Satellite-marked waterfowl reveal migratory connection between H5N1 outbreak areas in China and Mongolia

DIANN J. PROSSER,1* JOHN Y. TAKEKAWA,2 SCOTT H. NEWMAN,3 BAOPING YAN,4 DAVID C. DOUGLAS,5 YUANSHENG HOU,6 ZHI XING,6 DEHAI ZHANG,7 TIANXIAN LI,7 DELONG ZHAO,8 WILLIAM M. PERRY2 & ERIC C. PALM2

1United States Geological Survey, Patuxent Wildlife Research Center, Beltsville, MD 20705 USA and University of Maryland, College Park, MD 20742 USA
2United States Geological Survey, Western Ecological Research Center, Vallejo, CA 94592 USA
3EMPRES Wildlife Unit, Animal Health Service, Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, Italy 00153
4Chinese Academy of Sciences, Computer Network Information Center, Beijing, China 100080
5United States Geological Survey, Alaska Science Center, Juneau, AK 99801 USA
6Qinghai Lake National Nature Reserve, Xining, China 810003
7Chinese Academy of Sciences, State Key Laboratory of Virology, Wuhan Institute of Virology, Hubei, China 430071
8Chinese Academy of Sciences, Institute of Zoology, Beijing, China 100101

The role of wild birds in the spread of highly pathogenic avian influenza H5N1 has been greatly debated and remains an unresolved question. However, analyses to determine involvement of wild birds have been hindered by the lack of basic information on their movements in central Asia. Thus, we initiated a programme to document migrations of waterfowl in Asian flyways to inform hypotheses of H5N1 transmission. As part of this work, we studied migration of waterfowl from Qinghai Lake, China, site of the 2005 H5N1 outbreak in wild birds. We examined the null hypothesis that no direct migratory connection existed between Qinghai Lake and H5N1 outbreak areas in central Mongolia, as suggested by some H5N1 phylogeny studies. We captured individuals in 2007 from two of the species that died in the Qinghai Lake outbreaks and marked them with GPS satellite transmitters: Bar-headed Goose Anser indicus (n = 14) and Ruddy Shelduck Tadorna ferruginea (n = 11). Three of 25 marked birds (one Goose and two Shelducks) migrated to breeding grounds near H5N1 outbreak areas in Mongolia. Our results describe a previously unknown migratory link between the two regions and offer new critical information on migratory movements in the region.

Keywords: highly pathogenic avian influenza H5N1, satellite telemetry, waterfowl, wild birds.
marked a turning point in the evolution and spread of H5N1. For the first time since its emergence, wild birds were infected with H5N1 in a major epizootic, raising concerns over whether they have the ability to spread the disease along migratory corridors (Fergus et al. 2006, Normile 2006, Weber & Stilianakis 2008).

Radiation of H5N1 out of Asia and into Russia, Europe and Africa occurred subsequent to this event (Chen et al. 2006a, Gilbert et al. 2006, Sims & Brown 2008). In August 2005, H5N1 was reported in north-central Mongolia with deaths of an additional 89 waterfowl including Bar-headed Geese and Whooper Swans Cygnus cygnus at Erkhel and Khunt Lakes (OIE 2005, Wildlife Conservation Society 2005), 1200 km north of Qinghai Lake. The Qinghai and Mongolia outbreak sites were located in regions having few or no domestic poultry (FAO 2007a, 2007b), which raised questions about how the disease spread to these areas [an undocumented report of a Bar-headed Goose farm in Qinghai was suggested in a blog post (Butler 2006), however]. Several reviews have pointed to the domestic poultry system as the primary mechanism of H5N1 spread (Clark & Hall 2006, Muzaffar et al. 2006, Feare 2007, Gauthier-Clerc et al. et al. 2006), although wild bird involvement could not be ruled out and in certain events probably played a significant role (Gilbert et al. 2006). Phylogenetic analyses have been used to suggest routes of H5N1 movement based on genetic relationships of virus isolates (Chen et al. 2006a, 2006b, Kilpatrick et al. 2006, Janies et al. 2007, Wallace et al. 2007), although these analyses do not specify mechanisms underlying disease transmission. Large gaps in the knowledge of wild bird migratory patterns in Asia and the ecology of H5N1 in their populations have limited our understanding of how this disease spreads (Muzaffar et al. 2006, Olsen et al. 2006, Yasue et al. 2006, Alexander 2007).

