

The Epidemiology of H5N1 Avian Influenza in Wild Birds: Why We Need Better Ecological Data

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In 2005 and 2006, highly pathogenic avian influenza H5N1 infected wild birds or poultry in at least 55 countries in Asia, Europe, and Africa. Scientists still have limited understanding of how these wild birds were infected and of how the virus behaves in a field setting. Better ecological and ornithological data are essential to resolve these uncertainties. At present, information on species identity, location and habitat, and sampling and capture methodology, as well as details of the affected bird populations, are inadequate or lacking for most incidents of H5N1 in wild birds. Greater involvement by ornithologists and ecologists, who have extensive experience in conducting field research on wild animals, is vital to improve our ability to predict outbreaks and reduce the environmental and socioeconomic impacts of H5N1 avian influenza.

Keywords: avian influenza, H5N1, veterinary, ecology, virology

For nearly 10 years after its appearance in 1996, highly pathogenic avian influenza (HPAI) H5N1 was largely restricted to domestic poultry and to a small number of nonmigratory commensal wild birds that fed near infected poultry in Asia (Terakado 2004, Sims et al. 2005). In May 2005, an outbreak among wild birds occurred at Qinghai Lake, China, a site that was believed to be isolated from direct contact with poultry. Further outbreaks among wild birds, seemingly unrelated to poultry outbreaks, followed in 2005 and early 2006 at Erhel Lake in Mongolia and at a scattering of locations throughout Europe (Munster et al. 2006, Olsen et al. 2006).

These outbreaks have led to enhanced interest in the potential role of wild birds as vectors for H5N1 and in the behavior of the virus in natural environments. However, they have also highlighted the inadequacy of the available ecological data. Research and monitoring on avian influenza viruses are still largely the domain of veterinarians and virologists (Olsen et al. 2006). These scientists have expert knowledge in, for example, detecting avian influenza, identifying subtypes and strains, assessing virulence, and developing vaccines. However, most of their work is conducted with domestic or laboratory-reared animals in controlled laboratory settings. Excellent lab-based studies have answered important questions on topics such as host- or strain-specific pathogenesis of H5N1, the timescales of infection, and the routes of virus shedding (Guan et al. 1999, Perkins and Swayne 2003). For H5N1 outbreaks among wild birds, however, there needs to be much

greater input from field ornithologists and ecologists, as demonstrated repeatedly by the poor quality of data collected and reported on incidents of H5N1 in wild birds. For example, many outbreak reports to the World Organisation for Animal Health (OIE) identify wild bird species incompletely, incorrectly, or ambiguously. In peer-reviewed publications on H5N1 in wild birds, essential information on the field sampling methodology and the infected wild bird population is often missing, while laboratory methods, by contrast, are reported in great detail.

These deficiencies are not just of academic concern. Dealing effectively with the serious social, economic, and medical issues, together with the potential conservation issues, posed by H5N1 requires a base of sound and reliable information. Data that are incorrect or inadequate can lead to unwarranted assumptions and conclusions that in turn affect public perceptions, practical control and management measures, and the disposition of resources. Here we review some of the

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ornithological and environmental variables that have often been inadequately recorded or reported, and show how a more rigorous approach applied to future outbreaks of Asian HPAI H5N1 in wild birds could help improve scientists' understanding of the ecology of the virus.

Species identity and individual status

Even within a particular taxon, such as ducks or gulls, different wild bird species show significant variation in their ecology and behavior. To understand the role of wild birds in the epidemiology of avian influenza, knowing the exact species involved in particular incidents is fundamental. Unfortunately, many official reports and scientific papers do not identify bird species, referring only vaguely to, for example, "wild duck," "migratory duck," or even "wild bird" (see, e.g., Chen et al. 2006a, World Organisation for Animal Health 2006). The species was reported in only 15 percent of the 167 wild bird cases on the German national reference laboratory's list of confirmed outbreaks in February and March 2006 (Friedrich-Löffler-Institut 2006). If this is the standard in a western European country where there is advanced technology and numerous ornithologists, species identification is likely to be a much greater problem in less developed countries.

