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Satellite-tracking of Northern Pintail *Anas acuta* during outbreaks of the H5N1 virus in Japan: implications for virus spread

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We fitted Northern Pintail *Anas acuta* in Japan with satellite transmitters and monitored their spring migration movements relative to locations where the highly pathogenic H5N1 avian influenza virus was detected in Whooper Swans *Cygnus cygnus* in 2008. Pintails were assumed not to be infected with the H5N1 virus at the time they were marked because capture occurred between 2 and 5 months before reported outbreaks of the virus in Japan. We assessed spatial and temporal overlap between marked birds and occurrence of the virus and tracked Pintails after they departed outbreak locations. Eight of 66 (12.1%) Northern Pintails marked with satellite transmitters used wetlands in Japan where the H5N1 virus was detected in Whooper Swans. Apparent survival did not differ between Pintails that used H5N1 sites and those that did not. However, the proportion of Pintails that migrated from Japan was significantly lower among birds that used H5N1 sites compared with those that did not (0.50 vs. 0.79). Northern Pintails were present at the H5N1 sites from 1 to 88 days, with five birds present at the sites from 0 to 7 days prior to detection of the virus in Swans. The six Pintails observed to depart H5N1 sites did so within 2–77 days of the reported outbreaks and moved between 6 and 1200 km within 4 days of departure. Four Pintails migrated to eastern Russia. After their departure from outbreak sites, Northern Pintails made long-distance migrations within the period when newly infected ducks would shed the H5N1 virus. This supports a hypothesized mechanism by which a highly pathogenic avian influenza virus could be spread by migratory birds.

Keywords: *Anas acuta*, *Cygnus cygnus*, highly pathogenic H5N1 avian influenza, migration, Northern Pintail, Whooper Swan.

The highly pathogenic Asian H5N1 avian influenza virus (hereafter H5N1 virus) first appeared in Hong Kong in 1996 and has since spread throughout Asia and parts of Europe and Africa (World Health Organization 2009). The extent to which the virus has been spread by shipping of infected poultry or poultry products, the wild bird trade and migration of wild birds has been the subject of debate, and has implications for transmission of the virus to other parts of the world (Kilpatrick *et al.* 2006, Feare 2007, Flint 2007, Si *et al.* 2009). The H5N1 virus has been detected in wild birds that show no clinical signs of infection (Hesterberg *et al.* 2009, Kou *et al.* 2009, World Organization for Animal Health 2009), and migratory birds are believed to be responsible for movement of the virus into some areas of Asia and Europe (Li *et al.* 2004, Gilbert *et al.* 2006). Transmission of the H5N1 virus may be facilitated by migratory species that are asymptomatic carriers of the virus (Sturm-Ramirez *et al.* 2005, Brown *et al.* 2006, Keawcharoen *et al.* 2008), birds that shed the virus before they become ill (Brown *et al.* 2008), or

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when previous exposure to other avian influenza viruses reduces pathogenicity yet allows infected birds to shed the H5N1 virus (Pasick *et al.* 2007, Kalthoff *et al.* 2008, Khalenkov *et al.* 2009). For a migratory bird to spread the H5N1 virus between geographical regions, it must (1) occur in an outbreak area during the time when the virus is present, (2) contract the virus without suffering mortality or severe physiological impairment, and (3) migrate within approximately 1 week of contracting the virus, the period when a newly infected bird is most apt to shed the virus orally or in faeces (Brown *et al.* 2006, 2008). Studies of the movements of wild birds during outbreaks of the H5N1 virus would help to evaluate spatial and temporal overlap between migratory species and occurrence of the virus (Si *et al.* 2009). Gaidet *et al.* (2008a), Newman *et al.* (2009) and Prosser *et al.* (2009) studied movements of wild birds marked with satellite transmitters relative to locations of H5N1 outbreaks and provided useful information on migratory connections among outbreak sites. However, because their studies were conducted ≥ 1 year after outbreaks occurred, they could not directly measure temporal relationships among occurrence of the virus, use of an outbreak site by radio-marked birds and migration movements of marked individuals. Direct measures of the timing of migration relative to reported dates of outbreaks are needed to assess whether long-distance movements occur within the relatively short interval during which an infected bird would shed the virus. However, to date there are no studies of migrations of wild birds conducted during an H5N1 outbreak.

