A Surveillance Plan for Asian H5N1 Avian Influenza in Wild Migratory Birds in Hawai‘i and the U.S.-Affiliated Pacific Islands

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List of Abbreviations and Acronyms

CDC .....Centers for Disease Control and Prevention
CNMI .....Commonwealth of the Northern Mariana Islands
DLNR.....Hawai‘i Department of Land and Natural Resources
DOA.....Hawai‘i Department of Agriculture
DOH.....Hawai‘i Department of Health
DMWR.....American Samoa Division of Marine and Wildlife Resources
DU.....Ducks Unlimited
FSM.....Federated States of Micronesia
FTE .....Full-time Equivalent
FWS .....United States Fish and Wildlife Service (Dept. of the Interior)
HC&S.....Hawai‘i Commercial and Sugar Company
HPAI .....Highly Pathogenic Avian Influenza
KS .....Kamehameha Schools
LPAI.....Low Pathogenic Avian Influenza
NAHLN....National Animal Health Laboratory Network (USDA)
NBII .....National Biological Information Infrastructure (USGS)
NHP.....National Historical Park
NPS .....National Park Service
NVSL .....National Veterinary Services Laboratory (USDA)
NWHC .....National Wildlife Health Center (USGS)
NWR .....National Wildlife Refuge (USFWS)
NWRC.....National Wildlife Research Center (USDA)
RMI.....Republic of the Marshall Islands
RT-PCR....Reverse Transcriptase Polymerase Chain Reaction
SPC .....Secretariat of the Pacific Community
USDA.....United States Department of Agriculture
USGS .....United States Geological Survey
WHO.....World Health Organization
WS.....Wildlife Services (USDA)

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INTRODUCTION

Avian influenza is endemic in wild populations of waterfowl and many other species of birds. The emergence and spread of a Highly Pathogenic Avian Influenza (HPAI) H5N1 subtype in Asia over the past few years (hereafter called Asian H5N1) has elevated concerns about potential expansion of this virus to Pacific islands and the Americas. Apprehensions among government agencies and the public are based on a range of possibilities that include sickness and mortality in wild bird populations, introduction of a disease that could devastate the poultry industry, and potential mutation of the virus into a form that could be highly infectious and pathogenic to humans. Currently, public concern related to Asian H5N1 has been heightened due to extensive media coverage about this virus in Asia, its subsequent spread to Europe and Africa, and limited human infections. Much of the coverage includes speculation that migratory birds are a primary vector of Asian H5N1. Government agencies in the United States, particularly state and federal wildlife agencies, are being called upon to develop an early detection system to determine if and when the virus arrives.

Key assumptions on the status of avian influenza (as of March 2006) and terminology that relate to this plan are indicated below.

- Migratory waterfowl and shorebirds are the natural reservoir for most of the 144 possible subtypes of avian influenza, named for their protein components hemagglutinin (H) and neuraminidase (N). Most avian influenza types are not very pathogenic, but the H5 and H7 types seem to be more pathogenic to domestic poultry.
- The terms “highly pathogenic” and “low pathogenic” avian influenza (HPAI and LPAI, respectively) refer specifically to pathogenicity to domestic chickens—testing for HPAI is documented by mortality rates in dosed poultry or by genetic sequence analyses.
- Some avian influenza varieties may mutate into forms that become pathogenic to specific taxa (e.g., birds, swine, humans). The currently prominent Asian H5N1virus is highly pathogenic to some birds, particularly domestic poultry, but is not easily transmitted to people. This is primarily a bird disease that has infected a small number of people who have been exposed to infected poultry or raw poultry parts.
- The Asian H5N1 strain has not been detected in North America or the Pacific islands. Low pathogenic H5N1 and a wide variety of other types of avian influenza have been documented in poultry and wild waterbirds in North America over many years.
- The degree to which migratory birds may be agents in the spread of Asian H5N1 is poorly documented. In nearly all cases of expansion in Eurasia, movements of poultry and poultry products are suspected as the primary vehicle. Mortality of wild birds has been associated with contact or shared use of habitats with domestic birds. Some migratory waterfowl, however, are tolerant of Asian H5N1 and could be vectors.

- Currently, there is inadequate information about the virulence of Asian H5N1 in wild bird species, its persistence in wild populations, and the degree to which it can spread from bird to bird during seasonal and annual cycles. Fecal contamination is assumed to be the primary mode of transmission, and the virus can remain viable for many days in fresh water.
- It is not a given that Asian H5N1 is the most likely threat for a global influenza pandemic. The onset of a human pandemic could result if some form of avian influenza—Asian H5N1 or any other type—adapted into a form that was infectious among humans.

For two reasons, islands in the Pacific Ocean may facilitate the movement of Asian H5N1 from Asia to North America. First, air and sea transport link the islands in the Pacific, including Hawai‘i, to the U.S. mainland, so any appearance of Asian H5N1 in these islands increases its probability of reaching North America. Second, migratory birds from Asia overwinter on many Pacific Islands, and closely associate with people and local island wildlife. For these reasons, surveillance for Asian H5N1 in Hawaii and other Pacific islands is a national priority.

GOALS AND OBJECTIVES

This document is intended to serve as a comprehensive plan to detect Asian H5N1 in wild migratory birds within Hawai‘i and the U.S.-affiliated Pacific islands. This plan is a stepped-down adaptation of the U.S. Interagency Strategic Plan (Interagency HPAI Early Detection Working Group 2006). The goal of the national strategy and this Pacific Islands strategy is early detection of Asian H5N1 in wild migratory birds—not to assess its prevalence over time, monitor its rate of movement, or investigate the ecology of the disease. Response actions in the event of a detection of Asian H5N1 in wild birds are the subject of separate plans currently being drafted.

Early detection of Asian H5N1 in tropical Pacific islands that are part of, or affiliated with, the United States presents unique advantages and challenges that will shape the surveillance program. Among these are:

- *Unique migratory species or populations*: Migratory birds that stop in these islands may breed in Asia, or mingle with Asian birds during migration, increasing the chance that they would vector Asian H5N1. Some of these species are rare or absent in North America and so will only be adequately monitored by island-based surveillance programs.
- *Large distances between islands*: The small and widely separated islands of the Pacific extend over an area larger than North America (Figure 1). Movement of samples to laboratories can be hindered by the timing and logistics of air travel over such distances.
- *Small, dispersed migratory populations*: Small islands do not support the large migratory bird populations found on continents, making it more difficult to obtain adequate sample sizes. Most migrants are shorebirds which rarely aggregate in their wintering grounds.
- *Sensitive resident bird populations*: Fall and winter migrants from Asia and North America interact with large, dense populations of nesting seabirds on remote Pacific islands, and with endangered endemic ducks, geese, coots, moorhens, and stilts in

wetlands on several islands. Risks to these populations from H5N1 infection, and from harassment and injury during sample collection, must be considered.

