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Chapter 13

Development of a National Early Detection System for Highly Pathogenic Avian Influenza in Wild Birds in the United States of America

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IMPORTANCE OF NATIONAL DISEASE SURVEILLANCE SYSTEMS

It is generally recognized that countries conducting comprehensive disease surveillance in wildlife populations are more likely to understand the epidemiology of specific infectious pathogens and zoonotic disease outbreaks (1, 2). These countries are better equipped and prepared to develop solutions that will protect humans, agriculture, and wildlife. Consequently, active surveillance for diseases of animal or public health concern in wildlife is particularly beneficial to national and international interests. The World Organization for Animal Health (OIE) encourages all countries to develop and maintain wildlife disease surveillance systems, which complement and support human health and agricultural animal disease programs. These systems should be capable of detecting unusual disease events, involve proficient diagnostic laboratories and international reference laboratories, use molecular characterization tools, and have communication protocols in place for reporting results (3).

Disease surveillance systems should be designed to systematically collect relevant ecological and health-related data on the species of concern, efficiently assimilate and analyze data, and disseminate results of the analysis in an appropriate time frame to decision makers so that management actions can be rapidly implemented. It is this latter characteristic that distinguishes surveillance systems from the more commonly implemented monitoring

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systems in the study of wildlife diseases. Monitoring systems are developed to assess the health and disease status of a population and often are used to increase understanding of pathogens and their epidemiology in the environment. Alternatively, disease surveillance systems are more active by definition and incorporate thresholds for disease prevalence or incidence, above which, predefined management actions will be implemented (4, 5). Effective monitoring and surveillance systems (MOSS) form the foundation of all animal health programs and the most important component in strategies for preventing emerging infectious diseases.

There are numerous reasons for developing a MOSS (4, 6, 7, 8, 9). Most MOSS implemented for wildlife species have been designed to evaluate the progress of a disease control or eradication program (e.g., bovine tuberculosis, chronic wasting disease, rabies). Recently though, increased awareness by the international community of the important role wildlife species can have in the emergence and spread of infectious diseases, has led to recommendations that wildlife resources be included in nationally coordinated animal and human health surveillance programs (3, 9). In particular, there is a need for wildlife to be incorporated into early warning systems for emerging infectious diseases (10). Early warning systems are a specific type of MOSS focused on detecting the introduction of a pathogen into a novel population or region, and rapidly implementing control and communication strategies in target populations if an introduction is detected.

Relatively few nationally coordinated MOSS for wildlife, and fewer international ones, have been implemented. This paucity of wildlife MOSS is directly related to the difficulties associated with working with free-ranging animals, such as obtaining diagnostic samples and quantifying disease in a wildlife population (11). If biological samples are obtained, storage and transport from remote locations are challenging, especially if testing protocols require maintenance of a cold chain. The lack of validated diagnostic tests on wild species and laboratories skilled in conducting such tests also are limited (12, 13). All of these difficulties are further complicated by varying perceptions of ownership and management jurisdictions of wildlife (14).

Here we describe the development and implementation of a nationally coordinated, risk-based MOSS for detecting highly pathogenic avian influenza virus (HPAIV) into the USA through wild birds. We also demonstrate how collaboration with Canada and Mexico expanded each country's programs into an effective North American early detection system.

DEVELOPMENT OF A RISK-BASED MOSS IN WILD BIRDS FOR HPAI H5N1

At the direction of the USA Homeland Security Council, the United States Departments of Agriculture (USDA) and Interior (DOI) convened a working group comprised of wildlife biologists, veterinarians, virologists, and public health experts to develop and Interagency Strategic Plan (Strategic Plan) to address the threat of a potential introduction of HPAIV H5N1 into the USA by wild migratory birds (15). This Strategic Plan described the essential components of a unified national early detection system (NEDS) for HPAIV in migratory birds. While the immediate concern was a potential introduction of the H5N1 subtype into the USA, the NEDS was developed to detect any HPAIV in wild birds, and increase knowledge regarding low pathogenic avian influenza viruses (LPAIV) in these species. The Strategic Plan has been used to develop flyway and state-specific implemen-

tation plans for HPAIV surveillance by establishing guidelines consisting of standardized protocols for sampling wild birds, handling and shipping samples, diagnostic testing, and communicating results.

The Strategic Plan provides a conceptual framework for a risk-based MOSS. These MOSS apply risk assessment methods to traditional surveillance designs for early detection and management of diseases, and support strategic and operational decision making (4, 16, 17). The objectives are to identify the necessary components of surveillance to protect animal and human health, prioritize those requirements, and effectively and efficiently allocate available resources. The steps in the design of a risk-based MOSS are 1) selection of the disease agent, 2) selection of sampling strata, 3) selection of sample size and sampling methodology 4) selection or development of a data management system, and 5) development of an action plan if *a priori* conditions are realized.