In spring 2008, dead or dying Whooper Swans *Cygnus cygnus* infected with the H5N1 virus were detected at three locations in Japan (Uchida *et al.* 2008, World Organization for Animal Health 2008). The infected Swans were found in wetlands used by large numbers of waterbirds during a period when many species were staging for their northward migration. Prior to the outbreaks of the H5N1 virus, we had marked Northern Pintail *Anas acuta* on the islands of Honshu and Hokkaido with satellite transmitters as part of an ongoing study of the role of wild birds in transmission of avian influenza viruses. We studied movements of Northern Pintails because they frequently carry low pathogenic avian influenza viruses (Parmley *et al.* 2005, Ip *et al.* 2008), and because captive Northern Pintails experimentally infected with the H5N1 virus were asymptomatic but shed live virus orally after exposure (Brown *et al.* 2006). Northern Pintails can rapidly fly long distances, sometimes migrating between North America and Asia (Miller *et al.* 2005, Nicolai *et al.* 2005), and in Japan they often occur in close proximity to Whooper Swans. Furthermore, Flint *et al.* (2009) observed that Pintails from Asian and North American wintering areas were sympatric on breeding areas in eastern Russia, resulting in genetic exchange between continental populations. Additionally, through whole genome analysis, Koehler *et al.* (2008) found that low pathogenic avian influenza viruses of Northern Pintails in Alaska often had gene segments more closely related to Asian lineages, whereas Pearce *et al.* (2009) found that influenza viruses of Northern Pintails on their wintering areas in California were almost exclusively of North American ancestry. This suggests that intercontinental movement of viruses may occur via Northern Pintails that migrate between Asia and Alaska, but that Asian viruses are not maintained during southern migration to North American winter areas.

The marked sample of Northern Pintails enabled us to address whether this species satisfied the first two of the three conditions necessary for wild birds to transmit the H5N1 virus. We estimated the proportion of radio-marked Northern Pintails that used sites where the H5N1 virus was detected in northern Japan, their duration of use at those sites, and the timing of use relative to the reported occurrences of the virus in Whooper Swans. We also determined the date that marked Northern Pintails migrated from Japan, the timing of migration relative to their last use of outbreak sites, and identified migration routes they followed. We could not evaluate whether Northern Pintails contracted the H5N1 virus without suffering mortality or severe physiological impairment. However, we hypothesized that if Northern Pintails at H5N1 sites were at greater risk of contracting the virus, and as a result suffered an adverse physiological response, we would observe lower rates of survival or a reduced likelihood of migration among marked individuals that used H5N1 sites compared with those that did not.

METHODS

We captured Northern Pintails at four sites in Japan between 12 November 2007 and 6 March