- *No waterfowl hunting*: Hunter-killed birds allow collection of large samples in Alaska and the Pacific Flyway, but such opportunities do not exist in the islands, where waterfowl hunting is not legal. Similarly, there is no large-scale routine banding of migrants.
- *Inadequate flyway characterization:* The island groups covered in this plan lie within the East Asian-Australasian and the Mid-Pacific Flyways (Fig. 2). However, the breeding areas used and migratory routes taken by migrants in the Pacific islands are poorly characterized, due to a lack of extensive banding studies and hunter band returns.
- *Diverse governmental structures*: The region covered by this plan includes the State of Hawai‘i and multiple Territories, Incorporated and Unincorporated Possessions, a Commonwealth, and three independent Republics in free association with the United States. Implementing and coordinating an efficient and cost-effective surveillance program spanning multiple political entities will be challenging.
- *Tropical, rural human environments*: In parts of Hawai‘i and the U.S.-affiliated Pacific, interactions between people and wildlife, including free-ranging poultry, more closely resemble those in rural Asia rather than North America. Such environments have been shown to have a higher risk of avian-to-human H5N1 transmission than temperate zone environments. In these settings, free-roaming or feral chickens are potentially good indicators of Asian H5N1 circulation.

The flyway-level surveillance plans prepared elsewhere in the U.S. set broad priorities, but leave implementation planning to robust state and federal wildlife infrastructures with long histories of migratory waterfowl management. With fewer of these pre-existing structures and capacity, the collaborating agencies within the U.S-affiliated Pacific islands intend for this strategy document to contain much of the detail and guidance required for implementation of the program.

Surveillance efforts for Asian H5N1 will involve, by necessity, extensive cooperation at local levels among wildlife agencies, agriculture agencies, public health systems, and other entities. This plan focuses on wildlife surveillance, while acknowledging that surveillance of other species (domestic, feral, and zoo) and humans will also be important.

To accomplish the goal of early detection in the unique social, ecological and geographic setting of the Pacific islands, these collaborating agencies and governments must deploy available resources strategically, coordinate actions to avoid redundancy, and focus sampling effort on high-probability species and locations.

Objectives of this document:

1. Prioritize migratory species to be sampled for Asian H5N1 in the Pacific islands.
2. Recommend sampling approaches to effectively establish an Asian H5N1 detection system in wild birds.
3. Recommend procedures to integrate detection efforts within the islands, other flyways and with national programs.

4. Describe additional staffing and coordination necessary to establish and maintain an effective Asian H5N1 detection system in the Pacific.

APPROACHES

Species Prioritization

Efficient sampling for Asian H5N1 requires that migratory bird species be prioritized in terms of the relative probability that they could be exposed to the virus through contact with other migratory birds, domestic flocks, or habitats in Asia where Asian H5N1 has been documented. Sampling should focus on the highest priority species. The U.S. Interagency Strategic Plan identifies waterfowl and shorebirds as the taxonomic groups with the highest likelihood of meeting these criteria, and presents ranking information for North American species. Although seabirds nest throughout the Pacific islands and some forage at sea near Asia and North America, their contact with freshwater habitats and species from which Asian H5N1 has been described appears to be minimal.

Data on the migratory patterns of birds in the tropical Pacific islands is limited for most species compared to North America (Engilis and Naughton 2003). Except for those species with distinct Asian and American forms or subspecies, it is not possible to assign breeding locations to most Holarctic breeding migrants in the Pacific islands. Islands in the Western Pacific (e.g. Palau, Guam, CNMI) receive more identifiable Asiatic migrants, many of which are not available for sampling in North America.

In North America, a plethora of species and large numbers of migrating birds allowed for prioritization to optimize sampling efficiency. The five species ranking criteria used in the U.S. Interagency Strategic Plan were: (1) the degree of contact with Asia; (2) contact with any known Asian H5N1 outbreaks; (3) habitat preferences in relation to the occurrence of H5N1; (4) the proportion of the population that would be available for sampling; and (5) the probability of obtaining a sufficient number of birds for sampling. These criteria were applied to species common in North America and for which extensive population and migration route data are available.

For this plan, migratory waterfowl and shorebird species that are common to abundant in the U.S-affiliated Pacific islands were identified (Table 1). Compared to most species in North America, the total numbers of these birds available for sampling in the Pacific islands are quite small. These species were then designated as primary targets if some or all of their populations are known to breed in Asia. Secondary target species were those that breed only in North America or that will be well-sampled in the Pacific Flyway Plan. A third group of species includes birds which are known to commingle with migratory waterfowl and shorebird species in the islands, or predatory species which could depredate infected migratory birds.

Figure 1. The State of Hawai‘i and the U.S.-affiliated Pacific Islands.

