Journal of Applied Ecology

British Ecological Society

Journal of Applied Ecology 2010, **47**, 1147–1157 doi: 10.1111/j.1365-2664.2010.01845.x

Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: dispersal ranges and rates determined from large-scale satellite telemetry

Nicolas Gaidet[1]*, Julien Cappelle[1], John Y. Takekawa[2], Diann J. Prosser[3], Samuel A. Iverson[2], David C. Douglas[4], William M. Perry[2], Taej Mundkur[5,6] and Scott H. Newman[6]

[1]*CIRAD ES, UR Animal Gestion intégrée des risques, TA 30/E Campus international de Baillarguet, 34398, Montpellier, France;* [2]*USGS Western Ecological Research Center, Vallejo, CA, USA;* [3]*USGS Patuxent Wildlife Research Center, Beltsville, MD, USA;* [4]*USGS Alaska Science Center, Juneau, AK, USA;* [5]*Wetlands International, Ede, The Netherlands; and* [6]*FAO, Infectious Disease Group/EMPRES, Animal Health Service, Rome, Italy*

Summary

1. Migratory birds are major candidates for long-distance dispersal of zoonotic pathogens. In recent years, wildfowl have been suspected of contributing to the rapid geographic spread of the highly pathogenic avian influenza (HPAI) H5N1 virus. Experimental infection studies reveal that some wild ducks, geese and swans shed this virus asymptomatically and hence have the potential to spread it as they move.

2. We evaluate the dispersive potential of HPAI H5N1 viruses by wildfowl through an analysis of the movement range and movement rate of birds monitored by satellite telemetry in relation to the apparent asymptomatic infection duration (AID) measured in experimental studies. We analysed the first large-scale data set of wildfowl movements, including 228 birds from 19 species monitored by satellite telemetry in 2006–2009, over HPAI H5N1 affected regions of Asia, Europe and Africa.

3. Our results indicate that individual migratory wildfowl have the potential to disperse HPAI H5N1 over extensive distances, being able to perform movements of up to 2900 km within time-frames compatible with the duration of asymptomatic infection.

4. However, the likelihood of such virus dispersal over long distances by individual wildfowl is low: we estimate that for an individual migratory bird there are, on average, only 5–15 days per year when infection could result in the dispersal of HPAI H5N1 virus over 500 km.

5. Staging at stopover sites during migration is typically longer than the period of infection and viral shedding, preventing birds from dispersing a virus over several consecutive but interrupted long-distance movements. Intercontinental virus dispersion would therefore probably require relay transmission between a series of successively infected migratory birds.

6. *Synthesis and applications*. Our results provide a detailed quantitative assessment of the dispersive potential of HPAI H5N1 virus by selected migratory birds. Such dispersive potential rests on the assumption that free-living wildfowl will respond analogously to captive, experimentally-infected birds, and that asymptomatic infection will not alter their movement abilities. Our approach of combining experimental exposure data and telemetry information provides an analytical framework for quantifying the risk of spread of avian-borne diseases.

Key-words: avian influenza, disease ecology, dispersal, duck, H5N1, migration, pathogen, waterfowl, zoonosis

Introduction

Migratory birds engaged in repeated long-distance movements are major candidates for the dispersal of various zoonotic agents across national and intercontinental borders,

*Correspondence author. E-mail: nicolas.gaidet@cirad.fr

© 2010 The Authors. Journal compilation © 2010 British Ecological Society

including viral diseases (e.g. avian influenza and West Nile), fungal and bacterial diseases (e.g. salmonellosis), as well as infected arthropod vectors (e.g. tick-borne Lyme disease) (Reed *et al.* 2003). A better understanding of the dispersal potential of pathogens associated with wild bird movements, in particular during long-distance migration, is increasingly recognised as a major requirement for improving our ability to predict the risk of spread of avian-borne diseases (Nathan 2008).

Following the rapid spread of highly pathogenic avian influenza (HPAI) H5N1 virus over Eurasia and Africa in 2005–2006 and concurrent reports of mortality events in some migratory wildfowl, i.e. ducks, geese and swans (Anatidae) (Liu *et al.* 2005; Hesterberg *et al.* 2009), these birds have been suspected of contributing to the geographic spread of HPAI H5N1 virus. Today, whilst HPAI H5N1 outbreaks persist in these regions, the potential range and rate of long-distance dispersal of these viruses by wildfowl remains unknown. A direct investigation of virus dispersal by wildfowl is, however, challenged by the difficulty of detecting and monitoring the movements of naturally-infected free-living birds and by constraints on our ability to release experimentally-infected birds.

For a bird to be a long-distance vector of a viral disease, (i) it must be in contact with the virus, be receptive to infection and shed virus; (ii) infection should be asymptomatic, at least temporally, without hampering bird movements; (iii) it must be able to perform long-distance movements within a timeframe of asymptomatic infection; (iv) timing of asymptomatic infection must coincide with the time when it performs a long-distance movement; and (v) it must transmit virus infection to other susceptible hosts through direct contact or a shared environment.

Wildfowl are the primary reservoir of low pathogenic avian influenza (LPAI) viruses together with shorebirds (Olsen *et al.* 2006). These birds are generally infected asymptomatically, demonstrate no clinical signs or pathological lesions, and shed high-concentration of viruses in their faeces (Webster *et al.* 1978). World-wide surveillance studies have consistently revealed the occurrence of LPAI viruses in wildfowl, from boreal (Koehler *et al.* 2008) to tropical latitudes (Gaidet *et al.* 2007). Phylogenetic relationships and gene reassortment found between avian influenza viruses (AIVs) isolated from wildfowl world-wide indicate that inter-continental exchange of viruses via migratory birds does occur (Dugan *et al.* 2008; Koehler *et al.* 2008).

Prior to 2002, HPAI viruses responsible for severe mortality in domestic birds (i.e. gallinaceous poultry and ostriches) were generally not detected in wild birds (Olsen *et al.* 2006). The HPAI H5N1 virus that re-emerged in domestic birds in 2002 showed the capacity to infect a large diversity of wild birds, including wildfowl. Since 2002, HPAI H5N1 viruses have been reported in more than 120 species of wild birds (USGS 2008), usually found dead or diseased (Liu *et al.* 2005; Hesterberg *et al.* 2009). In a few cases however, HPAI H5N1-infection has been found in healthy free-living wildfowl, with no apparent clinical signs (Chen *et al.* 2006; Saad *et al.* 2007; FAO 2008; Hesterberg *et al.* 2009; OIE 2009), indicating that some healthy carriers may exist in the wild.

An increasing number of recent experimental infection studies have revealed that some wild species of ducks, geese and swans can replicate and shed HPAI H5N1 virus asymptomatically for several days without exhibiting any apparent clinical signs or prior to the onset of illness (Brown *et al.* 2006, 2007; Brown, Stallknecht & Swayne 2008; Kalthoff *et al.* 2008; Keawcharoen *et al.* 2008; Kwon, Thomas & Swayne 2010). Although there is heterogeneity amongst species in clinical susceptibility, these findings consistently suggest that some wildfowl could spread HPAI H5N1 virus during a period of asymptomatic infection (Brown, Stallknecht & Swayne 2008; Kalthoff *et al.* 2008; Keawcharoen *et al.* 2008; Kwon *et al.* 2010).

