

Chapter 12

Targeted Surveillance for Highly Pathogenic Avian Influenza in Migratory Waterfowl across the Conterminous United States

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ABSTRACT

Introduction of Asian strain H5N1 Highly Pathogenic avian influenza via waterfowl migration is one potential route of entry into the United States. In conjunction with state, tribe, and laboratory partners, the United States Department of Agriculture collected and tested 124,603 wild bird samples in 2006 as part of a national surveillance effort. A sampling plan was devised to increase the probability of detecting Asian strain H5N1 at a national scale. Band recovery data were used to identify and prioritize sampling for wild migratory waterfowl, resulting in spatially targeted sampling recommendations focused on areas with high numbers of recoveries. We also compared the spatial and temporal distribution of the 2006 cloacal and fecal waterfowl sampling effort to the bird banding recovery data and found concordance between the two. Finally, we present improvements made to the 2007 fecal sampling component of the surveillance plan and suggest further improvements for future sampling.

Key Words: Avian Influenza, Migratory Birds, Disease Surveillance, Bird Banding Data, Sample Design

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INTRODUCTION

Waterfowl (*Anatidae*), with subfamilies dabbling ducks (*Anatinae*), diving ducks (*Aythiinae*), and geese (*Anserinae*), along with members of the family Charadriiformes, appear to be the primary natural reservoir for type-A influenzas (AI) [1,2], which have been implicated as the progenitor of at least one human flu pandemic in 1918 [3]. Because influenza viruses can mutate and acquire the ability to infect and replicate within human host cells [4], there remains concern about the pandemic potential of highly pathogenic Asian strain H5N1 avian influenza (HPAI H5N1). The recent emergence of HPAI H5N1 in domestic poultry and humans in several countries resulted in an initiative by the United States (U.S.) government to establish a wild bird surveillance program [5] in 2006 to detect introduction of this pathogen. To enhance the surveillance effort we developed an approach that prioritizes sampling locations across the U. S. with respect to the collection of wild bird samples. Here, we describe the process behind the development of national-scale sampling to detect introduction of HPAI H5N1 and evaluate the data collected from the 2006 sampling effort to make refinements for sampling in future years.

We focused on migratory waterfowl, including dabbling ducks, swans and geese, which are thought to be the primary functional groups responsible for maintaining AI enzootic cycles and large-scale movements of AI virus [1]. Further, because some migratory waterfowl appear to be mildly affected or unaffected by HPAI H5N1 [6], certain species may act as competent long distance dispersers of this strain of the virus. In 2006, 13.8 million waterfowl and 3.6 million geese were harvested across the U.S. [7], a portion of which were migrants that originated from breeding grounds outside the conterminous U.S. Our sampling recommendations focused on locations within the conterminous U.S. where large numbers of waterfowl commingle on breeding grounds with birds originating in Southeast Asia and Europe, where HPAI H5N1 may be endemic in wild bird populations [8] that represent potential sources of introduction of HPAI H5N1 into the U.S.

In this paper, we first state the assumptions behind our proposed improvements to the sampling design and describe how we identified recommended sampling locations within each state. We follow with a comparison of the band recovery data used to identify sampling locations and the 2006 sampling effort. We conclude with the improved sampling guidelines implemented in the collection of fecal samples in 2007 and provide recommendations on future sampling refinements.

METHODS

Design Assumptions and Deductions

We developed our sampling design based on three assumptions. The first assumption was that migratory waterfowl could transport infectious HPAI H5N1 into North America. Because some wide-ranging migratory waterfowl may become infected and shed this virus without exhibiting morbidity or mortality [6] the potential for wide spread dispersal of the pathogen by wild birds cannot be discounted [1]. Experimental infection in mallards [8] has shown variability in the pathogenicity of HPAI H5N1 isolates for this species. Some virus isolates that were apparently nonpathogenic in mallards were replicated and transmitted efficiently to naïve contacts from infected birds. This suggests that infected ducks

with minimal signs of disease could spread the virus to other wild birds, poultry, or possibly humans. Our sampling design targeted sample collection from migratory waterfowl due to this potential for some waterfowl species to show resistance to certain strains of HPAI H5N1. Although shorebirds (Scolopacidae) have been found to harbor avian influenza [9], practical sampling constraints, such as trapping and handling methods, limit use of this group in a nation-wide sampling effort. In addition, there are no national-scale data on the distribution of shorebirds comparable to those existing for waterfowl.

Our second assumption was that there exist three major routes of introduction of HPAI H5N1 into North America by migratory waterfowl. Alaska and northeast Asia contain breeding grounds where species that winter in Southeast Asia, where HPAI H5N1 has been found in wild birds, converge with birds from the conterminous U.S. [10,11] and Canada. Similarly, migrant populations that breed on Baffin Island, along with those that winter in Central and South America, provide potential opportunities for transmission of HPAI H5N1 virus to North American waterfowl from Europe and Central/South America, respectively [12].