Selection of the Disease Agent

After movement of HPAIV H5N1 out of southeast Asia and into the Qinghai province of China, Mongolia, and eventually into Europe and Africa in 2005, considerable international effort focused on controlling HPAIV H5N1 in endemic countries and preventing further spread. The USA, along with many other countries, began evaluating the risk of HPAIV H5N1 introduction. In its National Strategy for Pandemic Influenza (18), the USA recognized that the HPAIV H5N1 virus had already obtained the ability to infect a wide variety of hosts and would likely continue to spread. Although it was impossible to determine whether HPAIV H5N1 would evolve into a human pandemic, the presence of the virus in domestic and wild birds in Asia and Europe increased the likelihood of its continued spread. Consequently, an implementation plan was developed to reduce the risk of the virus from entering the USA, limit its spread if the virus did enter the country, and sustain infrastructure and mitigate impacts if a pandemic developed (19).

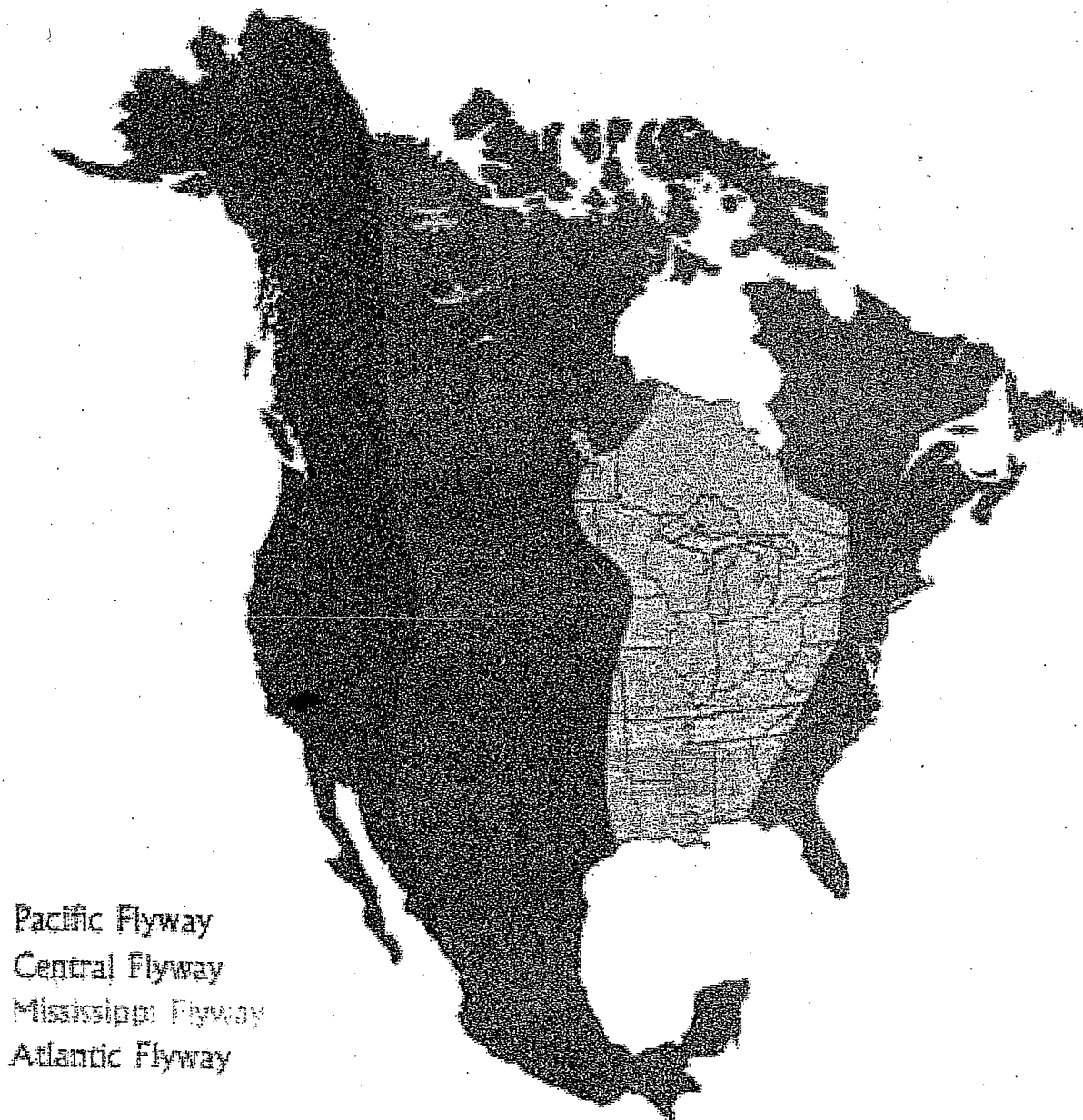
The USA recognized that the greatest risk of introduction was from the illegal importation of poultry and poultry products, and through the illegal trade of wild and exotic birds. Consequently, border protection and domestic bird surveillance programs already in place were strengthened to meet the increased risk of the rapidly spreading HPAIV H5N1 subtype (19). The risk that wild birds could move the HPAIV H5N1 subtype into the country also was identified; wild birds likely played a role in moving the virus into the Qinghai province of China, Mongolia and western Europe (20, 21, 22, 23, 24, 25, 26). While studies on avian influenza viruses (AIV) in wild birds have been conducted in North America (23, 27), these were limited in geographic scope and not designed to provide early warning of new virus introductions. Therefore, entry of the virus into the USA via wild birds was identified as a critical risk factor requiring increased surveillance (Homeland Security Council 2006).