In this study, we evaluated the dispersive potential of HPAI H5N1 viruses by wildfowl by an indirect approach, through an analysis of the movement ranges and rates of satellite-tracked birds in relation to the apparent asymptomatic infection duration (AID) measured in experimental studies. Satellite-based telemetry is increasingly used to monitor free-ranging animal movements over extensive and remote regions, but considerable advances in miniaturisation of satellite-tracking devices have only recently allowed medium-size birds such as small wildfowl to be equipped. Satellite telemetry offers various advantages over conventional methods in estimating long-distance dispersal (Nathan *et al.* 2003). It provides a direct measurement of individual movements over relatively long periods and at an intercontinental scale, including over the most remote areas of the world. In addition, location data obtained every 2–4 days allows for analysis of individual movements with a high temporal resolution compatible with the duration of viral infection.

Most migratory wildfowl species are known to make long-distance movements with a relatively high flight speed (50–80 km h^{-1}) (Clausen *et al.* 2002). From a disease transmission and spread standpoint, three types of relevant movements may be distinguished: (i) cyclical and predictable migration movements between non-breeding and breeding grounds; (ii) irregular climate-influenced movements performed in response to cold weather, drying of wetlands or temporarily available habitat; and (iii) daily movements amongst feeding, breeding or roosting sites. Range, frequency and orientation of these different types of movements vary amongst individuals, populations and species, seasons, latitude and ecological contexts, resulting in a gradient of mobility behaviour ranging from sedentary birds to nomadic and long-distance migrants.

We report here the analysis of the first large-scale data set of wildfowl movements monitored by satellite telemetry in the framework of an international programme coordinated by the Food and Agricultural Organisation of the United Nations. Birds from 19 wildfowl species were monitored during 2006–2009 over the main regions reporting occurrence of HPAI H5N1 viruses, i.e. South-East, East and Central Asia, Middle-East, East Europe and West Africa (Fig. 1). Several of the species monitored are some of the main candidates identified as potential long-distance vectors of HPAI H5N1 virus (e.g.

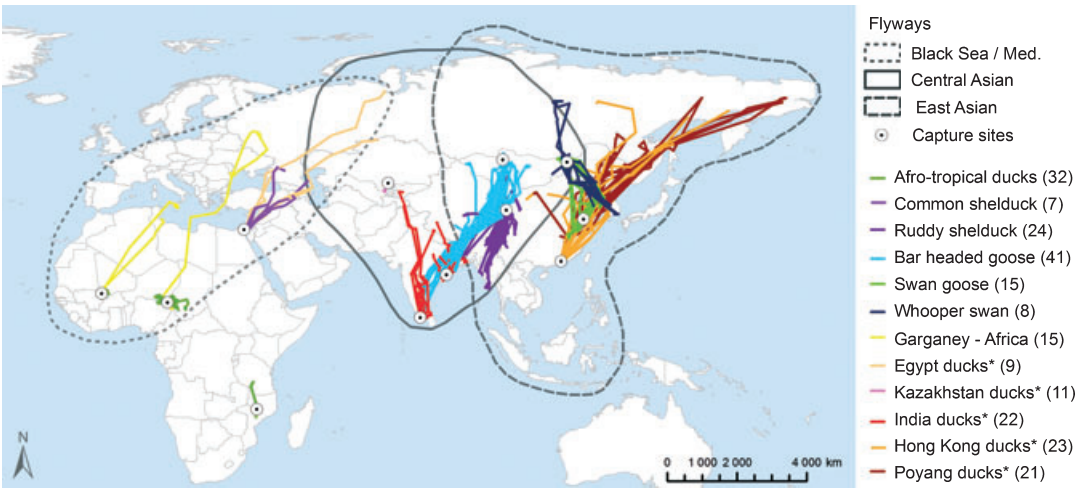

Fig. 1. Movements paths of wildfowl species (no. of birds) monitored by satellite telemetry during 2006–2009 over three main wildfowl migratory flyways (Black Sea-Mediterranean, Central Asian and East Asian flyways) and the inter-tropical African region. For a detailed list of birds monitored see Table S1 (Supporting information). *Afro-tropical ducks: spur-winged goose, comb duck, white-faced and fulvous whistling ducks; Egypt ducks: common teal, Northern pintail and Northern shoveler; Kazakhstan ducks: common teal, gadwall, mallard, Northern shoveler; India ducks: common teal, Eurasian wigeon, gadwall, garganey, Northern pintail and Northern shoveler; Hong Kong ducks: Eurasian wigeon and Northern pintail; Poyang Ducks: Baikal teal, Chinese spotbill duck, common teal, Eurasian wigeon, falcated teal, garganey, mallard and Northern pintail. (Map by M. Gély © CIRAD.)

mallard *Anas platyrhynchos*, bar-headed goose *Anser indicus*, whooper swan *Cygnus cygnus*; Brown, Stallknecht & Swayne 2008; Keawcharoen *et al.* 2008). Species monitored are also amongst the most abundant wildfowl species across Eurasia and/or Africa, representing 9 of the 16 species estimated to each have populations in excess of one million birds (Wetlands International 2006).

Our analysis included two primary components. We first compiled results of all available studies of wild species of ducks, geese and swans experimentally infected with HPAI H5N1 virus and determined the average and range values of the AID in wildfowl. Secondly, we estimated the maximum distance and rate of potential virus dispersal for each wildfowl species monitored by satellite telemetry. Through an iterative process over the entire monitoring period of each bird, we analysed the magnitude and frequency of individual movements during timeframes when birds could spread the virus, considering the minimum to maximum values of the AID.

Materials and methods

REVIEW OF EXPERIMENTAL INFECTION STUDIES OF WILDFOWL WITH HPAI H5N1 VIRUS

We restricted our review to studies of wildfowl experimentally infected with virus strains of the Qinghai lineage (clade 2·2) (WHO/OIE/FAO 2008), i.e. the lineage of all HPAI H5N1 viruses isolated in wildfowl since 2005 (Chen *et al.* 2006; Salzberg *et al.* 2007; with few exceptions, Uchida *et al.* 2008). Duration of shedding was computed from virus isolation data, considering the absolute duration of virus excretion detected in either the cloaca or the oro-pharynx after inoculation. We considered results from each species × strain inoculation trial as replicates of distinct potential infection events. We pooled results from birds across different treatment groups (e.g. inoculated/contact birds, naïve/pre-exposed birds, various concentrations of infectious doses) to represent individual variability within a population exposed to a natural infection.

