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# The Ecological Risks of Avian Influenza Virus Spread via Migratory Birds: A Case Study at Poyang Lake, China

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**Abstract.-**The highly pathogenic avian influenza H5N1 virus (here after H5N1) still produces devastating effects in humans, poultries and wild birds. Migratory birds were thought to play a role in the long-distance spread of H5N1. This study identified 7 high-risk species and 18 potential high-risk species that may transmit H5N1 into Poyang Lake and determined four resident bird species as “bridge species” that may disperse H5N1 around the lake. The high-risk species were selected according to the following behavioral and ecological factors: migratory status, abundance, degree of mixing of species and gregariousness, migration from or stopovers at epidemic areas, and previous infection status. Among the 25 high-risk and potential high-risk species, 22 belonged to the orders Anseriformes (n = 11) and Charadriiformes (n = 11). The risk of intra-species transmission was high for Anseriformes (ducks and geese) and Charadriiformes birds, and was relatively low for Gruiformes and Ciconiiformes birds. This study can be informative and useful in the surveillance of avian influenza epidemics at breeding, stopover, and wintering sites other than Poyang Lake along the East Asian-Australian Flyway.

**Key words**: Waterbirds, field studies, avian influenza, transmission, Poyang Lake

## INTRODUCTION

Following the unprecedented outbreak of highly pathogenic avian influenza (HPAI) H5N1 (here after H5N1) in 2005 at Qinghai Lake, China, the role of wild birds in the maintenance and transmission of H5N1 has been widely debated (Liu *et al.*, 2005; Olsen *et al.*, 2006; Munster *et al.*, 2007). Wetlands and lakes and the waterbirds they hosted have been considered to contribute a lot to the spread of H5N1 (Tracey *et al.*, 2004; Kilpatrick *et al.*, 2006; Liu *et al.*, 2007; Xiao *et al.*, 2007; Fang *et al.*, 2008). Information from bird ringing, satellite telemetry and remote sensing has been used to identify relationships between bird migration and the spread of H5N1 virus (Zhao, 2006; Jourdain *et al.*, 2007; Xiao *et al.*, 2007; Prosser *et al.*, 2011). Satellite tracking of wild waterbirds in Qinghai-Tibetan Plateau has shown that wild birds play a role in the spread of H5N1 (Prosser *et al.*, 2009; 2011). However, no matches of time and location have been found in studies at Poyang Lake, which is one of the most important areas in the East Asian-Australian Flyway (Takekawa *et al.*, 2010).

In March 2013, the new deadly H7N9 strain was reported in Shanghai, China, and the NA gene of the virus may have been transferred from migratory birds infected with avian influenza viruses along the East Asian-Australian Flyway (Liu *et al.*, 2013; OIE, 2013). Poyang Lake is an important wintering site for migratory birds using the East Asian-Australian Flyway. Tens of thousands of waterbirds, mainly the Anseriformes and Charadriiformes birds, travel to Poyang Lake for their wintering. Among them, several globally endangered species were included, such as Siberian Crane (*Grus leucogeranus*) and Oriental White Stork (*Ciconia boyciana*) (Ji *et al.*, 2007; Shao *et al.*, 2014). During their yearly migrations, birds can

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potentially disperse avian influenza virus at Poyang Lake. Although epidemics of H5N1 have not been reported at Poyang Lake, the H5N1 virus has been isolated from migratory ducks at the lake (Chen *et al.*, 2006). Within the Poyang Lake region, close interactions between migratory birds and domestic poultries are common and obviously increase the probability of virus transmission (Zhang *et al.*, 2013). In addition to its impacts on poultry and implications for human health, the spread of H5N1 also has significant implications for the conservation of several species of globally endangered waterbirds (Chen *et al.*, 2005). Most likely, cross-host infections in wild birds occur most frequently when birds of different species feed, drink, or bathe in water contaminated by the feces of infected birds. For this reason, we need more accurate understandings of the composition of mixed-species groups, especially for those including endangered species.