Selection of Sampling Strata

The Strategic Plan stratifies the USA wild bird metacommunity geographically, by species that are likely to exhibit heterogeneity in the probability of moving HPAIV into the USA, and by the heterogeneity in the severity of consequence if an individual is infected with HPAIV. State and federal wildlife management agencies further refined these sampling strata to prioritize areas and species within flyways.

Stratification by Geographic Region

Sampling for HPAIV in wild birds was stratified longitudinally to account for general migratory patterns across the continent. Although intraspecific and interspecific variability in migratory pathways are common (28, 29, 30), the traditional waterfowl flyways (i.e. Atlantic, Mississippi, Central and Pacific) were used as a template in evaluating the risk of HPAIV H5N1 introduction through migratory birds on a continental scale. These flyways are associated with major topographical features in North America, which also tend to be aligned along a north-south axis (31, 32). Their boundaries are defined administratively, and are not biologically fixed or sharply delineated (Figure 1). Some species such as the mallard (*Anas platyrhynchos*) use specific flyways, while individuals of other species such as the northern pintail (*Anas acuta*) migrate across flyways during fall and spring (33).



The Pacific and Central Flyways extend from the Russian Far East, Alaska, and western Arctic Canada, through coastal and western regions of Canada, the USA and Mexico, and on to Central and South America (Figure 1). These Flyways were considered the regions through which the introduction of HPAIV H5N1 most likely would occur by wild birds. Many migratory species that nest in the Arctic regions of the Russian Far East, Alaska, and Canada follow the Pacific and, to a lesser degree, the Central Flyways to wintering areas in North and South America (34, 35). The overlap at the northern end of these flyways, and in Hawaii and Oceania establishes a pathway for potential disease transmission across continents and for mixing, re-assortment, and exchange of genetic material among strains from Eurasia and North America (36).

The Atlantic and Mississippi Flyways extend from the arctic regions of eastern Canada and western Greenland through the eastern regions of the USA (31). While the risk of HPAIV H5N1 introduction through migratory birds was considered higher in the Pacific and Central Flyways, potential introduction of the virus through the Atlantic and Mississippi Flyways was also of concern. Some species such as the northern pintail and tundra swan (*Cygnus columbianus*) migrate across several flyways during fall and spring (31, 33, 37). Also, there is geographic overlap of breeding birds in these flyways with birds from the East Atlantic Flyway (23), although the degree of overlap of species and individuals is considerably less than occurs in flyways of the Pacific region (37, 38). However, birds that winter in Europe and Africa where HPAIV H5N1 occurs, or co-mingle with birds from infected areas, have overlapping breeding areas with birds from North America. Makarova et al. (38) and Spackman et al. (39) demonstrated that at least some North American isolates belonged to Eurasian lineages.

Finally, although HPAIV H5N1 had not been detected in the western hemisphere, the potential for wild birds to move the virus north if it was introduced into Central and South America was considered. Thus, the Strategic Plan provided a national framework for HPAIV H5N1 surveillance in wild birds, which recommended that regional flyway plans be developed. These flyway plans were further refined into individual state implementation plans, such that all the potential routes of entry for HPAIV H5N1 through migratory birds could be monitored.

Stratification by Species

Initially, species were prioritized by their relative risk of introducing HPAIV H5N1 into the USA. During the development of the Strategic Plan in 2005, no information was available on the ability of North American species to become infected with, shed, or spread the virus over short or long distances. While there was considerable research available on LPAIV in some species, the unprecedented effects of the H5N1 subtype in wild birds throughout Asia clearly challenged the conventional wisdom that HPAIV did not significantly impact these natural reservoir species. Consequently, migratory species from North America that spent part of their life cycle in endemic HPAIV H5N1 areas were of highest concern, regardless of whether they were known LPAIV reservoirs.