Our third assumption was that cloacal and fecal sampling was sufficient for determining if locations had HPAI H5N1 infected birds present at or near the time of sampling. Maintenance and transmission of AI virus in wild bird populations depends on fecal/oral transmission [13,14] from virus concentrated in fecal matter and excreted into water. Experimental infection of waterfowl has shown the presence of HPAI H5N1 in cloacal samples [6,15]. A reliable assay for detecting viral RNA in feces using rRT-PCR has recently been developed at the USDA-APHIS-Wildlife Services National Wildlife Research Center (unpublished data). Finally, based on rRT-PCR testing, fecal sampling has been shown to be comparable to cloacal sampling for determining the presence of several type-A influenzas [16].

If HPAI H5N1 does arrive in the United States, formal sampling theory could be used to estimate prevalence, spread, and occurrence probabilities, all of which would help inform the sampling design. In the absence of such information we focused on using the number of waterfowl originating from three broad geographic areas to identify sampling locations and we made no *a priori* assumptions regarding which geographic pathway for introduction was most likely.

Target and Sampled Population

Sampling from waterfowl across the conterminous U.S. should be guided in part by information on concentrations and seasonal movement patterns to increase the probability of detecting an introduction of HPAI H5N1. Sampling should also target locations and times when waterfowl are found in high numbers. However, in designing a sampling protocol it is important to consider practical constraints, such as access to sampling locations and data collection resources, incomplete knowledge about the distribution and movement patterns of waterfowl across the United States, and an absence of detailed information on host-pathogen dynamics of HPAI H5N1 in wild birds. Although data do not exist to develop a complete mapping of the distribution of waterfowl populations at a national scale, information on the spatiotemporal distributions for several species of interest can be acquired from band recovery data [17]. We considered the target population to include all waterfowl concentration areas in the conterminous U.S. used as overwintering grounds by birds that breed outside this area during the summer.

Knowledge of relative abundance, in terms of the number of bands recovered from a given location, cannot be the sole consideration in determining how to allocate sampling effort across the lower forty-eight states. Because of this, we also used the band recovery data to identify birds originally banded outside the conterminous U.S. and subsequently harvested within it. This allowed us to identify locations in the lower forty-eight states having relatively large numbers of bands recovered that were placed on individuals originating outside of this area. We defined the sampled population as all accessible locations where banded waterfowl of interest have been recovered in the conterminous states; including federal, state, and private land. Within this sampled population, sampling units are ranked in each state by the number of dabbling ducks, goose, and swan bands recovered from 1990 through 2005 (15 years) from the 10-minute band recovery blocks making up each sample unit. For all analyses we limited the band recovery data to this time period of intermediate length to balance annual variability due to environmental fluctuations associated with short time series versus bias associated with including older data, due to land use change such as habitat conversion.

Use of Band Recovery Data to Define Sampling Units

One critical attribute of our design is that bird banding data provide both the origin of the banded bird and the location where the bird was recovered, information many field personnel do not have access to. The data used were from waterfowl banded on their breeding grounds in northern latitudes (e.g., Alaska, northeast Asia, Canada, and Greenland) and then recovered, primarily through hunter harvest, during the fall migration and on their wintering grounds. Locations of band recoveries are reported at the 10 minute block scale of longitude and latitude, which is an area of approximately 100 km² in the conterminous states. Data from birds banded in the conterminous U.S. that subsequently migrate to countries lying south of the U.S. in the fall were too sparse, both spatially and temporally, to be useful in this effort, and hence were excluded from analyses. However, we recognized that these migrants may be important to consider in the future should the HPAI H5N1 become established in the western hemisphere. Finally, we did not include band recoveries from shorebirds and diving ducks because the band recovery data were too sparse [18] to be applied practically at the national scale.

The primary assumption underlying the use of these data to define waterfowl concentration areas on a national scale is that hunters select harvest locations and times corresponding to the presence of large numbers of waterfowl. In addition, band recovery data primarily represent the location of birds during the hunting season, which in the case of waterfowl is typically from September through February, a time period that coincides with peak migration into the conterminous states of the U.S. Because reporting rates are not expected to be uniform in time, using national scale data from the previous 15 years may smooth over much of this variability. Conditional upon the assumptions and data characteristics, band recovery data likely offer the best source of information on the spatial and temporal distribution of species of interest across the conterminous United States.

