Review

Understanding the transmission of H5N1

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Abstract

H5N1 influenza virus is now endemic in many regions of the world. Outbreaks continue to occur with devastating impact to local economies and threatening human health. The transmission of the virus involves a complex interplay between the spatio-temporal dynamics of the potential avian hosts, the ecology of the virus and poultry farming and trade. Despite increased monitoring of wild bird populations and poultry trade, control measures remain insufficient to mitigate the spread of the virus. Here, we focus on the influence of seasonality of migration and breeding in the transmission of H5N1. We also review recent findings on the interaction between migratory birds and poultry in the pattern of recurrence of H5N1 outbreaks. Finally, impacts of vaccination and co-circulation of avian influenza strains are discussed in the context of re-emergence and silent spreading of the disease.

Keywords: Avian influenza H5N1, Spatio-temporal patterns, Migratory birds, Poultry, Seasonality, Strain interaction, Silent shedding

Introduction

Outbreaks of highly pathogenic avian influenza (HPAI) H5N1 have led to the culling of 400 million domesticated birds since 2006 (e.g. [1]). In 2006, 63 countries were affected, resulting in economic losses of US$20 billion worldwide. To date, there have been more than 600 H5N1 human cases with a fatality rate of nearly 60%. Most casualties were in Indonesia, Vietnam, China and Cambodia. Most human cases were in close contact with poultry prior to diagnosis [2–4]. Despite the increasing number of human victims, the identification of cases of human-to-human transmission remains rare [5, 6]. However, recent works showed that HPAI H5N1 viruses have the potential to support transmission in mammals [7]. Moreover, there is now evidence that sufficient mutations leading to airborne transmission of the virus could occur within one mammalian host [8, 9]. Airborne transmission of a highly pathogenic avian flu virus could lead to a pandemic with devastating consequences. The fact that few mutations are needed to potentially trigger airborne human-to-human transmission is a reason for concern and continued intensive monitoring of flu outbreaks worldwide. In its current form, the cycle of transmission of H5N1 has a complex spatio-temporal dynamics, involving the entangled dynamics of bird migration, local farming activities, and pathogen dynamics and recirculation. Understanding such entanglement is a key to the prevention and control measures mitigating the impact of H5N1 outbreaks in both human and animal populations. Despite increased monitoring of various bird populations, the roles of migratory and wild birds in the transmission of HPAI remain unclear.

The goal of this review is to discuss various aspects of the complex chain of H5N1 virus transmission with a particular focus on the spatio-temporal interplay between the ecology of the migratory avian hosts and poultry. Throughout this review, we highlight the key factors leading to a seasonal pattern of outbreak worldwide. The review will focus on the role of avian populations in the transmission of H5N1. These include both wild birds and poultry. As an important caveat, we note that while this is a review article with limited number of citations, making a complete scholarly account of this subject is impossible. Wherever possible, we have cited the most recent articles and reviews on various subjects described. Although

we have tried to be objective in terms of topic selection, our review is to an extent idiosyncratic, reflecting our own areas of interest in the dynamics of the complex spatio-temporal spread of HPAI H5N1.

Low versus High Pathogenic Avian Influenza

Avian influenza is caused by a virus frequently affecting wild birds and poultry. In particular, birds of the order of Anseriformes (e.g. ducks, geese and swans) and Charadriiformes (e.g. gulls, terns and waders) are generally considered to be the virus reservoir in nature [10–13]. Although various definitions of reservoir can be found [14], here we use 'reservoir' to refer to the bird groups showing relatively low mortality and mild symptoms without which the sustainability and spread of the virus into other hosts would not be possible. The range of symptoms caused by the virus in chicken populations is often used to classify the virulence of avian influenza viruses. The mild form of infection is referred to as low pathogenic avian influenza (LPAI). Ducks, shorebirds and gulls were particularly well documented for their capacity to shed LPAI virus for a long period of time, with ducks being able to shed the virus via intestinal tract for up to 4 weeks [13].