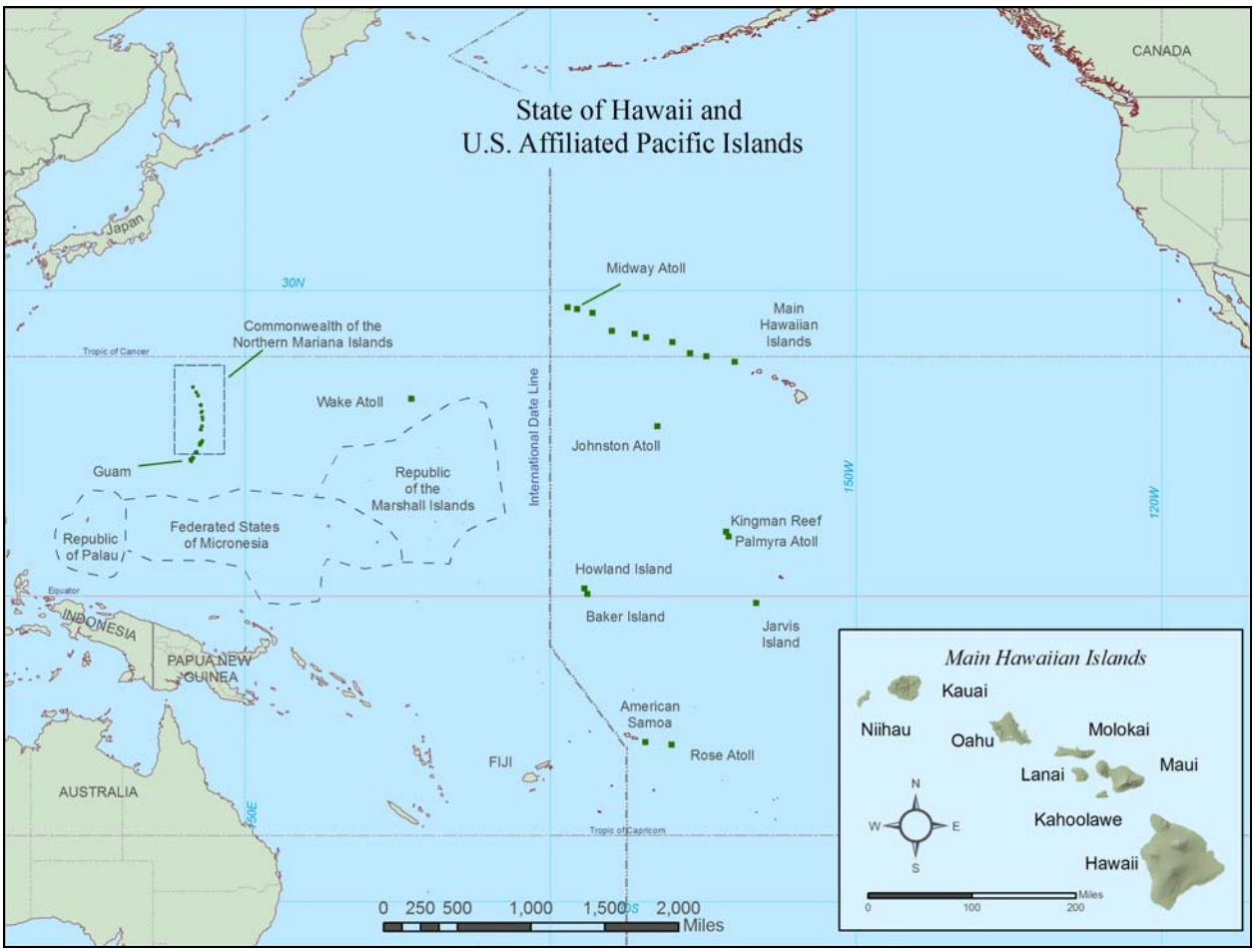

Figure 2. Flyways of Asia and the Pacific Basin.

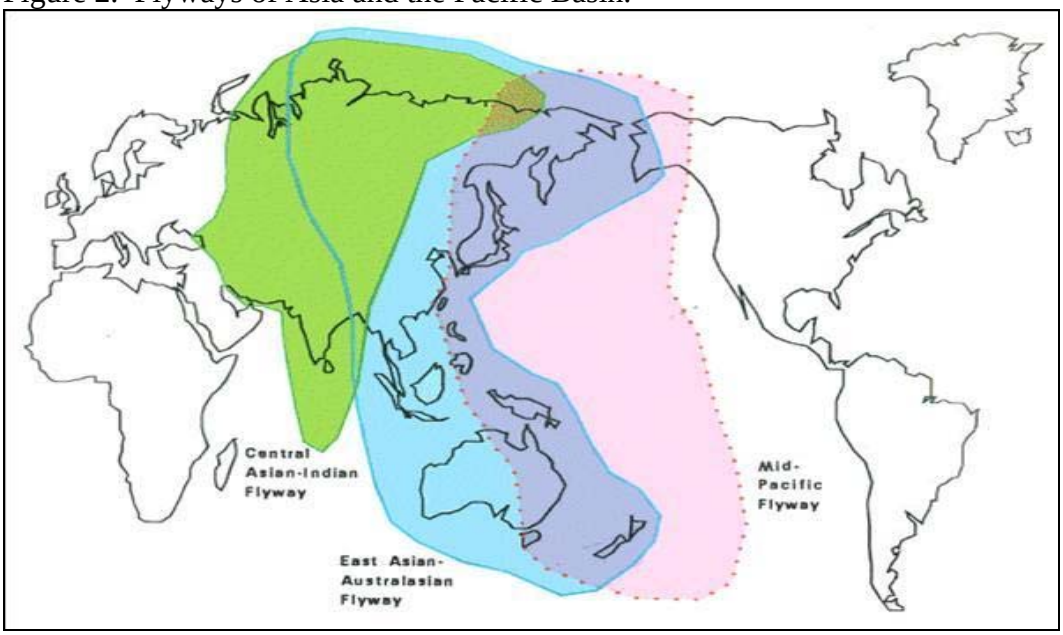

Table 1. Target species for live bird surveillance in the Pacific Islands, and estimates of wintering population size (P indicates peak numbers of passage migrants).

Primary Target Species (partly or fully Asian breeders)

Species	Hawaii	Midway	Palmyra	Guam & CNMI	Palau	RMI (Kwaj.)	Am. Samoa
Northern Pintail *Anas acuta*	200			100	<50		
Tufted duck *Aythya fuligula*	<20			100	<50		
Pacific Golden-plover *Pluvialis fulva*	15,000	900	200	3000		300-500	4500
Gray-tailed Tattler *Heteroscelus brevipes*				100	100		
Whimbrel (Eurasian) *Numenius phaeopus varigatus*				500 P	200 P		
Ruddy Turnstone *Arenaria interpres*	1500	250	100	500	<200	300-700	550
Mongolian Plover *Charadrius mongolus*				50			
Blackbellied Plover *Pluvialis squatarola*				25			
Rufous-necked Stint *Calidris ruficollis*				25	50		
Sanderling *Calidris alba*	2000	40		<20 P			
Sharptailed Sandpiper *Calidris acuminata*	100 P			500 P			
Pectoral Sandpiper *Calidris melanotos*				20 P			
Wood Sandpiper *Tringa glareola*				100	100		
Marsh Sandpiper *Tringa stagnatilis*				10 P			
Common Sandpiper *Actitis hypoleucos*				50	50		
Common greenshank *Tringa nebularia*					100		
*Blackwinged Stilt *Himantopus himantopus*				25			

Secondary Target Species (N. American breeders, or well-sampled in Pacific Flyway)								
Northern Shoveler *Anas clypeata*	340			<20	<20			
Bristle-thighed Curlew *Numenius tahitiensis*		300	50-200					
Wandering Tattler *Heteroscelus incanus*	1000	30		100				900

Tertiary Target Species (commingle with, or prey upon, primary or secondary species)			
Seabirds	Laysan Albatross *Diomedea immutabilis*	**Shorebirds**	Hawaiian Stilt (E) *Himantopus mexicanus knudseni*
	Wedge-tailed Shearwater *Puffinus pacificus*		Yellow bittern *Ixobrychus sinensis*
	Greater Frigatebird *Fregata minor*		Cattle Egret *Bubulcus ibis*
	Black Noddy *Anous minutus*		Intermediate Egret *Egretta intermedia*
	Brown Noddy *Anous stolidus*		Black-crowned Night Heron *Nycticorax nycticorax*
	White tern *Gygis alba*	**Raptors**	Barn Owl *Tyto alba*
Waterbirds	Hawaiian Coot (E = endangered) *Fulica alai*		Hawaiian Hawk (E) *Buteo solitarius*
	Hawaiian Moorhen (E) *Gallinula chloropus sandvicensis*	**Others**	*Common mynah *Acridotheres tristis*
	Koloa (E) *Anas wyvilliana*		*Red junglefowl *Gallus gallus*
	Mallard (and Mallard X Koloa) *Anas platyrhyncos*		*Philippine turtledove *Streptopelia bitorquata*
	Nene (E) *Branta sandvicensis*		*Eurasian tree-sparrow *Passer montanus*

* Not listed under the Migratory Bird Treaty Act

Potential Sampling Methods

The sections below examine potential sampling methods. The merits of specific sampling methods suggested in the U.S. Interagency Strategic Plan and others have been considered relative to the feasibility of implementation in this region. The recommended sampling regime is detailed in the section following this, "Strategic Species and Area Priorities." Four of the five sampling programs suggested in the U.S. Interagency Strategic Plan are discussed; the fifth, hunter-killed birds, is not relevant in this region.