The problem of poor identification was highlighted in April 2006 when a swan was found dead, positive for HPAI H5N1, in a coastal village in Scotland. The bird had been dead for some time and lacked its head, the markings on which constitute the simplest (though not the only) identification feature. It was initially misidentified as a mute swan (*Cygnus olor*), and only after several days was confirmed to be a whooper swan (*Cygnus cygnus*) after DNA comparisons. The two species have very different migratory behavior in western Europe: Mute swans are mainly sedentary, whereas whooper swans are migrants, spending the winter in northwestern Europe but breeding further north in Iceland, Scandinavia, and northern Siberia (Scott and Rose 1996). Thus, depending on the species of the dead swan, the origin of the infection could be local (in Scotland) or farther afield in Europe, where a series of H5N1 infections in swans had been recorded in early 2006. Species identity should therefore prompt different mitigation responses. In the case of an infected mute swan, biosurveillance at nearby poultry or wild bird congregation areas would be warranted, whereas identification of a whooper swan should lead to a greater emphasis on genetic research to assess its geographical origin.

The use of vague identification terms has also been incorporated into virus nomenclature. Recent phylogenetic trees for avian influenza viruses have included several ambiguous identifiers, including A/egret/HK757.3/03 (Chen et al. 2005) and A/gull/Maryland/704/77. (Six species of egret are known to occur in Hong Kong [Chalmers 1986], and nine species of gull are recorded in the Maryland area.) In A/MDk/Jiangxi/2136/05 (Chen et al. 2006a), MDk is an abbreviation for "migratory duck," but the actual species from which samples were taken is not recorded. The list of three possible species includes the spot-billed duck (*Anas poecilorhyncha*). Both

resident and migratory populations of spot-billed ducks exist at the sampling collection area, and it is thus possible that the birds sampled were not even migratory.

The importance of identifying the study species clearly is also exemplified by a study of avian influenza in Slovakia (Betacova et al. 2005). The authors present their results in the context of evaluating the risks of transmission of avian influenza viruses from wild migratory waterbird populations. However, they do not identify the species that were tested. Moreover, one of the two species in which they detected avian influenza was a long-eared owl, *Asio otus*, which is neither a waterbird nor, in Slovakia, migratory (Cramp 1992).

Within species, identification should if possible include the sex and age of the individual tested. In some birds, the sexes and age groups behave differently, migrating at different times and by different routes, and moult and winter in different locations. In addition, natal dispersal in male waterfowl may be several orders of magnitude greater than in females (Cramp 1992). Among waterfowl, age may be important because first-year juveniles have higher avian influenza infection rates than adults (Halvorson et al. 1985), and thus juveniles may play a more important role in the maintenance of avian influenza viruses in natural habitats.

There also needs to be much more precise reporting on the status of infected individuals. Official reports of the OIE usually categorize birds as "wild fauna" or "poultry." It is often not clear, however, whether "wild" birds are truly free-living or are captive in zoos, parks, or wildfowl collections (or possibly escapes from such collections), which was the case in a "wild flamingo" in Kuwait. The implications for H5N1 epidemiology are different in each case. In Southeast Asia, "merit release" of captive birds into the wild for religious reasons provides a further complication (Severinghaus and Chi 1999). The origin of these birds is untraceable, but many are likely to have been bought in bird markets where proximity to infected poultry is a risk. Careful examination of such birds will usually reveal signs, such as wear on the wing and tail feathers, that show they have been kept in captivity (Martin Williams, ornithologist, Hong Kong, personal communication, 9 September 2006). However, such checks are rarely performed. Conservation programs may release captive-bred birds to augment or restore wild populations: H5N1 infections among such birds could have been acquired in captivity. Similarly, in the United States and Europe, waterfowl and pheasants are bred in large numbers at game farms and released into the wild for hunting (Smith and Rohwer 1997, Draycott et al. 2002). Again, such information needs to be reported. The outbreaks of H5N1 among several thousand bar-headed geese in China in 2005 provide a case in point. It has only become evident one year after the outbreaks that there had been captive-breeding farms of bar-headed geese in Lhasa and Qinghai Lake, close to the wild bird outbreaks, since 2003 (Butler 2006) and possibly as early as 1997 (Division of Nature Reserve Management 1997).

Semidomesticated waterfowl can present particular problems because, although they often closely resemble their wild

conspecifics, they are almost completely dependent on humans for food. As a result, they are tolerant of close approach by people and thus may be more likely to be included in sampling programs than their wild counterparts. Dependence on humans may also, in areas of infection, expose these birds to the virus to a greater extent than that experienced by wild waterfowl. Even fully domesticated waterfowl often resemble wild birds, but competent ornithologists can generally distinguish domestic forms by subtle differences in plumage and posture.