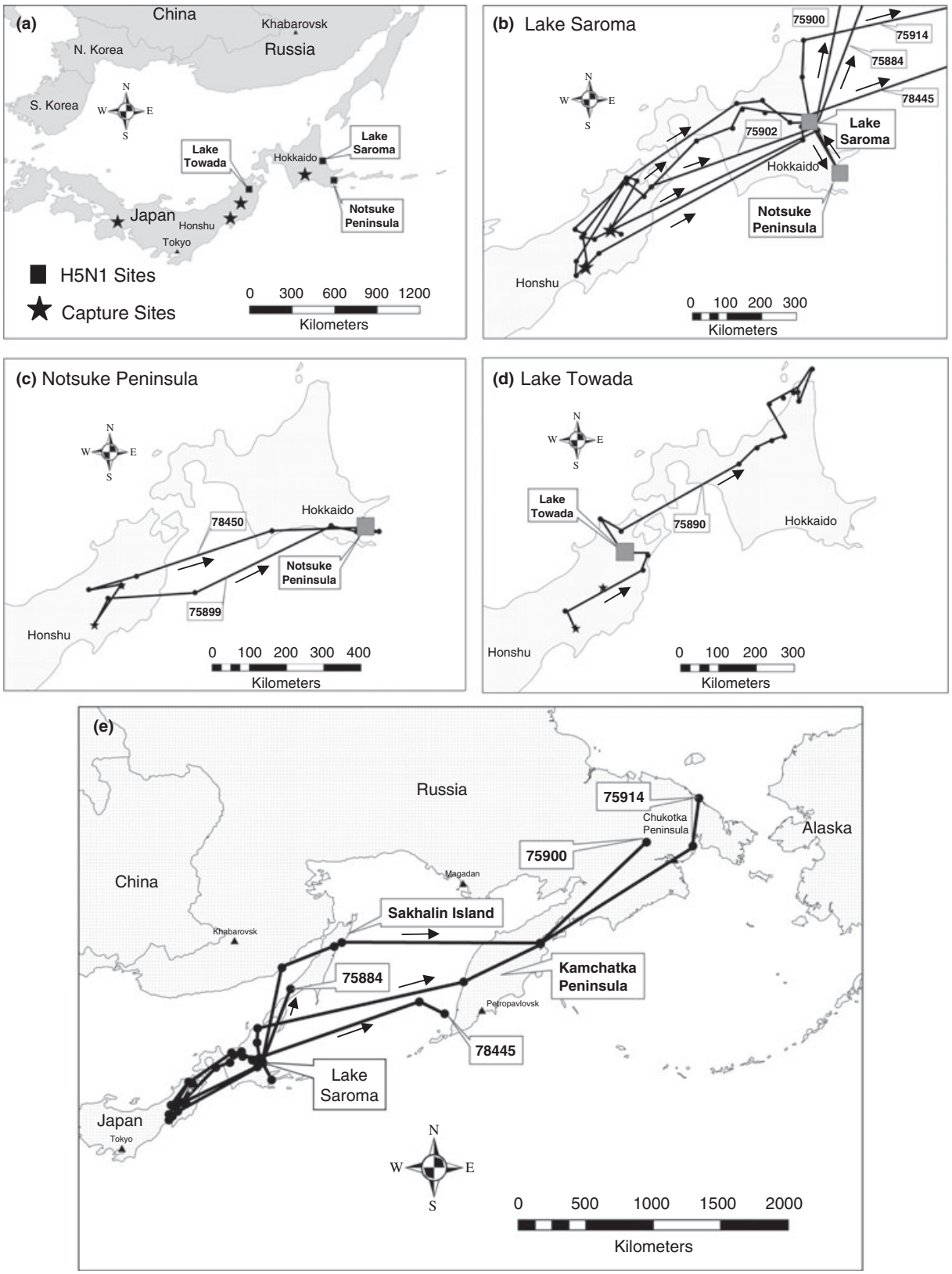
2008 (Fig. 1). Capture sites were near wetlands where Pintails congregated in winter. We captured birds by hand or with a 4 × 10-m net that was flipped over birds that were attracted to bait piles of grain. We determined the gender and age of trapped birds based on plumage characteristics (Carney 1992), and selectively marked adult birds (> 1 year of age) that were of above average body mass. Pintails were not tested for the presence of the H5N1 virus, but we assume they were not infected as capture occurred 2–5 months before the 2008 outbreaks were first reported in Japan. We attached Platform Transmitter Terminals (PTTs) to 6–35 Northern Pintails at each site, and marked a total of 92 birds (53 males and 39 females). We attached PTTs via a harness that consisted of a Teflon-treated ribbon that was secured with metal ferrules at attachment points on the anterior and posterior ends of the PTT. The ribbon crossed and was secured with knots at the keel of the sternum, and a small amount of glue was added to the knots (Miller *et al.* 2005). We used solar- and battery-powered PTTs. The solar-powered PTTs (n = 40) were manufactured by North Star Science and Technology, weighed 12 or 20 g, and transmitted for 10 h within each 34-h interval. The battery-powered PTTs (n = 52) were manufactured by Microwave Telemetry, weighed 20 g, had an expected transmission life of approximately 10 months, and transmitted for 6 h within each 72-h interval. The weight of a PTT plus harness materials was 1.1–2.8% of the body weight of the birds.