Until recently, it was thought that LPAI strains could only cause mild to no symptoms in migratory birds. This is changing as we will discuss hereafter. On the other hand, HPAI strains are highly contagious, can cause debilitating symptoms and up to 100% poultry mortality within days (e.g. [15–17]). Various waves of HPAI H5N1 have been continuously appearing since 1997 [18], but in 2005, a new highly pathogenic strain of H5N1 virus began to have unprecedented deadly effect on various bird species, including industrial poultry, domesticated resident birds and migratory wild birds. This outbreak led to the culling of up to 150 million domesticated birds and resulted in loss of billions of dollars in Asia [19]. The new highly pathogenic H5N1 strain also led to an anomalously high cumulative mortality of more than 6000 among wild birds usually considered reservoirs to AI in Central China's Qinghai Lake [20]. Among other species, the casualties included 3018 bar-headed geese *Anser indicus* [21] representing 5–10% of its global population [12, 22–25]. Outbreaks among wild birds and poultry are now regular in many regions of the world, including Russia, Mongolia, India, Indonesia, Israel, Nepal, Viet Nam, the Middle East, Europe and Africa (see [1, 26, 27]).

Endemicity: A Complex Chain of Transmission

The conditions favourable for H5N1 endemicity are still not well understood and remain controversial. Indeed, following the deadly outbreaks of 2005 and the dynamics of LPAI, migratory birds were designated as the source and spreader of HPAI H5N1. They were thought of as the source of contamination of poultry. This hypothesis was quickly adopted by various organizations such as the World Health Organization [28] based on their usually accepted status of LPAI reservoir. However, only a few studies supported this view. For example, in the Poyang Lake region, Chen *et al.* [29] identified H5N1 infected ducks, which were labelled as 'migratory'. However, the lack of proper identification of sub-species and location of capture of the duck was pointed out on several occasions (e.g. [30–32]). In particular, migratory birds sampled around the lake were said to involve falcated teal, spotbill and mallard ducks; however, only the first is surely migratory, the second is a common breeder around the lake, and mallards and their descendants are the most common domesticated species released in the vicinity of the lake by local farmers (e.g. [31]). Based on issues related to improper identification of the birds sampled, unreported location of capture and possible bias of the sampling itself, it was not possible to conclude whether the birds sampled were vectors exposed, importing the virus from prior travelling stopovers or if they acquired the disease locally upon arrival. One study reported the movement of one migratory wild bird infected with the HPAI strain over several hundred kilometres [33].

Recent studies showed that domesticated birds infected with LPAI can actually show respiratory symptoms, depression and egg production problems [34]. Van Gils *et al.* [35] determined that the feeding and migratory performance of LPAI infected Bewick's swans (*Cygnus columbianus* bewikii) was altered. Infected birds showed reduced bite and fuel storage rates and delayed migratory schedule when compared with their healthy counterparts. It was also found that LPAI infected mallards are leaner than their uninfected conspecifics [36]. Such observations challenged the status of reservoir generally attributed to Anseriformes and Charadriiformes birds for HPAI. Indeed, if LPAI causes such disturbances, worse is to be expected for more virulent HPAI strains. More recent work on the relation between fitness and virus shedding of LPAI led to counter-intuitive results, where concentration and duration of viral shedding were positively correlated with host condition [37]. Further work is needed to clarify this issue. Indeed, it is critical to improve our understanding of the link between body condition, shedding of AI virus and migration in order to gain further insight into the epidemiology of both LPAI and HPAI in the wild.

Besides avian species, specific farming practices and agroecological environments are also important to consider when trying to explain the occurrence, the spread and maintenance of HPAI virus in many countries [15]. In particular, the large-scale monoculture of domestic birds has facilitated the unprecedented evolution and dissemination of avian influenza viruses worldwide. The movement of infected poultry can introduce the virus in previously unaffected countries. Given the regulations in

Table 1 Spatio-temporal scales involved in H5N1 transmission and persistence

	Short timescale	Long timescale (with delays)
Large spatial scale	International trade networks	Seasonal migratory dynamics
Small spatial scale	Local stopover of birds	Recurrent interaction of migratory birds with local poultry