Detection and investigation of morbidity/mortality events

The highest probability of detecting Asian H5N1 in the Pacific islands is through investigation of avian mortality events. Globally, almost all detections of Asian H5N1 in a new locality have been through mortality events in wild birds or domestic poultry. To date, there has been no case where sampling apparently healthy birds has revealed the presence of Asian H5N1. Furthermore, the recent detections of H5N1 in swans in Europe and the Middle East show that large die-offs in wild birds should not be expected; tests of single dead individuals has allowed the virus to be detected in new localities. Because of the low density of migratory waterfowl and shorebirds on most Pacific islands, restricting investigations to unusual, multiple-mortality events as suggested in the U.S. Interagency Strategic Plan risks undersampling this crucial resource.

Due to the low numbers of wildlife professionals or veterinarians in the Pacific islands, increasing the frequency of surveillance in waterfowl and shorebird habitat in order to detect a high proportion of mortalities will require additional human resources. Requesting amateur ornithologists and other members of the public to report mortalities would increase the efficiency of this surveillance program. The program itself, however, will require dedicated people on the ground to systematically survey habitats, document mortalities, and collect and process carcasses for shipment to a diagnostic lab.

Poultry die-offs are key indicators of Asian H5N1 that have been linked to traffic of poultry products or live birds, and only hypothetically to infection from wild bird populations. Tropical Pacific islands often have robust feral chicken populations, as well as backyard poultry flocks. Multiple mortalities in these birds could result from illicit movement of infected fowl from Asia or inadvertent movement of virus by travelers returning from trips to Asia, as well as contact with infected migratory birds. Die-offs of backyard or free-ranging poultry are highly significant and should be promptly investigated by qualified personnel. While investigating such die-offs would not be the direct responsibility of personnel acting under this program, local publicity related to wildlife surveillance would increase the likelihood of Asian H5N1 detection in non-commercial poultry.

Sampling of Live Birds - Waterfowl

Migratory waterfowl in the region are restricted to a limited number of well-known wetlands, and the total numbers of migrants are low (Table 1). The two most abundant species in Hawai‘i, northern pintail and northern shoveler, have average wintering populations of less than 400 birds in the entire state (data from 1986-2000). Numbers of migrant ducks in the Mariana Islands (Guam and CNMI) and Palau are lower. Shovelers and pintails will be sampled extensively as part of the Pacific Flyway plan. However, trans-Pacific movements of individual pintails are well documented, suggesting that a portion of the wintering population in Hawai‘i, and certainly in the western Pacific, are Asian breeding individuals. With no current banding programs, and no legal waterfowl hunting, new programs would have to be instituted to capture and sample these species.

Potential capture methods include rocket nets and baited fixed traps. Netting or trapping would likely also result in capture of feral mallards and native, non-migratory ducks, coots and moorhens. Because these species could be secondarily infected by arriving migrants, they could act as sentinel species. Sampling of all birds captured, regardless of species, would increase the chance of detecting H5N1 as well as other avian influenza subtypes. Capture activities should coincide with the arrival of migratory ducks, because shedding of avian influenza in waterfowl occurs over a relatively brief period following infection. The target period would be mid-September through November for Hawai‘i (Engilis et al. 2003). Diving ducks such as the Tufted duck require more specialized trapping methods than do dabbling ducks, and the success rate is likely to be lower.

Sampling of Live Birds – Shorebirds

As a group, shorebirds represent an important potential source of information regarding the early detection of Asian H5N1 in the Pacific islands. The primary target species of shorebirds in the Pacific islands are not expected to be sampled in large numbers at continental sites, so

surveillance of these species at island locations will be an important aspect of the national program. As with waterfowl, capture of sufficient numbers of shorebirds for sampling will require deployment of dedicated crews, as the only banding program currently in the Pacific is a small research endeavor which bands less than 100 birds annually. Pacific golden-plovers aggregate in predictable locations and can be netted either using rocket nets or mist nets. Ruddy turnstones usually forage in small flocks or individually in predictable locations. Concentrations of this species could be captured with rocket nets. Both species, and other species that occur in lesser numbers, might be most efficiently sampled by fecal collection (see Environmental Sampling, below). Baited walk-in traps are quickly discovered and exploited by passerines and doves and do not achieve useful capture rates in most locations.

Sampling Sentinel Birds

Sentinel birds, which are domestic fowl monitored for infections originating in wild birds with which they have contact, are potential tools for Asian H5N1 surveillance. Due to the high lethality of this virus for chickens, good reporting and sampling of mortality events in backyard flocks would appear to be a more cost-effective monitoring tool than obtaining swabs from apparently healthy chickens.

Regular weekly sampling of captive sentinel ducks could detect Asian H5N1 in selected wetlands. Placement of disease-free domestic ducks into water bodies frequented by migratory species could result in higher detection probabilities than sampling wild birds, because regular sampling is more likely to catch the brief period of virus shedding. Only domestic duck breeds derived from mallards, but unable to hybridize with the endangered native koloa (*Anas wyvilliana*), would be suitable for use in Hawai‘i. Breeds derived from Muscovy ducks are not suitable due to risks of enteric disease introduction. Guidelines for such programs (Attachment 6 of the U.S. Interagency Strategic Plan) suggest 20-40 pinioned ducks be reared in pathogen-free conditions and stationed at each selected wetland in time for the arrival of wintering ducks. This would entail establishment of a full sentinel duck program with a dedicated staff person for husbandry, sampling, etc.

An alternative approach would use captive ducks currently deployed in Hawai‘i for control of pest apple snails in taro fields on the islands of Kaua‘i, Maui and Hawai‘i. Currently there are approximately 100, 45, and 25 of these ducks on the three islands respectively. These ducks roam free during the day and have some contact with wild migrants, and are penned at night. Their availability and the extent of contact with wild waterfowl and shorebirds will be variable among islands. Discussions with farmers and knowledgeable biologists would be required to establish this system. The utility of these captive ducks for surveillance would be dependent on their lack of previous exposure to avian influenza subtype H5, which could be assessed serologically. Sampling by cloacal swab would occur weekly, while husbandry would be the responsibility of the owners.