We collected data on date, time, latitude and longitude of each PTT location, as well as its quality (LC) through the Argos Data Collection and Location System (CLS America 2007). Estimated error was ≤ 1 km for locations of LC1 or better, and > 1 km for LC0 locations. We used a computer program to filter unlikely locations based on rate and distance of movement (Douglas 2006). We monitored movements of Northern Pintails relative to locations where the H5N1 virus had been detected in Whooper Swans in Japan (Fig. 1). In 2008, the H5N1 virus was discovered in Whooper Swans on the Island of Honshu at Lake Towada (18, 21 April, 8 May), and on the island of Hokkaido at the Notsuke Peninsula (24 April) and Lake Saroma (5 May). Whooper Swans wintered at Lake Towada and the Notsuke Peninsula, and used each of the sites as spring migration staging areas. We based locations and dates of H5N1 occurrences in Swans on reports from the World Organization for Animal Health (2008).

Data analyses

Analysis was restricted to Northern Pintails that were alive with functional PTTs on or after 18 April 2008, the first detection date of the H5N1 virus in Japan. Radio-marked individuals were monitored for as long as location data indicated that they continued to move or the activity sensor in the PTT indicated that the bird was alive. A total of 66 birds were included in these analyses. A marked Pintail was assumed to use an H5N1 site when one or more PTT locations of at least LC1 quality were recorded in the same waterbody where infected Swans were found, or if an LC0 location was present in the waterbody and there were no other wetlands within 10 km. We could not measure the distance separating Pintails from infected Swans because of the error surrounding PTT locations and because the World Organization for Animal Health (2008) reported only the approximate latitude and longitude of outbreak sites. Furthermore, Swans succumb to the H5N1 virus 4–14 days after exposure (Brown *et al.* 2008, Kalthoff *et al.* 2008) and the distance to point locations where dead or dying Swans were found would not account for movements of Swans during the period they were incubating the virus. Marked Pintails were used to derive an estimate of the proportion of the Pintail population that used

Figure 1. Northern Pintail movements through H5N1 areas. (a) Locations where Northern Pintails were captured and marked with satellite transmitters in Japan, November 2007–March 2008 (Obihiro, 42.933°N, 143.300°W; Iwate, 39.667°N, 140.983°W; Izunuma, 38.717°N, 141.083°W; Miyagi, 34.783°N, 135.900°W). Sites where the H5N1 virus was detected in Whooper Swans in April and May 2008 as indicated by the World Organization for Animal Health (Lake Towada, 40.253°N, 140.533°W; Notsuke Peninsula, 43.335°N, 145.212°W; Lake Saroma, 44.055°N, 143.544°W). (b–d) Movements of Northern Pintails marked with satellite transmitters through each of three sites where the H5N1 virus was detected in Whooper Swans in Japan. Dots represent locations of Pintails during transmission periods of transmitters. Lines connecting dots show sequence of movement and arrows indicate the general direction of movement relative to an H5N1 site. One Pintail used both the Notsuke Peninsula and Lake Saroma. (e) Movements of four Northern Pintails marked with satellite transmitters in Japan after they departed the H5N1 site at Lake Saroma. Arrows indicate direction of migration.



wetlands where infected Whooper Swans also occurred. Swans and Pintails were often observed foraging sympatrically in winter and it was expected that if both species used the same wetlands during migration they would probably be in close contact.