To map the risk of H5N1 transmission to Poyang Lake via bird migration, we explored bird data in an attempt to furnish general ideas on bird abundance, migration, and interspecies contact. We reviewed the factors influencing outbreaks of H5N1 at Poyang Lake, such as the species of birds, especially for the migrants known to have been infected by H5N1. In this study, we aimed to 1) identify the high-risk species most likely to transmit H5N1 to Poyang Lake, 2) determine the bridge species that are most likely to disperse H5N1 around Poyang Lake, and 3) assess the risk posed by H5N1 to the conservation of endangered bird species at Poyang Lake.

## MATERIALS AND METHODS

### *Study area*

The study was conducted at Poyang Lake National Nature Reserve (PLNNR), Jiangxi Province, China. PLNNR locates at the northwestern area of Poyang Lake, which is the largest freshwater lake in China and constitutes a major hydrological subsystem of the middle Yangtze basin in Central China (Fig. 1). Local communities are situated behind the dikes around the lake or on islands within or outside the lake basin. The villagers primarily engage in fishing and farming. Annual floods, beginning in late May or June and usually ending in October or November, fill up the lake, producing a large inland lake that is accessible only by fishermen. When the floods subside, the lake loses as much as 90% of its water, resulting in the exposure of extensive areas of flat marshlands. In Poyang Lake, a total of 125 waterbird species have been recorded, among which 19 were listed as threatened species by International Union for Conservation of Nature (IUCN) (Ji *et al.*, 2007; IUCN, 2013).

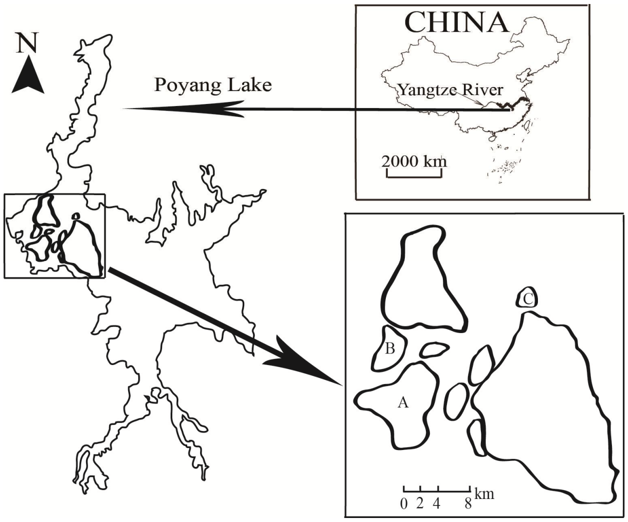

Fig. 1. Location of Poyang Lake and three small lakes: Dahuchi (A), Shahu (B) and Meixihu (C) in Poyang Lake National Nature Reserve.

### *Bird survey*

Data on waterbird population sizes was collected during the wintering season between 2003 and 2007. Field observations were conducted three times per month between September and next April, in total 22 surveys were conducted in one wintering season, and the number of species and individuals were recorded. Three most representative lakes in the Poyang Lake area, Dahuchi, Shahu, and Meixihu, were sampled for field observation of the bird abundance and diversity. Regarding the three lakes, Dahuchi and Shahu are well protected, and both are larger than 10 km$^2$. Meixihu is a small lake severely disturbed by fishing activities, with a size of approximately 2 km$^2$. All birds on the ground and those that flew in a direction opposite to the counting direction were recorded. Survey points where observations would not disturb birds on the lakes were selected. Binoculars (Olympus, 8×42) and spotting scopes (Swarovski ATS80) were used to count birds. Waterbird counts were performed under favorable weather conditions (no rain, wind speed ≤5 on Beaufort scale). Most of the birds were counted within 1000 m. The amount of time spent at each survey site varied according to the size of the survey area, and more time were spent for larger areas. Large flocks were counted by dividing them into groups of 10, 20, or 50 individuals and landmarks were used during the division procedure to improve the accuracy of bird counting. On these surveys, we recorded all waterbirds if possible to identify all birds to species. Groups of unidentified waterbirds were all composed of various duck species belonging to the family Anatidae. This information was used to create a database of the species regularly observed at Poyang Lake. Multi-year average population sizes should serve as the representative values for mapping the abundance of bird populations for each survey day at Poyang Lake. Accordingly, the counts of population sizes for the five years were averaged in the final analyses.