Environmental Sampling

U.S. Interagency Strategic Plan states, “An approach based on fecal sampling could be immediately implemented and may be the only reasonable approach in areas where bird capture is not practical.” The dispersed nature of shorebirds in the Pacific islands suggests this method as a preferential alternative to capture programs, especially if it is desired to sample birds in a

compressed timeframe upon their arrival in the islands. Such timing would probably coincide with maximal shedding of avian influenza viruses and thus detection probability. Careful observation of individual shorebirds for defecation could eliminate fecal identity issues and permit calculation of prevalence rates. In species such as the Pacific golden-plover, where nighttime single-species aggregations are predictable and observable, sampling of fresh feces in the morning could be an efficient technique, although multiple defecations per individual would obviate prevalence calculations. Tests are underway to determine whether this collection protocol would affect detection of influenza virus in fecal samples.

Implementation of fecal sampling would require dedicated observers to collect feces deposited by foraging individual shorebirds. For species that aggregate, identification of loafing sites would be needed. Sampling, shipping and analysis protocols for fecal samples parallel those of cloacal swabs, and the RT-PCR analytical techniques have recently been optimized and are ready for use. This technique could also be used to sample feces from any waterfowl observed loafing on land. At this time, a dependable technique for sampling water bodies for H5N1 is not available.

Sampling Locations

The islands covered in this plan stretch across an area of ocean approximately the size of North America, and include portions of the East Asian-Australasian and the mid-Pacific Flyways (Figs. 1 and 2). In addition to the State of Hawai‘i (including the Northwest Hawaiian Islands and Midway Atoll), the U.S.-affiliated Pacific islands include the Territory of Guam, the Territory of American Samoa, the Commonwealth of the Northern Mariana Islands (CNMI), the Republic of Palau, the Republic of the Marshall Islands (RMI), the Federated States of Micronesia (FSM), the unincorporated islands of Wake and Johnston Atoll, and the islands that together comprise the Remote Islands National Wildlife Refuge Complex (Howland, Baker, Jarvis, Kingman Reef and Palmyra Atoll).

Opportunities to sample migratory birds on these islands are limited by available habitat, the abundance of various target species, and the logistics of sampling, which include the presence of trained personnel and infrastructure to store and transport samples. Capture of waterfowl and shorebirds requires a substantial workforce in the field, and on many islands there are few or no trained biologists available to participate. Sampling live birds will require hiring, equipping and fielding small teams of biologists and technicians focused on avian influenza monitoring. Fecal sampling and surveillance for migrant mortalities require fewer people per site, and so can occur at more locations, yielding expanded spatial and taxonomic coverage.

Sampling Intensity

The U.S. Strategic Plan includes a hypothetical rationale for minimum rates of sampling that would be necessary to detect Asian H5N1 in a target population under assumed rates of virus prevalence (IAEDWG 2005; see Attachment 7). For general guidance, it was calculated that a minimum of 200 samples would be required to have a 95% probability of detecting Asian H5N1, if the virus had a prevalence of 1.5% in a population of >1000 individuals. Because most migratory populations of interest are larger than 1000 individuals, the prevalence of H5N1 within

the population is the key variable determining sample size, and is quite sensitive. For example, if the prevalence were 0.1%, 3000 samples would be needed for the same degree of confidence.

Most of the known strains of Asian H5N1 are pathogenic to some degree in ducks, some extremely so (Sturm-Ramirez et al. 2005, Hulse-Post et al. 2005). Even if a strain of HPAI H5N1 is non-pathogenic in a given species (i.e., 100% of infected birds survive and shed the virus), it would only be one of many avian influenza viruses circulating in the population, so its prevalence would be some fraction of the total influenza prevalence within each species (e.g. H5 was only 7% of all H subtypes in a recent North American duck survey; Hanson 2005). Of course, significant mortality rates from Asian H5N1 would cause lower prevalence within a population. A review of all avian influenza surveys published up to 2002 (Hanson 2003) showed that avian influenza prevalence is typically higher in ducks (approximately 9%, but often 20% in migrating juveniles) than shorebirds (approximately 2%). So while the 1.5% H5N1 prevalence assumption (which yields sample sizes of 200) is potentially valid for waterfowl, achieving high confidence of detection in shorebirds would require much larger sample sizes. Significantly, ruddy turnstones, a target species in this plan, consistently have higher avian influenza prevalence than other shorebird species tested (Hanson 2003).

Sample sizes obtainable in the Pacific islands will be limited by actual migrant population sizes in the case of waterfowl and some shorebirds, and by manpower and capture technology in the case of the relatively abundant shorebirds. Obtaining sample sizes that are statistically meaningful will require dedicated and experienced personnel. Opportunities to sample species that migrate directly from Asia and may not be sampled elsewhere in the U.S. should be taken despite the relatively low statistical power such samples may have. Estimation of statistical power will be possible as sampling progresses using total influenza prevalence rates in each species sampled.

SAMPLING PROGRAM

The species in Table 1 are divided into primary, secondary and tertiary sampling groups. Primary species will be sampled intensively by live capture and fecal sampling. Secondary species will be captured for sampling only to the extent that doing so does not interfere with sampling the primary species due to resource limitations. Tertiary species will be sampled if captured during other surveillance activities. All three groups are potentially informative in found dead, and will be targets of mortality surveillance.

Dedicated surveillance crews are anticipated to operate for extended periods within the main Hawaiian Islands, Guam and the CNMI. These crews will focus on capture of waterfowl and shorebirds. Fecal sampling and mortality recoveries will occur at those sites as well as at Midway, Palau, and American Samoa. An expedition of approximately two weeks' duration is proposed for Kwajalein Atoll in the RMI to capture shorebirds and do fecal sampling. No sampling is proposed for the FSM because other sites appear to sample the same migratory populations. The cost of air charters to Palmyra makes routine sampling there prohibitive.

Detection and investigation of morbidity/mortality events

Active searching for waterfowl and shorebird mortalities, the primary indicator for Asian H5N1, will focus on high-use areas in the main Hawaiian Islands, Guam, the CNMI, Palau, and American Samoa (Table 2). Rather than focusing only on "unusual" mortality events, all mortalities of migratory birds will be investigated as part of this surveillance effort. However, dense nesting colonies of seabirds have a background level of mortality, so in those locations where such colonies are closely monitored (e.g. Midway), only unusual mortality events involving these tertiary targets will be investigated.