To ensure that identity and status are correctly and precisely recorded, ornithologists should be included in teams that undertake virological sampling of wild birds. If this is logistically difficult, sampled specimens could be collected for subsequent identification by field ornithologists or in ornithological museums. Alternatively, high-quality digital photographs can be taken of significant plumage and other body markings. Personnel doing the sample would need training in how to select the appropriate features to photograph (SANCO 2006).

Location and habitat

Outbreak reports usually identify only the district or village where samples were collected. This is problematic because widely available atlases often do not include smaller villages, and because name changes or different spelling options can make it difficult to trace locations. When sampling wild birds, it is essential to report precise geographical coordinates. Geographical coordinates can be easily obtained and would reduce confusion and difficulties when people who are unfamiliar with local names or languages examine information on avian influenza outbreaks.

Details of the environment or habitat where an outbreak occurs, agriculture or aquaculture operations near the sampling area, and distances to (and extent of) roads and villages should also be documented, because these characteristics may be important to understand the potential sources of infection and risks of spread to new areas (Ellis et al. 2004). The many researchers and government scientists visiting Lhasa and Qinghai Lake in 2005 failed to record the presence of captive-breeding programs for bar-headed geese in these areas (noted above) and concluded that wild birds brought the disease to this region.

In a widely cited paper by Chen and colleagues (2006a), six "wild migratory ducks" tested positive for H5N1 in Poyang Lake, China, in January and March 2005. Poyang Lake is large (up to 3500 square kilometers, depending on seasonal water levels) and has different habitat types (ponds, rivers, marshland, mudflat, and grassland) (Markkola et al. 1999). There are several villages (and approximately 13,000 people) on the shores of Poyang Lake (Melville 1994), and in some regions domestic ducks, which may carry H5N1 asymptotically (Hulse-Post et al. 2005), share habitats with wild birds (Mark Barter, Wetlands International—China, Glen Waverley, Australia, personal communication, 26 September 2006; Li Fengshan, International Crane Foundation, Baraboo, Wisconsin, United States, personal communication, 4 Octo-

ber 2006). More exact information on the location would have greatly improved the epidemiological value of these study results. In particular, it would make it easier to assess whether infected poultry or other wild birds may have infected these six birds through shared water bodies (Gilbert et al. 2006) or whether these allegedly wild birds had carried the virus from infected breeding or stopover habitats. Depending on the species (as described above), the latter may be unlikely, because these birds were sampled in January and March, and thus probably wintered or were resident at Poyang Lake.

In contrast to nearly all other reports, an exemplary study by Ellis and colleagues (2004) illustrated the value of documenting location and habitat types of H5N1 outbreaks. This paper included detailed documentation of the location of the outbreaks, the distances between them, and the presence of potential sources of infection, such as chicken farms with genetically similar H5N1 strains or poultry markets.

Timing of sample collection

Precise documentation of timing is important for interpreting the results of surveillance testing. Most obviously, this can help to determine the pattern or direction of spread: Vague or incorrect dates can easily produce a misleading impression of the routes taken by the virus. Furthermore, official lists of veterinary authorities often only specify the date when test results are confirmed rather than when the dead or sick birds were detected, which may be several weeks earlier (Friedrich-Löffler-Institut 2006). Also, the prevalence of avian influenza is influenced by time of year (Li et al. 2004), linked to changes in congregation patterns of wildfowl and to the relative proportion of juveniles that have yet to develop antibodies to avian influenza (Halvorson et al. 1985, Stallknecht et al. 1990). Monthly variability in avian influenza infection rates may also result from the longer environmental survival times for the virus at low temperatures (Stallknecht et al. 1990). Two birds tested positive for avian influenza in an autumn surveillance study by Betacova and colleagues (2005), but the authors did not provide precise details on when the positive samples were taken.

Information on sampling dates, especially when coupled with information on marked individuals, may also help to establish where infected birds may have contracted the disease. For example, one of the whooper swans that tested positive for H5N1 in Rügen Island, Germany, had been ringed as a breeding adult in Latvia (an uninfected area). This bird was observed to be apparently healthy on Rügen Island on 28 January 2006 and then found dead on 14 February (Franz Bairlein, Institute for Avian Research, Wilhelmshaven, Germany, personal communication, 8 September 2006). It is crucial that all dead birds are checked for bird rings, wing tags, or nasal markers, and that the full inscription of the ring, and, if applicable, the color, location, and type of marks, are reported to the national bird ringing center or to the institution identified on the marking. The information on tags or rings can provide distributional data and potentially important information on the age and sex of the individual.