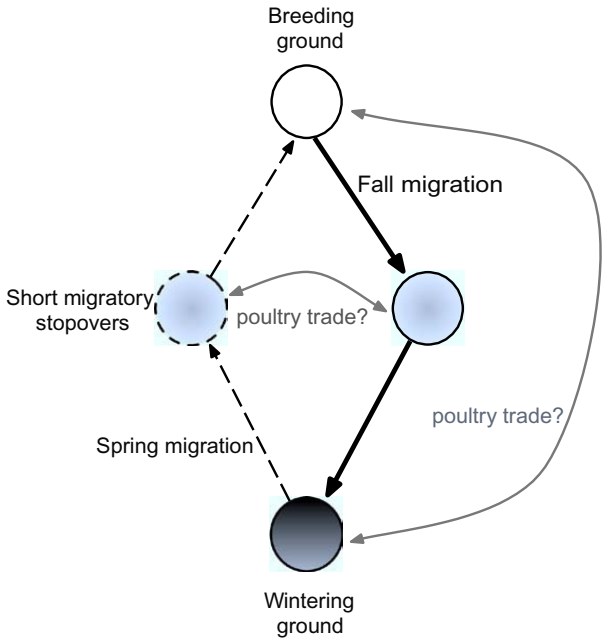

Figure 1 Illustration of the migratory route of birds with a summer breeding ground from which fall migration is initiated towards the wintering ground. Note that the birds stop at a multitude of regions along their migration. These stopovers are illustrated with one region. After the wintering season, the birds initiate their spring migration returning to their breeding ground. The spring migration is generally faster than the fall migration, during which weather and food conditions are better. For example for bar-headed geese migrations lasted 61 and 46 days on average for fall and spring, respectively [54]. The dynamics of H5N1 virus on the stopovers, the breeding and the wintering grounds are coupled by the cycle of migration; however, the role of poultry trade, occurring on a much faster timescale than migration could be the dominant coupling factor.

place, such movement of infected poultry can only occur in the phase prior to H5N1 diagnostics or during illegal trade. One case of such H5N1 introduction through live poultry movement was documented in 2003 and involved the Netherlands, Belgium and Germany [38]. Using mathematical modelling, the impact of illegal trade of poultry on the efficiency of vaccination programmes of poultry was examined [39]. The selective pressure of the vaccine was found to lead to the potential emergence of a vaccine-insensitive strain in another region connected via illegal poultry trade. Hence, only complete eradication in the vaccinated area can lead to complete eradication in the connected other areas.

The chain of transmission of H5N1 involves more players than just avian species. Carnivorous animals (e.g. cats and foxes) fed with carcasses of infected dead birds or poultry were observed to excrete the virus. Although infected cats showed symptoms and were able to cause horizontal disease transmission, foxes showed few symptoms (e.g. [40, 41]). In other words, the disease spreads and returns yearly as a result of the coupling between global and local dynamics (see Table 1 and Figure 1). Clearly, the local dynamics on migratory stopovers appears critical not only to the understanding of the onset of an outbreak in some geographical location but also to the understanding of the ecological impact of the endemicity of H5N1 on various species involved. For Asia, the likelihood of poultry trade being the cause of H5N1 introduction events was estimated to be three times as high as migratory bird movement [42]. However, the opposite was true for Europe. There, migratory birds were found to be most likely to introduce H5N1.

Seasonality of Migration and H5N1 Recurrence

LPAI strains are often detected in the wild with a peak prevalence in waterfowl observed during pre-migratory staging in late summer and early fall; followed by a rapid decrease on autumn migration, reaching its lowest point on wintering grounds [43]. Various hypotheses explaining the repeated outbreaks of LPAI in aquatic birds have been proposed. These could involve continuous circulation and persistence of the virus within one species or group during migration, circulation and exchange between different avian species or groups at stopover locations of the migration, the persistence of the virus in water or ice or its persistence in a single individual host [13].

Figuerila and Green [44] found that migratory birds in general have a higher risk than sedentary birds of becoming infected by parasites. Hence, mapping migratory trajectories can help gain insight into the factors leading to the persistence of HPAI worldwide (e.g. [12]). Surprisingly, migratory routes have been mapped with precision only very recently. Most migratory birds are observed to return yearly to some known stopovers, breeding and wintering sites [45]. Despite the complexity of their migratory routes, birds are observed to follow similar migration paths yearly [46]. In order to understand the parameters determining such paths, various models were used. One of the first model used optimization where birds, going through a succession of stopover sites, had to choose their migration schedule and fuelling to maximize reproductive success [47]. Others focused on the optimization of female bird choices between reproduction, foraging, moulting or migrating [48]. The influence of