*Selection of high-risk species most likely to transmit H5N1 to Poyang Lake*

A modification of the method from the European Food Safety Authority was used to select the high-risk species most likely to transmit H5N1 to Poyang Lake. The high-risk species were selected based on the criteria in Figure 2. Resident species were selected as bridge species for the dispersion of H5N1 within Poyang Lake based on the following criteria: (1) resident at Poyang Lake, (2) common species at Poyang Lake, (3) species that have opportunities to contact high-risk species that may transmit H5N1 to Poyang Lake, (4) species that mix with other species and are gregarious, and (5) species that have opportunities to contact poultries in the surrounding areas.

The candidate species were checked against the list of species affected by H5N1 established by United States Geological Survey (USGS) and against published information to determine the occurrence of previous infection by H5N1 (USGS 2013a). The bird would be identified as a known user of areas with a history of H5N1 infection, when it was known to winter, breed, or stop over in the specific area where poultries or wild birds infected with H5N1 had been identified before.

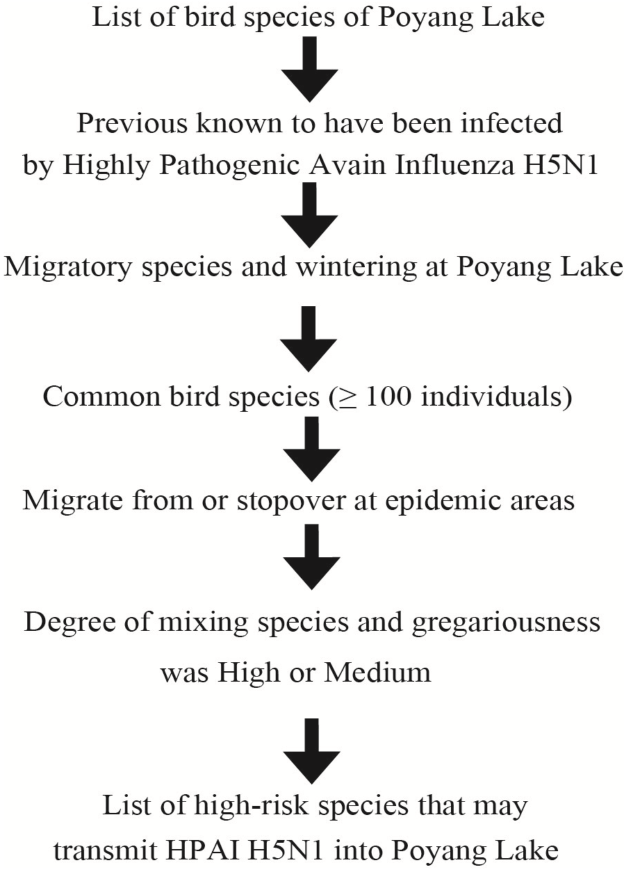

Fig. 2. Decision tree for selection of migratory species that may transmit Highly Pathogenic Avian Influenza H5N1 virus into Poyang Lake, China.

The OIE 2013 disease outbreak maps were used to locate the H5N1 epidemic areas (OIE, 2013). Information on bird migration (where the birds begin their migration or stop over) was obtained from bird ringing or satellite tracking data available in published papers or books (Zhang and Yang, 1997; Zhao, 2006; Takekawa *et al*., 2009; 2010; USGS 2013b). Migration information for many species is insufficient to establish whether these species migrate from or stop over at the areas where H5N1 has been identified. In these cases, we synthesized information on the distribution of the species and estimated the migration routes based on related species whose migration information was available (Zhang and Yang, 1997; Zhao, 2006; Avibase, 2013).