SATELLITE TELEMETRY PROCEDURES

We captured wildfowl during 2006–2009 in eight African, Asian and European countries (Fig. 1, see Table S1, Supporting information) in collaboration with a number of national and international teams (Appendix S1, Supporting Information), using baited funnel traps, mist nets, cannon-nets, Indian and Nigerian traditional leg nooses, or by driving flightless moulting birds into corrals. Birds were equipped with solar-powered Platform Terminal Transmitters (PTTs; Microwave Telemetry Inc., Columbia, MD, USA) attached dorsally with a Teflon harness-attachment. PTTs of various size (9·5–70 g) were used according to species body mass (Table S1, Supporting information), each programmed to transmit every 24 or 48 h. Battery-powered coelomic cavity implants (26 g) were also used for a few ducks (4 h/76 h duty cycle, $n = 18$). The transmitters represented ≤4% of the body mass of each bird. We evaluated the potential detrimental effect of harness-attached transmitters on captive birds (at Montpellier Zoo, France) equipped with exact replicas of 12, 18 and 30 g PTTs. Monitoring of three garganeys *Anas querquedula*, three fulvous whistling ducks *Dendrocygna bicolor* and two comb ducks *Sarkidiornis melanotos* every two days during 7 weeks revealed no body mass reduction, feather damage or skin irritation.

Movements were monitored until June 2009 using the Argos satellite tracking system. We considered all locations recorded from time of release until June 2009 or until PTTs stopped transmitting. Only birds that had transmitted after the first-30 days post-release were considered in our analysis, in order to discard potentially aberrant movements of birds that may have been affected by the capture, handling or harness. Some birds ($n = 4$) remained within their wintering areas beyond the departure dates generally recorded for these species. They were excluded from the analysis after a time limit corresponding

to the departure date of the last successfully tracked bird from this species.

We selected locations according to spatial accuracy classes estimated by Argos CLS, based on the number of transmissions received from the PTT during a satellite overpass. We used only locations from classes 3–0 (based on ≥4 satellite transmissions) with an estimated accuracy of < 150, 150–350, 350–1000 and > 1000 m, respectively (Argos 1996). We removed all aberrant locations typical in satellite telemetry studies (erroneous Argos calculated locations) using both quantitative and qualitative criteria based on implausible flight speed, angle and rate to previous and subsequent locations as well as unrealistic habitat. On rare occasions (< 1% of locations analysed), we used validated locations of unspecified accuracy (A and B classes, based on three and two transmissions, respectively) to complete the description of the flight path for some long-distance movements (only when they indicated meaningful flight speeds and angles).

ANALYSIS OF SATELLITE-TRACKED BIRD MOVEMENTS

We distinguished two groups of birds (see Table S1, Supporting information) according to scale, timing and orientation of movements: (i) long-distance migratory birds undertaking extensive, seasonal and latitudinal-orientated movements; (ii) sedentary or nomadic birds performing only local to regional year-round movements, generally irregular and multidirectional, within the main lake or river basin in which they had been caught. In long-distance migratory birds, we distinguished four stages in their annual cycle: non-breeding, spring migration (i.e. northward boreal spring migration), breeding–post breeding and autumn migration (i.e. southward). We defined the onset of spring and autumn migration for each bird as the first set of sequential locations indicating a persistent latitudinal-orientated long-distance (> 100 km) movement. Spring and autumn migrations lasted until the last of a series of successive long-distance movements, interrupted by staging periods. The end of migration was defined as the first date of a series of nearby locations, generally < 50 km apart, situated within the species' breeding and wintering ranges respectively, in accordance with available ornithological information (Scott & Rose 1996; Miyabayashi & Mundkur 1999). Long-distance movements apparently associated with moult migration were attributed to either post breeding, autumn migration or non-breeding stages according to the period and sites where they took place.

EVALUATION OF POTENTIAL DISPERSAL DISTANCES AND RATES

We assumed each location recorded during the monitoring period of satellite-tracked birds as a site and time of potential infection. We evaluated the potential dispersal distance of HPAI H5N1 viruses by wildfowl as the maximum distance (D_{max}) covered by a satellite-tracked bird during a timeframe corresponding to the AID. For each bird, we first calculated the D_{max} between all locations recorded on a defined day of potential infection and every location recorded in successive days of the AID period. We calculated this D_{max} for each day for which at least one valid location was available, through an iterative process using a sliding timeframe over the entire bird monitoring period. We then calculated the maximum dispersal distance from the maximum value of all D_{max} calculated over each stage of the annual cycle. We repeated this analysis for each AID-considered. Distances between locations were calculated with the Great Circle distance (WGS84 ellipsoid) method, using R (http://www.R-project.org).

We estimated the individual rate of potential long-distance dispersal as the proportion of days of potential infection associated with a maximum dispersal distance ≥100 and 500 km within a mean AID of 4 days. We first calculated the percentage of days for which we calculated a D_{max} with a value > 100 and 500 km over each stage of the annual cycle. We then estimated the number of days associated with a D_{max} > 100 and 500 km over the entire period of each stage, considering the distribution of long-distance movement to be homogenous over each stage. We estimated the annual rate from the sum of days with D_{max} > 100 and 500 km calculated for each stage of the annual cycle.

Satellite transmissions were interrupted in some birds during some periods that exceeded the AID timeframes. To avoid under-estimating maximum dispersal distance, in each season we excluded birds for which 90% of the maximum dispersal distance that could have been performed during an interrupted transmission period (i.e. the distance between the last and first consecutive locations recorded) exceeded the maximum dispersal distance performed in a mean AID-timeframe throughout the remainder of their migration. This resulted in excluding birds from each group including the spring (n = 21, 22%), breeding (n = 20, 17%), autumn (n = 28, 30%), and wintering (n = 14, 15%) stages, as well as sedentary (n = 3, 2%) and nomadic (n = 4, 11%) birds.

Results

ASYMPTOMATIC INFECTION DURATION

We compiled results of 135 inoculated birds from 23 distinct inoculation trials, representing 18 wildfowl species and four clade 2·2 virus strains (Table 1). The response to infection differed between species in terms of clinical susceptibility (morbidity and mortality rates varying from 0 to 100%) and in duration of viral shedding (1–8 days). However, 90% (122/135) of all inoculated and contact birds were successfully infected and generally began excreting virus one day post inoculation.

All infected birds showed a period of asymptomatic but productive infection (with the exception of some black swans *Cygnus atratus*): birds excreted virus either without any clinical signs or before the onset of illness (i.e. pre-clinical shedding) from which some recovered and others died. We calculated the AID for each species × strain trial from the time between inoculation and either (i) the end of viral shedding in asymptomatic birds or (ii) the onset of detectable clinical signs in symptomatic birds (Table 1). We estimated minimum, mean and maximum AID values across all species of 1, 4 and 8 days, respectively.

MAXIMUM POTENTIAL DISPERSAL DISTANCE

We analysed the movement patterns of 228 satellite-tracked birds from 19 species. Birds were monitored for an average period of 188 days (26–838 days, Table S1, Supporting information), over three main wildfowl migratory flyways (Fig. 1).

