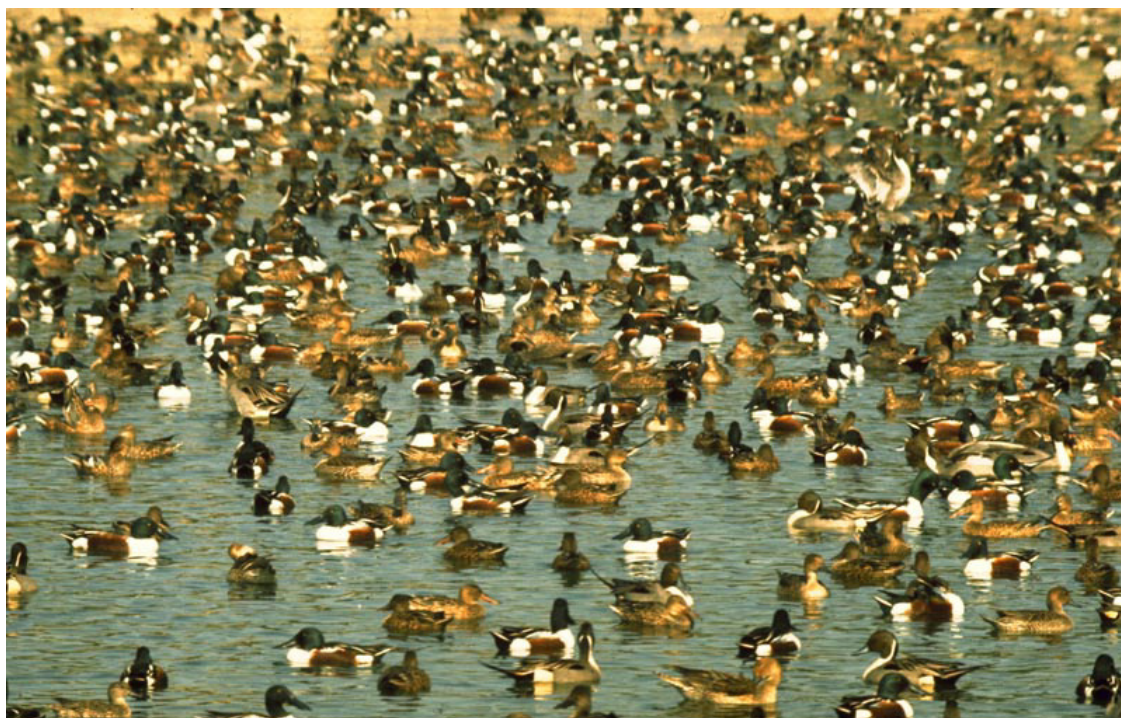




## Surveillance of wild birds



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# Surveillance of wild birds

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Better quality data collection, and reporting of both positive and negative avian influenza (AI) surveillance, is crucial to understanding general patterns in outbreaks, possible routes of transmission and the potential impacts on migratory bird populations (Butler 2006). This information can be used to focus contingency efforts, to predict future outbreaks, and to guide effective policy to reduce the economic and conservation impacts of avian influenza.

Information on recent outbreaks of H5N1 avian influenza from sources such as the OIE, Pro-MED-mail or the media is often presented without important details such as species, collection methods or the number of individuals sampled.

Wild bird surveillance needs funding but this is not viewed by many authorities as a priority action against H5N1. An example of this is the fact that EU matched funding is made available for laboratory testing of samples but no funding is available for collection of samples, or for structured surveillance for sick or dead birds. Ornithological NGOs could help play a vital role in wild bird surveillance, given adequate resources.

This document provides guidance to BirdLife Partners and public authorities on appropriate sampling, data collection and reporting strategies to monitor avian influenza in wild bird populations.