To further focus sampling, five criteria were employed to quantitatively rank these migratory species. Ranking criteria included proportion of the population occurring in Asia, contact with an area known to have HPAIV H5N1, habitats used in Asia, population size, and likelihood of obtaining a representative sample of sufficient size (15). These

rankings were used to produce priority species lists for apparently healthy bird sampling in Alaska and the North American flyways. Flyways and agencies refined these lists by adding additional species of concern.

Stratification by Severity of Consequence

The HPAIV H5N1 subtype has caused the death of individuals from a wide variety of wild species (40), and most detections in wild birds have been through morbidity and mortality events (23, 41). Systematic investigation of these events in wild birds seems to offer the highest and earliest probability of detecting HPAIV H5N1 if it is introduced by wild birds (8, 15, 42). Benefits gained from conducting disease investigations of wildlife mortality events are not unique to AIV. Many diseases have been identified through the wildlife disease investigation process (43, 44, 45). Investigation of morbidity and mortality events also provides management recommendations that can mitigate or reduce additional deaths in wildlife.

Unfortunately, comprehensive surveillance of morbidity and mortality events is problematic, even in countries with established programs. This is primarily due to the difficulties associated with the detection of sick and dead wild animals, and the submission of carcasses or samples to a diagnostic laboratory (11, 12). Most morbidity and mortality events in wild birds go undetected because they involve few individuals, occur in areas of low human densities, or quickly become unavailable for sampling due to predation, scavenging, or rapid autolysis (46, 47, 48, 49, 50, 51, 52, 53). Such biases can significantly influence the perceived distribution of disease (54). Additionally, evidence for the evolution of HPAIV H5N1 strains that are not pathogenic to particular species of wild birds is mounting (21, 55, 56, 57, 58). Recent experimental research demonstrated that some species are resistant to developing clinical signs from HPAIV H5N1 infection (59, 60), while other studies documented even susceptible species can be clinically protected by previous exposure to AIV (61, 62). For these reasons, it is difficult to quantify and assess morbidity and mortality surveillance with regard to disease detection. Consequently, surveillance systems also should employ active (e.g., apparently healthy bird) as well as passive (e.g., morbidity/mortality event) sampling techniques (4, 9, 63, 64).

Selection of Sample Size and Sampling Methodology

Sample Size

Determination of sample size depends on statistical (e.g., population size, power, confidence, test accuracy, etc.) as well as non-statistical factors (e.g., funding, human resources, access to sampling units, etc.). The Strategic Plan provided guidance for agencies conducting surveillance for HPAIV H5N1, but recognized that specific implementation plans should be developed for each flyway and state. Population estimates for species of interest vary by location, time of year, and sampling method employed. Sample stratification by flyway and state within flyway reduces the effects of this variability. However, such stratification requires agencies to coordinate their sampling efforts to assure that adequate sample sizes are obtained from each target species within each flyway and state.

Estimation of sample size for morbidity and mortality events were not recommended in the Strategic Plan. Agencies were encouraged to identify and sample as many of these events as possible. Guidance on determining the number of samples for apparently healthy

list until state-wide target numbers were achieved. Agencies within each state decided which species from the flyway priority list to sample.

Total sample sizes ultimately were determined by available funding and prioritization of states. During the first year of the Wild Bird HPAIV NEDS, \$29 million dollars were made available through the USDA and DOI, resulting in a national target of 150,000 wild bird and environmental fecal samples. Unfortunately, available funding decreased in subsequent years resulting in a corresponding decrease in target sample numbers to 64,000 by the 2009 sampling year.

Sampling Methodology

Investigation of Morbidity and Mortality Events The success of this strategy requires early detection and assessment of events, rapid submission of samples to qualified diagnostic laboratories, rapid testing, immediate reporting of diagnostic results, and rapid implementation of pre-established response protocols. The USA strategy capitalizes on existing morbidity and mortality surveillance programs by state and federal agencies; some of these programs have been in place for decades (e.g., surveillance at migratory waterfowl refuges), and others (e.g., West Nile virus monitoring programs) are relatively new (65, 66, 67). These programs use agency personnel as well as the public to detect and report events to trained wildlife disease investigators. Investigations related to morbidity and mortality events are conducted regardless of the time of year, type of species involved, number of species involved, or the number of samples previously collected in the state. Assessment of these events, and collection and shipment of samples to diagnostic laboratories are usually made within 24 hours of identifying the incident. Diagnostic testing and reporting results are completed within an additional 72 hours allowing for rapid implementation of response protocols.