Our analysis reveals that migratory wildfowl have the potential for dispersal of HPAI H5N1 virus over extensive distances: during spring and autumn migrations, satellite-tracked birds covered up to ~300 km to 1700 km in a 4-day timeframe on average depending on the species (Table 2), with individual maximum values of up ~2500 and 2900 km (Table S2,

Table 1. Review of asymptomatic infection duration (AID) of wildfowl experimentally infected with HPAI H5N1 viruses

						Viral excretion			Clinical response**				AID††	
Species*	No.	Age (months)	Treatment group†	Strains‡	Dose§ $\log_{10}$	Infect %‡‡	Onset dpi	Duration days	Onset dpi	Sick/ Total	Dead/ Total	MDT dpi	days	R
Asymptomatic														
North. pintail	3	2·5–4	In	WS/Mg/05	6_a	100	1	1–2		0/3	0/3		2·5 (2–3)	1
Comm. teal	3	2·5–4	In	WS/Mg/05	6_a	100	1	2		0/3	0/3		3 (3–3)	1
	8	8–11	In	Tk/Tk/05	4_b	38	1	1–5		0/8	0/8		3·5 (2–6)	5
Eur. wigeon	8	8–11	In	Tk/Tk/05	4_b	50	1	1–2		0/8	0/8		2·5 (2–3)	5
Mallard	8	8–11	In	Tk/Tk/05	4_b	100	1	1–4		0/8	0/8		4 (2–5)	5
	3	3	In/Ct	Ck/Kr/06	6_a	100	1	2–3		0/3	0/3		3·5 (3–4)	6
Gadwall	8	8–11	In	Tk/Tk/05	4_b	88	1	1–6		0/8	0/8		4 (2–7)	5
Redhead	3	2·5–4	In	WS/Mg/05	6_a	100	1	1–4		0/3	0/3		3·5 (2–5)	1
Symptomatic														
Wood duck	3	2·5–4	In	WS/Mg/05	6_a	100	1	4–6	5	2/3	2/3	7·5	5·5 (5–6)	1
	20	3–4	In	WS/Mg/05	$1·5–6_a$	95	2	2–4	NA	18/20	18/20	5·5	5 (4–6)	2
Mandarin duck	3	2	In/Ct	Ck/Kr/06	6_a	100	1	5–6	4	1/3	1/3	5	6 (4–7)	6
Eur. pochard	7	8–11	In	Tk/Tk/05	4_b	100	1	2–5	3–6	4/7	1/7	4	3·5 (3–6)	5
Tufted duck	7	8–11	In	Tk/Tk/05	4_b	86	1	2–4	3·5	7/7	3/7	4	3·5 (1–4)	5
Ruddy sheld.	3	3	In/Ct	Ck/Kr/06	6_a	100	4	6	5	3/3	3/3	7	5 (5–5)	6
Bar-hd. goose	5	3	In/Ct	WS/Mg/05	6_a	100	1–2	5–8	3–7	5/5	2/5	6·5	4·5 (3–7)	3
Cack. goose	4	3	In/Ct	WS/Mg/05	6_a	100	1–3	4–6	3–7	4/4	3/4	6	5 (3–7)	3
Greylag goose	3	1·75	In/Ct	Ck/Kr/06	6_a	67	1	5–6	5–6	3/3	0/3		5·5 (5–6)	6
Black swan	5	1–1·5	In/Ct	WS/Mg/05	6_a	100	1	2–3	1–2	5/5	5/5	2·5	1·5 (1–2)	3
Trump. swan	5	1–1·5	In/Ct	WS/Mg/05	6_a	100	1	4–6	2	5/5	5/5	4·5	2 (2–2)	3
Whooper swan	4	1–1·5	In/Ct	WS/Mg/05	6_a	100	1	4–6	2–4	4/4	4/4	4	3 (2–4)	3
Mute swan	5	1–1·5	In/Ct	WS/Mg/05	6_a	100	1	3–7	5–7	5/5	5/5	6·5	6 (5–7)	3
	14	12–48	In/Ct/Px	WS/Gm/06	$4–6_a$	100	1–3	6	4–8	12/14	11/14	9	5·5 (3–8)	4
	3	1·75	In/Ct	Ck/Kr/06	6_a	100	1	3–5	3–4	3/3	3/3	4·5	3·5 (3–4)	6
Total 18 sp.	135	1–48	3	4	1·5–6	90	1–4	1–8	1–8	0–100	0–100	2·5–9	4 (1–8)	

*Northern pintail *Anas acuta*, common teal *Anas crecca*, Eurasian wigeon *Anas penelope*, mallard *Anas platyrynchos*, gadwall *Anas strepera*, redhead *Aythya americana*, wood duck *Aix sponsa*, mandarin ducks *Aix galericulata*, Eurasian pochard *Aythya ferina*, tufted duck *Aythya fuligula*, ruddy shelduck *Tadorna ferruginea*, bar-headed goose *Anser indicus*, cackling goose *Branta hutchinsii*, greylag geese *Anser anser*, black swan *Cygnus atratus*, trumpeter swan *Cygnus buccinator*, whooper swan *Cygnus cygnus*, mute swan *Cygnus olor*.

†Treatment group: Inoculated (In) and Contact (Ct) immunologically naïve birds, or birds with Pre-exposure AIV- antibodies (Px)

‡ A/Whooper swan/ Mongolia/244/2005 (WS/ Mg/05), A/turkey/Turkey/1/2005 (Tk/Tk/05), A/Cygnus cygnus/Germany/R65/2006 (WS/Gm/06), A/chicken/South Korea/IS/06 (Ck/Kr/06)

§Titre of viral infectious dose: a) median Embryo Infectious Dose (EID_{50}) or b) Tissue Culture Infectious Dose ($TCID_{50}$)

¶Viral shedding measured by virus isolation (maximum values between pharyngeal/cloacal excretions)

**Susceptibility to infection characterised by the onset of detectable clinical signs, morbidity (No. birds with clinical signs/total) and mortality (No. dead birds/total) rates, and Mean Death Time (MDT)

††Mean (min.-max.) Asymptomatic Infection Duration (AID) calculated as the number of days from inoculation to (i) the end of viral shedding in asymptomatic birds or (ii) the onset of detectable clinical signs in symptomatic birds

References (R): Brown *et al.* 2006 (1), Brown *et al.* 2007 (2), Brown, Stallknecht & Swayne 2008 (3), Kalthoff *et al.* 2008 (4), Keawcharoen *et al.* 2008 (5), Kwon *et al.* 2010 (6). dpi, day post inoculation; NA, non available.

‡‡Rate of productive infection (% birds that excreted virus).

© 2010 The Authors. Journal compilation © 2010 British Ecological Society,

Table 2. Estimated mean (+/− SE) maximum potential dispersal distance – $D_{max}4$ (km) of HPAI H5N1 viruses for each species of satellite-tracked birds (*n*) for a mean AID (4-day timeframe)