### *Degree of mixing species and threats posed by H5N1 to endangered bird species*

Eight lakes with areas from 0.2 km$^2$ to more than 10 km$^2$ were surveyed to record the level of gregariousness and degree of mixing species when the birds were feeding, bathing, or resting together in January 2009. Four categories were used to assess the degree of mixing (high degree: often mixed with more than 5 species which co-habitat; medium degree: often mixed with 3–5 species which co-habitat; low degree: often mixed with 1–2 species which co-habitat; zero degree: hardly any mixing with other species). There were also four categories to assess the gregariousness of species (high degree: often several hundreds to thousands of individuals group together; medium degree: often several tens to a few hundred individuals group together; low degree: often up to a few tens of birds group together; zero degree: usually solitary or a few birds group together). Bird habitats supporting a greater number of species, especially a greater number of high-risk species, were expected to show a high risk of interspecies virus transmission. If only a few individuals (less than 100 individuals) were recorded at a lake, then the risk of inter-species transmission was considered to be low. The globally threatened species according to the IUCN Red List were particularly recorded during the survey, and their world population sizes were obtained from published data (IUCN, 2013). The risk of spread of H5N1 of these threatened species was analyzed.

## RESULTS

### *Bird abundance and diversity*

A total of 58 species were recorded in Shahu, Dahuchi, and Meixihu. Birds began migrating to Poyang Lake in September and became abundant in early November. Both the species abundance and diversity were high from November to the following February, with a peak in December (Figs. 3-4). Anatidae birds were the most abundant in Poyang Lake, and totally 18 species were noted, including 6 species of geese and 12 species of ducks. Charadriiformes birds were also abundant (Fig. 4). Four species of Gruiformes were found, however, compared with ducks relatively few crane individuals were recorded.

### *Selection of high-risk species most likely to transmit H5N1 to Poyang Lake*

Seven species belonging to 3 orders were selected as the high-risk species that were most likely to transmit H5N1 into Poyang Lake (Table I). Another 18 none host species were also identified as potential high-risk species because they would most likely have the opportunity to transmit H5N1 to Poyang Lake once they became infected at breeding or stopover sites outside the Poyang Lake (Table II). Among the 25 species, 22 belonged to the orders Anseriformes (n = 11) and Charadriiformes (n = 11). Five of these high-risk host species were members of the family Anatidae, order Anseriformes. Four resident species were selected as the bridge species that are most likely to disperse H5N1 in the Poyang Lake area (Table III).

### *Risk of inter- and intra-species transmission and threats to endangered bird species*

Inter- and intra-species bird groups were frequently recorded at large lakes, such as Shahu and Dahuchi (Table IV). Among the eight lakes surveyed, the risk of inter-species transmission was the highest at Shahu and Dahuchi. The risk of intra-species transmission was high for Anseriformes (ducks and geese) and Charadriiformes birds, and was relatively low for Gruiformes and Ciconiiformes birds.

Six globally threatened species were recorded in the five-year survey, and 5 of them (except the Lesser White-fronted Goose *A. erythropus*) represented a large fraction of the world population of their species (Table V). Of the 5 relatively abundant threatened species, the risk of infection by H5N1 was relatively high for Siberian Crane, Oriental White Stork, and Swan Goose (*Anser cygnoides*), and the risk was relatively low for White-naped Crane (*G. vipio*) and Hooded Crane (*G. monacha*) in consideration of the degree of mixing with other species and the gregariousness of species (Table V).

## DISCUSSION

We developed a semi-quantitative method to select the migratory species that were most likely to introduce the H5N1 virus to Poyang Lake. We also identified the bridge species that were most likely to

**Table I.– The identified high-risk species that may transmit highly pathogenic avian influenza H5N1 virus into Poyang Lake, China.**

| Order, family and species name | Scientific name | Host species | Migratory and wintering at Poyang Lake | Population >100 | Migration from or stopover at epidemic areas | Degree of mixing species | Gregariousness |
|---|---|---|---|---|---|---|---|
| **Anseriformes** | | | | | | | |
| Anatidae | | | | | | | |
| Graylag Goose | *Anser anser* | Y | Y | Y | Y | H | H |
| Pintail | *Anas acuta* | Y | Y | Y | Y | H | H |
| Green-winged Teal | *Anas crecca* | Y | Y | Y | Y | H | H |
| Mallard | *Anas platyrhynchos* | Y | Y | Y | Y | H | H |
| Wigeon | *Anas penelope* | Y | Y | Y | Y | H | H |
| **Gruiformes** | | | | | | | |
| Rallidae | | | | | | | |
| Coot | *Fulica atra* | Y | Y | Y | Y | H | M |
| **Charadriiformes** | | | | | | | |
| Laridae | | | | | | | |
| Black-headed Gull | *Larus ridibundus* | Y | Y | Y | Y | M | M |