A recent study by Ducatez and colleagues (2006) demonstrated that strains of H5N1 isolated in Nigeria were closely related to strains in Egyptian poultry and Russian poultry and wild birds. From these data, the authors suggest that wild migratory birds may have introduced H5N1 to Nigerian poultry operations, because migratory birds travel between Egypt and Nigeria (Ducatez et al. 2006). However, the Nigerian outbreaks began in mid-January 2006, whereas wild bird migration from the Middle East to Nigeria would have been completed in the fall. The timing of the outbreaks thus suggests that the virus is unlikely to have been transported to Nigeria by migrating wild birds. Similarly, in 2006, viruses isolated in India have been related, through sequencing, to viruses isolated from swans in Iran and Italy, leading Pattnaik and colleagues (2006) to surmise that the long-distance spread of avian influenza between these countries is through wild aquatic bird migration. However, the dates of the Indian (January–February) and Italian and Iranian (February) outbreaks did not occur during a time of seasonal migration, suggesting that other modes of transport were involved. These two examples illustrate the importance of accurate reporting of dates for the interpretation of wild bird involvement in virus spread.

Avian influenza viruses progressively degrade when outside of hosts, at rates that depend on the temperature and the type of substrate (Stallknecht et al. 1990). Viruses that remain in the environment may be able to infect new birds, even after an outbreak has apparently died down. Dates of separate H5N1 surveillance studies or outbreaks should be recorded accurately so that it is possible to evaluate whether two outbreaks had the same or different sources of infection.

Capture method

There are many methods of catching wild birds. For Anseriformes (ducks, swans, and geese), these include baited swan tunnels and other cage traps, swan hooks, cloverleaf traps, mist nets, cannon nets, and roundups of flightless birds during molt, as well as destructive methods such as shooting or poisoning (Kear 1990, Wildlife Conservation Society 2005). Appropriate selection and documentation of the capture method is important to evaluate whether there are sample biases in the individuals that are tested (Pollock 1981).

Assessing infection rates in wild birds is crucial to understanding the mechanisms of transmission in natural ecosystems and the potential threats to biodiversity. Studies on infection rates can give misleading results if either healthy or unhealthy birds are preferentially captured. Methods that rely on baited traps or close approach distances between people and birds probably bias samples toward individuals that are habituated to people (Carney and Sydeman 2000), sick, or in poor physical condition (Beale and Monaghan 2004); these techniques can preferentially select for younger, inexperienced birds. Cannon netting of large congregations at feeding, moult, or roost sites is likely to capture a more representative sample of birds and thus may provide a more accurate indication of H5N1 prevalence.

A fundamental question relating to the transmission of H5N1 by wild birds is whether they can carry the virus asymptotically. As yet, most outbreaks in wild birds have resulted in localized mortalities, usually of relatively few individual birds, over a short period and with very limited spread to nearby regions (Terakado 2004). However, if healthy migratory birds carry and actively shed HPAI H5N1, they have the potential to transport the virus over the distances covered along their migration route during the two to three weeks that they shed the virus.

Asymptomatic infection with a distinctive H5N1 strain has been demonstrated in domestic poultry (Hulse-Post et al. 2005, Nguyen et al. 2005) and in one resident species in China, the tree sparrow (*Passer montanus*), which commonly lives in towns and is in frequent contact with domestic poultry (Kou et al. 2005). Unfortunately, inadequate documentation makes it difficult to interpret the few claims of asymptomatic infection in wild birds. Problems with the interpretation that wild birds can carry H5N1 on long-distance migration, on the basis of unidentified “migratory ducks” (Chen et al. 2006a), have been highlighted above. Recent Russian studies have also alleged that asymptomatic infection in apparently healthy wild birds may occur. However, these studies relied largely on samples obtained from birds shot by hunters (Lvov et al. 2006). An assessment of health requires researchers to conduct necropsies or to examine features such as body or feather condition or irresponsiveness in the field. These methods do not appear to have been used in these studies. Very sick birds may be forced to fly because of the intense disturbance stimuli created by loud gunshots (Madsen and Fox 1995), and so short flights of heavily disturbed birds cannot be used to assess health.