Species	Non-breeding		Spring migration		Breeding–Post breeding		Autumn migration			Annual cycle	
LDM*	$D_{max}4$	*n*	$D_{max}4$	*n*	$D_{max}4$	*n*	$D_{max}4$	*n*	SN†	$D_{max}4$	*n*
North. pintail	109 (123)	26	1444 (465)	16	302 (243)	4	NA		Mallard‡	66 (24)	5
North. shoveler	111 (111)	12	919 (363)	5	88 (58)	3	322	1	Spotbill duck	83 (42)	8
Comm. teal	98 (92)	8	886 (369)	4	357 (373)	5	323 (124)	3	Gadwall	67 (56)	2
Falcated teal	8	1	928 (25)	3	176 (106)	3	969	1	F Wh. duck	135	1
Baikal teal	NA		1303	1	NA		NA		Wf Wh. duck	137 (151)	9
Eur. wigeon	45 (50)	7	1673 (842)	6	90 (71)	3	1461	1	Comb duck	196 (151)	12
Mallard‡	26	1	NA		55 (26)	2	331	1	Spwg. goose	47 (14)	10
Garganey	123 (119)	22	1269 (772)	11	286 (293)	2	1002	1			
Ruddy sheld.	101 (52)	19	1106 (454)	14	203 (249)	20	1263 (380)	16			
Comm. sheld.	107 (53)	7	767 (178)	4	65 (79)	3	NA				
Swan goose	105 (41)	5	1007 (236)	4	79 (45)	15	753 (332)	6			
Bar-hd. goose	94 (103)	25	633 (245)	11	160 (167)	33	625 (226)	33			
Whooper swan	30	1	723	1	122 (66)	8	1008	1			

D_{max} was estimated as the maximum distance covered by a bird during every 4-day timeframes to evaluate the potential dispersal range of H5N1 HPAI virus by wildfowl. D_{max} was estimated for four distinct stages in long-distance migratory birds (LDM) and throughout the annual cycle in sedentary or nomadic birds (SN).

*LDM: Northern pintail *A. acuta*, Northern shoveler *A. clypeata*, common teal *Anas crecca*, falcated teal *A. falcata*, Baikal teal *Anas formosa*, Eurasian wigeon *A. penelope*, mallard *A. platyrhynchos*, garganey *A. querquedula*, ruddy shelduck *Tadorna ferruginea*, common shelduck *T. tadorna*, swan goose *Anser cygnoides*, bar-headed goose *Anser indicus*, whooper swan *Cygnus cygnus*.

†SN: mallard *A. platyrhynchos*, spotbill duck *Anas poecilorhyncha*, gadwall *A. strepera*, fulvous whistling duck *Dendrocygna bicolor*, white-faced whistling duck *D. viduata.,* comb duck *Sarkidiornis melanotos*, spur-winged goose *Plectropterus gambensis*.

‡ Sympatric populations of sedentary and migratory mallards.

Mean values per species are presented here (for max. individual values see Table S2 (Supporting information). D_{max} estimates for min. and max. AID (1 and 8 days, respectively) are available in Table S3 (Supporting information). NA, no data available.

Supporting information). Potential virus dispersal appears to also be extensive when one considers the minimum AID value (1 day), reaching up to ~350–800 km on average in some species during the spring and autumn migration respectively (Table S3, Supporting information), with maximum distances up to ~1700 km for some individuals (Table S2, Supporting information). During the breeding–post breeding and non-breeding periods, potential virus dispersal was restricted on average to distances of < 350 km and < 100 km respectively, although dispersal of up to ~1000 km was occasionally recorded during the post-breeding period. Maximum potential virus dispersal by sedentary and nomadic birds was < 200 km, with occasional 4-day dispersals of up to ~400–550 km.

In most species, we observed only a small increase in the maximum dispersal distance for an AID-timeframe of 4 versus 8 days. We then re-ran our analyses to plot the maximum dispersal distance as a function of AID. We considered a maximum duration of 17 days, corresponding to the longest period of detectable virus shedding after inoculation with HPAI H5N1 virus measured in domestic ducks (Hulse-Post *et al.* 2005). Results reveal a threshold in the maximum dispersal distance: in most species, birds completed ≥75% of their maximum distance after only 1–4 days of a 17-day timeframe (Fig. 2; Fig. S1, Supporting information). This indicates that long-distance migratory flights are completed in a few rapid and direct long flights, interspersed with relatively long periods of staging. In addition, staging duration exceeds the duration of asymptomatic infection and shedding. This finding was consistent across all wildfowl species in all stages of their life cycle.

RATE OF POTENTIAL LONG-DISTANCE DISPERSAL

We estimated that for an individual bird, on average, in migratory species there are only 5–15 days per year when infection could result in a dispersal of HPAI H5N1 virus over 500 km (Table 3). This rate was consistent across species, with days of potential dispersal occurring almost exclusively during seasonal long-distance migration. Days of potential virus dispersal > 100 km occurred also during the non-breeding and post-breeding periods, and mean individual rates ranged from 26 to 64 days per year depending on the species (Table 3). On the other hand, when we considered the ensemble of migratory wildfowl we monitored, we found a potential for long-distance virus dispersal throughout the year. In both East and Central Asian flyways, during 10 months per year, from 20 to 80% of birds of all species performed at least one 4-day movement > 100 km per month (Fig. 3). The potential for virus dispersal of > 500 km was restricted to migration periods, i.e. mostly during March-June and September-November. However, as a consequence of asynchrony in departure and arrival time between species within the same flyway, we found that a significant proportion of birds (~20–50%) performed at least one 4-day movement > 500 km per month during six to seven

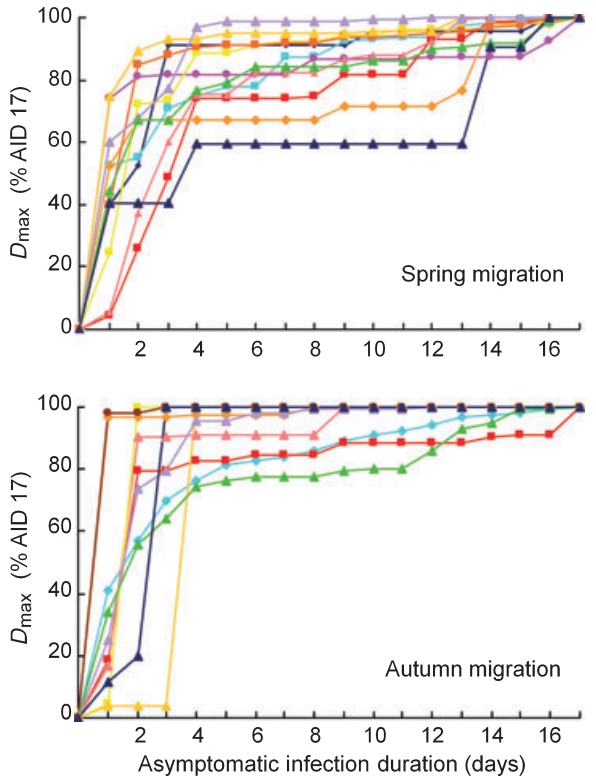

Fig. 2. Maximum potential dispersal distance of HPAI H5N1 virus (D_{max}) estimated for long-distance migratory birds during spring and autumn migration as a function of asymptomatic infection duration (AID). Mean D_{max} per species was calculated for every AID-time-frames of 1–17 days, and presented as a percentage of D_{max} for AID 17. For non-migration stages and for sedentary or nomadic birds see Fig. S1 (Supporting information). Northern pintail (orange, square), Northern shoveler (light orange, triangle), common teal (red, square), falcated teal (orange, diamond), Eurasian wigeon (pink, triangle), mallard (maroon, circle), garganey (yellow, square), ruddy shelduck (purple, triangle), common shelduck (pink, circle), swan goose (green, triangle), bar-headed goose (light blue, diamond) and whooper swan (dark blue, triangle).

months per year. Nomadic and sedentary birds rarely moved more than 100 km in a timeframe when they could disperse viruses (0–16 days per year, Table S4, Supporting information), and the potential for virus dispersal of > 500 km was observed in only a single bird.