The Asian flyways are the least studied in the Palaearctic (Mundkur 2006) with little known about specific waterfowl migration routes (Miyabayashi & Mundkur 1999, Kear 2005, Popovkina 2006). The goal of our work is to document wild bird migration patterns in Asian flyways to inform hypotheses regarding H5N1 transmission. In 2007, we began satellite telemetry studies at Qinghai Lake on migration pathways of two waterfowl species extensively affected in the outbreaks: the Bar-headed Goose and Ruddy Shelduck. Migration data for these species are generally lacking (Miyabayashi & Mundkur 1999, Javed et al. 2000, Kear 2005), particularly from the Qinghai Plateau (Uttangi 1987, Zhang 2006). Here we tested a null hypothesis that no migratory connection exists between Qinghai Lake and Mongolia in these species.

**METHODS**

**Study area**

We conducted research at the Qinghai Lake National Nature Reserve, Qinghai Province, in north-central China (36.82°N, 99.81°E). The 495 000 ha reserve was established in 1975 as a provincial reserve and listed as a Wetland of International Importance in 1992 (Ramsar Convention Secretariat 2007). Qinghai Lake, located on the eastern edge of the Qinghai-Tibet Plateau, is China’s largest salt-water lake. Elevation of the lake is 3200 m and annual precipitation is 35 cm, with the majority of rainfall occurring from May to September (Xu et al. 2007). The Qinghai Lake climate is characterized by long, cold, dry winters (October to April), strong winds, high solar radiation, and average annual temperatures of −0.7 °C (Xu et al. 2008). Qinghai Lake has long been recognized for its important position in the intersection of the East Asian and Central Asian flyways, and has been designated a key breeding site for colonial nesting waterbirds such as the Bar-headed Goose (more than 15% of the global population use this area; Miyabayashi & Mundkur 1999), Ruddy Shelduck, Brown-headed Gull Larus brunnicephalus, Pallas’s Gull Larus ichthyaetus, and Great Cormorant Phalacrocorax carbo (Li 2001).

**Capture and marking**

We captured Bar-headed Geese and Ruddy Shelducks in March 2007 and September 2007, respectively, on the western and southern edges of Qinghai Lake, China. Birds were captured outside the breeding season to reduce disturbance during nesting and to increase the opportunity of marking potential migrants using Qinghai Lake as a migratory stopover. We captured individuals with monofilament leg nooses (made by Indian trappers) and a remotely activated net launcher (Coda Enterprises, Mesa, AZ, USA). Upon capture, birds were immediately removed from the nooses and nets, placed in individual cloth bags, and processed. We recorded mass, flat wing chord, short tarsus (diagonal length
of the tarsometatarsus, measured along its outer edge; Dzubin & Cooch 1992), sex, and age for each bird. Adult birds and equal numbers of males and females were targeted for marking. Virology sampling was conducted for birds marked in September only (due to logistical constraints) and included cloacal and tracheal swabs and blood samples for each bird following standard sampling and transport procedures (FAO 2007c). Analyses included: (1) type A influenza with an ELISA test (OD630 above 0.23 as positive), (2) H5 subtype with RT-PCR (Fouchier et al. 2000), and (3) H5, H7, H9, and H10 antibodies with hemagglutinin inhibition following OIE standards (OIE 2004). Laboratory analyses were conducted by the Chinese Academy of Sciences, Wuhan Institute of Virology. Birds were marked with 45 g (Geese) or 30 g (Shelducks) GPS solar-powered Platform Terminal Transmitters (PTTs: solar-GPS PTT-100, Microwave Telemetry, Inc., Columbia, MD, USA) affixed with Teflon harnesses (Bally Ribbon Mills, Bally, PA, USA). Transmitter packages averaged 2.1% and 2.4% of the bird’s body weight (Geese and Shelducks, respectively). Birds were released as close to capture locations as possible within 1 h of capture. Procedures for capture, handling and marking were reviewed and approved by the USGS Patuxent Wildlife Research Center Animal Care and Use Committee and University of Maryland Baltimore County Institutional ACUC (Protocol EE070200710).

