Surveillance for highly pathogenic avian influenza (H5N1) in live captured tundra swans in Northwest Alaska

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ABSTRACT To examine relationships between highly pathogenic avian influenza (HPAI H5N1) prevalence and migration patterns of tundra swans (*Cygnus columbianus)* breeding and molting in Northwest Alaska, we captured and marked a total of 230 molting tundra swans (including 3 recaptures) from areas surrounding Kotzebue, Alaska (Selawik, Buckland, Kobuk Delta, Noatak Delta, and Baldwin Peninsula; 22-28 July 2008) and implanted 10 of these individuals (5 breeding and 5 molting females) with satellite transmitters. In total, we sampled 224 individuals for highly pathogenic avian influenza (HPAI H5N1). In addition we collected whole blood, serum, breast and head feathers for genetic, contaminant, seroprevalence, and stable isotope analyses. Thus far, no samples have tested positive for HPAI H5N1, but final results are still pending. As of 24 October 2008, 9 of the 10 tundra swans marked in Northwest Alaska were alive and migrating south through Southern Alberta and Saskatchewan (Canada), Central Montana, and Northern Utah. Continuing movement data from satellite transmitters will provide information on migration routes, wintering areas, and seasonal movement patterns for tundra swans breeding and molting in Northwest Alaska and help clarify population structure among aggregations of tundra swans statewide. Given continued evidence of HPAI H5N1 infection in swans worldwide, interest in their role as carriers of the disease, and large sample sizes and ancillary data achievable in Northwest Alaska, continued (and possibly expanded) sampling in 2009 is recommended.

KEY WORDS Alaska, highly pathogenic avian influenza, H5N1, migration, tundra swan, satellite telemetry, *Cygnus columbianus*

Inter-continental and inter/intra-specific transmission of highly pathogenic avian influenza (hereafter referred to as HPAI H5N1) by migratory birds could have catastrophic impacts on both national and global economies, as well as human health. Modeling the potential spread of HPAI H5N1 is dependent on understanding the probability of interacting with birds that are infected, in addition to knowledge of the proportion of populations of interest that frequent areas where HPAI H5N1 is present or is likely to occur. However, for many migratory bird species, even basic information on distribution and movement patterns is unknown (Convention on Migratory Species 2005, Nairobi Conference 2005).

Since 2003, a virulent strain of avian influenza (AI), identified as H5N1, emerged and spread throughout Southeast Asia, killing more than 210 people worldwide. The role of migratory birds in spreading HPAI H5N1 is unknown, but migratory birds are considered a possible vector for the virus. Alaska is a crossroads for bird migration between Asia and North America and as a result, thousands of migratory birds from Alaska have been tested for HPAI H5N1 (Ip et al. 2008). To date, none have tested positive for HPAI H5N1, but several species, including tundra swans (*Cynus columbianus;* Ip et al. 2008) have shown a proclivity for carrying other AI viruses. Of these species, swans appear to frequently carry low pathogenic AI (Harless, 28 Aug 2006, Ip et al. 2008) and have been one of the most common wild birds found dead in association with outbreaks of HPAI H5N1 in Europe. The recent outbreak of HPAI H5N1 in swans in the UK (Morales, 16 Jan 2008), coupled with experimental dosing in captivity (Kalthoff et al. 2008), suggest that exposure to HPAI H5N1 may not be as highly pathogenic to swans as previously thought (i.e., not all swans with the HPAI H5N1 die), and implies that swans may be effective carriers of the disease.

If tundra swans in Alaska migrate inter-continentally, a better understanding of AI virus prevalence in tundra swans in Alaska will be important in determining their role in the dispersal of AI viruses such as HPAI H5N1. In 2006, the Alaska Interagency APAI H5N1 Bird Surveillance Working Group completed a strategic plan for early detection of HPAI H5N1 in wild migratory birds in Alaska (Alaska Interagency Avian Influenza Working Group 2006). In the strategic plan, the tundra swan was identified as one of 29 species at high risk of contracting HPAI H5N1, given that a segment of the North American subspecies of tundra swan is believed to breed in eastern Asia and winter in North America. Between 2006 and 2007, 267 tundra swans were captured and sampled for AI in Northwest Alaska (an average of 133 swans per year).