Discussion

Combining satellite telemetry and experimental infection data reveals that migratory wildfowl have the potential for dispersal of HPAI H5N1 viruses over long distances. Experimental infection studies indicate that all wildfowl species studied are receptive to HPAI H5N1 infection and show a period of asymptomatic infection and viral shedding. Birds from all of the migratory species monitored by telemetry were able to perform movements of several hundred to a few thousand kilometres within the mean period of asymptomatic infection. Potential virus dispersal could also be extensive (up to 1700 km) when considering a minimum asymptomatic period of one day. In addition, we found that the potential for long-distance dispersal by wildfowl exists for a large part of the year as a result of migration asynchrony between individuals and species.

Virus dispersal requires asymptomatic infection to coincide with the onset of a long-distance movement. Our results indicate that the probability of an individual performing a long-distance movement at the time of asymptomatic infection is low. We estimated that in migratory birds there are, on average, only 5–15 days per year during which infection could result in a dispersal of HPAI H5N1 virus over 500 km. In addition, overall migration, which commonly reached 4000–6000 km (Fig. 1; Table S1, Supporting information), followed a sequential rather than a continuous process. Migration is performed in a series of a few rapid long flights, generally undertaken in 1–4 days, interrupted by staging periods longer than the period of infection and viral shedding. This would prevent a bird from dispersing a virus over several consecutive but interrupted long-distance movements, and would limit the potential for virus dispersal to single movements of ≤2000 km (occasionally up to ~3000 km).

Intercontinental virus dispersal by wildfowl would therefore require a relay transmission amongst a series of birds successively infected. More data are needed to estimate the probability of HPAI H5N1 transmission amongst birds sharing the same site, but the large abundance and species diversity of wildfowl congregating at stopover sites along a migratory flyway, as well as the asynchronous timing of their arrival and departure, may facilitate such relay transmission. However, it would require a large number of birds to be infected at a congregation site to compensate for the low individual rate of long-distance movement. These requirements are fulfilled for LPAI viruses that circulate at high prevalence in migratory ducks during the autumn (Olsen *et al.* 2006). Accordingly, such frequent transfer of LPAI viruses between continents connected by migratory flyways has been evidenced by phylogenetic analysis (Dugan *et al.* 2008; Koehler *et al.* 2008).

The timing, routes and ranges of migration vary according to species, latitude and populations (Scott & Rose 1996; Miyabayashi & Mundkur 1999), but also between individuals according to age and sex (Clausen *et al.* 2002). The number of birds we monitored per species is small relative to population size because of high-costs associated with satellite telemetry and limitations in transmitter performances. However, we estimated dispersal for 19 species across three continents from tropical to boreal latitudes and, although only a fraction of global species diversity (~12%), they represent a wide range of body masses (300 g to 8 kg), sub-families (4 out of 5) and geographical ranges. Although greater movement distances and rates may have been over-looked, we consider our samples of species and regions to provide a valuable evaluation of movement rates in wildfowl.

Although we aimed to minimise any effect of capture, handling and transmitter attachment, transmitters may have affected flight performances of at least some individuals (Roshier & Asmus 2009). Birds for which the signal was lost during the first month or which failed to migrate were accordingly

Table 3. Number of days per year and per season of individual potential for dispersal of HPAI H5N1 virus over a distance > 100 and > 500 km estimated for each species of long-distance migratory (LDM) birds for a mean AID (4-day timeframe)

Species	Annual cycle			Non-breeding			Spring migration			Breeding–Post breeding			Autumn migration		
LDM	*N*	> 100	> 500	*n*	> 100	> 500	*n*	> 100	> 500	*n*	> 100	> 500	*n*	> 100	> 500
North. pintail		NA	NA		NA	NA	16	26	12		NA	NA		NA	NA
North. shoveler	13	56	8	12	4	0	5	24	8	3	22	0	1	6	0
Comm. teal	9	64	7	8	11	0	4	15	4	5	26	2	3	12	0
Falcated teal	4	33	9	1	0	0	3	13	2	3	9	0	1	10	7
Eur. wigeon	9	32	13	7	2	0	6	16	10	3	4	0	1	10	3
Garganey	22	35	12	22	5	0	11	16	10	2	12	0	1	3	3
Ruddy sheld.	24	26	9	19	6	0	14	8	5	20	7	1	16	4	4
Comm. sheld.		NA	NA		NA	NA	5	9	5		NA	NA		NA	NA
Swan goose	15	54	15	5	4	0	4	26	9	15	3	0	6	21	6
Bar-hd. goose	41	38	8	25	3	0	11	12	4	33	3	0	33	20	4
Whooper swan	8	40	5	1	0	0	1	13	3	8	14	0	1	13	2

Results correspond to the number of days per season/year when a bird if it gets infected could disperse virus over a distance > 100 and 500 km, i.e. the number of days that have been followed by such long-distance movements during the 4 following days. NA, no data available. For sedentary or nomadic birds see Table S4 (Supporting information).

excluded from the analysis. Departure and arrival dates of successfully-tracked birds, as well as the migration routes and the locations used during wintering and breeding periods, are consistent with field observations on population migration timing and location of key sites recognised for these species (Scott & Rose 1996; Miyabayashi & Mundkur 1999). The flight distance and speed of successfully-tracked birds are also similar to the few instances of rapid long-distance movements evaluated from direct band recoveries or resightings (Clausen *et al.* 2002; Kleijn *et al.* 2010). Though a detrimental effect of transmitter on long-distance flights cannot be excluded, we consider that the birds that were successfully tracked characterised migration patterns with sufficient accuracy to meet our objectives.

There is also a potential difference in host response to HPAI H5N1 infection according to species, bird age and virus strain (Hulse-Post *et al.* 2005; Brown *et al.* 2006). A majority of species which we monitored through satellite telemetry have been found to be receptive to natural (Liu *et al.* 2005; Chen *et al.* 2006; Saad *et al.* 2007; FAO 2008; Uchida *et al.* 2008; Hesterberg *et al.* 2009; OIE 2009) and/or experimental infections (Table 1). Our review of experimental infection studies also indicates that all infected birds have shown a period of asymptomatic viral infection and shedding, regardless of species, age or virus strain (except in some black swans). In addition, the AID was consistent amongst species and strains, ranging on average between 3 and 5 days in 65% of inoculation trials.

Captive versus free-living wildfowl

Our estimates of virus dispersive potential by wildfowl rest on the assumption that free-living birds will respond analogously to captive experimentally infected birds kept under controlled experimental conditions. Environmental constraints, such as adverse climatic conditions, episodic high concentration of birds, resource limitation, predation or hunting pressures, as well as concurrent physiological stress or infections with other pathogens, may increase the impact of HPAI H5N1 virus infection in free-living birds. In a few cases, free-living wildfowl have been found naturally infected with HPAI H5N1 virus without apparent clinical signs during large-scale surveillance studies (Chen *et al.* 2006; Saad *et al.* 2007; FAO 2008; Hesterberg *et al.* 2009; OIE 2009), indicating that asymptomatic HPAI H5N1 infection, though scarce, does occur in nature.