We determined the first and last dates a marked Pintail was detected at an H5N1 site and calculated how many days a bird stayed at an H5N1 site based on those dates. Most PTTs transmitted once every 3 days, so our measures of the duration of use at a site were minimum estimates. Timing of use and departure from a site were evaluated relative to the dates Swans infected with the H5N1 virus were discovered. We measured the total distance Pintails moved within 4 days of their last observed use of an H5N1 site. This provided a measure of the potential range of virus movement during the period when a newly infected bird would be likely to shed the virus (Brown *et al.* 2006). We noted the last date that marked Pintails were located in Japan before their migration elsewhere, recorded the location of the first stopover Pintails made after leaving Japan, and their final location on 31 July 2008 or when the PTT transmission was no longer received.

It was assumed that a bird migrated if it departed the islands of Honshu or Hokkaido and was detected at least once elsewhere, and that a bird did not migrate if its final location was on Hokkaido or Honshu on the date the transmitter was last operable, or if the bird had not departed Japan by 31 July 2008. A randomization test (Manley 1997) was used to evaluate whether the proportion of marked Pintails that migrated from Japan was smaller for the group of birds that used H5N1 sites compared with the group that did not. We computed the proportion of marked Pintails that migrated from Japan for each H5N1 exposure group and calculated the observed difference in proportions between groups and then randomly assigned individual birds to exposure groups without replacement 1000 times and for each random iteration maintained the sample size originally found in each group. We computed the difference in proportions of migrants between randomly assorted exposure groups for each iteration and report a one-tailed test based on the proportion of random iterations in which the difference between exposure groups was less than the observed difference.

It was assumed that if there were higher rates of mortality among marked Pintails that used H5N1 sites, there would be higher rates of signal loss because PTTs of dead birds commonly end up such that signals are blocked or solar panels obscured. For radios that continued transmitting, a lack of movement in activity sensor data would have been detected. To evaluate whether the apparent survival time (i.e. longevity of PTTs in which activity sensors indicated movement) was lower among birds that used an H5N1 site vs. those that did not, a randomization test was used. It was assumed that signal loss due to transmitter or battery failure was independent of whether a bird used an H5N1 site or not. We calculated the difference in the number of days between 18 April and 31 July 2008 for which birds were indicated to be alive with a functioning transmitter by subtracting the average days for birds that used H5N1 sites from the average days for birds that did not. We followed the same randomization procedure described above and for a one-tailed test report the proportion of 1000 random iterations in which the difference between exposure groups was less than the observed difference. Where randomization tests indicated significant differences, a 95% confidence interval surrounding the difference was computed following Manley (1997). All randomization analyses were carried out using the PopTools macro in program Excel (Hood 2008).

RESULTS

Eight of 66 (12.1%, 95% CI = 6.2–20.8%) Northern Pintails marked with satellite transmitters used one or more of the three sites where the H5N1 virus was detected in Whooper Swans (Table 1). One female Pintail used both outbreak sites on Hokkaido. Northern Pintails arrived at H5N1 sites a median of 9 days prior to the detection of the virus in Whooper Swans (Table 2). PTTs of three Northern Pintails ceased transmitting due to either transmitter failure or death of the birds 1–42 days after they arrived at an H5N1 site. We do not know the length of stay at outbreak locations or dates of departure for those individuals. Five Pintails remained at outbreak locations a median of 8 days, and departed a median of 17 days after the outbreaks were first detected. Three marked Northern Pintails were present at H5N1 sites on the dates that the virus was detected in Whooper Swans, and two birds were last detected at outbreak sites 2–7 days before the virus was detected. Among other Pintails, a 24–26-day interval

Table 1. Northern Pintails marked with satellite transmitters (PTTs) that used sites where the highly pathogenic H5N1 avian influenza virus was detected in Whooper Swans in Japan, 2008.