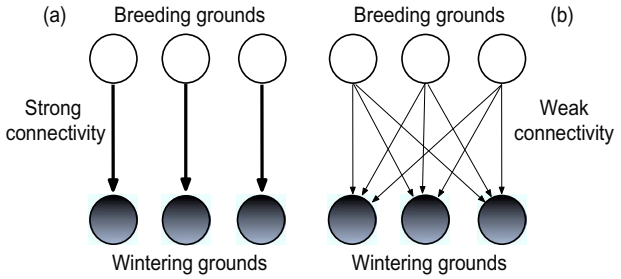

Figure 2 Illustration of strong connectivity (a) and weak connectivity (b). The former occurs when most individual members of a breeding ground move to the same wintering region. The latter occurs when individual members of a breeding ground migrate to several wintering regions and can mix with groups originating from different breeding grounds

climate on the conditions at the stopover sites on the schedule of migration was also examined from an optimization point of view (e.g. [49], for Pinkfooted geese). Until recently, the details of bird migratory paths were unknown. The use of satellite tracking allowed for the identification of migratory trajectories and the potential factors influencing their change. For example, the tracking of bar-headed geese showed that the sub-groups of geese from the Central Asian Flyway were found to breed in Mongolia, Central China, India, Pakistan, Kyrgyzstan and Tibet (e.g. [24, 50]).

Once the migratory paths of the avian hosts are identified, one can examine the correlation between migratory birds, density of poultry populations and local potential hosts, and the recurring outbreaks of influenza worldwide. The correlations between the presence of migratory birds in a given location and H5N1 outbreaks were the subject of numerous studies (e.g. [50–53]); however, such studies cannot infer causality between outbreak and migration. Using mathematical models of the full spatio-temporal dynamics of migration, the causalities between the presence of migratory birds, poultry, and H5N1 outbreaks and persistence was examined. Figure 1 illustrates the migratory routes and connectivity between key stopovers (e.g. [54, 55]). Combining mathematical models of migration and epidemics with local data on poultry density and migration patterns allowed for assessing the complex nonlinear interplay between poultry and migratory birds in dense areas such as Poyang Lake. The important role of seasonality introduced by bird migration was highlighted. In particular, the timing of migration, the ecological properties encountered on each stopover and the demographic changes in the migratory bird populations along the migration route were examined. The arrival of migratory birds in the winter was found to be able to introduce avian influenza to poultry in disease-free periods. Once H5N1 becomes endemic in that region, the disease becomes endemic in the migratory bird population itself. Despite the regular pattern of migration, the direct nonlinear coupling between migratory birds and poultry was shown to possibly lead to the emergence of irregular patterns of outbreaks [55].

Finally, migratory connectivity, which is the extent to which individuals from the same breeding area migrate to the same non-breeding area, was also thought to explain the recurrence of outbreaks from one season to the next. Given that wintering populations may share and exchange genes, it follows that they should also be expected to share and exchange diseases and parasites [56]. Connectivity as illustrated in Figure 2, occurs when most individuals from one breeding population move to the same non-breeding location to form a non-breeding population, with a relatively small proportion of individuals migrating to other winter locations. Weak connectivity occurs when individuals from a single breeding population migrate to several different wintering regions [13]. The weak connectivity network allows for a larger mixing of pathogens than strong connectivity. The dynamics of the migratory bird host immune response is influenced by the ecology of the pathogen and the location of its host; hence, the patterns of infection vary from region to region [57]. Using a population model, the role of such infection-induced delays in migration was examined for mallard populations (e.g. [58]). Delay in migration of infected birds was found to possibly result, in turn, in an overall reduction of the yearly number of cases among mallards. In terms of connectivity between regions, a delay in onset of migration would result in a lower encounter of other birds in connected regions.

Variability in Susceptibility

The immunity of birds to a given influenza virus varies greatly from one species to the other. This disparity is due to the diversity of metabolisms and hence susceptibility of the birds. Some species or individual birds show symptoms of infection within 1–2 days from initial virus inoculation (such as Trumpeter Swan in [59]), while others can take up to 5 days to show symptoms (such as Mute Swans inoculated with a low dose [59]). Mallards, or Common Teals were reported not to show any symptoms post-inoculation [60]. Note that most inoculated species had a very high case fatality rate. The shedding rate and duration are examples of critical parameters that are key to improve the modelling and understanding of persistence of H5N1.