Sampling units were defined as collections of 10-minute band recovery blocks within refuge and management area boundaries where large numbers of waterfowl are harvested. Typically, a sample unit would be defined as a discrete management unit, for example a single U.S. Fish and Wildlife National Wildlife Refuge. The band recovery data were inter-

sected with maps of National Wildlife Refuges and State Management Areas. We then summed the total number of band recoveries from the previous fifteen years from all 10-minute blocks encompassed by a refuge or management area's boundaries. We used two criteria to ordinally rank the top twenty sampling units (refuges) based on band recovery counts. First, the top ten refuges and management areas in a state were identified as those having the greatest number of bands originating in Alaska, Wrangel Island, or northeast Asia (upper Pacific flyway), or Greenland, Baffin Island, and far Eastern Canada (upper Atlantic flyway). The next grouping of ten refuges and management areas were then identified as those having the greatest number of bands originating in the remainder of central Canada (upper Central flyways). These top twenty sampling units (refuges) in each state were provided to USDA-APHIS-Wildlife Services personnel to assist in planning sample collection. In addition, a list of all remaining refuges within each state, ranked in order of band recovery abundance from functional groups of interest, was produced to augment sampling from the top twenty locations should resources be available.

AI Samples Collected in 2006

Because the route by which Asian strain H5N1 might enter the U.S. is not known, the USDA, in conjunction with state partners and tribes, collected 124,603 fecal, cloacal, and tracheal swab samples from all 50 states and U.S. territories between April 20 and December 31, 2006. Contrary to previous assertions [19] regarding the focus and extent of avian influenza sampling in 2006, personnel from USDA-APHIS-Wildlife Services and its partners (e.g., state wildlife agencies and tribes) used the national strategic plan [5] to guide sampling, with a goal of collecting a minimum of 1000 fecal samples and between 1250 and 3000 cloacal samples within each state. This effort resulted in the collection of 50,184 fecal samples and 74,419 cloacal samples. Sampling in the conterminous United States began in April from resident and breeding ground waterfowl populations and continued through December, while migrants were arriving from northern breeding grounds and becoming established on their wintering grounds. Sampling locations and times where species of interest could be found were identified through discussions between state wildlife agencies and USDA-APHIS-Wildlife Services personnel, but did not include prior information from band recovery data.

Comparison of Band Recoveries and 2006 Sampling

We compared the locations and timing of sampling chosen by field personnel who did not have prior knowledge of band recovery locations in 2006 with our subsequent design based on the band recovery analysis. To accomplish this, we made statistical and graphical comparisons of the spatial and temporal distributions of wild birds sampled for AI in 2006 and the band recovery data aggregated over the 15-year period (1990 through 2005). We characterized differences in the number of bands recovered over the previous fifteen years from blocks with and without wild bird AI samples collected in 2006. In addition, we used χ^2 tests to determine the relationship between band recoveries and wild bird sampling locations based on three different threshold values for the number of bands recovered in a 10-minute block. For each threshold value, we made 2×2 χ^2 comparisons of blocks with and without band recoveries and blocks with and without sampled birds. We graphically assessed the temporal relationship between band recoveries and wild birds sampled for AI by producing a map of both data sets at the one-degree block scale.