PTT	Sex	H5N1 site used	Arrival date[a]	Departure date[a]	Days between use and H5N1 detection[b]	Region where last located
75884	Male	Saroma	1 May	23 June	0	Sakhalin
75890	Male	Towada	23 March	23 March	−26	Hokkaido
75899	Female	Notsuke	17 April	29 May[c]	0	Hokkaido
75900	Female	Notsuke	13 April	13 April	−11	Chukotka
75900[d]	Female	Saroma	20 April	3 May	−2	Chukotka
75902	Male	Saroma	12 May	12 May[c]	7	Hokkaido
75914	Male	Saroma	9 April	9 April	−26	Chukotka
78445	Male	Saroma	26 April	21 July	0	Okhotsk Sea
78450	Female	Notsuke	29 May	31 May[c]	35	Hokkaido

[a]Dates of first and last detection of a Northern Pintail PTT in the same wetland where H5N1 was detected in Whooper Swans.
[b]The minimum number of days that separated observed use of a site by a Northern Pintail and reported detection of the H5N1 virus in Swans. Negative values indicate use occurred before H5N1 was reported, a 0 indicates the Pintail was present on the date H5N1 was detected, and positive values indicate use occurred after detection of the virus in Swans. Detection dates of the H5N1 virus in Swans used to compute values were 18 April for Lake Towada, 24 April for the Notsuke Peninsula, and 5 May for Lake Saroma.
[c]Date of final location. Departure from the site was not observed.
[d]Female 75900 was detected at two sites where H5N1 was reported in Whooper Swans.

Table 2. Chronology of Northern Pintail use of sites where the H5N1 virus was detected in Whooper Swans, and the distance Pintails moved after departure from outbreak locations. Chronology is expressed as the number of days that separate an event and the dates that the H5N1 virus was detected in Whooper Swans. Negative values indicate an event occurred prior to detection of the virus, and positive values indicate an event occurred after dates the virus was detected in Swans. Data are based on movements of eight Northern Pintails marked with satellite transmitters in Japan, 2009. Detection dates of the H5N1 virus in Swans used to compute values were 18 April for Lake Towada, 24 April for the Notsuke Peninsula, and 5 May for Lake Saroma.

	N[a]	Median	Minimum	Maximum
Days between Pintail arrival at H5N1 site and detection of the virus[b]	8	−9	−26	35
Days separating detection of virus and the last date a Pintail was at an H5N1 site	6	−2	−26	77
Days Pintails remained at H5N1 sites	6	8	1	87
Days separating final detection of Pintails in Japan and occurrence of the H5N1 virus	4	51	−2	83

[a]Number of radio-marked Northern Pintails used to derive estimates for an event.
[b]Date of arrival was the first date the PTT of a Northern Pintail was detected at an H5N1 site.

separated use of an H5N1 site and reported occurrence of the virus in Whooper Swans.

The six Pintails that departed H5N1 sites travelled from 6 to 1215 km (median = 59 km) within 4 days of their departure (Fig. 1). PTTs of two Pintails failed in northern Hokkaido after their departure from H5N1 sites but before their migration from Japan was detected. Four Pintails migrated from Japan after using Lake Saroma. Migration occurred from 2 days before until 83 days after the detection of the H5N1 virus in Swans. A female (75900) last located at Lake Saroma 2 days before an infected Whooper Swan was discovered migrated 600 km directly from Lake Saroma to the coast of the Khabarovsk region of Russia within 3 days, and ultimately migrated an additional 2800 km to Sakhalin Island and the Chukotka Peninsula, Russia, over the next 22 days (Fig. 1). After being detected at Lake Saroma 24 days before the H5N1 virus was detected in a Swan, a male (75914) remained within 10 km of the site during the following 13 days before migrating 180 km to northeastern Hokkaido, then 3600 km to the Chukotka Peninsula over a 61-day interval. Two males (75884 and 78445) departed Japan between 22 and 27 July after spending 53–87 days at Lake Saroma. Both were present at that site when the H5N1 virus was reported in a Swan, but migrated 77–83 days after the date the virus was detected. In the week prior to migration, 75884 moved approximately 30 km from Lake Saroma to the northern coast of Hokkaido before

migrating 600 km to Sakhalin Island, whereas 78445 migrated directly from Lake Saroma approximately 1200 km toward the Kamchatka Peninsula before the signal was lost. Of 58 marked Northern Pintails not detected at H5N1 sites, 46 (79.3%) were observed to migrate from Japan.