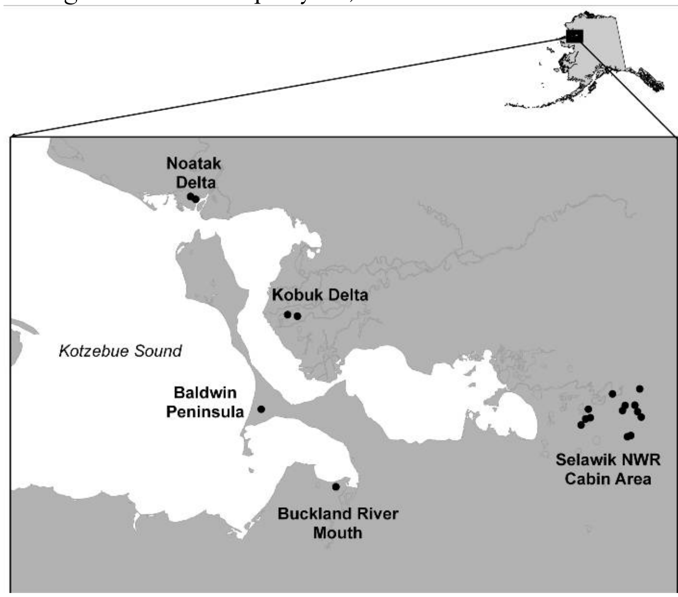

Figure 1. Capture locations of molting tundra swans near Kotzebue, Northwest Alaska (22-28 July 2008).

Although the relationship between low pathogenic AI and highly pathogenic H5N1 is not well understood, recent experimental infection of swans with highly pathogenic H5N1 demonstrated that individuals could be clinically protected from pathogenicity via historical exposure to other low pathogenic AI subtypes (Kalthoff et al. 2008). In other words, swans with historical exposure to AI viruses may serve as asymptomatic carriers of HPAI H5N1. Overall, the prevalence of AI in swans in North America and elsewhere, coupled with consistent outbreaks of highly pathogenic H5N1 in swan species throughout the world, implies that swans are an important indicator species. Increased monitoring of swans in Alaska is a critical component of understanding the prevalence and potential inter-continental movement of AI viruses such as HPAI H5N1 via wild birds.

In addition to examining potential patterns of inter-continental movement of AI, this study was designed as part of a larger effort to integrate information on the historic and contemporary relationships among populations of tundra swans based on yearly movement patterns, genetic, and isotopic data in order to evaluate the likelihood of HPAI H5N1 spreading amongst breeding birds within Alaska (Ely et al. 2008). As part of this larger effort, winter movements of tundra swans captured throughout Alaska will be characterized in relation to avian influenza prevalence and potential routes of transmission (Ely et al. 2008). Herein, we report the preliminary results for Northwest Alaska, 2008.

STUDY AREA

Our study was conducted at several locations in Northwest Alaska, near Kotzebue (66°09'N, 162°0'W), Alaska (Fig. 1). The wetland areas in which we captured molting swans were composed of extensive lake systems, often surrounded by upland tundra habitat. Large aggregations of molting swans were primarily found on interconnected lake systems in low-lying coastal wetlands. In contrast, molting adults in family groups tended to utilize smaller, more isolated lakes in upland tundra, several miles inland from the coast.

METHODS

Capture methods followed those used by Selawik NWR personnel during historical swan banding operations (Spindler and Hall 1986), as modified by Fischer (2007). Specifically, crews located molting flocks and family groups from float equipped aircraft. When swans were sighted, larger aircraft (e.g., C-206's) landed and deployed personnel and small inflatable boats with 8 hp motors, while lighter aircraft (PA-18) maintained the flock in position for capture. Teams of two biologists (per boat) captured flightless swans with dipnets, bound their feet with electrical tape, and transported them to shorelines for processing (Figs. 2 and 3). Many captured swans were also restrained with custom made harnesses, modified from Evans and Kear (1972; Fig. 3). After processing, all swans were subsequently released at the capture site.

All captured swans were marked with U.S. Geological Survey (USGS) metal leg-bands and sampled for avian influenza (1 oral-pharyngeal and 1 cloacal swab each).

Additionally, 200 of the captured birds were marked with unique, alpha-numerically coded plastic neck collars (Fig. 4). From each bird, crews collected 6 mL of blood via jugular venipuncture, feather samples (12 head and 6 breast) and digital photos (e.g., Fig. 4). All birds were weighted to the nearest 0.1 kg and sexed by cloacal examination. Birds with gray feathers on the neck and head were classified as second year (SY) birds (i.e., one year old), while birds with all white feathers were considered after second year (ASY) birds (Limpert et al. 1987). Dial calipers were used to measure length of the ninth primary, middle toe, tarsus, culmen, and length and width of lore.