## **1. General sampling strategies to detect outbreaks**

### **1.1. Monitoring and sampling of sick or dead birds**

This is the most cost-effective method for rapid detection of the presence of H5N1. So far, H5N1 has been detected almost entirely in dead wild birds despite sampling of many tens of thousands of healthy wild birds across Europe, Asia, North America and Africa. The weakness of this approach is that, by definition, it will not help to identify asymptomatic carriers of the disease. Although previous outbreaks indicate that a wide range of bird species can be affected by H5N1, surveillance efforts should focus on waterfowl because they have been the dominant group affected in major wild bird H5N1 outbreaks in China, Mongolia, Azerbaijan and Germany (Liu *et al.* 2005, Chen *et al.* 2005, 2005). For a list of affected species, see <http://tinyurl.com/fbbdu>

Areas with high waterfowl densities should be monitored regularly to scan flocks for sick or dead birds. The frequency of monitoring should be increased during periods of extensive bird movement between sites (i.e. during migration, immediately after birds

arrive at new sites or when harsh environmental conditions cause dispersal movements of birds). Even if no dead birds are observed, the exact location, time, the number of birds of each species, and the presence of any colour-ringed or otherwise individually identifiable birds should be systematically recorded, so that it is possible to quantify monitoring sample effort, and better understand the movement patterns of birds. This information can be important if an outbreak in the area occurs later.

Censuses of sick or dead birds can be carried out most easily by government or NGO staff based at a site. However, site coverage and census frequency can be improved by training members of the public who frequently visit wetlands (e.g. birdwatchers, hunters, members of conservation organisations, student volunteers (Table 1 and 2)). Moreover, many of the recent outbreaks of H5N1 in Europe have involved small numbers of birds that were not always in areas of high bird densities. Thus information on how the general public can quickly report sick or dead birds to authorities (such as contact phone numbers) needs to be widely advertised. To minimise the risk of infection, the public should be discouraged from handling carcasses.

## **1.2. Sampling of apparently healthy birds**

This approach can help assess background levels and identify strains (both high- and low-pathogenicity) of avian influenza circulating in wild bird populations. Monitoring of healthy birds could help identify any strains of H5N1 that can be asymptotically carried by some species of wild birds, and the species that can carry them. However, this method cannot serve as an effective 'early warning system' because of the large sample sizes required to be confident of a negative result. Results need to be interpreted with caution, because most trapping methods are biased towards weak or inexperienced individuals, and it cannot therefore be assumed that captured birds found with the virus were necessarily 'healthy'.

So far, two studies have demonstrated the presence of H5N1 in healthy wild birds (Kou *et al.* 2005, Chen *et al.* 2006). Previous studies have indicated differences in the severity of infections between bird species and between strains of H5N1 (Perkins & Swayne 2003). Moreover, some strains of low-pathogenicity (LP) avian influenza may be precursors to high-pathogenicity (HP) strains (Webster 1998). Monitoring changes in the genetic structure of the HP and LP strains of the virus may help to assess risks and predict the likelihood of major HP outbreaks (Hulse-Post *et al.* 2005).

Sampling near areas of wild bird or poultry outbreaks may help to assess how easily the particular strain can be transmitted to other birds (Terakado 2004). To reduce the additional costs of sampling, and enable sampling of a larger number of birds, H5N1 sampling should be coupled, where possible, with ongoing ringing studies. This approach may be particularly informative if birds are being individually colour-banded.

Individually identifiable birds can be followed in the field over time and may help to provide information on local and migratory movements of birds, and determine the location and timing of infections.

Sampling should cover both migratory and sedentary species. Dispersal movements by species usually considered 'sedentary' can also spread the disease over some distance – as in the outbreak among Mute Swans *Cygnus olor* in Europe. Such dispersal movements may be less predictable in direction and timing than long-distance seasonal migration,

and may be influenced by extreme cold weather events, droughts or other important changes in habitat quality (Scott & Rose 1996).

Catching birds can be a costly and time-consuming process, and this may be a particularly significant limitation in countries where there are few experienced ringers. Information and assistance for capturing waterfowl, and country-specific advice on the species that are most vulnerable to infection, may be obtained by contacting local ringing groups, Birdlife International's national Partner organisations, Wetlands International or the Wildfowl and Wetlands Trust.

Cannon nets can be used to catch large numbers of waterfowl. However, they may disturb birds and could thus risk spreading infected birds over a larger area. Baited swan pipes, cage traps or swan hooks (long poles with a hook on the end that can be placed around a swan's neck to bring it close enough to handle) may be effective, especially during periods of cold weather. These methods may be less likely to disturb birds than cannon nets, and for some species such as Mute Swans that can be easily habituated to people, baiting and keeping the birds contained over a small area may allow repeat sampling and prevent dispersal to surrounding areas. Cloverleaf traps may be useful for catching birds on water, while mist nets may be used to capture shorebirds. Efforts should be made to capture a representative sample of the population. For example, using baited traps may be more likely to capture younger or unhealthy birds whereas cannon nets may capture a more representative sample of birds.

