focus on wild bird species known or suspected to be susceptible to infection by influenzas of interest. Preliminary information from the current outbreak suggests that Canada geese and raptor species likely to feed or scavenge on waterfowl may be particularly affected, and therefore should be included in any passive surveillance activities. Additionally, morbidity and mortality events of other groups of birds known to be affected or carry influenzas such as waterfowl, wading birds, geese, and swans all represent potential sources of information regarding the geographic and host range expansion of novel influenza viruses.

We recommend conducting passive surveillance as a complementary effort to the active surveillance activities. The integration of these two efforts will provide the highest likelihood of early detection of influenzas of interest in new geographic locations and wildlife species.

Appendix 6. Wildlife Conservation Concerns

The statistical design of this surveillance plan has drawn from current knowledge about the biology and movements of focal waterfowl species (e.g., dabbling ducks) as well as current knowledge about distribution and diversity of influenza viruses within those species to target specific geographic regions for surveillance. This has provided a comprehensive strategy for maximizing the likelihood of detecting influenzas of interest in waterfowl and establishing key locations for monitoring the intensity of infection post-detection. However, given the breadth of movements of migratory waterfowl, they can potentially carry and spread influenza viruses of concern into locations that are home to species of high conservation value and negatively impact their resident populations. Therefore, in addition to the geographic regions selected for surveillance described previously, specific locations within these regions or additional regions with significant species conservation value should be incorporated into the implementation of this surveillance design.

To identify these specific areas of high conservation value, we recommend consulting with tribal, State, and Federal wildlife biologists with specific knowledge of State or Federal species of conservation concern to identify any deficiencies of the surveillance program's current geographic coverage. Such deficiencies should then be remedied prior to final implementation of the surveillance program. Specific groups of conservation species to target are wetland dependent species, wading bird species, gallinaceous birds, and raptors that potentially feed or scavenge on waterfowl. Examples of such species that may be at risk include:

- Whooping cranes (*Grus americana*): wetland dependent ; maintain specific migratory routes and stop-over sites to target for surveillance;
- Piping plovers (*Charadrius melodus*): wading birds that rely solely on gravel or sandy shoals for nesting and make large migratory movements;
- Attwater's prairie chicken (*Tympanuchus cupido attwateri*), lesser prairie chicken (*Tympanchus pallidicinctus*), and Gunnsion sage grouse (*Centrocercus minimus*): gallinaceous conservation species that likely have a low risk of influenza infection, but may be susceptible if waterfowl stop-over sites are also used by these species;
- California condors (*Gymnogyps californianus*) and peregrine falcons (*Falco peregrinus*): raptor species of high conservation value that potentially feed or scavenge waterfowl where their distributions overlap.

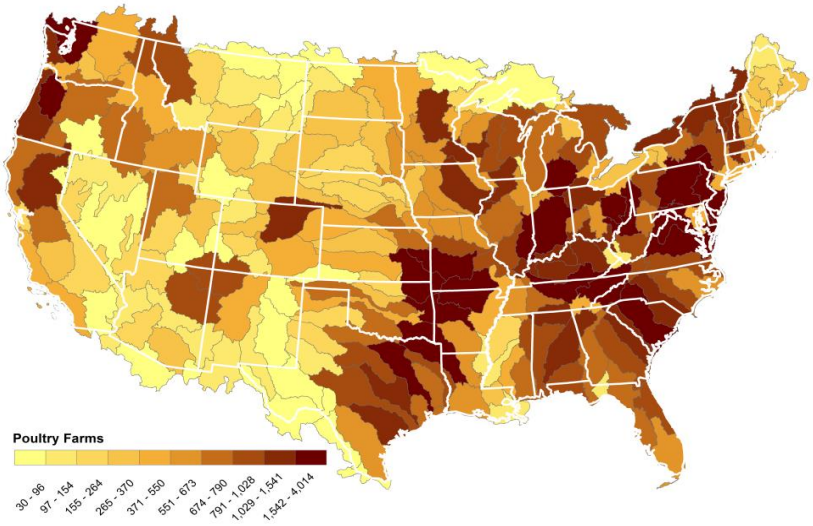
These examples are not exhaustive, but illustrate the important need to address conservation concerns during surveillance activities.

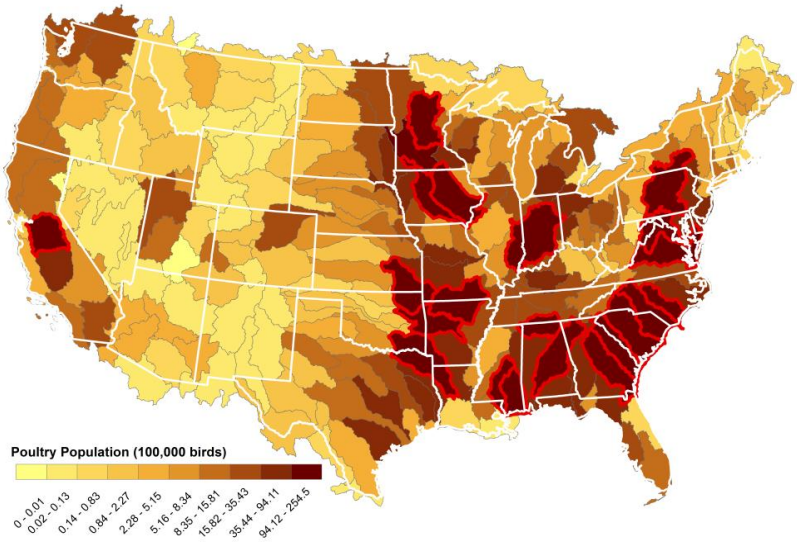
We recommend adding identified areas of conservation concern to the geographic sampling frame if they are not already included within a selected HUC; or, if they are already included in the selection, targeting these locations for focused sampling within the HUC. Including these high-value areas in the surveillance for influenzas of interest will increase the probability of early detection of the pathogen in populations of these critical species, and help mitigate negative impacts while management actions are still feasible.

