

Forming of regional network for surveillance and monitoring of avian influenza viruses in migratory birds

Policy brief on issues related to wild birds obtained from APEIR studies

- Until such time as H5N1 viruses are eliminated from Asia (and this will not occur in the next 5 to 10 years) long distance transmission of H5N1 avian influenza viruses by wild birds is expected to recur. This has potentially serious implications for virtually all of the countries where poultry have been infected previously, including those currently free from infection.
- To provide early warning, systems based on strategic testing of wild birds in key concentration points along migratory pathways in mainland Asia, especially investigations of abnormal mortality in migratory birds, need to be maintained and strengthened. This should include systems for detection and investigation of disease outbreaks in remote locations on the Qinghai Tibetan plateau. The global public good of these investigations needs to be recognised and supported.
- Early warning systems require results of testing of poultry and wild bird outbreaks (including sequences of all genes from isolated viruses) to be shared regionally and made available on public databases as soon as they are available (not longer than one month after virus isolation). This is readily achievable given the network of laboratories providing these services both regionally and internationally.
- Infection in wild birds will probably disappear once infection in poultry is controlled, as has been demonstrated in Thailand. Action on wild bird habitats or wild bird populations is not required to achieve this result.
- Song birds sold as pets (or for religious release) represent a potential source of virus. Farms raising wild birds (such as farms rearing or housing bar headed geese and song birds) could transmit virus between free flying and farmed wild bird populations (in both directions). They should be monitored closely and measures implemented to minimise this risk in line with those proposed below for poultry.
- The evidence gathered in these studies provides further justification for separating poultry from wild birds. For management systems that do not allow segregation (such as free running ducks) other ways of preventing or minimising the risk of infection or onward transmission of infection such as well-managed vaccination programs and/or movement controls (as used in Thailand for ducks) must be considered.
- Regional approaches to wild bird monitoring and sharing of information should be retained and strengthened, bringing in additional partners from South Asia, South East Asia and East Asia.
- A number of questions remain unanswered about the relative contribution of different wild bird species to the movement of H5N1 avian influenza viruses. Additional research aimed at answering these outstanding questions is recommended especially if the expected results will also help to improve targeting of surveillance programs, control the disease and/or prevent new outbreaks in both poultry and wild birds.

Brief summary of what was known about H5N1 highly pathogenic avian influenza viruses and wild birds prior to the APEIR project

When highly pathogenic influenza viruses of the H5N1 subtype (H5N1 HPAI) first emerged as a serious health problem for poultry and people in Hong Kong in 1997 there was no evidence to suggest that wild birds were involved in the transmission of these viruses (although, presumably, an ancestral low pathogenicity virus

from wild birds was the initial source of the virus). However, in late 2002 and early 2003 wild bird cases of H5N1 HPAI were detected in Hong Kong in two zoological collections and related wild birds (Ellis et al 2004). This was the first time since 1961 that an outbreak of disease in wild birds had been attributed to an HPAI virus, when terns in South Africa were infected (Becker 1966).

The spread of H5N1 to multiple countries in 2003-04 resulted in considerable discussion on how the viruses had dispersed. The epidemiological



characteristics and timing of outbreaks in the Republic of Korea and Japan strongly pointed to wild bird introductions although there were still doubts (Kilpatrick et al 2006), with some arguing that trade in wild birds, poultry or poultry meat were also probable routes, especially given that virus had been detected in duck meat imported to Korea in 2001 (Tumpey et al 2004). Genetic evidence from viruses (and evidence from a subsequent outbreak in 2007 in the Republic of Korea) provided additional support for wild bird introduction.

The means of introduction to South East Asian countries in 2003-04 remained unclear. Different strains of virus were introduced to the different countries/sub-regions (e.g. Clade 2.1 to Indonesia, Clade 1 to Thailand, Vietnam, Laos and Cambodia) at least demonstrating that there were no direct links between the two.

In 2005, H5N1 HPAI viruses caused a severe disease outbreak in wild birds at Qinghai Lake in China. When disease and virus were reported in wild birds in Mongolia and Russia shortly afterwards it was evident that wild birds were playing a role in the dissemination of the virus. Although there was considerable debate about the mode of long distance transmission, the lack of poultry in the area around the sites in Mongolia pointed strongly to transmission by migratory birds (Promed 2005, Sims and Brown 2008). Similarly, spread across Russia and through the former Soviet states and onwards to Turkey, Europe and also north and west Africa by late 2005 and early 2006 provided further strong circumstantial evidence for wild birds as the mode of transmission (Kilpatrick et al 2006, Sims and Brown 2008), although even at this time there were many who still believed trade in poultry was the most likely means of introduction (Feare 2007)).