Careful precautions should be taken to prevent transmission of H5N1 when handling birds (see: <http://www.bto.org/ringing/diseases-from-birds.doc>). Close attention to hygiene is essential. Any signs of illness such as droopy posture, fluffed feathers, poor feather condition, or cloudy eyes with discharge should be carefully noted. Cloacal, salivary or tracheal swabs and blood samples can be taken from captured birds.

Whenever possible, blood samples should be taken in order to detect antibodies. This is because faecal or salivary swabs may fail to detect viruses because infected birds excrete avian influenza viruses for only a short period (less than 2 weeks) and at a very low titre level (Lu et al 2003). All types of samples should be clearly labeled with relevant information on species, age and condition, or with the ring number where these data are recorded in a ringing book.

As infection rates in wild populations can be quite low, many wild birds would have to be sampled to be confident that a population is free of H5N1. Recent outbreaks in Rügen Island, Germany, involved very high densities of waterfowl. Out of the 4,000 birds found dead, only 3 % were infected with H5N1. As 10,000 healthy birds were also tested near Rügen Island and found to be negative, the disease appears to be transmitted even when overall population infection rates are very low.

The sample size required needed to show statistically (i.e.  $\alpha = 0.05$ ) that a population does not have H5N1 can be calculated by:

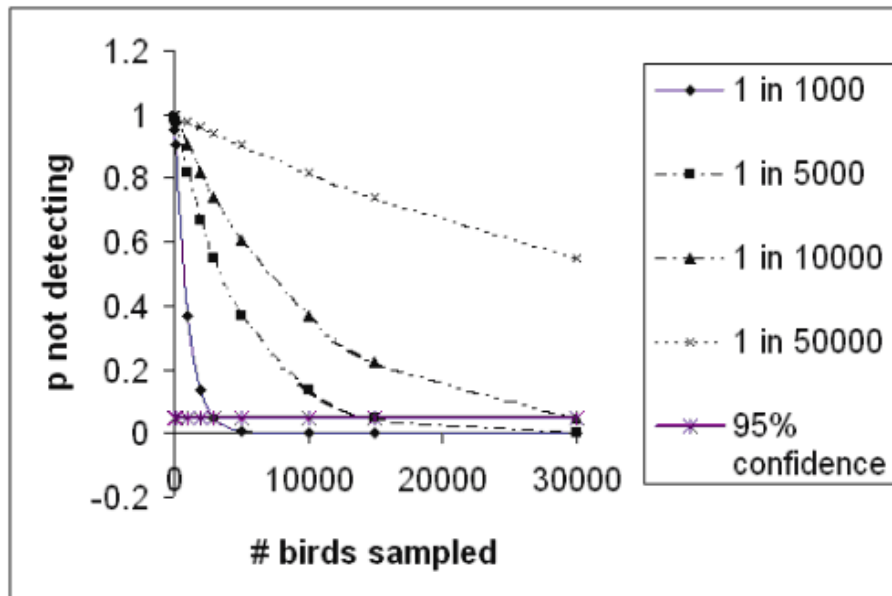
$$Pf = (1 - Ir)N$$

**Where Pf = Probability of failing to detect an infection that is present (a 'type 2' error)**

**Ir = infection rate**

**N = Sample size**

**Example:** Suppose the rate of asymptomatic infection in an area is one in a thousand ( $=0.001$ ) birds. You would need to sample 3000 birds to be 95% confident that the virus wasn't present in a given population ( $0.9993000 = 0.0497$ ).