**Satellite telemetry locations**

PTTs were programmed to record GPS locations every 2 h and data were uploaded to the Argos satellite tracking system every 2 days (CLS America Inc., Largo, MD, USA). For this analysis, we used a subset of locations that examined connectivity between Qinghai and Mongolia from March through June 2007 and September 2007 through July 2008 for Shelducks. We used ArcGIS 9.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) and Google EARTH 4.3 (Google, Mountain View, CA, USA) to plot and analyse the telemetry locations. Migratory stopover sites were defined as areas where birds moved less than 20 km within a 24-h period or longer.

**RESULTS**

We marked 25 birds with PTTs at Qinghai Lake in 2007: 14 Geese (12 adults: four males, eight females; two first-year: one male, one female) and 11 Shelducks (six adults: three males, three females; five hatch-year: two males, three females). Three of the birds (12%) migrated to breeding grounds in central Mongolia, including one Goose and two Shelducks.

Of the Geese, female #67693 migrated from Qinghai Lake to central Mongolia, whereas the other 13 marked Geese remained at Qinghai Lake through the 2007 breeding season (April–June). Goose #67693 was captured and marked at a creek on the western edge of the lake on 26 March. It remained at Qinghai Lake for 25 days using freshwater wetlands and wheat fields before migrating north on 20 April. Between then and 7 May, the bird flew 1200 km to a complex of small lakes 200 km south of Erkhel Lake (47.99°N, 99.88°E), Arhangay Province, Mongolia (Fig. 1). The 17-day migration included three stopovers ranging from 1 to 7 days (Table 1) and distances flown between stopovers ranged from 108 to 755 km. Goose #67693 remained on the Mongolia breeding grounds within an area of 200 km² until the PTT ceased transmitting on 5 June 2007.

Of the 11 Shelducks, two migrated to breeding grounds in central Mongolia (Fig. 1). Female #74808 and male #74810 were caught and marked on 13 September, and remained within 5 km of the capture site until commencing separate fall migrations. On 11 November, the female migrated 59 km southwest for a 12-day stopover, and then flew 1300 km to wintering grounds (25.15°N, 97.22°E) in Kachin State, Myanmar arriving on 23 November (Table 2). On 18 November, the male Shelduck flew 763 km southwest to a riverine floodplain where it remained for 16 days before migrating an additional 576 km south to wintering grounds in the same vicinity as the female. From December to March, both birds used riverine wetlands and agricultural fields within a 370-km² area in northern Myanmar (25.15°N, 97.22°E). On 12 March 2008 they began the northward spring migration together, flying 1378 km to an area of wetlands 100 km west of Qinghai Lake. They remained here for 47 days before flying 1423 km north to breeding grounds in Mongolia (49.30°N, 99.93°E), 70 km southwest of Erkhel Lake (49.93°N, 99.93°E).

Virology results for the 11 Shelducks sampled revealed no positives for type A influenza and two positives for H5 antibodies (inhibition at serum dilution of 1 : 32 and 1 : 64, respectively). All
birds handled appeared healthy, showing no symptoms of influenza infection, and virology tests of the two Shelducks that migrated to Mongolia (74808, and 74810) were negative for avian influenza (results unavailable for goose 67693).

**DISCUSSION**

Disease models have offered different hypotheses to explain H5N1 movements from China to Mongolia, Russia and Kazakhstan in late 2005 (Chen et al. 2006a, Kilpatrick et al. 2006, Webster & Govorkova 2006, Janies et al. 2007, Wallace et al. 2007). Wallace et al. (2007) were among the first to use phylogenetic analyses to map patterns of hypothesized H5N1 dispersal within and beyond Asia from available genetic sequences of viral isolates. They reported on two significant H5N1 movement patterns from Asia including Qinghai Lake to Novosibirsk, Russia, and Qinghai Lake to