Figure 2. Molting tundra swan capture sequence; dipnetting from a small inflatable boat (Photos: Brad Scotton).

In addition to the 200+ swans captured from molting groups, 10 swans (5 molters and 5 local breeders) were implanted with satellite transmitters (platform transmitting terminals: PTTs) by a team of veterinarians in the field (Cheryl Scott and Juliana Milani, U.C. Davis; Fig. 5).

Breeders were defined as those individuals with a mate and cygnets at the time of capture. These birds were captured individually, using a dipnet off the floats of a small float plane (PA-18), rather than using boats. All swans slated for surgery were processed (e.g., banded, bled, photographed) prior to surgery and placed under anesthesia within 1 h of trapping. Veterinarians followed standard implant techniques developed by Korschgen et al. (1996), as modified by Petersen et al. (1995, 1999), and others. Birds recovered in portable dog-carriers post-surgery and were subsequently released at their respective capture lakes via float plane.

Figure 3. Captured tundra swans awaiting processing on a shoreline, Kobuk Delta, Alaska, 2008. (Photo: Brad Scotton).

Figure 4. Photo of swan head with collar used to assess morphological measurements. (Photo: Karen Bollinger).

All satellite transmitters were programmed to transmit one pulse every 3 days (5 h transmission period with 72 hrs between transmissions) from time of deployment through 20 Sept. Beginning ~20 Sept. (through 1 Dec. 2008), the transmission cycle increased to once per day (i.e., 5 h transmission periods, with 18 hrs between transmissions) in order to better examine rapidly changing migration locations.

Table 1. Numbers of samples, markers, and measurements obtained during the tundra swan highly pathogenic avian influenza H5N1 surveillance program in Northwest Alaska, 2008.

Location	Date	Capture & Band	Recap	AI Swab	Collar	PTT	Morph Measure	Serum	Whole Blood	Feathers	DNA
Selawik NWR Cabin	Jul 22-24	22	1	22	13	7	22	21	19	22	19
Baldwin Peninsula	Jul 25	1		1	0	1	1	1	1	1	1
Noatak Delta	Jul 26	66		66	65	1	66	61	64	66	65
Buckland River Mouth	Jul 27	53	2	51	51		53	52	46	47	50
Kobuk Delta	Jul 28	88		82	71	1	88	54	56	55	57
Total		**230**	**3**	**224**	**200**	**10**	**230**	**189**	**186**	**191**	**192**

After 1 Dec. 2008, the frequency of transmissions has been programmed to change again, returning to an every 3 day cycle during winter (2 Dec.-15 Mar. 2009), then back to a once/day cycle during spring migration (18 Mar.-15 May 2009). This schedule of alternating duty cycles should result in a total of 1500 potential PTT hours of transmission (using two 750 h batteries). Thus, transmitters could potentially last until early summer of 2010.

Figure 5. A female tundra swan undergoing satellite transmitter implantation surgery by veterinarians Cheryl Scott and Juliana Milani of U.C. Davis.

All satellite transmitters were equipped with sensors to monitor the body temperature of individuals (indicating survival) and battery voltage (to indicate likelihood of future transmitter failure). Data are received through the ARGOS data collection and location system in Landover, Maryland (Service Argos 2001). The Douglas Argos-Filter Algorithm (Douglas 2007) will be used to assess the plausibility of every ARGOS location according to: 1) distances between consecutive locations, and 2) rates and bearings among consecutive movement vectors.

RESULTS

Captures, sampling, and satellite implants

Field crews captured and marked a total of 230 molting tundra swans (148 adult females and 82 adult males, 3 of which were recaptures; Table 1) from wetlands surrounding Kotzebue, in Northwest Alaska. Specific locations included the Buckland River Mouth, areas surrounding the Selawik NWR cabin, the Noatak Delta, Kobuk Delta, and the Baldwin Peninsula (Fig. 1). Crews sampled 224 individuals for HPAI H5N1, 186 individuals for whole blood, 189 for serum, 192 for DNA, and 191 individuals for breast and head feathers (see Table 1). Additionally, 10 of the 230 individuals (5 breeding and 5 molting females) were implanted with satellite transmitters.