On the other hand, prior natural exposure to LPAI viruses (in particular those of H5Nx or HxN1 subtypes) may result in partial acquired immunity and could modulate the outcome of an HPAI H5N1 infection. Wildfowl with naturally (Kalthoff *et al.* 2008) or experimentally (Pasick *et al.* 2007; Fereidouni *et al.* 2009) acquired LPAI-specific antibodies showed no or reduced clinical signs and a lower, delayed and shorter period of viral shedding compared to immunologically naïve birds. This suggests that pre-existing immunity may increase the proportion of subclinical infections in wildfowl populations, but would not increase the timeframe in which birds are capable of dispersing viruses.

Dispersal of HPAI H5N1 virus over long-distance also assumes that asymptomatically infected wildfowl, i.e. with no apparent clinical signs, will attempt to migrate. Activation of the immune system in response to infection comes with costs in terms of energy and time that may compete with other physiologically demanding activities such as long-distance flights (Buehler & Piersma 2008). Several studies indicate that the physiological stress, in terms of metabolic rate (Kvist *et al.* 2001), flight-induced muscle damage (Guglielmo, Piersma & Williams 2001) and energy cost (Butler, Woakes & Bishop 1998) resulting from long-distance migration may be lower than previously assumed, and that long flights may not reduce concurrent immune response (Hasselquist *et al.* 2007), at least for birds in good physiological condition, though interpreta-

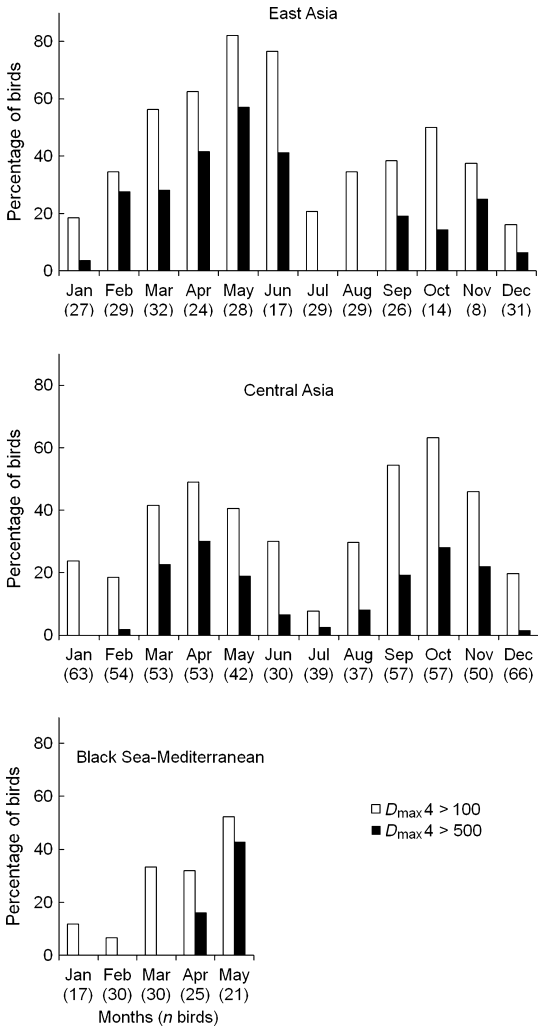

Fig. 3. Monthly variation in the percentage of long-distance migratory birds (n) monitored by satellite telemetry that showed a potential for HPAI H5N1 virus dispersal > 100 and 500 km within each flyway, i.e. that performed at least one movement > 100 and 500 km per month during a 4-day timeframe of asymptomatic infection.

tion of immune response measurement in wildfowl remains complex (Matson *et al.* 2006). These results suggest that migratory birds are physiologically well-adapted to demanding long flights, without compromising the capacity of their immune function. This may result from an adaptive response of migrants to exposure to a wide diversity of pathogens in various environments throughout their annual cycle (Møller & Erritzøe 1998).

Few studies provide direct evidence of the effect of natural AIV infection on migration performance in free-living wildfowl. In two multi-year studies of banded migratory mallards *Anas platyrhynchos* (Latorre-Margalef *et al.* 2009) and greater white-fronted goose *Anser albifrons* (Kleijn *et al.* 2010), dispersal distance of LPAI-infected birds (estimated from banding recoveries or resightings) did not differ from that of uninfected birds, including during the first days after testing (Kleijn *et al.* 2010). Infected mallards had slightly lower body mass (< 2% of the body mass) than uninfected ducks, whilst infected and uninfected geese did not differ in body mass in three of four years (body mass of infected geese < uninfected geese in the fourth year). In two telemetry studies, some marked birds were found naturally infected with AIVs at the time of capture, without clinical signs: two LPAI infected-Bewick's swans *Cygnus columbianus bewickii* showed reduced subsequent migratory performances compared to uninfected swans (van Gils *et al.* 2007); conversely, one white-faced whistling duck *Dendrocygna viduata* performed long-distance movements after it had tested positive for HPAI H5N2 virus (Gaidet *et al.* 2008). However, in both cases, during the first days after release, infected birds performed similar movements to concurrently monitored uninfected birds. These were only short-range movements, precluding our ability to examine the actual impact of infection on long-distance movements during the period of asymptomatic infection. Similarly, no clinical signs were observed during the initial days following inoculation in experimentally infected birds (Table 1). Results from our study, where birds were capable of achieving their maximal dispersal distances in a timeframe of 1–4 days, suggests that wildfowl may disperse the virus over great distances before the effects of infection, if any, would hamper their migration. The delayed effect of infection may impose a longer staging period at a stopover, supporting our conclusion that this virus is unlikely to be dispersed by a single individual over successive but interrupted long-distance movements.

Effective dispersal

Finally, for virus dispersal to be effective, it must be shed at a sufficiently high concentration, in a location with appropriate environmental conditions for virus survival, and in a location with suitable density and species assemblages for a successful transmission to another host. Asymptomatically infected birds generally excreted virus at lower concentrations than symptomatic birds (Brown *et al.* 2006; Kalthoff *et al.* 2008; Keawcharoen *et al.* 2008), although exceptions exist (e.g. mallard, Keawcharoen *et al.* 2008). Several studies have, however, shown that even low concentrations of inoculated virus can produce productive infections in captive wildfowl which subsequently contaminated contact birds (Brown *et al.* 2007; Kalthoff *et al.* 2008). In addition, inoculated swans and geese (Brown, Stallknecht & Swayne 2008), as well as mallards (Kwon *et al.* 2010), shedding virus asymptomatically for several days successfully transmitted virus to contact birds before showing (or not) clinical signs of disease. These results suggest that asymptomatically infected birds can disseminate the virus. Outside the breeding season, wildfowl are generally gregarious, particularly at stopover sites during migration where birds from various species, geographic origins and destinations aggregate in large numbers, offering suitable locations for transmission and dispersion over extensive regions.