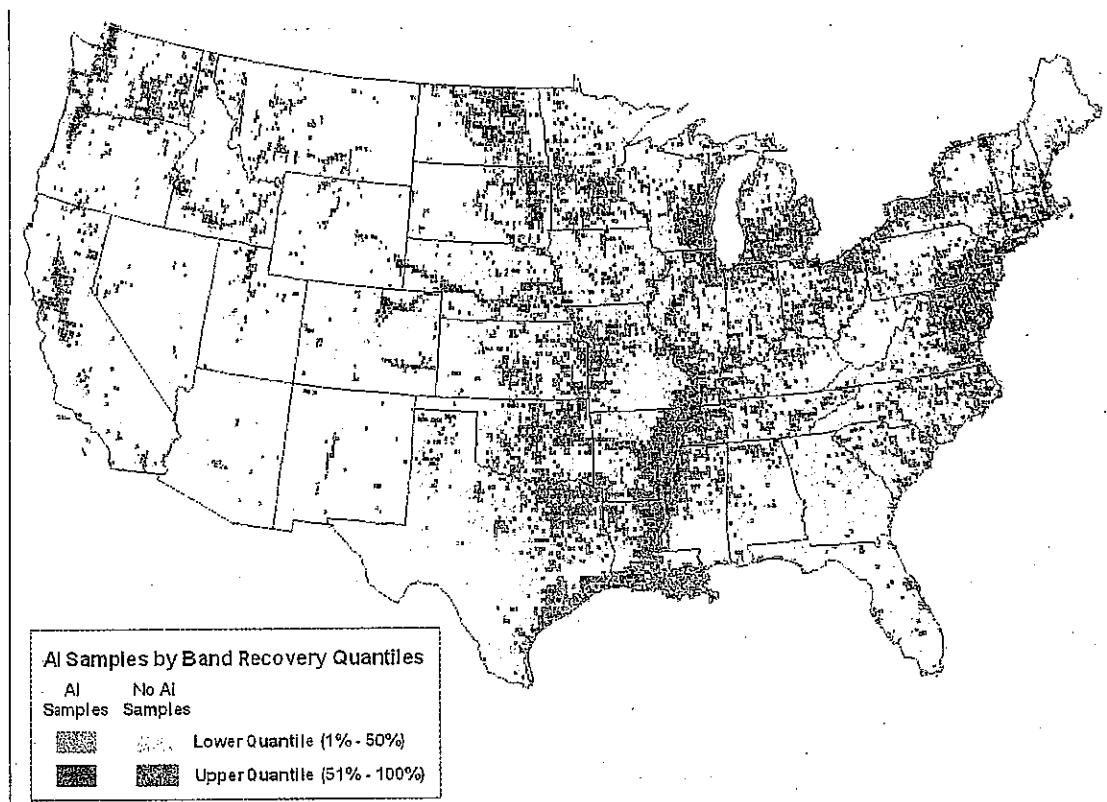


Figure 1: Comparison of locations where wild birds and their feces were sampled by USDA personnel for avian influenza (AI) in 2006 and numbers of waterfowl band recoveries in 10-minute blocks across the contiguous 48 states in the U.S. Numbers of band recoveries from 1990 through 2005 are grouped by upper and lower 50% quantiles. Blocks where AI samples were collected in 2006 are indicated in dark red for upper quantile band recovery blocks and light red for lower quantile band recovery blocks. Gray areas represent recovery blocks without AI sampling in 2006. Note the increased sampling for AI in recovery blocks where the number of band recoveries occurs in the upper quantile.

RESULTS

There were a total of 30,478 10-minute blocks across the entire contiguous 48 states, with 14,797 (48.5%) of these blocks containing band recoveries. In comparison, 85% of the 1,886 10-minute blocks where AI samples were collected also had band recoveries (Figure 1). Considering only blocks with band recoveries, the number of recoveries was greater for 10-minute blocks sampled for AI ($\bar{x} = 42.71$, $SE = 2.43$, $n = 1,642$) than for blocks not sampled for AI ($\bar{x} = 11.61$, $SE = 0.27$, $n = 14,797$). Based on χ^2 tests, AI samples were preferentially collected from locations with relatively high numbers of band recoveries for all the threshold values we examined (Table 1). In addition, field personnel sampled from band recovery blocks having relatively large numbers of band recoveries of migrants of interest and in a manner that provided representative coverage of these locations across the contiguous 48 states of the U.S. even though they had no prior knowledge of band recovery locations (Figure 1).

There was also a positive association between bi-weekly frequency distributions for AI samples and the previous fifteen years of band recoveries (Figure 2) and a positive association between the spatial distribution of peak AI sampling and band recovery times (Figure

Table 1. Results of χ^2 tests comparing 10-minute blocks which were sampled and not sampled for avian influenza (AI) in relation to three different threshold levels of numbers of band recoveries within blocks; blocks with 0 recoveries compared to those with ≥ 1 recoveries, blocks with < 10 versus ≥ 10 recoveries, and blocks with < 100 versus ≥ 100 band recoveries. OR = Odds Ratio; if rows and columns are independent the expected OR = 1. 95% CI = 95% confidence interval.

Threshold Comparison ¹	Proportion of Blocks		n	p-value	Odds Ratio (95% CI)
	AI Sampled	Not AI Sampled			
Blocks ≥ 1 BR	0.11	0.89	14797	<0.001	7.90 (6.88, 9.10)
Blocks 0 BR	0.02	0.98	15681		
Blocks ≥ 10 BR	0.22	0.78	3954	<0.001	7.43 (6.73, 8.20)
Blocks < 10 BR	0.04	0.96	26524		
Blocks ≥ 100 BR	0.45	0.55	399	<0.001	13.38 (10.85, 26.47)
Blocks < 100 BR	0.06	0.94	30079		

¹Threshold comparisons are for 1) 10-minute blocks with at least 1 band recovery (BR) versus blocks with no band recoveries, 2) 10-minute blocks with > 10 BR versus < 10 BR, and 3) 10-minute blocks with > 100 BR versus < 100 BR.