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**Figure 1.** Migration routes of one Bar-headed Goose (yellow) and two Ruddy Shelducks (red) from Qinghai Lake to central Mongolia. Qinghai, Erhel, and Khunt Lakes were the locations of large wild bird HPAI H5N1 outbreaks in 2005. Insets provide enlarged view of local habitats for the following areas: (a) Ruddy Shelducks breeding grounds, (b) Bar-headed Goose breeding grounds, (c) Qinghai Lake local bird movements. Circles and lines demarcate breeding, wintering, and migration locations. Poyang Lake, the location of suspected progenitors to Qinghai HPAI H5N1 isolates, is indicated in the lower right of the map.
Table 1. Location, duration of stopover, duration of flight, and habitat used by Bar-headed Goose #67693 during 2007 spring migration between HPAI H5N1 outbreak areas of Qinghai Lake (QL), China and central Mongolia.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>No. of days at location</th>
<th>No. of flight days</th>
<th>Province, Country</th>
<th>km flown</th>
<th>Co-ordinates (decimal degrees)</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 March</td>
<td>Capture site, SW edge of QL</td>
<td>&lt; 1</td>
<td>Qinghai</td>
<td>China</td>
<td>0</td>
<td>36.76°N 99.77°E</td>
<td>Small stream, oxbow lake</td>
</tr>
<tr>
<td>26 March–20 April</td>
<td>W edge of QL</td>
<td>25</td>
<td>Qinghai</td>
<td>China</td>
<td>0</td>
<td>37.22°N 100.02°E</td>
<td>Freshwater wetlands and wheat fields</td>
</tr>
<tr>
<td>20 April</td>
<td>Stopover A</td>
<td>1</td>
<td>Qinghai</td>
<td>China</td>
<td>108</td>
<td>38.13°N 99.53°E</td>
<td>100 m lakes</td>
</tr>
<tr>
<td>22 April</td>
<td>Stopover B</td>
<td>1</td>
<td>Qinghai</td>
<td>China</td>
<td>196</td>
<td>39.71°N 99.32°E</td>
<td>Riverine wetlands</td>
</tr>
<tr>
<td>27 April–4 May</td>
<td>Stopover C</td>
<td>7</td>
<td>Bayanhongor</td>
<td>Mongolia</td>
<td>755</td>
<td>46.45°N 100.21°E</td>
<td>150 m lakes</td>
</tr>
<tr>
<td>7 May–5 Junea</td>
<td>Breeding grounds</td>
<td>28</td>
<td>Arhangay</td>
<td>Mongolia</td>
<td>172</td>
<td>47.99°N 99.88°E</td>
<td>150–700 m lakes</td>
</tr>
</tbody>
</table>

aLast signal received from PTT on 5 June 2007.

Table 2. Location, duration of stopover, duration of flight, and habitat used by Ruddy Shelducks #74808 and #74810 during fall 2007 and spring 2008 migrations between HPAI H5N1 outbreak areas of Qinghai Lake (QL), China and central Mongolia.

<table>
<thead>
<tr>
<th>Date 2007–2008</th>
<th>Location</th>
<th>No. of days at location</th>
<th>No. of flight days</th>
<th>Province, Country</th>
<th>km flown</th>
<th>Coordinates (Decimal degrees)</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruddy Shelduck #74808, female – 2007 fall migration</td>
<td>Capture site, Hei Ma He</td>
<td>28</td>
<td>Qinghai</td>
<td>China</td>
<td>0</td>
<td>36.74°N 99.80°E</td>
<td>Lake edge</td>
</tr>
<tr>
<td>13 September–11 November 2007</td>
<td>Stopover A</td>
<td>12</td>
<td>Qinghai</td>
<td>China</td>
<td>59</td>
<td>36.64°N 99.18°E</td>
<td>Freshwater wetlands</td>
</tr>
<tr>
<td>11–23 November 2007</td>
<td>Wintering site</td>
<td>109</td>
<td>Kachin</td>
<td>Myanmar</td>
<td>1300</td>
<td>25.15°N 97.22°E</td>
<td>Riverine wetlands, agricultural fields</td>
</tr>
<tr>
<td>Ruddy Shelduck #74810, male – 2007 fall migration</td>
<td>Capture site, Hei Ma He</td>
<td>35</td>
<td>Qinghai</td>
<td>China</td>
<td>0</td>
<td>36.74°N 99.80°E</td>
<td>Lake edge</td>
</tr>
<tr>
<td>13 September–18 November 2007</td>
<td>Stopover A</td>
<td>16</td>
<td>Tibet</td>
<td>China</td>
<td>763</td>
<td>30.19°N 97.44°E</td>
<td>Riverine wetlands</td>
</tr>
<tr>
<td>18 November–5 December 2007</td>
<td>Wintering site</td>
<td>98</td>
<td>Kachin</td>
<td>Myanmar</td>
<td>576</td>
<td>25.15°N 97.22°E</td>
<td>Riverine wetlands, agricultural fields</td>
</tr>
<tr>
<td>Ruddy Shelducks #74808 and #74810 – 2008 paired spring migration</td>
<td>Capture site, Hei Ma He</td>
<td>47</td>
<td>Qinghai</td>
<td>China</td>
<td>1378</td>
<td>36.86°N 98.50°E</td>
<td>Wetlands and small lake</td>
</tr>
<tr>
<td>14 March–30 April 2008</td>
<td>Stopover A</td>
<td>2</td>
<td>Hovsgol</td>
<td>Mongolia</td>
<td>1423</td>
<td>49.30°N 99.57°E</td>
<td>Riverine wetlands, mountain cliffs</td>
</tr>
<tr>
<td>2 May–15 July 2008</td>
<td>Breeding grounds</td>
<td>74</td>
<td>Arhangay</td>
<td>Mongolia</td>
<td>172</td>
<td>47.99°N 99.88°E</td>
<td>150–700 m lakes</td>
</tr>
</tbody>
</table>