The USA has enhanced its capabilities to respond to morbidity and mortality events by increasing personnel and resources dedicated to detection, investigation, and reporting of sick and dead birds. Training courses designed to increase the number of wildlife professionals qualified to investigate morbidity and mortality events were conducted, educational materials were provided to sportsmen, bird watchers, and the general public to increase reporting of events, and a national telephone hotline was established to report dead birds.

Surveillance in Apparently Healthy Birds Two strategies for sampling apparently healthy wild birds are recommended in the USA Strategic Plan: hunter-harvest and live-bird sampling. Similar to morbidity and mortality event sampling, each of these strategies has advantages and disadvantages. Successful implementation of these strategies is time and location specific.

Hunter-Harvest Sampling Regulated hunting of wild migratory birds by sportsmen and subsistence harvests by Native Americans occur throughout most of North America. The primary advantage to hunter-harvest sampling is its cost-effectiveness; most of the waterfowl species in North America are classified as game birds, existing infrastructure (e.g., game check stations) is in place in many migratory and wintering areas, and sufficient numbers of birds are harvested by hunters, decreasing the amount of time and resources required to obtain samples.

The main disadvantages to hunter-harvest sampling are that not all species are harvested and hunting seasons only occur at specific times of the year (e.g., September through

January). Also, although sport hunting is widely distributed throughout North America, specific areas receive little to no hunting pressure because of low hunter density or it is prohibited by regulation (e.g., urban areas, preserves, private property). Finally, managers may not have access to all harvested birds, and reliable collection site information may not be available for samples collected.

Live-Bird Sampling Live-bird sampling involves capturing, sampling, and releasing wild birds. This strategy is often time and labor intensive requiring trained personnel, which can result in a significant financial investment. However, if implemented properly, live-bird sampling provides valuable data toward a comprehensive surveillance system.

An important advantage of this strategy is that it can be implemented at specific sites and at any time of the year birds are present. For example, many species of Charadriiformes are not hunted and hunting of game species within urban areas is not possible. Virtually any species of interest can be targeted, but the technique requires trained biologists to operate specific trap types as well as properly handling targeted species to prevent injury and death.

Sentinel Species Sentinel ducks have been used effectively to determine the presence of AIV and timing of infection associated with the arrival of wild migratory waterfowl in wetland habitats (68, 69, 70, 71, 72, 73, 74, 75). An additional advantage of using sentinel bird surveillance is its applicability in areas (e.g., urban areas) in which other methods can not be used. Disadvantages include the expense of rearing disease-free birds, pen construction, and husbandry. Sentinel flocks also are subject to predation and human disturbance. Another important consideration is that the susceptibility of individuals and species vary among AIV subtypes (59, 60, 62, 76, 77)

As with the other methods used for apparently healthy bird surveillance, targeting a sentinel system for HPAIV H5N1 can be challenging given the lack of knowledge about this virus in North American species and ecosystems. Consequently, it was recommended that agencies use AIV-free birds from species on the flyway priority list, and target specific locations where, and periods when, sentinel animals are likely to interact with free-ranging individuals of high priority species.

Fecal Sampling Avian influenza viruses are generally transmitted by waterfowl through the intestinal tract and viable virus can be detected in feces (78, 79). Analyses of fecal material from waterfowl habitat can provide evidence of AIV circulating in wild bird populations, the specific subtypes present, pathogenicity, and possible risks to poultry and susceptible livestock (80, 81, 82). Collection of fecal samples from waterfowl habitat is a reasonably cost effective method of surveillance compared to live bird sampling. Fecal sampling does not require the same level of skill to implement as live-bird sampling and can be implemented in rural and urban habitats. However, wild bird fecal samples must be fresh (e.g., before desiccation and extended exposure to sunlight), may contain environmental contaminants that adversely impact diagnostic analyses, and can be difficult to obtain from some species of waterfowl that spend considerable amount of time foraging and defecating in water. Exceptions are species such as Canada geese (*Branta canadensis*) and Snow geese (*Chen caerulescens*) that primarily forage and defecate on land. Additionally, while detection of AIV in fecal samples are useful in determining the presence of these viruses in the environment, the species infected may be difficult to determine if the collector does not observe the birds defecating. In the event of a HPAIV detection in feces, these limitations

will require subsequent sampling of the wild bird populations in the area to allow for predictions of viral spread and assessing risks of transmission to poultry and humans.