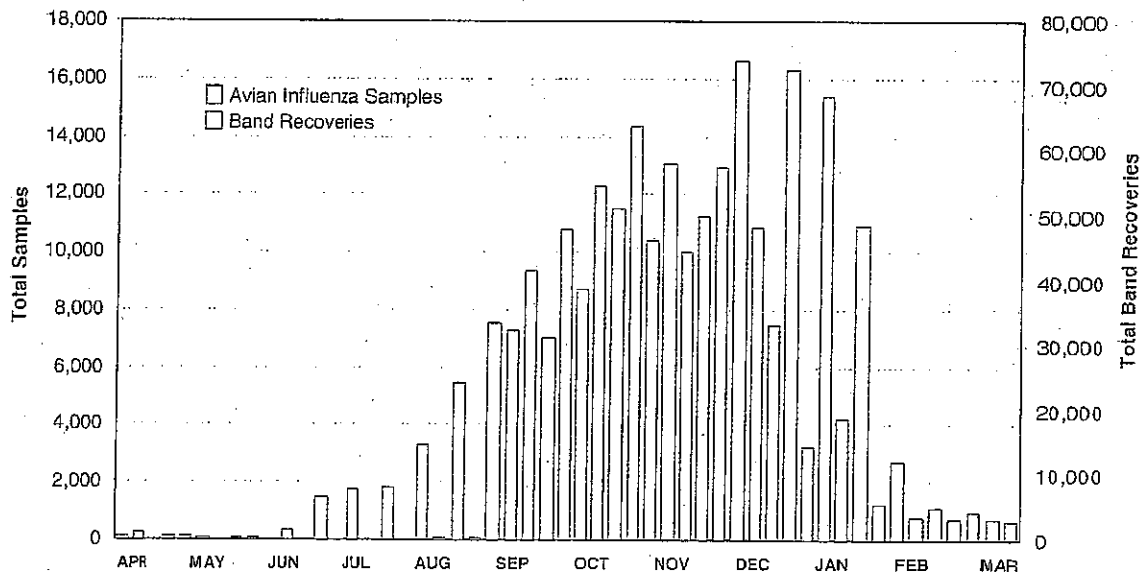


Figure 2: Monthly comparison of the timing of avian influenza samples collected by USDA in 2006 with peak band recoveries from 10-minute blocks summed across the years 1990 through 2005.

3). Thus, the statistical analysis and mapped associations suggest good concordance between spatial and temporal AI sampling in 2006 and band recoveries from the previous 15 years.

Improvements to Sampling Design for 2007

Based on the ability of field personnel to sample for AI from most areas with high band recoveries, we ultimately recommended a two-stage sampling approach. First, we allocated the total number of samples to be collected within a state proportionate to the number of bands recovered from birds potentially originating outside the U.S. Second, we target-