§ abbreviations: Y = yes, H = high degree, M = medium degree.

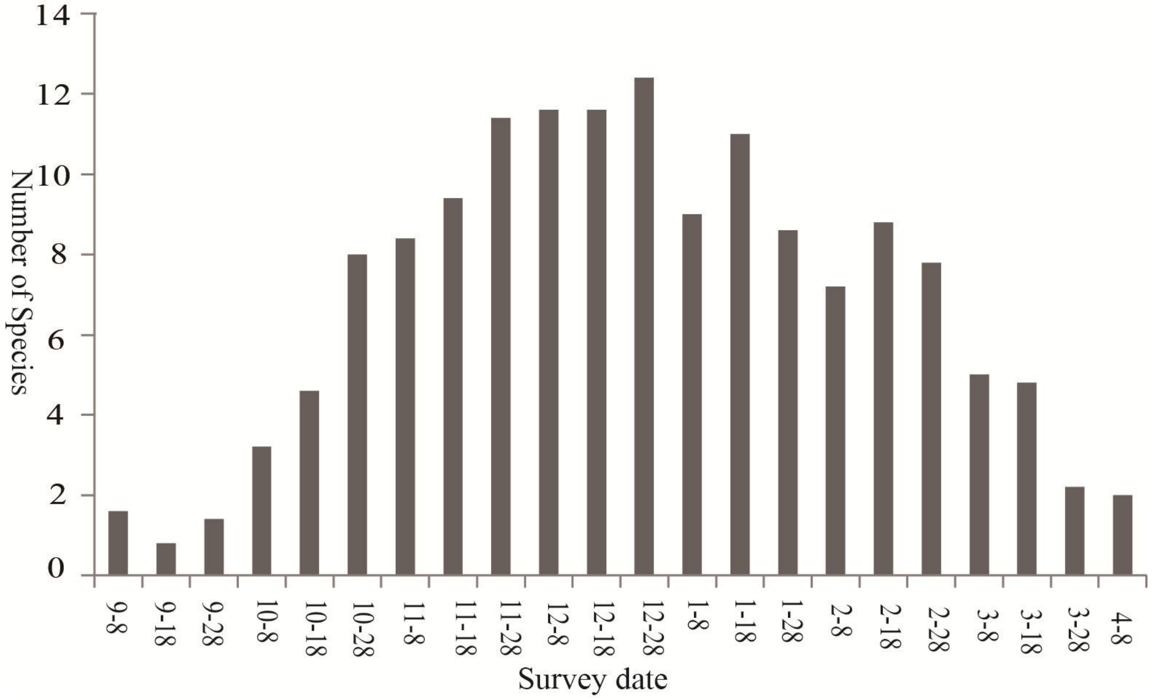

Fig. 3. Changes in waterbird species diversity at Poyang Lake (Data were obtained from field surveys in Dahuchi, Shahu, and Meixihu between 2003 and 2007, and data for each survey day were averaged in the analyses).