Sampling method and pseudoreplication

Several studies that use cloacal or tracheal swabs do not indicate whether samples were taken from the same or different individuals (Wildlife Conservation Society 2005, Chen et al. 2006a). Where birds congregate in the same area for long periods, repeat samples could inadvertently be taken from the same individuals, violating the assumption of independence. It may be difficult to prevent pseudoreplication, especially when there are limited resources available for sampling, but it is crucial that researchers clearly define the time and area over which infected birds were sampled, so that the robustness of results can be better assessed.

Although Chen and colleagues (2006a) state that six ducks tested positive for H5N1 over two sampling periods, they do not state whether any of these birds were clustered in the same area or whether the birds were sampled and released. If birds were unmarked, released and sampled at another date, it is possible (depending on the population size and on the degree of site fidelity of the “migratory duck” involved) that the same infected individual may have been sampled twice. For this reason, details of the sample population should be recorded, or individuals should be ringed.

Fecal sampling is a convenient method that is frequently used for monitoring H5N1 in wild birds (Wildlife Conservation Society 2005), but its weaknesses in epidemiological studies must be recognized. In most cases, without detailed behavioral observations, droppings that are sampled cannot be associated with an individual bird, and thus the species identity and state of health of the bird are unknown. Even when flocks of a single species have been seen at a particular site, droppings could have been deposited by other birds that used the site before the observed flock did. In addition to these weaknesses, recent research, presented at an FAO (Food and Agriculture Organization of the United Nations) conference in Rome, demonstrated that in some species of dabbling ducks H5N1 may only be detected using throat swabs, as the virus is excreted primarily in the upper respiratory tract. Fecal or cloacal swabs may show false negative results because of the short duration of viral shedding and the low viral replication rates within the oral–digestive tract (FAO/OIE 2006).

Population characteristics

Details about the study population are frequently not reported in scientific papers or outbreak reports on avian influenza. An example is the study of the major outbreak in 2005 in Qinghai Lake, where several thousand waterbirds died, some of which tested positive for H5N1 (Chen et al. 2005). Chen and colleagues (2005, 2006b) noted that bar-headed geese (*Anser indicus*), ruddy shelducks (*Tadorna ferruginea*) brown-headed gulls (*Larus brunnicephalus*), great black-headed gulls (*Larus ichthyaetus*), great cormorants (*Phalacrocorax carbo*), whooper swans (*Cygnus cygnus*), black-headed cranes (*Grus nigricollis*) and common pochards (*Aythya ferina*) died, but they did not document the number of healthy individuals of each species at the lake, nor the identity and numbers of other species that were present but not apparently infected. This species-specific information is essential to estimate infection rates for each species, detect changes in the virulence of the disease in wild bird populations, and measure the possible impacts of avian influenza on species of conservation concern.

This paucity of relevant information on the infected population was also demonstrated in an H5N1 outbreak at Ubsunur Lake in Russia and Mongolia in June 2006. Although the media reported that thousands of birds had died in the area, and that some had tested positive for H5N1, no details have been provided on the number of each species infected or the wild bird population in the region.

Two studies provide noteworthy exceptions, showing how good data on populations can help in the assessment of infection rates. In outbreaks at Erhel Lake (Wildlife Conservation Society 2005), and Rügen Island (Marcus Nipkow, Naturschutzbund Deutschland, Bonn, Germany, personal communication, 27 September 2006), both the total population size and the number of dead birds of each species present were clearly documented. At Erhel Lake, 110 dead birds were found among 6500 apparently healthy individuals. Of

these 110 dead birds, 4 were tested, and of these only 1 proved positive for H5N1. These data suggest that many of the mortalities could have been due to other natural causes, and that H5N1 infection rates may have actually been low (Wildlife Conservation Society 2005). Similarly, as of 22 March 2006, in the Rügen Island outbreak, only 3 percent of the dead birds found and tested were actually infected with H5N1 (Marcus Nipkow, Naturschutzbund Deutschland, Bonn, Germany, personal communication, 27 September 2006). As Rügen Island is an important wintering area with high densities of waterbirds, this low infection rate suggests that the virus cannot yet transmit efficiently in wild populations. The scale of mortality—about 4000 dead individuals, both from H5N1 and from other causes—approximated the normal annual winter mortality on the island (Lingenhöhl 2006). Moreover, recent cases in Europe have demonstrated that in some cases dead birds that tested positive for H5N1 may not have died from the disease (Richard Hoop, National Reference Center of Poultry Diseases, Zurich, Switzerland, personal communication, 20 September 2006). Detailed population counts from the field coupled with current laboratory studies on the pathogenesis and virulence of H5N1 can act as a warning signal and help researchers detect significant changes in the behavior of the virus (Li et al. 2004).