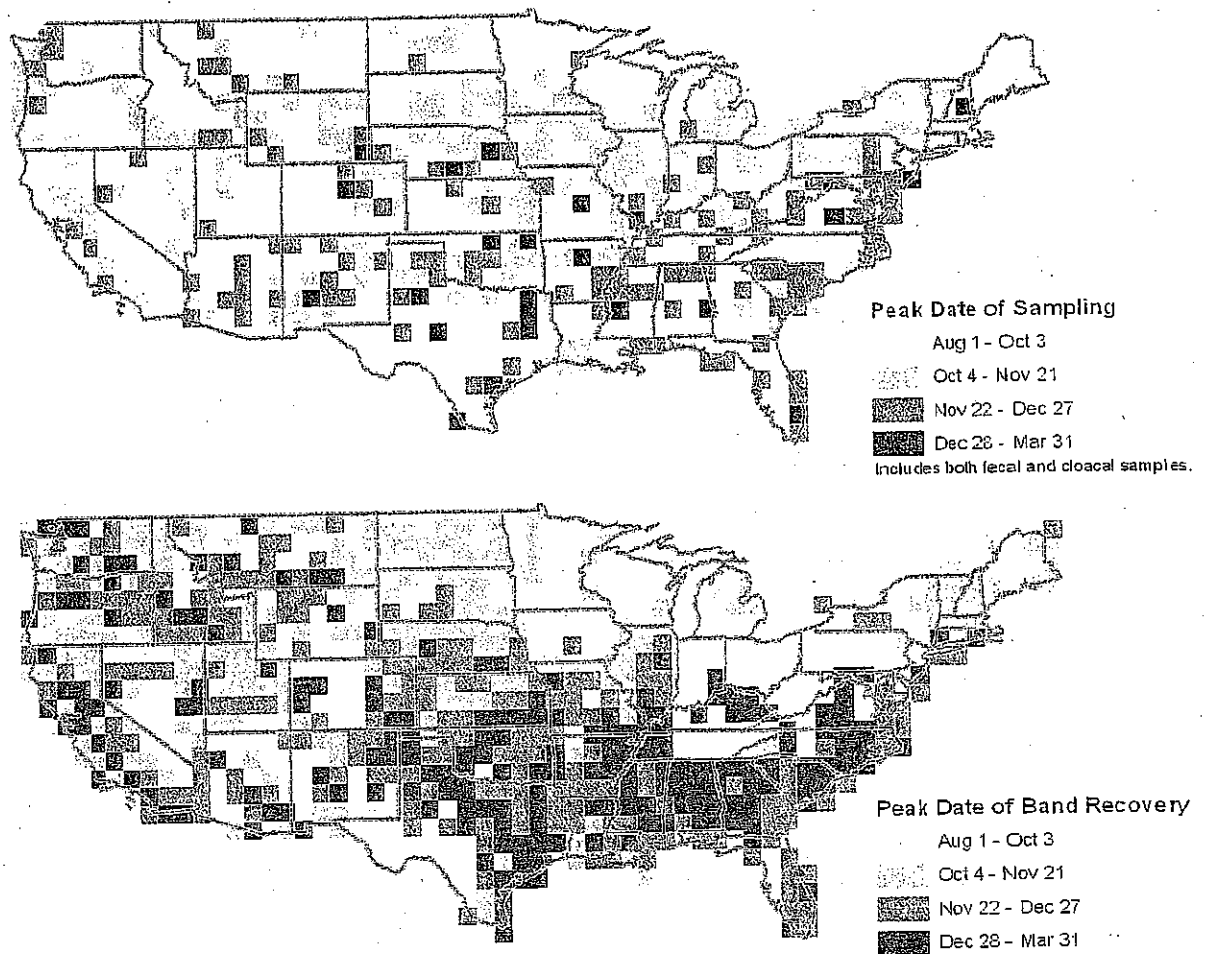


Figure 3: Maps of the temporal distribution of AI sample collection from wild birds in 2006 and waterfowl band recovery numbers for the years 1990 through 2005 at the 1-degree block scale. Note the overall concordance between timing of sample collection and band recovery.

ed band recovery blocks within the same state based on the number of bands recovered in each block. The original design using refuges and management areas with high numbers of band recoveries was obviated by the ability of field personnel to sample from those blocks, independent of land ownership and other limitations we perceived as problems. However, that design might still be useful for future national scale sampling efforts.

In 2006, USDA-APHIS-Wildlife Services personnel, and a few state wildlife agencies in the Pacific flyway, were directed to collect fecal samples within each state. For the 2007 sample year (1 April 2007 through 31 March 2008), USDA-APHIS-Wildlife Services implemented a state-level plan (Table 2) to distribute 25,000 fecal samples in Alaska and across 30 states in the conterminous U.S. based on 4 categories of origin for the band recoveries, which represented potential points of origin for HPAI H5N1 to enter the U. S. via wild migratory birds. These categories of the origin where birds were initially banded, were 1) the Upper Atlantic Flyway (Greenland, Europe, Queen Elizabeth Islands, Baffin Island, and the Labrador sea coast of Newfoundland and Quebec), 2) the Upper Pacific Flyway (Alaska, northeast Asia, and Wrangel Island), 3) the Upper Midcontinent Flyway (Mississippi and Central flyways within Canada), and 4) Central and South America

Table 2. Recommended number of fecal samples to be allocated to each of 31 selected states in the U.S. based on the proportion of bands recovered in each state that originated from locations outside the conterminous U.S. As a potential source of HPAI H5N1, Alaska was allocated 1,500 samples while the remaining 23,500 samples were allocated to 30 of the 48 contiguous states based on band recovery data from the different potential source areas for introduction of HPAI H5N1.

State	No. of Samples	% of Samples
California	2089	8.4%
Alaska	1500	6.0%
Washington	1215	4.9%
Oregon	1076	4.3%
Pennsylvania	1005	4.0%
Delaware	953	3.8%
Arkansas	902	3.6%
New Jersey	865	3.5%
Maryland	860	3.4%
Texas	853	3.4%
North Dakota	848	3.4%
New York	846	3.4%
Colorado	781	3.1%
South Dakota	753	3.0%
Louisiana	710	2.8%
Minnesota	682	2.7%
Nebraska	676	2.7%
Montana	650	2.6%
Utah	649	2.6%
Illinois	645	2.6%
Iowa	604	2.4%
Michigan	601	2.4%
Missouri	599	2.4%
Kansas	594	2.4%
North Carolina	590	2.4%
Wisconsin	589	2.4%
Mississippi	580	2.3%
Idaho	578	2.3%
Tennessee	576	2.3%
Ohio	567	2.3%
Oklahoma	563	2.3%
TOTAL	25000	100.0%

(including birds banded in the U. S. and subsequently recovered in Central or South America). As a potential point of origin, Alaska was considered separately and assigned 1, 500 samples. The 30 states selected for sampling in the conterminous U.S. were derived by sorting all states by the number of recoveries within each category of origin and including states until 75 percent of the recoveries within each origin category were accounted for. Number of samples was then allocated to each state by summing and then normalizing the percentages to derive a relative weight for each state. Each state was initially assigned 500 samples to ensure adequate sampling and the relative weights were then used to allocate additional samples. Although the block-level allocation within each state was not implemented, it remains a goal for fecal sampling in the 2008 sample year.