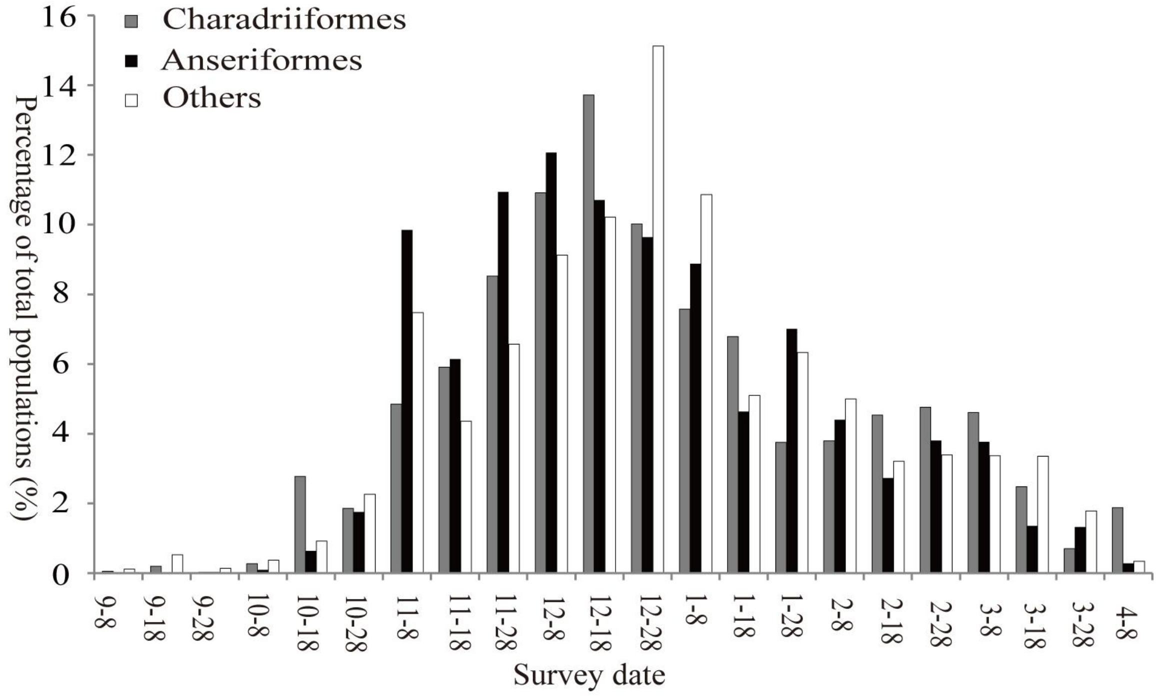

Fig. 4. Changes in waterbird species abundance at Poyang Lake (Data were obtained from field surveys in Dahuchi, Shahu, and Meixihu between 2003 and 2007, and data for each survey day were averaged in the analyses).

disperse H5N1 within Poyang Lake. Additionally, the risk of spread of H5N1 within the local wild bird populations at each bird aggregation site was evaluated based on data on mixed-species groups and gregarious species. The results indicates that ducks and geese are most likely to introduce H5N1 into the lake, and the globally threatened bird species would encounter a survival risk if an outbreak of H5N1 virus occurs at Poyang Lake.

The migratory birds were hypothesized to be responsible for the transmission of the H5N1 virus at both continental and local scale (Rappole and Hubalek, 2006; Jourdain *et al.*, 2007; Peterson *et al*., 2007; Winker *et al*., 2007). In this study, information on the distribution, species diversity, and abundance of birds was used to screen high-risk species that might introduce the H5N1 virus into Poyang Lake. Data on bird diversity and abundance showed that Poyang Lake is an important wintering site for waterbirds, especially for cranes, geese, and ducks. Under the hypothesis that the spread of H5N1 is correlated with the seasonal migration of waterbirds, our analyses help to identify the high-risk species that could introduce H5N1 into Poyang Lake (Tracey *et al.*, 2004; Xiao *et al.*, 2007; Fang *et al.*, 2008). Although the risk of H5N1 outbreaks is also influenced by various factors, such as environmental and climatic factors (Garamszeji and Møller, 2007; Fang *et al*., 2008), this study preliminarily evidenced the potential risks of outbreaks was associated with wild bird movements in Poyang Lake by a semi-quantitative description.