Waterbirds frequently exist in mixed-species flocks (Silverman et al. 2001). Good information on populations at an outbreak site may help in identifying species or taxa that are relatively resistant to some strains of H5N1 and could be preferred asymptomatic carriers of the virus. Although several laboratory studies have indicated differential virulence in taxa of domesticated or laboratory-reared birds (Perkins and Swayne 2003, Hulse-Post et al. 2005), vulnerability to diseases such as avian influenza may relate not only to birds' genetic or physiological resistance but also to their behavior, a characteristic that can only be examined in the wild. As species that flock together may have different foraging behaviors, life histories, and patterns of local movement and migration, this information may provide opportunities to conduct experimental tests in a field environment and help to determine where and how birds become infected with avian influenza. In particular, details of local daily movement patterns of different species are important to identify potential sources of infection, potential risks to nearby areas, and appropriate areas for heightened biosecurity after an outbreak. Some waterbirds will travel tens of kilometers during the day between roost sites and foraging areas (Owen and Black 1990). With the possible exception of a study on H5N1 outbreaks among large-billed crows (*Corvus macrorhynchos*) in Japan (Terakado 2004), local movement patterns do not seem to have been documented in previous research on H5N1 outbreaks in wild birds.

In addition to species-level differences, information on individually marked birds, or on sampled birds' sex or age, may also help to identify where birds were infected, since at molt or wintering sites birds of the same species may have originated from different breeding areas (Veen et al. 2005).

The outbreak of H5N1 among wild birds in May to July of 2005 at Qinghai Lake is frequently cited as evidence that the virus can be carried vast distances by wild birds (Chen et al. 2005, Liu et al. 2005, FAO 2006). However, studies of this outbreak did not adequately examine the life history of the infected population. For example, the majority of the birds that died at Qinghai Lake were bar-headed geese. This species is an obligate migrant and winters in India (where there were no H5N1 outbreaks during the preceding winter; Javed et al. 2000). Because bar-headed geese arrive in March at Qinghai Lake, these birds had been in the area for several weeks before the first H5N1 mortalities, suggesting that it is more likely they were infected at or near Lake Qinghai rather than during migration or on their wintering grounds (Baker 1921). The outbreak at Qinghai Lake, like those at Erhel Lake, was characterized by synchronized and localized deaths of a proportion of individuals from larger flocks, the hallmark of a locally acquired infection (Feare 2006a). At the least, this appears to warrant ecological and epidemiological investigation of potential local routes of infection, such as poultry feed, captive bar-headed geese breeding programs, and poultry manure used to fertilize fish farms or agricultural crops (Feare 2006b). Such studies do not appear to have been undertaken.

Conclusions

Each outbreak of H5N1 in wild birds provides a new opportunity to extract information on the behavior of the virus and its hosts. Standardized, detailed, and accurate data collection, including key ecological variables, from outbreaks around the world will help to focus mitigation efforts, predict future outbreaks, and reduce the socioeconomic and environmental impacts of avian influenza. To this end, we make a plea for greater involvement by ornithologists and ecologists in H5N1 research, monitoring, and management, as members of response teams, research advisors, and journal referees.

The basic data requirements at every outbreak of HPAI H5N1 are as follows:

- Full and accurate identification of wild birds involved, including age, sex, status (wild, semidomesticated, captive, or released), and, if possible, subspecies
- Location and habitat, including geographical coordinates, and distance from human habitation, agriculture, wildlife habitats, and any other potential source of infection
- Date of collection of samples (rather than date of sample analysis)
- Capture method (how sampled birds were procured; e.g., trapped, caught by hand)
- Sampling method (cloacal, oropharyngeal, or fecal) and whether birds sampled were dead or alive
- Population characteristics (total number of every species present at site, number of sick or dead birds of each species, number of these sampled, number of apparently healthy birds sampled, and species not affected by H5N1)

In encouraging the collection of these basic data at every outbreak of the virus among wild birds, we hope that our paper may be a first step in encouraging a fruitful collaboration among ecologists, veterinarians, and virologists that can significantly improve our understanding of the H5N1 virus and its control.

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