Diagnostics and Case Definition

Swab samples were collected from wild birds (i.e., tracheal, oropharyngeal, cloacal) and wild bird fecal samples. Bird samples were screened at one of 43 participating National Animal Health Laboratory Network facilities. This network is a partnership of state and federal laboratories that have been certified by the National Veterinary Services Laboratories (NVSL), which is the OIE Reference Laboratory for AIV diagnostics in the USA. Swabs were initially tested by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) using the matrix gene assay (83). The matrix gene rRT-PCR assay was capable of detecting all 16 hemagglutinin and nine neuraminidase subtypes. Matrix gene rRT-PCR-positive samples were further characterized by H5- and H7-specific rRT-PCR assays (84). Positive H5 or H7 rRT-PCR samples were express shipped to the NVSL within 24 hrs of a presumptive finding (85). Specific rRT-PCR assays, virus isolation, subtyping, and pathogenicity tests were performed according to international guidelines (9, 86, 87).

Wild bird fecal samples were screened by rRT-PCR at the USDA Wildlife Services National Wildlife Research Center using a modified assay based on Spackman et al. (88). Positive H5 and H7 samples were forwarded to the NVSL for virus isolation, subtyping, and pathogenicity testing as described above. Additional subtyping was performed by amplifying hemagglutinin genes and sequencing analysis (89).

A confirmed positive sample, was one from which a HPAIV was isolated and resulted in a positive pathogenicity test. A sample collected from a mortality event and which tested positive on the rRT-PCR matrix and H5 or H7 assay were considered presumptive positive until virus isolation and pathogenicity testing could be performed by the NVSL. A case was considered suspect if a sample from an apparently healthy bird tested positive on the rRT-PCR matrix and H5 or H7 assay.

Development of a Data Management System

One of the biggest challenges of developing a national MOSS for the early detection of a disease is the rapid assimilation of data and the transmission of relevant information to decision makers. Sampling and diagnostic testing often requires the coordination of numerous government agencies and laboratories, which have pre-existing data management programs they are required to use. Differences in the objectives of these databases, security requirements and protocols, and software upon which they are based often inhibit the ability to electronically transfer data among the MOSS participants. These constraints can severely limit the integration, analysis, and dissemination of information to decision makers in an appropriate time frame. In the case of an early detection system, rapid identification and dissemination of information related to an introduction of an exotic disease is critical for effectively and efficiently implementing a management plan to control and eliminate a pathogen. Thus, a system that manages input of data through multiple routes and provides a secure platform for contributing partners is a necessity.

The DOI and USDA developed the Highly Pathogenic Avian Influenza Early Detection Data System (HEDDS) to manage animal and specimen data taken by federal and state wildlife management agencies participating in the Wild Bird HPAIV NEDS. The HEDDS allows for either direct data entry or the electronic transfer of surveillance data from exist-

ing databases. Also, a listing of wild bird mortality events nationwide details information on causes of wildlife mortalities. If HPAIV is detected in wild birds, the system could be used to provide specific information regarding the number of species and individuals sampled and their infection status. While HEDDS maintains a secure platform for compiling specific agency data, it also has an open-access internet site that enables the public to track the progress of the Wild Bird HPAIV NEDS (90).

Development of Action Plans

An Implementation Plan for the National Strategy for Pandemic Influenza (19) clarifies the roles and responsibilities of government and non-government entities in the event of a HPAIV H5N1 detection in the USA. This Implementation Plan also outlines actions and expectations of federal, state, and tribal entities for preparedness and communication, surveillance and detection, and response and containment for protecting animal as well as human health.

Based on this Implementation Plan, the USDA, DOI, Departments of Health and Human Services, Defense, and Homeland Security, and state and tribal partners have adopted a common response strategy for an outbreak of HPAIV H5N1 in domestic and wild birds (86). This strategy involves intensified surveillance, containment, coordinated interagency situational assessment, and activating or supporting an interagency unified command when needed. The USDA and DOI have incorporated the principles of this strategy into agency specific response plans (86, 91). These response plans were tested through tabletop exercises of hypothetical HPAIV H5N1 outbreaks in wild birds independent of, and in association with, poultry outbreaks. Exercises tested and integrated field, laboratory, and policy resources along with Incident Command Systems at the local, regional, and national levels.

Unlike actions developed to protect domestic animal and human health, management actions in wild birds are limited. Wild birds, are not subject to disease containment controls as are domestic birds and people. Therefore, management options for wild birds after a detection of HPAIV should focus on mitigating the potential spread to domestic birds and humans.

If a HPAIV outbreak in poultry is detected, wildlife managers should assess the presence of susceptible wildlife in the affected area, the potential for spread of the virus to those species, develop a surveillance protocol to determine if the virus has spread to wildlife, and determine if wildlife are moving the virus outside of the affected area. Managers should minimize the risk of wildlife becoming infected on positive farms, by integrating a variety of techniques (e.g., hazing, fencing, etc.) to exclude wildlife from those premises. Additionally, managers must evaluate whether public land closures are prudent to prevent dispersal of the virus by humans visiting areas where the virus is known to occur.