Our analyses showed that the greatest known threat of H5N1 virus introduced into Poyang Lake was from ducks and geese, followed by Charadriiformes birds. Ducks and geese were the most abundant waterbirds, and the prevalence of the virus was relatively high in these species (Kou *et al*., 2009). Previous studies have reported that several ducks carry the H5N1 virus without any clinical signs (Chen *et al.*, 2006; Keawcharoen *et al*., 2008). In view of the continuous outbreak of H5N1 in Southeast Asia during the recent past, we suggest that the risk of the introduction of H5N1 into Poyang Lake by migratory birds still exist (Brown *et al*., 2006, 2008; OIE, 2013).

**Table II.– Eighteen potential high-risk species that may transmit highly pathogenic avian influenza H5N1 to Poyang Lake, China†.**

| Order, family and species name | Scientific name | Host species | Migratory and wintering at Poyang Lake | Population >100 | Degree of mixing species | Gregariousness |
|---|---|---|---|---|---|---|
| **Ciconiiformes** | | | | | | |
| Ciconiidae | | | | | | |
| Oriental White Stork | *Ciconia boyciana* | N | Y | Y | H | H |
| Threskiornithidae | | | | | | |
| European Spoonbill | *Platalea leucorodia* | N | Y | Y | H | H |
| **Anseriformes** | | | | | | |
| Anatidae | | | | | | |
| Swan Goose | *A. cygnoides* | N | Y | Y | H | H |
| Bean Goose | *A. fabalis* | N | Y | Y | H | H |
| White-fronted Goose | *A. albifrons* | N | Y | Y | H | H |
| Falcated Teal | *A. falcata* | N | Y | Y | H | H |
| Spot-billed Duck | *A. poecilorhyncha* | N | N | Y | H | H |
| Tundra Swan | *Cygnus columbianus* | N | Y | Y | H | H |
| **Charadriiformes** | | | | | | |
| Charadriidae | | | | | | |
| Lapwing | *Vanellus vanellus* | N | Y | Y | M | H |
| Scolopacidae | | | | | | |
| Spotted Redshank | *Tringa erythropus* | N | Y | Y | H | H |
| Black-tailed Godwit | *Limosa limosa* | N | Y | Y | H | H |

| | | | | | | |
|---|---|---|---|---|---|---|
| Bar-tailed Godwit | *Limosa laponica* | N | Y | Y | H | H |
| Spotted Redshank | *Tringa erythropus* | N | Y | Y | H | H |
| Redshank | *T. totanus* | N | Y | Y | H | H |
| Marsh Sandpiper | *T. stagnatilis* | N | Y | Y | H | H |
| Greenshank | *T. nebularia* | N | Y | Y | H | H |
| Dunling | *Calidri alpina* | N | Y | Y | H | H |
| Recurvirostridae | | | | | | |
| Avocet | *Recurvirostra avosetta* | N | Y | Y | H | H |

† For the gap of bird migration information, the migration source of these 18 species was not checked.
§ abbreviations: N = no, Y = yes, H = high degree, M = medium degree.

From late September to November of each year, waterbirds fly to Poyang Lake from Siberia and northeastern China where H5N1 epidemics occur (Sharshov *et al*., 2010). These species may carry avian influenza virus, including H5N1, to Poyang Lake during their autumn migration. At Poyang Lake region, close interactions between wild migratory birds and domestic poultry are common which provide an opportunity for the transmission of HPAI virus (Zhang *et al*., 2013). Between March and September, water level of Poyang Lake rises, and almost all parts of the lake converge into a single water body. Some resident bird species, such as Little Grebe (*Tachybaptus ruficollis*) and Grey Heron (*Ardea cinerea*), have to disperse to small lakes that are close to human habitats around Poyang Lake, and these movements increase the risk of H5N1 transmission to poultry and human beings.

Regarding to the 5 relatively abundant globally threatened species recorded at Poyang Lake, White-naped Crane and Hooded Crane generally used separated grass habitats, so the risk of infection is relatively low. However, the other 3 species, *i.e.* Siberian Crane, Oriental White Stork, and Swan Goose, generally live together with the high-risk species identified, thus their risk of infection is relatively high.