Within one species of bird, the susceptibility of the group is also seasonal. Indeed, birds breed at a very particular time of the year (summer months) and the resulting juvenile birds are highly susceptible to both LPAI and HPAI H5N1. Hence, the impact of mass mortality from a potential AI outbreak varies depending on the moment of the outbreak and the age-group that is affected. A sudden decrease of the number of adult birds, which generally have relatively high survival rates, can have a greater impact than a similar decrease in juveniles,

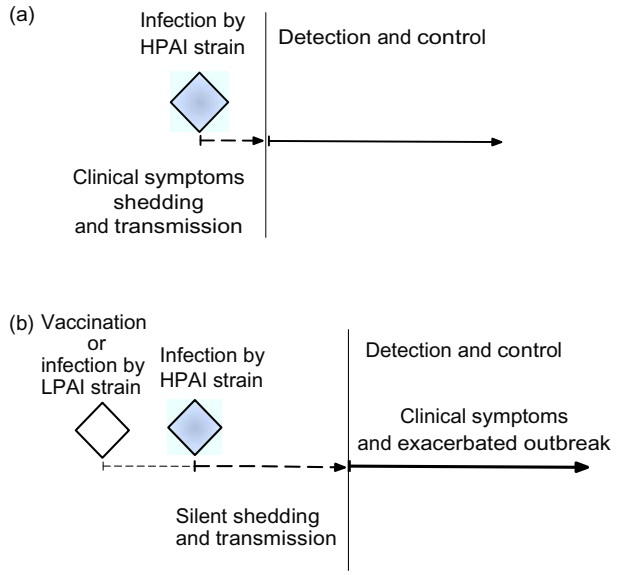

Figure 3 (a) Silent H5N1 shedding and transmission in poultry can occur due to prior infection with LPAI strains or vaccination. This effect is known to be temporary only, but can last for a few months. The shedding and transmission in (b) can be prolonged by the lack of detection of obvious clinical symptoms in poultry populations. Indeed, detection of clinical symptoms differ from detection of shedding. The former is the method most commonly used to implement control measures.

especially when this happens during the breeding season and results in a year of low reproduction. Moreover, seasonal migration, population and disease dynamics on stopovers can be combined in order to assess the overall impact of repeated H5N1 outbreaks on the ecology of the migratory birds according to their yearly demographic cycle. For example, repeated H5N1 induced death toll was shown to incur major ecological implications for the long-term survival of a species like bar-headed geese [54]. Indeed, female bar-headed geese do not reach maximum reproduction efficiency before age 4 [61, 62]; hence, repeated H5N1 outbreaks on this species can greatly influence the internal migratory and reproductive dynamics of the flock. This was illustrated by the results of a two-age mathematical model of bar-headed geese combined with ecological data [54]. Note that the effect of age-structure arises from the difference between immune system maturation and reproduction maturation.

H5N1 Virus Shedding and Routes of Transmission

Two competing routes of virus shedding are reported for H5N1 [63]: cloacal and oropharyngeal shedding. The former is associated with indirect environmental transmission of the pathogen referred to as faecal–oral route. The latter is associated with direct transmission and involves a close contact between the shedder and the susceptible birds. When examining HPAI H5N1, using a combination of data and mathematical modelling, outbreaks were found to lead to higher death tolls when indirect transmission dominated. Indirect transmission mechanisms can result in a two-step mechanism with one bird-to-environment segment; and a second environment-to-bird segment. Hence, a delay and extension of the 'effective' infectious period could be expected. Depending on the parameters governing the persistence of the virus in the environment, new infections can be generated during a longer time than when compared with direct contact transmission. The role of the contamination of the environment in the chain of disease transmission can be critical in determining the outcome of an epidemic. Ducks, shorebirds and gulls are particularly well documented for their capacity to shed the low pathogenic strain of avian influenza (LPAI) for long times. For example ducks can shed the virus via intestinal tract for up to 4 weeks [13] leading to LPAI peak prevalence to vary from a few percent in the winter months to up to 30% during the weeks preceding fall migration (e.g. [64]). Focusing on LPAI, it was noted that if the size of a population is small, the environmental transmission can become a key in explaining the persistence of the epidemic in small size communities [65]. Using stochastic modelling, key properties of the epidemics such as explosiveness and duration were found to be caused by environmental contamination. Faecal–oral route is dominant in LPAI epidemics; however, oropharyngeal shedding is dominant for HPAI (e.g. [59, 60, 66, 67]). Although environmental transmission rates are hundreds of times lower than direct transmission rates, it was found that the virions can remain viable for more than 100 days at 4 °C [68]. Indeed, environmental transmission apparently provides a possible persistence mechanism in situations where an epidemic would not be sustained by direct transmission alone [65, 69]. Golbig *et al.* [66] discussed the impact of such a finding as a possible explanation for the relatively localized and surprisingly species-limited H5N1 outbreaks observed in Germany during 2006 and 2007. Finally, note that in a recent study examining the influence of body condition on LPAI infection in mallard ducks, the concentration of viral shedding was observed to be highly variable among wild and captive bred birds [37]. Two of the fourteen mallards tested demonstrated four times as much viral shedding than the other treatment groups. Interestingly enough, this variability in shedding can also be seen in the form of supershedding of flu in humans (e.g. [70]).