DISCUSSION

The concordance between the location and timing of birds sampled for AI in 2006 and the total number of bands recovered in a 10-minute block over a 15-year period implies strong knowledge on the part of field personnel regarding the timing and locations of concentrations of waterfowl across the conterminous United States. Given this strong local knowledge of field personnel, it might be possible to further refine the spatial allocation of sampling to occur at the scale of the 10-minute blocks having the highest concentrations of waterfowl that breed outside the conterminous U.S.

Although we concluded that there is agreement at the national scale between the 2006 sample locations and the recommendation to sample from refuges with high numbers of band recoveries, field personnel were not restricted from access to other waterfowl concentration areas for sampling. For example, in Louisiana (Figure 4), large concentrations of ducks and geese that breed in Alaska winter in the southwestern part of the state where

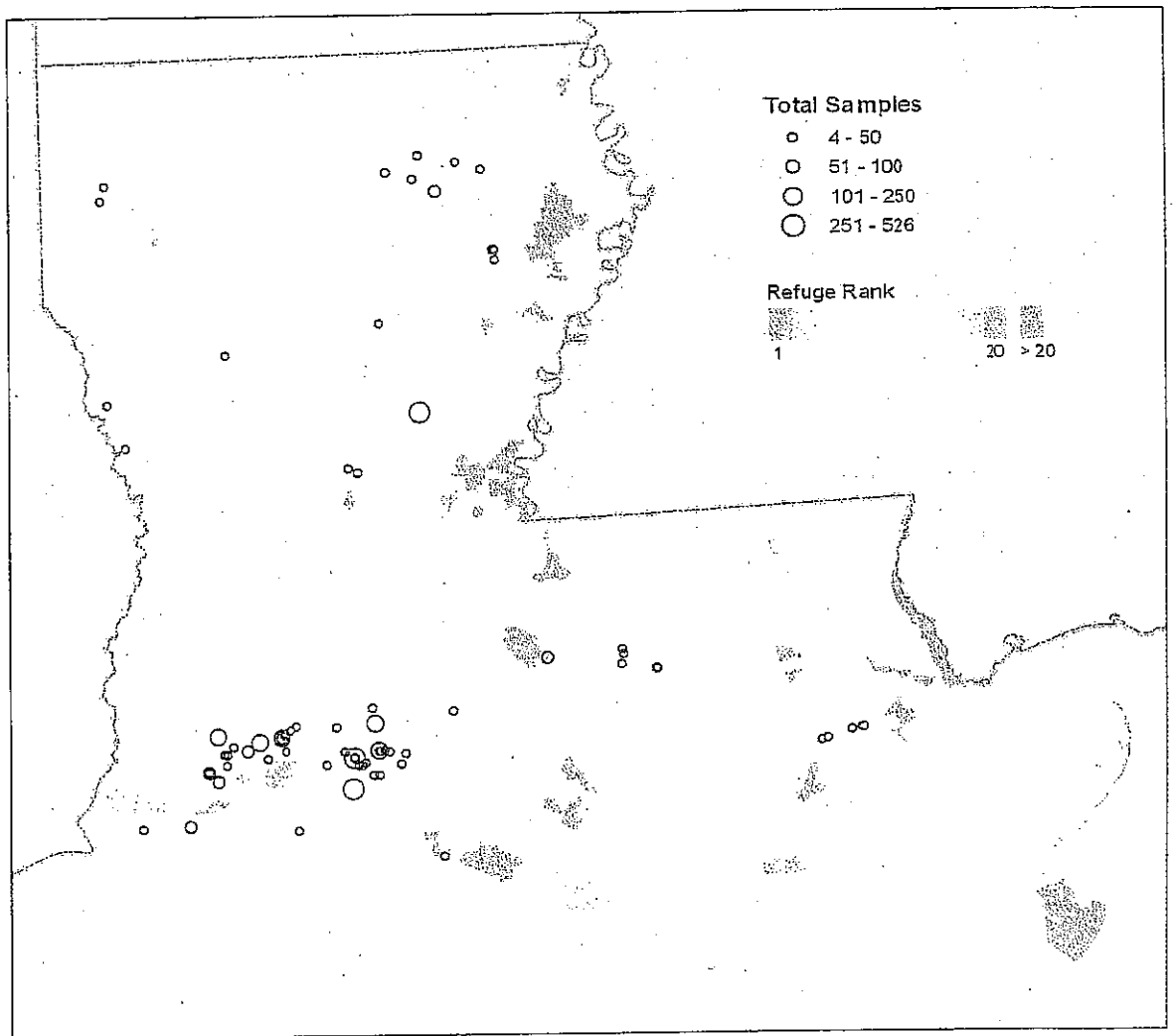


Figure 4: Locations of Louisiana refuges ranked in order of sampling importance for avian influenza (AI) based on the number of waterfowl bands recovered from each refuge relative to locations sampled for AI by field personnel. This type of map was produced for all 48 states in the conterminous U.S.

most bands were recovered. Thus the committee recommended concentrating sampling in refuges and management areas in this part of the state, which corresponds to the portion of the state where the majority of birds sampled for AI in 2006 occurred based on the knowledge of field personnel. However, a number of the samples were collected in areas outside of refuge boundaries, indicating that field personnel were not restricted solely to sampling public refuges.

Within the United States, there exist locations where high-concentration waterfowl areas are represented by relatively few band recovery data. Feedback from state waterfowl biologists and field personnel in each state regarding the relative contribution of these locations to total waterfowl numbers will be critical to future refinements of this design. Understanding where there are gaps in our knowledge with regard to prioritizing sampling locations is a necessary component of any successful surveillance program. In addition, reliable data on the movement of birds between North and South America should be sought out and included in future designs, particularly in the event Asian strain H5N1 HPAI is introduced into the western hemisphere by countries lying to the south of the U.S.

Although sampling for AI as described in the U.S. Interagency Strategic Plan [5] is targeted primarily at first introduction of HPAI H5N1 by migrant waterfowl entering the U.S. after breeding, samples are also being collected from resident species prior to arrival of summer migrant waterfowl from outside the conterminous United States. For these resident populations the highest AI infection rates can be expected to occur in spring [20] with the production of young that are naïve to avian influenza viruses. Because it might be possible for HPAI H5N1 to go undetected in certain species it remains important to have a portion of the sampling effort targeted at resident populations. Additionally, the goal of early detection in wild birds allows surveillance to detect spill-over that may occur from illegal poultry shipments, illegal wild bird trade, and legal trading and importing pathways.