All avian influenza samples were sent to the USGS National Wildlife Health Center in Madison, Wisconsin for analysis. As of October 2008, no samples had tested positive for AI, but final results are still pending. Serum samples have undergone initial screening for historical exposure to AI (Wilson and Hall 2008) and to date, 20% of sera samples appear to be seropositive and 14% borderline-seropositive for AI antibodies (J. Hall pers. comm.). Examination of Northwest Alaska tundra swan serum biochemistry, hematology, bacteriology, and general health evaluation is currently underway at the University of California, Davis (Milani et al. 2008). Whole blood and DNA samples have been archived at the USGS Alaska Science Center and will be analyzed for elevated lead levels, as well as used to assess population structure within Alaska (Ely et al. 2008). Additionally, a short documentary film, highlighting the 2008 Northwest Alaska capture work is being produced by a Montana State University Natural History Filmmaking Master's Student (Stephani Gordon).

In addition to avian influenza sampling, we implanted 10 of the 230 individuals (5 breeding and 5 molting females) with satellite transmitters. Captured tundra swans slated for PTT implants were transported to veterinarians by small floatplane (PA-18) within 0.5 hrs of capture. Average surgery time (from initial isoflurane gas, until extubation) was 1 h 2 min (SE ± 9 min) and ranged from 0:36-1:45 (hrs:min). There was 1 mortality as a result of surgery; an adult female swan from the Selawik NWR Cabin area, captured from a family group, died just after surgery, but before recovering from anesthesia. Although no direct cause of death could be determined, this bird dove repeatedly during her capture, and exhaustion may have played a role in her negative response to surgery. The transmitter from this individual was removed post-mortem, resterilized, and used in another bird.

As of early September 2008, all 10 satellite transmitters were active (i.e., no deaths or equipment failures had occurred post release) and all satellite-tagged tundra swan females remained in the vicinity of their capture location. However, by 23 September 2008, 1 transmitter indicated low body temperature over a 7-10 day period, suggesting the individual had died (C. Ely pers. comm.). Aerial reconnaissance of the area revealed a mass of white feathers at the mortality-signal location, but crews were unable to attempt retrieval of the transmitter due to poor landing conditions (T. Moran pers. comm.). As of 24 October 2008, 9 of the 10 tundra swans marked in Northwest Alaska were alive and migrating south through Southern Alberta and Saskatchewan (Canada), Central Montana, and Northern Utah (Fig. 6).

DISCUSSION

Movement data

This tundra swan project was coordinated with sampling and marking efforts on the Alaska Peninsula, Yukon Delta, and North Slope of Alaska (Ely et al. 2008). Together, leg and neck markers, satellite transmitters, and feather samples