Appendix 7. Poultry Production Regions

Livestock distribution in the United States (U.S.) can only be mapped at a county-level or worse resolution. This results in conflicts of scale when using farm production data with other data sources such as waterfowl band recovery data. To address this problem we used the Farm Location and Agricultural Production Simulator (FLAPS) that simulates the distribution and populations of individual livestock farms throughout the conterminous U.S. [5]. FLAPS uses iterative proportional-fitting algorithms for the hierarchical structure of the U.S. Census of Agriculture and imputes unpublished state and county-level livestock population totals that are redacted to ensure confidentiality. The FLAPS model is based on a data representing the presence and absence of farms and is used to develop a national-scale distribution model that predicts the distribution of individual farms at a 100 m resolution. Microsimulation algorithms are used to simulate the populations and locations of individual farms using output from the imputed Census of Agriculture dataset and distribution model. The model is validated using aerial photography that confirms the presence or absence of livestock farms at 10,238 locations across the U.S. The model has demonstrated good predictive capacity using cross-validation methods. Verification of the model shows that FLAPS accurately imputes and simulates Census of Agriculture data based on absolute percent difference value of < 0.01% at the state-to-national scale and 0.03% for the individual farm-to-county scale. The FLAPS model and data is freely available at http://flaps.biology.colostate.edu. The watershed level distribution of poultry farms and inventory generated by FLAPS used in this plan is illustrated in Figure 8. These data were used to identify watersheds with an estimated annual average inventory greater than 10 million birds.

Figure 8. Distribution of U.S. poultry production in the United States visualized by the Farm Location and Agricultural Production Simulator (FLAPS). (Top) Number of poultry farms per watershed; (Bottom) Mean number of birds per watershed. Red outline indicates watersheds with an annual average inventory greater than 10 million birds based on the 2012 NASS census.





Appendix 8. 2015 AI Surveillance Plan – Modifications and Feedback

The 2016 Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Migratory Birds in the United States has two slight changes that differentiate it from the 2015 Surveillance Plan. The first is a reduction in overall sample size from approximately 41,000 birds down to 31,550. This sample size reduction reflects a reduction in resources available for wild bird avian influenza surveillance in 2016. The second change was the inclusion of domestic chicken and poultry production areas as a criteria for determining which watersheds to sample. In 2015, sampling occurred across 139 watersheds. Overlaying these sampled watersheds with USDA:VS:CEAH:FLAPS data on domestic chicken and turkey production reveal that the 2015 wild bird surveillance effort covered 73% of areas with more than 10,000,000 chicken and turkeys (all production types). This ensured that wild bird sampling for HPAI was not only in areas that maximized the chance of HPAI detection, but also in areas that could suffer catastrophic losses in the event of HPAI introduction into domestic poultry operations. In 2016, the resources available allow for wild bird HPAI surveillance in approximately 105 watersheds. In order to counter this reduced coverage, areas with domestic chicken and turkey production exceeding 10,000,000 birds were included in selection criteria. This has allowed wild bird HPAI surveillance in 2016 to cover a similar level (68%) of high density commercial poultry areas as was covered in 2015. The trade-off is that states with concentrations of domestic poultry production end up with large target numbers for wild bird samples.

Additional feedback received on the 2015 Surveillance Plan for Highly Pathogenic Avian Influenza in Wild Migratory Birds in the United States has been incorporated into the 2016 Surveillance Plan. Input from Wildlife Services personnel and state natural resource agencies regarding watersheds that were unable to be sampled in 2015 has been incorporated into the 2016 plan. This resulted in modifications to surveillance in 19 states; however, the majority of watersheds remained unchanged, providing the ability to investigate changed in AI within sampled watersheds through time.

It was also suggested in 2015 that wild bird HPAI surveillance should be built around the areas of greatest continental significance for ducks, geese, and swans identified in the North American Waterfowl Management Plan (NAWMP). NAWMP is a collaboration between the US, Canada, and Mexico to manage waterfowl through habitation protection, restoration, and enhancement. Calculations on surveillance activities in 2015 and 2016 demonstrate that 28 out of 30 areas (with boundaries in the continental US and Alaska) classified as “significant” for ducks, geese, and swans are already covered by the surveillance plans. There are known shortcomings to the data used to identify the areas of significance for ducks, geese, and swans and so it will not be built into watershed selection metrics, but the existing coverage of the surveillance plans demonstrate that the data used to identify targeted sample areas already incorporated areas known to have high waterfowl concentrations.

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