Individuals of all species in Table 1 are considered suitable for collection and processing. This surveillance will be a corollary duty to the live bird sampling and fecal sampling performed by dedicated surveillance personnel. Revisit intervals should be 3 days or less, to avoid decomposition rendering specimens unusable. Biologists at all federally- and locally-managed wetlands will be asked to participate by notifying the surveillance teams if morbidity or mortality is discovered during routine duties, but current staffing levels at most sites are insufficient for the intensity of searching and observation required to consistently retrieve useful carcasses.

Table 2. Areas for migratory bird mortality surveillance

Hawai‘i	Kaua‘i	Hanalei NWR, Huleia NWR, Mana plains, Hanalei Trader taro fields, Hanalei Post Office taro fields, Waipa taro fields, Kipu Reservoir, shorelines
	O‘ahu	James Campbell NWR, Pearl Harbor NWR, Amorient Aquaculture, Chevron USA ponds, Kawainui Marsh, Hamakua Marsh, Nu‘upia Ponds (Marine Corps Base), Honolulu Int’l Airport reef runway, Kualoa State Park, shorelines
	Maui	Kanaha Pond State Wildlife Sanctuary, Kealia Pond NWR, HC&S reservoirs 70, 71, 72, 80, 81, shorelines
	Moloka‘i	Kaunakakai wastewater treatment plant, Kualapu‘u reservoir, Kaluakoi golf course, Ohi‘apilo Marsh, shorelines
	Lana‘i	Lanai City oxidation ponds, shorelines
	Hawai‘i	Shipman Pond, Paiakuli Reservoir, Kehena Ponds, Waiakea Ponds, Kaloko-Honokohau NHP, Opae‘ula Pond, Keanakolu Road stock ponds, Kealakehe wastewater treatment plant, shorelines
Midway		Sand Island, Eastern Island
Guam		Fena Reservoir, Agana Marsh, shorelines
CNMI	Saipan	Susupe wetlands, shorelines
	Tinian	Lake Hagoi, shorelines
Palau	Koror	Shorelines
	Babeldaob	Ngardok Lake, Ngerkall Lake, shorelines
Am. Samoa	Tutuila	Pala Lagoon, shorelines
	Aunu‘u	Pala Lake, Faimulivai Marsh, shorelines

Staff at Midway, Palau and American Samoa will be able to revisit sites during the week by driving or short boat rides. Teams in Hawai‘i and the Marianas will need to focus on specific islands on a rotational basis, for example flying to Maui for one week and visiting all targeted wetlands and shoreline sections 2-3 times that week while also doing live captures and fecal collections of dispersed birds.

Several localities have ongoing dead-bird reporting programs or are considering establishing such programs, with hotline numbers and public awareness campaigns as key features. These programs will increase the number of potential carcasses, but careful screening will be needed to prevent responses to calls from consuming time better used for direct surveillance work. Local project personnel will be points of contact and responders to appropriate reports. This aspect of surveillance will be expanded upon in “Coordination and Communication,” below.

Numbers of target birds obtained for mortality sampling are unpredictable at this time because no such system has been implemented previously. Table 3 lists predicted sample sizes for this and other sampling modes, but caution should be used in projecting the mortality numbers.

Sampling of Live Birds – Waterfowl

Migratory ducks will be caught at selected wetlands in Hawai‘i, Guam, and the CNMI using baited swim-in traps or by rocket net if groups are predictably located. Traps will be based on published designs for dabbling ducks, modified to contain diving ducks as well. Traps are best deployed in areas out of view of the public, with controlled access, and where predatory mammals are controlled to some degree. Pre-baiting is usually required to achieve consistent results. Trapping will begin in mid-September and continue through mid-January, approximately one month after the arrival of the latest-arriving common migrant duck (Engilis et al. 2004). Fecal collections from loafing ducks may be obtained but are not expected.

Sampling of Live Birds – Shorebirds

Trapping of shorebirds at territories and aggregation sites, and fecal sampling of birds without trapping, will be the sampling modes for this group. Trapping is dependent on the presence of multiple experienced personnel, and so will occur only in the Mariana Islands and Hawai‘i, while fecal collection can occur at all sites. Exact locations will be determined by surveillance personnel in consultation with local experts. Secondary and tertiary target species that commingle with primary targets will be sampled if caught.

Pacific golden-plovers will be mist-netted, but this technique is not very successful with most other shorebird species. Rocket netting will occur in Hawai‘i, Guam, CNMI, and Kwajalein at locations where mixed or single-species flocks congregate for feeding or loafing.

Collection of observed fecal deposits is suitable for sampling all shorebirds, and will occur at all sites. Individual birds will be observed through binoculars or spotting scope, and feces will be collected within a few minutes of deposition. Locations of each sample will be recorded so that repeat sampling of territorial individuals is avoided. Personnel at Midway, Palau and American Samoa will focus on this sampling mode because they will lack the number of staff required to

safely capture birds in nets. Collection of feces from aggregations introduces potential error in species assignment unless the aggregation is consistently monospecific. Fecal deposits from mixed species groups will therefore be avoided unless other collection methods yield insufficient sample sizes.

Shorebird sampling will begin in late August and continue until most shorebirds migrate north in April. The sampling at Kwajalein in the RMI will occur over two weeks in September, both on Kwajalein Island and Roi-Namur Island.

Sentinel Ducks

With the cooperation of their owners, at least one flock of captive ducks currently used for apple snail control on Kaua'i will be used as sentinels. Ducks will be checked for antibodies to avian influenza H5 prior to beginning the program in September. Weekly swabbing of at least 20 birds per location will be required to maintain a functional sentinel surveillance program. This program will continue through January.

Table 3. Summary of projected sample yield by site, species and technique.

Location	**Capture samples**†	**Sentinel samples**	**Mortality samples**‡	**Fecal samples**	**Time window**
Hawaii	100 ducks + 200 mallards + 300 PGPL + 50 RUTU = **650**	20 ducks * 18 wks (Sept-Jan) = **360**	H carcasses/mo * 9 mos * 2 swabs = **18H H=20?**	600 PGPL+300 RUTU +100 tattler = **1000**	Aug-Apr
Midway	0	0	M carcasses/mo * 9 mos * 2 swabs = **18M M=10?**	4/day * 20 dy/mo * 9 mos = **720**	Aug-Apr
RMI (Kwajalein)	50 PGPL + 50 RUTU = **100**	0	0	100 PGPL + 100 RUTU = **200**	Sept (2 weeks)
Guam & CNMI	5 ducks + 150 PGPL + 25 RUTU +15 misc. shorebirds = **195**	0	G carcasses/mo * 9 mos * 2 swabs = **18G G=10?**	300 PGPL+ 50 RUTU + 20 Whimbrel + 20 tattler + 70 sandpiper = **460**	Aug-Apr
Palau	0	0	P carcasses/mo * 9 mos * 2 swabs = **18P P=6?**	4/day * 20 dy/mo * 9 mos = **720**	Aug-Apr
Am. Samoa	0	0	S carcasses/mo * 9 mos * 2 swabs = **18S S=6?**	4/day * 20 dy/mo * 9 mos = **720**	Aug-Apr
Totals	945	360	est. 936	3820	

† PGPL: Pacific golden-plover; RUTU: Ruddy turnstone; BTCU: Bristle-thighed curlew
‡ Each carcass yields one tracheal and one cloacal sample.