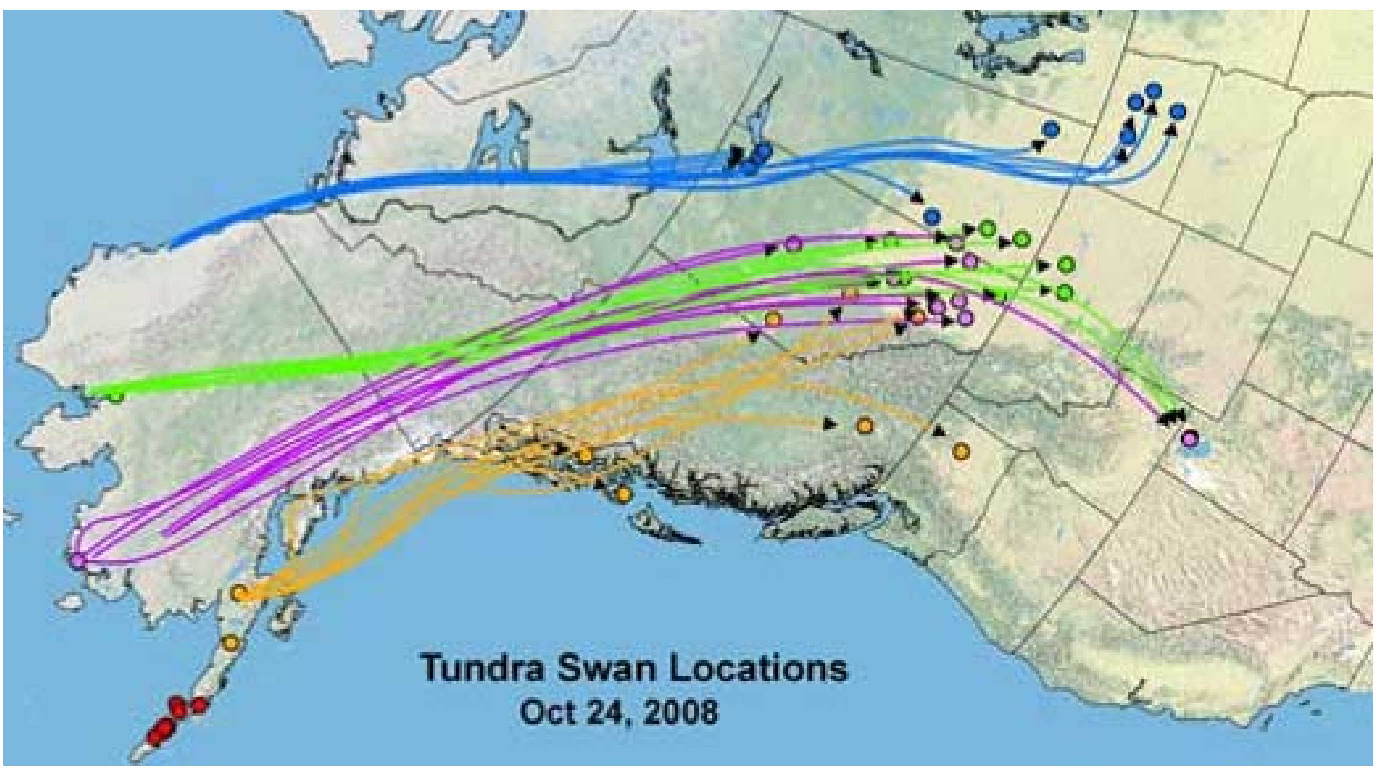

Figure 6. Movements of tundra swans satellite-tagged in Alaska in summer 2008, as of 24 October 2008. Swans marked in Northwest Alaska are denoted in green.

will provide new information on migratory pathways, timing of migration, and winter destinations through a multi-Flyway-wide resighting campaign, band returns, satellite locations, and isotope analyses. Forthcoming movement data and genetic analyses should help elucidate the breeding and winter site fidelity of Northwest Alaska breeders and molters and allow examination of genetic differentiation between breeding and molting aggregations in Alaska. Updated movement data for the tundra swans marked in Northwest Alaska (and at other locations) will be available on-line at http://alaska.usgs.gov/science/biology/avian_influenza/TUSW/index.html.

Avian Influenza

Preliminary screening has not detected H5N1 virus from oral-pharyngeal or cloacal samples collected in Northwest Alaska in 2008, but official H5N1 results will not be available until winter 2008 or later. Initial screening of swan sera detected historical exposure to avian influenza viruses in approximately 20-35% of the samples examined (J. Hall pers. comm.). Seropositive samples are currently being sent to the National Veterinary Services Laboratories (Ames, Iowa) for viral subtyping.

Continuing movement data from the satellite transmitters deployed in Northwest Alaska will help elucidate routes of possible HPAI H5N1 transmission on wintering grounds. Further, the information on migration routes and wintering areas for tundra swans breeding and molting in Northwest Alaska will help clarify population structure and possible transmission routes among breeding aggregations of tundra swans statewide.

Future sampling

Given continued evidence of H5N1 infection in swans worldwide (Harless 2006, Morales 2008), interest in their role as carriers of the disease (Kalthoff et al. 2008, Wilson and Hall 2008), and the large sample sizes and ancillary data achievable in Northwest Alaska (this report), I suggest continued (and possibly expanded) sampling in 2009.

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Northwest Alaska tundra swan field crew, 2008. Pictured left to right: Brad Scotton (Koyukuk NWR), Karen Bollinger (MBM), Bill Larned (MBM), Rachael Schively (Selawik NWR), Paul Anderson (in red, MBM), Juliana Milani (in white, U.C. Davis), Nate Olson (front, Selawik NWR), Heather Wilson (in orange, MBM), Rob MacDonald (in brown vest, MBM), Dennis Marks (in yellow pants, MBM), and Tina Moran (in yellow raincoat, Selawik NWR). Not pictured, veterinarian Cheryl Scott (U.C. Davis) and filmmaker Stephani Gordon (Montana State Univ.). (Photo courtesy of Karen Bollinger).

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