Experimental infections suggest that HPAI H5N1 is more readily detected [15] in oropharyngeal swabs than cloacal swabs of infected Canada Geese, probably because HPAI H5N1 is excreted in greater concentrations orally than via the cloacal route of shedding [6] for several species of North American waterfowl. However, these same studies have demonstrated that detectable levels of HPAI H5N1 are recoverable using cloacal and fecal sampling [6,15]. Complementing the current surveillance efforts that use cloacal and fecal sampling with oropharyngeal or tracheal sampling may result in a higher likelihood of detecting HPAI H5N1 on a per sample basis; however the total number of samples that could be collected would be substantially diminished. By using a mixed strategy for sampling it might be possible to sample a large enough number of birds to ensure that detection thresholds are met while introducing a more sensitive method for detecting this particular strain.

Surveillance for HPAI H5N1 in wild birds across much of Europe [21, 22] has included both cloacal sampling and morbidity and mortality events where birds are observed exhibiting clinical symptoms or are found dead. However, the exclusive use of morbidity and mortality events (i.e., passive surveillance) suffers from a strong bias towards only detecting virus in hosts that are most susceptible to disease induced morbidity or mortality [22]. It is increasingly recognized that there exists variation in susceptibility among wild bird host species [6,23], and that some species may exhibit minimal or no clinical signs while maintaining the capacity to amplify and spread HPAI H5N1 [6,23,24,25]. Further, it

has been shown that exposure of Canada Geese to an endemic North American H5N2 followed by exposure to a Vietnamese H5N1 HPAI strain allowed some individuals to amplify the H5N1 virus with minimal clinical signs, suggesting some level of cross immunity [15]. For these reasons a surveillance strategy that includes sampling of apparently healthy birds remains crucial to the goal of detecting introduction and movement of this pathogen into North America by migratory species of interest.

Finally, because conditions that trigger migration into and through the conterminous United States are variable in time and space, the exact timing and intensity of sampling must be determined by personnel within each state who are familiar with local patterns and have the ability to adapt sampling efforts to changing conditions. Thus, although this work has identified locations and times where targeted surveillance might provide the best trade-off between resources and first detection of HPAI H5N1, the specific time and location for data collection is a decision that should be guided by experienced field personnel.

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