Following introduction of H5N1 virus to Thailand in 2003, a number of cases of infection and disease were detected in wild birds covering 16 species, both migratory and local, with almost equal numbers of positive waterbirds and other types of bird (including passerines) (Siengsanon et al 2009). Testing of wild birds in markets in Thailand in 2006 and 2007 found virus in moor hens (*Gallinula* spp) and water cocks (*Gallicrex cinerea*) sold there (Amonsun et al 2008). In Cambodia wild bird cases were seen in a zoological collection in a wildlife sanctuary (and probably reflected spillover from infected poultry) in late 2003 (Desvaux et al 2009). Reports of positive samples from wild birds were rare from Indonesia. Studies conducted in Indonesia in 2006 and 2007 (but not published until 2009) (Stoops et al 2009) found a few infected wild or pet birds but no evidence of a role for migratory birds in disease transmission.

Studies conducted in China from 2004 onwards resulted in detection of H5N1 avian influenza viral RNA in 10 provinces from both live and dead wild birds. Mallards were the species with the highest percentage of positive samples. More positive cases were detected in Qinghai than all other provinces combined (Kou et al 2009).

By 2006 when APEIR held its first workshop in Vietnam, the following key points were already evident:

- i) Wild birds, including migratory species, represented a highly likely source of H5N1 HPAI virus for poultry, and were already recognised as a source of low pathogenicity avian influenza (LPAI) viruses. Recommendations had already been made to segregate poultry from wild birds so as to avoid direct and indirect contact (via feed and water) through improvements to farm biosecurity measures.
- ii) In some places, measures had already been introduced to minimise the contact between wild birds and poultry. However, many poultry, especially domestic waterfowl, were reared in a manner that allowed direct and indirect contact with wild birds.
- iii) Concerns had been raised about possible action being taken against wild birds or wild bird habitats to prevent the disease (Birdlife International 2006).
- iv) Long distance transmission of H5N1 HPAI virus had occurred and was almost certainly the result of movement of migratory birds.

At this time there were also a number of gaps in knowledge about the role of wild birds including the species that were involved in long distance virus transmission, how they got infected and whether there was a permanent cycle of infection in wild birds or if infection depended on reinfection from poultry.

Questions were also being asked about how viruses managed to move from East Asia to West Asia, and then into Europe, especially given most migratory pathways for wild birds tend to be aligned on a north-south axis, albeit with crossover between the pathways. It was not known whether wild birds were maintaining virus in places such as Indonesia and Thailand or whether wild bird cases were secondary to persistence of virus in poultry. The project examined these issues.

Main findings from APEIR activities

The group, comprised of Cambodian, Thai, Indonesian and Chinese scientists, formed a regional network for the surveillance and monitoring of avian influenza in wild birds, to share information, and to help understand the role



of wild birds in transmission and persistence of H5N1 HPAI and other avian influenza viruses¹.

The group consolidated findings about the role of wild birds in the transmission of HPAI and collected additional samples from selected wild birds in known high risk areas. The group was recognised by ASEAN as an important regional resource.

The work conducted included major studies on birds in the Central Asian flyway that connects areas in South Asia, where H5N1 HPAI viruses have been endemic since 2007, with major spring and summer breeding grounds in North West China, Russia and Mongolia. The evidence gathered, in association with other wild bird investigations, suggested that the main direction of viral spread was from north to south (to South Asia) despite most wild bird outbreaks occurring at or towards the northern end of their spring migration. A strong spatial link between outbreaks of HPAI in poultry in Tibet and migratory patterns was found. Temporal links were weaker between the cases in poultry and those in wild birds, with the former usually occurring several months before the latter (Prosser et al 2010). A lack of information on viral gene sequences from poultry outbreaks in Tibet prevented epidemiological links with those in wild birds from being proven but for the few cases for which sequence data were available, the strains involved in poultry outbreaks were not the same as those in wild birds. Nevertheless, wild birds were strongly suspected of transmitting new strains of virus to poultry in South Asia on several occasions, based on the genetic characteristics of the poultry viruses and those isolated in migratory birds prior to the disease occurring in South Asian poultry.

Migratory wild bird species considered likely to bring virus to Qinghai Lake, a vital site of congregation where a major wild bird HPAI outbreak occurred in 2005, were ranked based on a number of criteria and field studies, providing guidance on targets for future viral surveillance (Cui et al 2011). Studies in Thailand and Indonesia found evidence of infection in wild birds but no evidence of long distance transmission as detected in China. In Thailand a link was found between provinces that had poultry outbreaks and those where positive wild birds were detected but spread via poultry appeared to be the predominant route of viral dispersal nationally. Fifty percent of positive samples collected from 2004 to 2007 (largely completed before the project) were from apparently healthy birds but,

on a percentage basis, significantly more dead birds tested positive than live birds. In 2008-09 some 2,200 samples were tested but only four were positive. As the prevalence of HPAI in poultry fell so too did the number of positive wild birds, with no wild bird samples testing positive in 2009, corresponding to a period when there were no reported poultry outbreaks. Only Clade 1 H5N1 viruses or their derivatives were detected in wild birds in Thailand – the clade that became established there in 2003-04. In Indonesia more local species were found to be infected with H5N1 virus (based on tests that detect viral nucleic acid) than migratory species and a number of the positive samples were in species sold as song birds. This finding raised concerns about the possible role of trade in song birds in spread of the virus. No viruses were isolated in Cambodian studies but it is evident from field observations that wild birds and free ranging ducks share the same ecosystems providing opportunities for cross infection.