Public support for response activities is essential for success. The general public, including various constituency groups such as consumptive and non-consumptive wildlife users, farmers, and animal welfare advocates, will be affected by a HPAIV outbreak. Informational brochures on the role of wild birds in the transmission of AIV have been developed by USDA and have been distributed to producers, and industry and hunter organizations. Communication plans for notifying state and federal partners of HPAIV findings were developed and distributed to state agriculture and wildlife agencies, and diagnostic laboratories.

IMPLEMENTATION OF THE STRATEGIC PLAN

The USDA and DOI were the lead federal agencies responsible for working with tribal and state partners to implement the Strategic Plan. Facilitation and coordination of all partners in the Wild Bird HPAIV NEDS was through a Steering Committee consisting of representatives from the USDA Wildlife Services, DOI Geological Survey and Fish and Wildlife Service, Centers for Disease Control, Association of Fish and Wildlife Agencies, and National Flyway Council. In addition to ensuring that all partners were kept apprised of the progress of each agency's efforts, the Steering Committee also served as the primary body through which partners discussed proposed changes in the Wild Bird HPAIV NEDS. Through the Steering Committee, consistency in the multi-agency Wild Bird HPAIV NEDS was maintained.

From 1 April 2006 through 31 March 2009, the USA collected 367,834 wild bird and wild bird fecal samples for testing as part of the Wild Bird HPAIV NEDS (85). More than 250 species of wild birds in all 50 states, Puerto Rico, and the USA Pacific and Caribbean territories were sampled (85, 92, 93, 94). No HPAIV was detected in any wild bird sample. DeLiberto et al. (85) conducted a post hoc freedom of disease analysis (95) to test the null hypothesis that HPAIV was present in the USA wild bird metacommunity. Given an estimated population size of 50 million ducks, geese, and swans (96, 97, 98), the freedom from disease analysis indicated that the probability of observing a HPAIV positive reactor in a sample of 367,834 wild birds with a disease prevalence of 0.001% was $p = 0.000000$ (85).

About 52% of the samples were collected from the Pacific (including Alaska and Oceania) and Central Flyways and 42% from the Mississippi and Atlantic Flyways. Sampling in Alaska alone, accounted for 15% of all samples collected throughout the USA. Several authors implied the USA Wild Bird HPAIV NEDS focused entirely on the Asia-Alaska route of entry, with little to no wild bird surveillance in other potential pathways of introduction (99, 100, 101). These results demonstrate that the Wild Bird HPAIV NEDS effectively achieved its goal of targeting a potential entry of the virus from Asia, but not at the exclusion of other possible routes (e.g., Europe).

While sick and dead birds accounted for only 2% of total samples collected, they represented a significant and increasing investment by the agencies to identify morbidity and mortality events each year. This increased effort, though, resulted in the collection of relatively consistent sick and dead bird samples during 2006 (2,224 samples) and 2007 (2,276 samples), and a 38–40% decrease in samples during 2008 (1,382 samples) over previous years. These relatively low sample sizes exemplify the problems associated with using morbidity and mortality events in a wildlife MOSS. These events are generally stochastic, unpredictable, and when they do occur, are difficult to detect especially in areas of sparse human population (54).

Freedom from disease analyses conducted using only the morbidity and mortality surveillance data concluded that the USA wild bird population was free from HPAIV infection at a minimum prevalence of 0.21% in 2006 and 2007, and 0.34% in 2008 ($p \leq 0.002$). Although this is a relatively good level of detection compared to other early detection systems (42), there could have been 100,000 birds in 2006 and 2007, and 170,000 birds in 2008 infected with the HPAIV that went undetected if the USA relied solely on morbidity and mortality event surveillance.

Apparently healthy bird sampling (i.e., live-bird and hunter-harvest) comprised 75% of all samples collected. There has been much debate concerning the value of apparently healthy bird surveillance for the detection of HPAIV H5N1 (8, 63, 102, 103). Freedom from disease analysis using only apparently healthy bird data provided the capability of detecting the disease at a much lower prevalence ($P < 0.00001\%$; $p \leq 0.000000$) than morbidity and mortality surveillance. Consequently, apparently healthy bird surveillance allowed for the detection of HPAIV if < 5 birds out of a population of 50 million were infected.