In this study, 25 high-risk and potential

**Table III.– Resident species that may disperse highly pathogenic avian influenza H5N1 virus around Poyang Lake, China.**

| Order, family and species name | Scientific name | Resident | Population size >100 | Potentially contact with high-risk migratory species | Degree of mixing species | Gregariousness | Potentially contact with poultry |
|---|---|---|---|---|---|---|---|
| **Podicipediformes** | | | | | | | |
| Podicipedidae | | | | | | | |
| Little Grebe | *Tachybaptus ruficollis* | Y | Y | Y | M | M | Y |
| **Ciconiiformes** | | | | | | | |
| Ardeidae | | | | | | | |
| Grey Heron | *Ardea cinerea* | Y | Y | Y | M | H | Y |
| **Passeriformes** | | | | | | | |
| Sturnidae | | | | | | | |
| Crested Myna | *Acridotheres cristatellus* | Y | Y | Y | M | M | Y |
| Turdidae | | | | | | | |
| Blackbird | *Turdus merula* | Y | Y | Y | M | M | Y |

§ abbreviations: N = no, Y = yes, H = high degree, M = medium degree.

**Table IV.– Bird species diversity and abundance in the January 2009 survey in Poyang Lake National Nature Reserve, China.**

| Lake name | Area (km$^2$) | Number of species | Number of species in different abundance grade§ | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | D |
| Meixihu | 2.00 | 3 | 2 | 1 | 0 | 0 |
| Xianghu | 2.79 | 4 | 3 | 1 | 0 | 0 |
| Dongjiahu | 2.98 | 7 | 2 | 5 | 0 | 0 |
| Zhonghuchi | 6.00 | 5 | 2 | 2 | 1 | 0 |
| Changhuchi | 7.00 | 3 | 0 | 3 | 0 | 0 |
| Shahu | 14.00 | 19 | 0 | 6 | 4 | 9 |
| Bafenchang | 14.31 | 9 | 4 | 4 | 1 | 0 |
| Dahuchi | 30.00 | 22 | 2 | 8 | 4 | 8 |

§A <100 individuals; B 101-1000 individuals; C 1001-5000 individuals; D >5000 individuals.

high-risk species that may transmit H5N1 into Poyang Lake were identified and the potential risk of H5N1 to endangered bird species was analyzed. Four resident birds were identified as bridge species. This understanding would be meaningful and instructive for monitoring avian influenza in breeding, stopover, and wintering sites other than Poyang Lake along the East Asian-Australian Flyway. Further studies on the local and long-distance transmission of avian influenza virus should focus on these determined high-risk species, incorporating those studies addressing the surveillance, maintenance, and spread of the H5N1 virus.

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**Table V.– Globally threatened species recorded in Poyang Lake from 2003 to 2007.**

| Order, family and species name | Scientific name | Habitat | Degree of mixing species* | Gregariousness* | Population numbers at Poyang lake§ | Estimated flyway population | Status in the IUCN Red List¶ |
|---|---|---|---|---|---|---|---|
| **Gruiformes** | | | | | | | |
| Gruidae | | | | | | | |
| Siberian Crane | *Grus leucogeranus* | shallow water, mudflat | H | M | 4577 | 3000† | CR |
| White-naped Crane | *Grus vipio* | grassland, mudflat | M | M | 885 | 3000 † | VU |
| Hooded Crane | *Grus monacha* | shallow water, mudflat | M | M | 2713 | 1000 † | VU |
| **Ciconiiformes** | | | | | | | |
| Ciconiidae | | | | | | | |
| Oriental White Stork | *Ciconia boyciana* | shallow water, mudflat | H | H | 3120 | 3000‡ | EN |
| **Anseriformes** | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Anatidae | | | | | | | |
| Swan Goose | *Anser cygnoides* | grassland, shallow water | H | H | 70830 | 60000-100000 ‡ | EN |
| Lesser White-fronted Goose | *Anas erythropus* | grassland, shallow water | H | M | 98 | 20000 ‡ | VU |

† FromWetlands International 2013.
‡ From Wei and Mundkur 2004.
§ Reviewed from Ji *et al.*, 2007 and Li *et al.*, 2012.
¶ From IUCN 2013.
* H = high degree, M = medium degree.

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