Tracking studies helped to fill in gaps in knowledge about movement patterns of migratory birds beyond the broad flyways that had been developed previously. For example, it was demonstrated that southern Tibet is a major wintering ground for bar headed geese with only one of the geese fitted with a transmitter flying over the Himalayas to South Asia. The rest of the birds remained in the area around Lhasa during winter.

Testing of healthy wild birds resulted in a low proportion of positive samples in all countries, again demonstrating that shedding of H5N1 HPAI virus via the oral/respiratory or faecal route by these birds occurs infrequently. Some additional influenza virus subtypes were detected, other than H5N1 viruses, and this information helps in understanding the transmission of other influenza viruses by wild birds.

The information obtained confirmed the need to segregate poultry from wild birds (although this is a difficult task in places where large numbers of free running ducks share habitats with wild birds). It also demonstrated that, even in places where migratory birds and poultry are co-located, the wild birds may not necessarily be the source of infection for poultry or that poultry were necessarily infecting wild birds, although both could occur. It showed the need to target surveillance in wild birds (so as to minimise the cost and maximise the benefits) given the large number of negative samples if healthy wild birds are tested. Testing of dead birds also has its problems because they are not always easy to find (Siengsanon et al 2009). The results also provided indirect evidence to suggest that other means of testing that increase the likelihood of virus

¹ Further details of this project are available on the APEIR website
<http://www.apeiresearch.net/smenupage.php?menuid=4&submain=no>

detection should be considered in future programs given the low success rate with oral and faecal swabs. This might include collection of feathers as a sample for virus detection as has been proposed by others (Yamamoto et al 2009). Overall only a small percentage of samples positive for H5N1 virus by polymerase chain reaction yielded a virus suggesting low levels of viral RNA or non-viable viruses in the samples.

The studies also reinforced the importance of sequencing of genes of all avian influenza virus isolates and rapid uploading of the sequences onto public databases. It demonstrated that important gaps in knowledge remain about migratory pathways and the precise role played by different avian species in long distance transmission of H5N1 HPAI viruses.

Evidence gathered from the Central Asian flyway suggests that H5N1 viruses may not persist for an extended period of time (more than several years) in wild bird populations and their associated environment, based on the change in virus clade from clade 2.2 (present in wild birds between 2005 and 2007) to Clade 2.3.2.1 from 2009 onwards in wild birds in this flyway. This finding raises questions about the long term survival of H5N1 avian influenza virus in summer breeding grounds which has been proposed by others as a potentially important mechanism for viral persistence (Sakoda et al 2011).

The absence of introduction of new strains of H5N1 virus to Indonesia over the past 8 years (only Clade 2.1 viruses and their derivatives have been detected there since the initial introduction in 2003) demonstrate that if migratory birds were the source of the original introduction of virus to Indonesia such events occur rarely.

Capacity building

The major areas where capacity was built through the APEIR network included the following:

- Staff in each country received training in bird capture, bird banding and sample collection, in the use of geographical information systems and risk assessment.
- A number of graduate students were provided with projects for post graduate degrees. Some of these students were required to produce research papers published in international refereed journals.
- Improvements in wild bird identification schemes such as the Indonesian bird banding
- Translation of guides from international agencies on wild bird studies into local languages
- Building of transdisciplinary teams comprising veterinarians, foresters, ecologist and ornithologists and learning how to get the most from these teams
- APEIR was not the only group funding wild bird research but it played a vital role in bringing national groups together both within and between countries to share information and forming bridges between groups funded by different agencies.

Policy advocacy

Each of the groups provided information to national and state authorities (e.g. Agriculture and Conservation/Forestry Ministries) and to regional bodies (ASEAN) on the role of wild birds in the transmission of the disease. Findings from the studies were used by international agencies such as FAO in the development of policies and guidance related to control of infection in countries where the virus remains endemic to poultry (FAO 2011).

The following section contains information on the key messages that should be provided to policy makers from this work distilled from the findings of the country studies.