INTEGRATION AND SUPPORT FUNCTIONS

Personnel

Because of the large distances involved and the lack of available capacity in many island jurisdictions, establishing effective surveillance for Asian H5N1 will require positioning of personnel at a local level. The primary responsibilities of field personnel will be to:

1. Conduct live bird surveillance through capture and fecal sampling
2. Regularly patrol areas considered high risk for introduction of Asian H5N1 for presence of avian mortalities and document extent and nature of mortalities
3. Serve as a central point of contact in the area for reporting of avian mortality
4. Respond to avian mortality events
5. Collect, package, and properly store freshly dead specimens from mortality events
6. Submit specimens to appropriate diagnostic laboratories for HPAI testing.

Based on surveillance workload, geographic isolation, and available infrastructure, the following personnel needs have been identified:

Hawaiian Islands: Three biologists. One FTE from USDA-Wildlife Services is being dedicated to HPAI surveillance of live birds in Hawaii. Two additional FTEs will be contracted by WS through DLNR to assist in those efforts and form a three-person surveillance team. The primary responsibility of this team will be coordination and execution of wild bird captures, sentinel sampling, and fecal collections, and submittal of specimens to diagnostic laboratories. These personnel can also conduct and assist in dead bird surveillance and recovery. We anticipate that existing infrastructure in Hawaii will assist greatly in detecting and reporting dead migratory birds. Examples of such infrastructure include the DOH West Nile Virus hotline, National Wildlife Refuges on O‘ahu, Kaua‘i, and Maui, National Parks on Hawai‘i, and DLNR’s Kanaha Pond Wildlife Sanctuary on Maui.

Midway Atoll NWR: Two half-time biologists. These staff will be funded by FWS and are spouses of personnel already stationed on Midway. The primary responsibility of these biologists will be to conduct mortality surveillance and fecal sampling of shorebirds, and to submit specimens to diagnostic laboratories. Because of the small size of Midway, these tasks will not require a full time position, but having two biologists working together will improve efficiency.

Mariana Islands: Three biologists. One FTE from WS is being dedicated to HPAI surveillance of live birds in Guam. Two additional FTEs will be contracted through WS by FWS to assist in those efforts and form a three-person surveillance team. The primary responsibility of this team will be coordination and execution of wild bird captures and fecal collections, and submittal of specimens to diagnostic laboratories. These personnel can also conduct and assist in dead bird surveillance and recovery. Through cooperative agreements, this crew of three will conduct surveillance in both Guam and the CNMI.

American Samoa and Palau: One FTE biologist will be funded by FWS for each location. The primary responsibility of these biologists will be to document and respond to mortality events, to sample fecal material from shorebirds, and to submit specimens to diagnostic laboratories.

Coordinator and support: This surveillance effort will span multiple geographically disparate islands and involve numerous state, territorial, federal, and private organizations in different legal jurisdictions and countries. Effective coordination of such an effort will require the undivided attention of an individual who can facilitate interagency collaborations and help troubleshoot sensitive issues that may arise, particularly at the jurisdictional level. A coordinator at the GS-11/12 level will need to be hired to fill this role. This person will be based in Honolulu because it houses the administrative headquarters of all major federal agencies that are involved in AI surveillance regionally (FWS, USDA, USGS). Honolulu also serves as a central hub for communications between Pacific territories and the contiguous 48 states. In addition, a support biologist (GS-7/9) would be hired to assist the coordinator and facilitate logistical support for the field personnel.

Table 4. Summary of activities, outputs, and costs by location.

Location	Activities	Agency	Partners	Sample output*	Cost
Hawaii	Mortality surveillance, fecal sampling, live bird capture, sentinel duck sampling, Regional coordination and training.	WS, DLNR, FWS, USGS	FWS Refuges, Ducks Unlimited, KS, HC&S, HDOH, HDOA, NPS, private property owners	Cloacal: 1800 Fecal: 1000 Mort: 360?	$108,000 WS $100,000 WS (to DLNR) $269,660 FWS $42,300 USGS
Midway	Mortality surveillance, fecal sampling	FWS, USGS		Fecal: 720 Mort: 180?	$70,250 FWS $3,300 USGS
Kwajalein	Live bird capture, fecal sampling	WS, DLNR, USGS, FWS	US Army	Cloacal: 300 Fecal: 100	(salaries from Hawaii WS) $25,908 FWS
Guam & CNMI	Mortality surveillance, fecal sampling, live bird capture	WS, FWS, USGS	Guam DLNR, CMNI DLNR, NPS	Cloacal: 400 Fecal: 460 Mort: 180?	$72,000 WS $177,600 FWS $40,014 USGS
Palau	Mortality surveillance, fecal sampling	FWS, USGS	Gov't of Palau	Fecal: 720 Mort: 108?	$73,650 FWS $30,800 USGS
American Samoa	Mortality surveillance, fecal sampling	FWS, USGS	DMWR, NPS	Fecal: 720 Mort: 108?	$73,650 FWS $19,650 USGS
			Total costs	WS	$180,000
				WS through DLNR	$100,000
				FWS	$690,718
				USGS	$136,064
				Project total	$1,106,782

*Note: To insure that the WS quota of 2,500 cloacal samples is collected, capture of additional primary, secondary and tertiary species will be conducted opportunistically - in addition to what is indicated above

- to insure total requested cloacal output is produced. Every effort will be made to collect samples in a biologically meaningful manner utilizing DOI (FWS & USGS) funds. Because of the limited data available to determine optimal sampling, slightly differing sampling goals between Federal funding agencies, and the broad geographic scope/complexity of implementing this project regionally, it should be made clear that the Regional Interagency Coordinator and Leads of Hawaii & Mariana Sampling Teams will need to be given a fair degree of latitude and discretion in achieving the goals indicated in this plan. Sites discussed in this plan, including Hawai'i, have far greater complexities in implementation compared to most areas in the mainland U.S. or Alaska. Reviewers, readers, and funders of this plan are requested to keep this consideration in mind and not to underestimate the coordination required to implement this program successfully.

Training

Biologists conducting the field work for this project in Hawai'i and the Marianas will need to have significant previous experience in capture and banding of birds, preferably shorebirds and waterfowl. In addition, all personnel will need demonstrated expertise in identification of shorebirds to ensure the reliability of the fecal sampling component. Field personnel will undergo additional training in Hawai'i in August prior to beginning the project. This training will have three components:

1. Mist netting technique for plovers, conducted by Dr. Wally Johnson.
2. Rocket net technique for shorebirds and waterfowl, conducted by FWS personnel from the mainland U.S.
3. Sample collection, sample handling, and personal protection, conducted by Dr. Thierry Work of USGS.