Silent Shedding

Strains of LPAI can induce partial immunity to HPAI in poultry and wild bird populations. In Hong Kong's 1997 H5N1 virus outbreak, most chickens did not show clinical signs despite a 20% prevalence. Documentation of the outbreak indicates that chickens in most markets shed

virus via the cloacal route. At the time, the second most prevalent virus in the market was LPAI H9N2, which was isolated to about 5% of the chickens examined. Distinguishable lineages of H9N2 and H5N1 were present. Sea *et al.* [71] argued that the H9N2 infection caused a cross immunization in chickens, leading to a reduction of clinical signs and death rates. They observed that such acquired immunity due to a previous LPAI is only temporary. More importantly, such results indicate that the co-circulation of LPAI and HPAI can lead to a silent spreading of HPAI H5N1 and delay detection. Similarly, vaccinated poultry could be free of clinical signs, but continue to shed the virus. The monitoring of the shedding is rarely implemented; hence, it is argued that vaccinated poultry, even free of clinical signs, should not be traded to avoid all risk of silent shedding and transmission (see [38], and Figure 3 for illustration).

In wild birds, it was also observed that H5N2 induced a reduction in H5N1-induced lethality and reduced or suppressed H5N1 clinical signs of infection in Canada geese [72]. Adult birds responded to H5N2-immunization and subsequent H5N1 infection better than juveniles, and the survival rate for both adult and juvenile birds was 100%. Results supporting cross-immunity were also observed for Mute Swans [73]. These studies support the hypothesis that pre-exposure of birds to LPAI virus strain minimizes the mortality and symptoms of a subsequent H5N1 infection, although shedding can still occur. A lot remains to be understood to capture the role of pre-exposure to LPAI epidemic outcome among birds. How silent spreading due to strain co-circulation affects the pattern of H5N1 outbreaks worldwide remains to be determined.

Discussion

H5N1 continues to be a serious threat to global health. The transmission, persistence and recurrence of H5N1 outbreaks involve a complex spatio-temporal dynamics where several species (wild or farmed), spatial and temporal scales are at play. Recent developments of global health surveillance using satellite-tracking and GIS techniques allowed a novel combination of data and mathematical modelling to examine H5N1 transmission among wild birds. Combining outbreak and shedding data with a new mathematical modelling framework can help elucidate both the influence of H5N1 on the ecology and migration of birds and the role of birds in the recurrence or introduction of the virus in new or endemic regions. The patterns of interaction between the virus and the wild avian hosts are bound to change rapidly with climate change already observed to modify migration. The modification of migratory routes will in turn change not only the current coupling between stopovers within one migratory route but also the coupling between migratory routes of distinct flocks or species. As a result, the H5N1 outbreak patterns induced by migratory birds are likely to change rapidly (e.g [74, 75]). Poultry trade and farming are also bound to change in the context of a rapid climate change or rapid economic development due to the modification of the farming pattern. Such modification includes the continued expansion of currently densely populated and exploited regions of Asia. It remains important to continue monitoring of the presence of H5N1 in both poultry and wild birds which are likely to interact with high density poultry. This should preferentially be done using detection of shedding rather than detection of clinical symptoms. The sampling of migratory birds for various strains of H5N1 has now become the main control measure put in place in some countries such as Japan and Canada. Recently, conjunctiva sampling was identified as an efficient tool of detecting signs of HP H5N1 infections in species such as whooper swans [76]. More direct means to evaluate shedding will allow for tremendous improvement in the monitoring, modelling and mitigation of the transmission of H5N1.

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