Southward fall migrations were flown separately. Birds were paired on wintering grounds, during the northward spring migration, and on the breeding grounds.

Astrakhan, Russia. None of the 25 Geese and Shelducks tracked in our study migrated along these pathways. Instead, three of our 25 birds (12%) followed a migration route from Qinghai Lake to Mongolia, a pathway that Wallace et al. (2007) reported as a possible route for movement of
H5N1, but one which they found statistically non-significant compared to expected values using Monte Carlo distributions.

The timing of migration found in our study also provides supporting information for hypotheses put forth by Kilpatrick et al. (2006). Their integrated analyses combined molecular phylogenies, poultry routes, and wild bird patterns to suggest that H5N1 infection in Mongolia occurred through wild bird movements from China a few months before the outbreaks. The Geese and Shelducks in our study moved from Qinghai Lake to central Mongolia in early May, approximately 3 months before outbreaks in wild geese and swans were reported.

Establishment of the migratory connection between these two regions, however, does not prove that an infected bird could survive and shed virus along the 1200-km migration route (Weber & Stilianakis 2007). Laboratory trials have shown varying rates of mortality and viral shedding among species, including geese shedding virus for several days before dying and some migrant ducks and previously exposed swans shedding virus without any clinical signs of infection (Sturm-Ramirez et al. 2005, Brown et al. 2008, Keawcharoen et al. 2008). Despite extensive global surveillance efforts over the past 5 years (Alexander 2003, Ellis et al. 2004, Chen et al. 2006a, Globig et al. 2006, Buranathai et al. 2007, Gaidet et al. 2007a, 2007b, Munster et al. 2007, Nagy et al. 2007, Wallensten et al. 2007, Wildlife Conservation Society 2007), detection of H5N1 in healthy wild birds is rare (Chen et al. 2006a, L’Vov D et al. 2006, Saad et al. 2007). However, successful migration of an HPAI (H5N2) infected White-faced Whistling Duck Dendrocygna viduata recently documented in Nigeria demonstrates the possibility for wild bird movement of HPAI (Gaidet et al. 2008).

Two of the 11 Shelducks exhibited H5 antibodies, suggesting that these individuals survived a prior infection of a type A influenza with H5 haemagglutinin (although this does not indicate that the subtype was H5N1 or that the birds migrated while infected). Challenge studies by Brown et al. (2008) suggest relatively low susceptibility of Bar-headed Geese to A/whooper swan/Mongolia/244/2005 (H5N1) with exposed birds shedding virus for 5–8 days, and three of five inoculated geese making full recoveries. If these results from captive-reared geese relate to wild birds, geese that survived the 2005 outbreaks could have moved the virus to other places. The marked birds in our study migrated in multiple segments between stopover areas within a few days; conditions that could permit viral transmission among individuals sharing these stopover habitats.

Our work documents existence of a migratory connection between Qinghai Lake and Mongolia and provides new insights into the ecology of these species as well as informing hypotheses of H5N1 spread. Only when we have a greater understanding of the ecology of migratory populations in these Asian flyways will the possible role of wild birds as vectors for H5N1 be revealed.

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REFERENCES
Migratory route connects H5N1 outbreak locations


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