Training for mortality investigation, and shipment protocols for swab samples and carcasses will be conducted on-site by USGS in Hawai'i, Guam, Palau and American Samoa. This will allow larger numbers of local cooperators to be trained and to understand the needs of the program, as well as allow USGS personnel to troubleshoot any potential problems with sample shipment from each locale.

An independent, parallel system for Asian H5N1 surveillance is being established in the southwestern Pacific under the auspices of the Secretariat of the Pacific Community (SPC), using the WHO-certified laboratory in Australia as the diagnostic facility. Personnel identified as appropriate by the SPC will be invited to attend these trainings.

Flow of Diagnostic Samples

Samples from this program will be in the form of cloacal swabs from live birds, swabs of fecal deposits, and carcasses of dead birds. Basic protocols for taking and handling avian influenza samples have been developed in cooperation with NWHC, USDA, and other cooperators. The U.S. Interagency Strategic Plan includes procedures and protocols for safely handling bird carcasses (Attachment 1) shipping carcasses (Attachment 2), taking cloacal swabs (Attachment 3), taking and shipping fecal samples (Attachment 4), and operating a sentinel surveillance system (Attachment 6).

All samples from all field sites will first be sent to the NWHC Hawai'i Field Station in Honolulu. There, swabs obtained in the field will be consolidated for shipment to laboratories in the

mainland U.S. Necropsies of carcasses will be performed in Honolulu and tracheal and cloacal swab samples taken from the carcasses. Samples from live birds and from necropsies will be shipped from Honolulu to the NWHC laboratory in Madison, Wisconsin for RT-PCR screening and virus isolation. Fecal samples and duplicate samples from necropsies will be submitted to the NAHLN-certified laboratory operated by the State Laboratory Division of the Hawai‘i Department of Health, in order to reduce turnaround time and shipping costs. Fecal samples may also be shipped to the NWRC laboratory in Ft. Collins, Colorado. Samples from any lab that test positive for HPAI will be sent to the NVSL laboratory in Ames, Iowa, where definitive typing of AI viruses will be done.

Swabs taken for HPAI surveillance must either be chilled and shipped fresh to arrive at a laboratory within 48 hours, or frozen and maintained at -70°C or colder during shipment. Maintaining this cold chain is crucial to allow effective molecular RT-PCR testing and virus isolation. Because of the distances samples must travel within the Pacific region, special transport media and nitrogen vapor shipping containers will need to be purchased, distributed and maintained as part of this program.

Nitrogen vapor shippers will be charged with liquid nitrogen in Honolulu and swapped with shippers in Guam, Samoa and Palau on a weekly to bi-weekly basis, depending on sample accumulation. Carcasses submitted for diagnostics will need to be chilled or frozen as appropriate, properly labeled and bagged, and properly packaged for shipping to Honolulu. Refrigerators and freezers to store carcasses prior to shipping will be purchased on site. Coolers and ice packs will be purchased in Honolulu and supplied to the field. Coolers and nitrogen vapor shippers will be pre-labeled and prepaid, so that shipments of carcasses and swabs will require the minimum of effort by field personnel.

To ensure a seamless process for submittal of samples, it will be necessary to draft memoranda of agreement with major air carriers (e.g. Hawaiian Airlines for Samoa and Guam and Continental Air Micronesia for Palau) to ensure that appropriate agencies gain “Known Shipper” status. In addition, memoranda of agreement will need to be drafted with appropriate regulatory agencies (DOA, USDA, FWS) so that appropriate permits are in place for reception of diagnostic specimens.

Data Management and Reporting

All samples obtained as part of the Interagency Strategic Plan for HPAI surveillance will be logged into the NBII Wildlife Disease Information Node by the receiving laboratory. This is a web-based system that ensures complete tracking and data security for all samples. Data that will be needed for each sample include species, sample type, location (from GPS), any cold chain gaps, etc. Training of field biologists and monitoring of incoming sample data sheets will be required to maintain data quality. All shipments of samples to NWHC in Honolulu will be accompanied by hard copy data sheets as well as an email notification to the lab with all relevant sample data included.

The NBII system has a variety of permission levels, so field personnel may not be able to determine the diagnostic results from their samples. Because of the significant national impact

of a positive Asian H5N1 detection, notification of local authorities will occur by official communication channels rather than through simple posting of the result in the NBII database.

As described in the U.S. Interagency Strategic Plan, positive tests will result in immediate notification to the agency submitting the sample, the state veterinarian, the USDA area veterinarian in charge, the chief state public health official, and the CDC/USDA Select Agent program. Because of the importance and public impacts of a confirmation of Asian H5N1, notification will go first to top federal and state officials (e.g., Secretaries of Agriculture and Interior, Governors, Directors, etc.).

Coordination and Communication

Given the high level of concern among the public and wildlife agencies, and the logistical and jurisdictional complications of Asian H5N1 surveillance in the Pacific, effective coordination and communication will be critical. Key issues that will require focused attention by the Interagency Coordinator include:

- Integration and coordination of HPAI surveillance and reporting activities with agriculture and public health agencies within State, Territorial, Commonwealth, and Republic governments. Open, two-way channels of communication need to be established early in the project and maintained, and any conflicts resolved expeditiously.
- Informing the three lead agencies of progress and issues with the program.
- Stepping down the guidelines in this plan to the local level, with the assistance of cooperating agencies and field personnel. This will include resolving issues such as lack of veterinary capacity for response to poultry mortalities in most areas outside of Hawai‘i.
- Reviewing and monitoring surveillance activities by field personnel, quality control of sampling and reporting of activity.
- Obtaining and maintaining assistance and access for sampling activities, which will require forming relationships with land and facility managers and other partners, and resolving conflicts that arise.
- Establishing and maintaining a productive collaboration with the SPC surveillance effort in other Pacific island countries.
- Assisting in outreach and communication activities that affect the surveillance program. This includes acting as the public face for HPAI surveillance in the region, encouraging reporting of dead migratory birds, helping craft messages to enhance public cooperation and convey accurate information on HPAI, and making sure that such messages are in harmony with messages from health and agriculture authorities in the various jurisdictions.
- Assisting with emergency response planning and contingency actions in the event of a detection of Asian H5N1, either locally within the region or nationally. The latter could involve redeploying surveillance personnel or activities.

Implementing this plan in time to begin surveillance by September 2006 will be a formidable task and will require intense effort by all personnel involved. Early detection of Asian H5N1 may well occur in the Pacific, however, and the importance of this for island wildlife, the economy, and human health makes success in the effort critical.

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