References

- Becker, WB. (1966) The isolation and classification of Tern virus: influenza A-Tern South Africa—1961. *Journal of Hygiene*, 64:309-320.
- BirdLife International, 2006, BirdLife Statement on Avian Influenza, retrieved 6th November 2012, <http://www.birdlife.org/action/science/species/avian_flu/>
- Cui P, Hou Y, Xing Z, He Y, Li T, Guo S, Luo Z, Yan B, Yin Z, Lei F. (2011) Bird migration and risk for H5N1 transmission into Qinghai Lake, China, *Vector Borne and Zoonotic Diseases*, 11:567-576.
- Desvaux S, Marx N, Ong S, Gaidet N, Hunt M, Manuguerra JC, Sorn S, Peiris M, Van der Werf S, Reynes JM. (2009) Highly pathogenic avian influenza virus (H5N1) outbreak in captive wild birds and cats, Cambodia, *Emerging Infectious Diseases*, 15:475-478.
- Ellis, TM, Bousfield, RB, Bissett, LA, Dyrting, KC, Luk, GS, Tsim, ST, Sturm-Ramirez, K, Webster, RG, Guan, Y, Peiris, JS (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002, *Avian Pathology*, 33:492-505.
- Food and Agriculture Organization (2011) Approaches to controlling, preventing and eliminating H5N1 Highly Pathogenic Avian Influenza in Endemic Countries.
- Feare CJ (2007) The role of wild birds in the spread of HPAI H5N1', *Avian Diseases*, 51:440-447.
- Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P. (2006) Predicting the global



- spread of H5N1 avian influenza. *Proc Natl Acad Sci U S A*. 103:19368-73.
- Kou Z, Li Y, Yin Z, Guo S, Wang M, Gao X, Li P, Tang L, Jiang P, Luo Z, Xin Z, Ding C, He Y, Ren Z, Cui P, Zhao H, Zhang Z, Tang S, Yan B, Lei F, Li T (2009) The survey of H5N1 flu virus in wild birds in 14 Provinces of China from 2004 to 2007, *PLoS One*, 4, e6926
- ProMED Mail (2005) Avian influenza – Asia (23) Migratory birds, 20050907.2657 retrieved 6 November 2012.
<<http://www.promedmail.org/direct.php?id=20050907.2657>>
- Prosser DJ, Cui P, Takekawa JY, Tang M, Hou Y, Collins BM, Yan B, Hill Li T, Li Y, Lei F, Guo S, Xing Z, He Y, Zhou Y, Douglas DC, Perry WM, Newman SH. (2011) Wild bird migration across the Qinghai-Tibetan plateau: a transmission route for highly pathogenic H5N1. *PLoS One* 6:e17622.
- Sakoda Y, Ito H, Uchida Y, Okamatsu M, Yamamoto N, Soda K, Nomura N, Kuribayashi S, Shichinohe S, Sunden Y, Umemura T, Usui T, Ozaki H, Yamaguchi T, Murase T, Ito T, Saito T, Takada A, Kida H. (2012) Reintroduction of H5N1 highly pathogenic avian influenza virus by migratory water birds, causing poultry outbreaks in the 2010-2011 winter season in Japan. *Journal of General Virology*, 93:541-550.
- Siengsanon J, Chaichoune K, Phonaknguen R, Sariya L, Prompiram P, Kocharin W, Tangsudjai S, Suwanpukdee S, Wiriyarat W, Pattanarangsarn R, Robertson I, Blacksell SD, Ratanakorn P (2009) Comparison of outbreaks of H5N1 highly pathogenic avian influenza in wild birds and poultry in Thailand, *Journal of Wildlife Diseases*, 45:740-747.
- Sims LD, Brown IH, 2008, 'Multicontinental epidemic of H5N1 HPAI virus', in D. Swayne (ed) *Avian Influenza, USA*, Blackwell Ames, p. 251-286.
- Stoops AC, Barbara KA, Indrawan M, Ibrahim IN, Petrus WB, Wijaya S, Farzeli A, Antonjaya U, Sin LW, Hidayatullah N, Kristanto I, Tampubolon AM, Purnama S, Supriatna A, Burgess TH, Williams M, Putnam SD, Tobias S, Blair PJ. (2009) H5N1 surveillance in migratory birds in Java, Indonesia', *Vector Borne and Zoonotic Diseases*, 9:695-702.
- Tumpey TM, Suarez DL, Perkins LE, Senne DA, Lee JG, Lee YJ, Mo IP, Sung HW, Swayne DE. (2002) 'Characterization of a highly pathogenic H5N1 avian influenza A virus isolated from duck meat', *J Virol*, 76:6344-6355.
- Yamamoto Y, Nakamura K, Yamada M, Mase M. (2010) Persistence of avian influenza virus (H5N1) in feathers detached from bodies of infected domestic ducks. *Appl Environ Microbiol*. 76:5496-9.