Based on the randomization test, the proportion of radio-marked birds that migrated from Japan was significantly lower ($P = 0.014$) for Northern Pintails that used the H5N1 sites than for those that did not (0.50 vs. 0.79; 95% CI for the difference between groups: −0.589; −0.002). However, there was no difference in the apparent survival time (based on the duration a PTT transmitted) between birds that used an H5N1 site and those that did not (77 vs. 73 days, $P = 0.57$).

DISCUSSION

This is the first study in which a migratory species was tracked during an H5N1 outbreak in wild birds. Although wild birds other than Swans were suspected as the source of H5N1 outbreaks in Japan in 2008 (Uchida *et al.* 2008), it is unwise to speculate on whether Pintails played a role in transmission of the H5N1 virus to outbreak sites, or on possible mechanisms of virus transmission among Whooper Swans, Northern Pintails or other species present at outbreak sites. Nor do we imply that radio-marked Northern Pintails contracted the H5N1 virus. Instead, our study demonstrates that two of the three criteria required for a migratory bird to spread the virus were satisfied during a relatively limited occurrence of the H5N1 virus in Japan: Northern Pintails used outbreak sites during times when the virus was present, and at least one bird migrated directly from an outbreak site to a location outside of Japan within 1 week of detection of the virus in a Swan.

We defined Northern Pintail use of outbreak sites based on their occurrence in wetlands where infected Whooper Swans were detected. This is likely to be valid as our objective was not to evaluate the likelihood that marked Pintails actually came into contact with infected Swans, but rather to assess the proportion of marked Pintails that used wetlands where the H5N1 virus was present, and the migration movements of birds following their departure. Survey data on the numbers of Northern Pintails at outbreak sites are not available for all locations. However, if the percentage (12.1%) of marked Pintails that used H5N1 sites was representative of the 130 000 Northern Pintails that winter in Japan (Miyabayashi & Mundkur 1999), approximately 15 000 (95% CI = 8060–27 040) Northern Pintails may have used wetlands where the H5N1 virus was detected in Swans. This is plausible as the estimate is based on a relatively large sample of Pintails of both sexes marked with satellite transmitters at multiple locations in Japan, and because the outbreak locations on Hokkaido were < 40 km from major staging areas used by up to 21 000 Northern Pintails (Miyabayashi & Mundkur 1999). The Hokkaido wetlands where the H5N1 virus was discovered were also important migration habitat for up to 11 000 Eurasian Wigeon *Anas penelope* and 8000 Greater Scaup *Aythya marila* (Miyabayashi & Mundkur 1999). This demonstrates the capacity for large numbers of wild birds to pass through sites where the H5N1 virus is present if outbreaks occur at stopover areas during the period of migration.

Most Northern Pintails (five of eight marked individuals) used H5N1 sites within 7 days of dates Whooper Swans were discovered to be dead or dying from the virus. Some Swans survive infection by the H5N1 virus and the mortality of those that die occurs up to 14 days after their exposure to the virus (Brown *et al.* 2008, Kalthoff *et al.* 2008). Therefore, the H5N1 virus was likely to be present but undetected in Swans prior to the dates dead or dying birds were discovered, making temporal overlap with Northern Pintails more likely. Furthermore, the true duration and extent of outbreaks was poorly known because detection of infected Swans was opportunistic and was not the result of a systematic survey for the H5N1 virus. Furthermore, if the H5N1 virus persists in wetland environments, as has been documented for avian influenza viruses (Brown *et al.* 2007, Lang *et al.* 2008), Northern Pintails could have been exposed to the virus outside the dates when the virus was detected in Whooper Swans.