### 1.3. Sampling of apparently healthy birds shot by hunters or wildfowlers

Hunters may also contribute to surveillance activities by submitting rectal swabs of shot birds for laboratory testing. This can determine whether birds are infected with H5N1, but the approach has some weaknesses. First the health of the bird before it is shot cannot be assessed. Second, there can be no repeated analysis of the same individuals. Third, loud sudden disturbances such as gunshots will flush birds (Madsen & Fox 1997) and could cause infected individuals to be dispersed to other areas where they may infect other birds. Moreover, such intense disturbance could permanently change birds' movement patterns, impeding predictions of potential areas of future outbreaks (Béchet *et al.* 2004). Finally, similar to the problems associated with bait-traps in the above section, there may be a strong capture bias because younger or unhealthy individuals may be more likely to be shot.

Because of these weaknesses, governments should not use this method as the only means of sampling and should not advocate increasing waterfowl hunts in order to assist with AI surveillance. Hunters should be trained to ensure that they collect essential background information for the samples they take, such as species, sex, age and location, as well as any signs of sickness in the bird before it was shot).

## **2. Data recording**

If infected dead birds are detected, ecologists, ornithologists or trained student volunteers should assist veterinarians and visit outbreak sites to collect relevant details on the presence of other birds, and location of the outbreaks (see Table 1 and 2 below). If the outbreaks occur in areas close to villages, local people should be interviewed because they may be able to provide information on possible sources of infection and other information to complete Table 2. Moreover, during these interviews public authorities may be able to provide greater guidance and information on how to minimise the risks of infections to people or poultry, as well as discouraging people from inappropriate measures such as culling or disturbing wild birds.

Many sampling programmes to date have been hampered by frequent lack of identification to species level. A digital photograph of each individual bird sampled should be included in a standard protocol.

Although faecal or salivary swabs or serological tests may be sufficient to detect the virus, more detailed necropsies should be conducted on a subset of the carcasses to determine the actual cause of mortality. In some cases dead birds infected with H5N1 could have died from other causes, such as starvation.

For all three types of sampling methods there must be a labelling system so that each carcass/swab sample could be linked by a reference number to the sampling details of an individual bird (age, sex, sampling location, sampling date etc). All relevant details of *negative* test results must also be recorded (Table 1). Negative test results are a crucial element of predicting patterns in outbreaks and assessing virulence or transmission rates between hosts.

### **2.1. Laboratory Diagnosis**

Details of methods used to detect the presence of H5N1 in swab or blood samples can be obtained at <http://www.influenzareport.com/ir/ai.htm> (Harder & Werner 2006) or on the OIE manual at [http://www.oie.int/eng/normes/mmanual/A\\_00037.htm](http://www.oie.int/eng/normes/mmanual/A_00037.htm).

A digital photograph of each individual bird sampled should be included in a standard protocol during the laboratory diagnosis. Measurements of the bill and wing length should be undertaken, especially if the identity of the species is in doubt.

### **2.2. Data reporting**

At present report to OIE, Pro-MED-mail, AI watch (Avian influenza discussion group) and Birdlife International in Cambridge: [science@birdlife.org](mailto:science@birdlife.org). In the future there may be a global web-based data-base so that people can rapidly report details of outbreaks or surveillance testing.

**Table 1.** Variables that should **ALWAYS** be collected during both positive and negative H5N1 surveillance tests.

<b>Timing</b>	Dates of collection
<b>Specimen description</b>	Alive or dead when collected
	If alive, capture method (eg. shot, cloverleaf traps, baited swan pipes or cage traps, mist-net, cannon net at roost sites)
<b>Location</b>	County, City
	Habitat type
<b>Population</b>	No. of birds of each species (scientific name) found dead
	No. of birds of each species tested
	No. of birds of each species that tested positive
<b>Context</b>	Details of nearby outbreaks (timing, distance between sites, human/poultry/wild bird outbreaks)

**Table 2.** Important variables that should be collected if there are sufficient resources during H5N1 surveillance tests.