While sampling sick and dead birds has detected the vast majority of the wild bird infections to date, the freedom from disease analyses demonstrate the value of incorporating apparently healthy bird surveillance into NEDS. Comprehensive MOSS for HPAIV H5N1 in wild birds that use morbidity and mortality, and apparently healthy bird data are also important given the emergence of several strains of this virus (104) and the ability of some individuals from some wild bird species to survive infection (57, 59, 75,). These systems also are useful in identifying carrier species, potential bridge species and contribute holistic knowledge of AIV ecology (23, 85, 94, 105, 106, 107, 108).

Development and implementation of the USA Wild Bird HPAIV NEDS has provided important ancillary benefits toward improved comprehensive wildlife disease surveillance. The number of wildlife biologists trained to investigate morbidity and mortality events, and to conduct active surveillance programs for diseases was increased nationwide. Diagnostic laboratories certified to conduct AIV testing as part of the National Animal Health Laboratory Network were increased, improving the capability of the USA to rapidly detect introductions of HPAIV as well as other exotic diseases. Enhanced communication protocols for reporting test results of diseases of concern in wildlife were developed and implemented. Critical field equipment necessary for conducting disease surveillance in wildlife and to respond to disease outbreaks was purchased. A national wild bird tissue archive was created by USDA to provide a resource for future studies on AIV and other diseases. Finally, the benefits of improved coordination among wildlife biologists and veterinarians, agricultural veterinarians, laboratory diagnosticians, public health officials, and researchers can not be underestimated. This coordination has already proved invaluable in detecting, diagnosing, and improving our understanding of the epidemiology of other wildlife diseases (109). These enhancements to the wildlife disease surveillance efforts in the USA will continue to safeguard the health of wild and domestic animals, as well as the public.

The USA effort, combined with the Canadian (110) and Mexican NEDS, represented the largest, coordinated wildlife disease surveillance program ever implemented. During 2006–09, over 379,000 samples were collected from wild birds throughout North America and results were shared among all three countries. Coordination of each country's surveillance system was accomplished through the establishment of a trilateral HPAIV working group in 2006. This group met periodically to re-evaluate the continental surveillance of AIVs in wild birds, and ensured an appropriate sampling distribution in all four flyways given available resources.

The North American NEDS were supplemented with collaborative surveillance systems in eastern Russia, Greenland, and Iceland. The USDA worked closely with the Russian Federal Centre for Animal Health and Ministry of Natural Resources to conduct sampling for AIV in snow geese on Wrangel Island Nature Reserve. Most snow geese that breed on Wrangel Island migrate through Alaska and Canada, and winter in the western USA (110,

111). In Greenland, the USDA collaborated with the Technical University of Denmark, Aarhus University, the Danish Veterinary and Food Administration, and the Greenland Home Rule authorities to conduct AIV surveillance of wild birds in the western and southern portion of the country; since 2007, over 3,000 birds have been tested. Finally, the Canadian Cooperative Wildlife Health Center conducted surveillance for AIVs in wild birds in Iceland (112). These efforts, combined with the programs in Canada, Mexico, and the USA, provided comprehensive surveillance of migratory birds in the North American flyways.

SUMMARY

The USA Strategic Plan successfully developed and implemented the Wild Bird HPAIV NEDS. This nationally coordinated system was a targeted, risk-based MOSS that systematically collected relevant ecological and AIV data on wild birds, efficiently assimilated data, and rapidly disseminated results to partner agencies, decision makers, and the public. This system capitalized on existing infrastructure and expertise at state and federal agriculture and natural resources agencies. The integrated, targeted approach used several parallel surveillance activities that provided statistically-based evidence on the absence of the HPAIV from the wild bird metacommunity.

Standardized data collection protocols were developed to ensure the consistency and quality of samples collected. The NAHLN facilities were used to implement rapid screening for H5 and H7 viruses, which were molecularly characterized and tested for pathogenicity by the NVSL. Partner agencies provided collection data to a common database, which was used to provide status updates to the public and decision makers on the progress of the system in achieving annual sampling targets.

Interagency cooperation and communication was maintained through the Steering Committee, ensuring consistency and reliability in data collection, processing, and reporting. Flexibility in the Wild Bird HPAIV NEDS also was ensured by the Steering Committee, which periodically met to discuss recent advances in knowledge of HPAIV H5N1 virology, detection, and ecology. Proposed changes to the system were vetted through the Steering Committee and agreed upon by representatives from the partner agencies.

Continued implementation of the Wild Bird HPAIV NEDS will provide the USA with confidence that if introduction of a HPAIV virus in these species occurs, predefined management activities will be effectively employed to limit viral spread to poultry and humans. Future analyses of the surveillance data also will improve our knowledge of AIV in wild birds at large geographic and temporal scales. This knowledge will dramatically improve our ability to assess risk of HPAIV introductions to poultry and human populations.

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