An evaluation of the distance a wild bird moves within its first week after infection with the H5N1 virus is important to assess how migratory species may contribute to geographical spread of the virus. Although it is not possible to assess whether radio-marked pintails carried the H5N1 virus, our data demonstrate the potential for an infected migrant to travel long distances during the period of viral shedding. Northern Pintails moved up to 1200 km within 4 days of departure from an H5N1 site, and

at least two Pintails migrated directly from an H5N1 outbreak site to locations outside Japan. Of particular interest was a 600-km migration of a female that departed Lake Saroma within 2 days of the discovery of a Whooper Swan that had died from the H5N1 virus. This is the first instance in which a radio-marked bird has been detected at an H5N1 outbreak location at approximately the same time the virus was present, then observed to depart the site and migrate a long distance within the period that an infected bird would shed the virus. Northern Pintails that migrated from Japan first flew to coastal areas of eastern Russia, including the Khabarovsk Territory, Sakhalin Island and the Kamchatka Peninsula. Two Pintails ultimately migrated to the Chukotka Peninsula. Although the H5N1 virus has not been reported in those regions, the same clade of the virus found in Japanese Swans was detected in Eurasian Teal *Anas crecca* and domestic poultry in the Primorye Territory of eastern Russia, approximately 500 km west of Hokkaido and immediately south of the Khabarovsk Territory in April 2008, about the same time as the outbreaks in Japan (Lvov *et al.* 2008). Whether the virus reached that area via migratory birds or through other mechanisms is not known. The likelihood of detecting the H5N1 virus in many areas of far eastern Russia may be low because the region is sparsely populated by humans.

The likelihood that a radio-marked Pintail migrated from Japan differed between Northern Pintails that used known H5N1 sites and those that did not. Also, two of the Northern Pintails that migrated from Japan after using H5N1 sites did so > 30 days after other birds had departed. Because few Pintails remain in Japan during summer (Brazil 1991), such a delayed migration is unusual. We cannot rule out the possibility that differences in migration behaviour between Pintails that used H5N1 sites and those that did not were due to exposure of the former to the virus. However, this result is equivocal due to the small sample, its correlative nature, and because there are other potential causes for delayed migration, such as an adverse behavioural response to transmitter attachment. Although migration capabilities of birds infected with the H5N1 virus have been questioned (Feare 2007), migration by waterfowl infected with a highly pathogenic avian influenza virus has been documented (Gaidet *et al.* 2008b). Based on duration of transmission by PTTs we found no evidence that survival of Pintails that used H5N1 sites was lower than among birds that did not use outbreak sites. It is quite possible that if Northern Pintails contracted the virus they would not have been adversely affected (Brown *et al.* 2006). Northern Pintails and other waterbirds that were apparently healthy yet infected with the H5N1 virus have been detected in wild populations (Hesterberg *et al.* 2009, Kou *et al.* 2009).

Spatial and temporal overlap between occurrence of the H5N1 virus and migratory birds is necessary for wild birds to spread the virus. Our finding that Northern Pintails made long-distance movements shortly after using sites where the H5N1 virus was present supports a hypothesized mechanism by which the virus could be spread by migratory species (Gilbert *et al.* 2006, Lee *et al.* 2008, Si *et al.* 2009). There was evidence that Northern Pintails migrated to the Kamchatka and Chukotka peninsulas of eastern Russia after they departed H5N1 outbreak sites in Japan. These are also summer breeding and moulting areas for many North American migrants (Henny 1973, Dau *et al.* 2000, Hupp *et al.* 2007), in a region where Northern Pintails from Asia are likely to come into contact with Pintails from North America (Flint *et al.* 2009). Because Northern Pintails in Alaska show evidence of intercontinental exchange of influenza viruses (Koehler *et al.* 2008), movement of the H5N1 virus into eastern Russia would increase the risk for transmission of the virus to North America via species that migrate between Alaska and Asia.

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