<b>Timing</b>	Dates of collection
<b>Specimen description</b>	Alive or dead when collected
	If alive, capture method (eg. shot, cloverleaf traps, baited swan pipes or cage traps, mist-net, cannon net at roost sites)
	If dead state of decay when found
	Digital photo of specimen (open wings, spread tails, dorsal and ventral surfaces, Attach to document)
<b>Location</b>	
General	County, City
	Size of the area of land characterised below (eg. 2 km radius of outbreak)
	Habitat type: Coastal, estuary, salt marsh, tidal flat, inland (km to coast), river (water flow), island (size), fresh water wetland, approximate size of each type of contiguous habitat
	Water body description: Water source, size of lake/reservoir/swamp, water depth
<b>Human uses</b>	Surrounding land-use: Urban, agriculture (specify types),

	Poultry (distances and density), Aquaculture (specify types), Tourism, Villages (approximate number of households, and main source of income)
	Approximate percentage break-down of land use and habitat types (eg. 30 % salt marsh, 10 % sandy beach, 10 % roads or tourism infrastructure, 30 % banana plantations, 20 % tiger prawn aquaculture)
	Distances to major railways, highways, airports
	Signs of animal waste or carcasses in area
<b>Population details</b>	
General	No. of each species (Latin name) in affected area
	Resident / migratory breeding / wintering habitats
	If migratory, approximate timing of arrival or departure
	Details of known diurnal / tidal movement of species to nearby habitats
	Movement details of ringed individuals (Location and time of previous observations)
Outbreak	No. of dead birds of each species
	Details on sex, age (adult versus juvenile), rings of dead birds may help to identify migration patterns
<b>Sample type</b>	Faecal, blood, cloacal, tracheal, salivary, tissue
	No. of each species tested
	No. of each species tests positive
	Info on strain of H5N1 to assess whether wild healthy birds can carry strains of H5N1 lethal to poultry or people (Sturm-Ramirez, 2005. J of Virology).
<b>Conservation implications</b>	Description of threatened species in area (population size, movement patterns)
	Important Bird Areas or regions of high waterfowl density in area (distance to IBA from outbreak areas, information on movement between infected areas to IBA).
<b>Context</b>	Nearby outbreaks (timing, distance between sites, human/poultry/wild bird outbreaks)
	Details on future testing to be conducted in region

## Example

This is a hypothetical, fictitious record: it does **not** refer to an actual incident of H5N1.

### Sample date:

10 Jan – 14 Jan 2006

### Location

(12° 14' N 101° 45' E) 10 km stretch of beach + 1 km x 1 km salt marsh 3 km inland in Prachuap Khiri Khan Province, Khao Sam Roi Yod National Park, Thailand. Salt marsh, water sources tidal and also out flow from adjacent shrimp aquaculture ponds (Tiger prawns), and salt evaporation ponds

Surrounding land use (within 5 km): shrimp aquaculture (tiger prawn) (60 %), fish aquaculture (10%), salt evaporation ponds (20%), tourism infrastructure hotels and restaurants along beaches (10 %).

Three small villages 100 households within 5 km, only small-scale domestic poultry. Beach 40 km from main highway between Bangkok to southern Thailand. Closest airport 100 km Hua Hin.

### Population data

20 Common redshanks, 10 little terns, 3 Malaysian plovers, 5 Nordmann's greenshanks apparently healthy captured by mist-netting on mudflat near roost sites and tested with cloacal swab tests on beach. Reference labels on swab samples were affixed to matched each sample to individual tested.

According to Round et al 2003, 100-150 Common redshank migratory and found in sampling area between Dec – Feb, 100-200 little terns and 80 Malaysian plovers are resident and 5-20 Nordmann's greenshank stopover in January.

15 dead brown-headed gulls found on salt marsh 10 Jan – appeared fresh, 50 BH gulls alive in salt marsh within 1 km of dead birds. Little know about ecology of species, park superintendent says BH gulls feed in salt marsh during low tide but move elsewhere during high tide – Round, P for details on Thai waterfowl.

2 Cattle egret found dead on beach. Dead brown-headed gulls and cattle egrets were collected and tested for H5N1 in trachea.

### Test results:

20 Common redshank 20 all negative  
10 little terns 15 negative 5 positive  
3 Malaysian plovers all negative  
5 Nordmann's greenshank 2 positive  
15 Brown-headed gulls 13 positive  
2 Cattle egrets all positive

Subtype of one test: A/Ph/HK/NT123/03, Highly Pathogenic

### Conservation concerns

Nordmann's greenshank – critically endangered species  
Malaysian plover – near threatened species  
20 km from Khao Sam Roi Yod freshwater Ramsar Reserve

### Relevant previous notes and future testing

Outbreak in city of Hua Hin, Prachuap Khiri Khan 10 Nov 2005

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