In recent decades, the emergence and spread of zoonotic pathogens with a wildlife origin, including wild birds (e.g. West Nile, HPAI H5N1), have caused a major impact on global

health and economies (Jones *et al*. 2008). Our measurement of the potential dispersal range and rate of virus by wildfowl according to species and seasons should allow better evaluation of the risk of spread from an HPAI H5N1 outbreak site according to the period and the presence of wildfowl species when and where it took place. Our approach of integrating data on individual migratory movements and response to experimental infection also provides a novel analytical framework for quantifying the risk of dispersion of pathogens vectored by wild birds.

Acknowledgements

This telemetry programme has been coordinated (SH Newman) and funded by FAO through the generous contributions of the Governments of the Kingdom of Sweden, of the United Kingdom of Great Britain and Northern Ireland, of France and of the Kingdom of Saudi Arabia. We thank J. Domenech (FAO), J. Lubroth (FAO) for support of this large scale disease ecology initiative, and the permissions granted by national and local agencies in the participating countries. We are also grateful to the numerous ornithologists, veterinarians, and volunteers who participated in field operations (for a detailed list see Appendix S1, Supporting information).

References

Argos (1996) *User's Manual*. Argos, Inc., Landover, MD. Available from http://www.argosinc.com/.

Brown, J.D., Stallknecht, D.E. & Swayne, D.E. (2008) Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerging Infectious Diseases*, **14**, 136–142.

Brown, J.D., Stallknecht, D.E., Beck, J.R., Suarez, D.L. & Swayne, D.E. (2006) Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerging Infectious Diseases*, **12**, 1663–1670.

Brown, J.D., Valeika, S., Stallknecht, D.E. & Swayne, D.E. (2007) Susceptibility of wood ducks to H5N1 highly pathogenic avian influenza virus. *Journal of Wildlife Diseases*, **43**, 660–667.

Buehler, D.M. & Piersma, T. (2008) Travelling on a budget: predictions and ecological evidence for bottlenecks in the annual cycle of long-distance migrants. *Philosophical Transactions of the Royal Society of London B*, **363**, 247–266.

Butler, P.J., Woakes, A.J. & Bishop, C.M. (1998) Behaviour and physiology of Svalbard barnacle geese, *Branta leucopsis*, during their autumn migration. *Journal of Avian Biology*, **29**, 536–545.

Chen, H., Li, K.S., Wang, J., Fan, X.H., Rayner, J.M., Vijaykrishna, D., Zhang, J.X., Zhang, L.J., Guo, C.T., Cheung, C.L., Xu, K.M., Duan, L., Huang, K., Qin, K., Leung, Y.H.C., Wu, W.L., Lu, H.R., Chen, Y., Xia, N.S., Naipospos, T.S.P., Yuen, K.Y., Hassan, S.S., Bahri, S., Nguyen, T.D., Webster., G., Peiris, J.S.M. & Guan, Y. (2006) Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proceedings of the National Academy of Sciences*, **103**, 2845–2850.

Clausen, P., Nolet, B.A., Fox, A.D. & Klaassen, M. (2002) Long-distance endozoochorous dispersal of submerged macrophyte seeds by migratory waterbirds in northern Europe: a critical review of possibilities and limitations. *Acta Oecologica*, **23**, 191–203.

Dugan, V.G., Chen, R., Spiro, D.J., Sengamalay, N., Zaborsky, J., Ghedin, E., Nolting, J., Swayne, D.E., Runstadler, J.A., Happ, G.M., Senne, D.A., Wang, R., Slemons, R.D., Holmes, E.C. & Taubenberger, J.K. (2008) The evolutionary genetics and emergence of avian influenza viruses in wild birds. *PLoS Pathogens*, **4**, e1000076.

FAO (2008) *AIDE News, no. 52*. Food and Agricultural Organisation, Rome, Italy. ftp://ftp.fao.org/docrep/fao/011/aj094e/aj094e00.pdf.

Fereidouni, S.R., Starick, E., Beer, M., Wilking, H., Kalthoff, D., Grund, C., Häuslaigner, R., Breithaup, A., Lang, E. & Harder, T.C. (2009) Highly pathogenic avian influenza virus infection of mallards with homo- and heterosubtypic immunity induced by low pathogenic avian influenza viruses. *PLoS ONE*, **4**, e6706.

Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., Cattoli, G., Lamarque, F., Hagemeijer, W. & Monicat, F. (2007) Avian influenza viruses in water birds, Africa. *Emerging Infectious Diseases*, **13**, 626–629.

Gaidet, N., Cattoli, G., Hammoumi, S., Newman, S.H., Hagemeijer, W., Takekawa, J.Y., Cappelle, J., Dodman, T., Joannis, T., Gil, P., Monne, I., Fusaro, A., Capua, I., Manu, S., Micheloni, P., Ottosson, U., Mshelbwala, J.H., Lubroth, J., Domenech, J. & Monicat, F. (2008) Evidence of infection by H5N2 highly pathogenic influenza viruses in healthy wild waterfowl. *PLoS Pathogens*, **4**, e1000127.

van Gils, J.A., Munster, V.J., Radersma, R., Liefhebber, D., Fouchier, R.A.M. & Klaassen, M. (2007) Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza a virus. *PLoS ONE*, **2**, e184.

Guglielmo, C.G., Piersma, T. & Williams, T.D. (2001) A sport-physiological perspective on bird migration: evidence for flight-induced muscle damage. *Journal of Experimental Biology*, **204**, 2683–2690.

Hasselquist, D., Lindström, Å., Jenni-Eiermann, S., . Koolhaas, A. & Piersma, T. (2007) Long flights do not influence immune responses of a long-distance migrant bird: a wind-tunnel experiment. *Journal of Experimental Biology*, **210**, 1123–1131.

Hesterberg, U., Harris, K., Stroud, D.A., Guberti, V., Busani, L., Pittman, M., Piazza, V., Cook, A. & Brown, I.H. (2009) Avian influenza surveillance in wild birds in the European Union in 2006. *Influenza and Other Respiratory Viruses*, **3**, 1–14.

Hulse-Post, D.J., Sturm-Ramirez, K.M., Humberd, J., Seiler, P., Govorkova, E.A., Krauss, S., Scholtissek, C., Puthavathana, P., Buranathai, C., Nguyen, T.D., Long, H.T., Naipospos, T.S., Chen, H., Ellis, T.M., Guan, Y., Peiris, J.S. & Webster, R.G. (2005) Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proceedings of the National Academy of Sciences*, **102**, 10682–10687.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P. (2008) Global trends in emerging infectious diseases. *Nature*, **451**, 990–993.

Kalthoff, D., Breithaupt, A., Teifke, J.P., Globig, A., Harder, T., Mettenleiter, T.C. & Beer, M. (2008) Highly pathogenic avian influenza virus (H5N1) in experimentally infected adult mute swans. *Emerging Infectious Diseases*, **14**, 1267–1270.

Keawcharoen, J., van Riel, D., van Amerongen, G., Bestebroer, T., Beyer, W.E., van Lavieren, R., Osterhaus, A.D., Fouchier, R.A. & Kuiken, T. (2008) Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases*, **14**, 600–607.

Kleijn, D., Munster, V.J., Ebbinge, B.S., Jonkers, D.A., Müskens, G.J., Van Randen, Y. & Fouchier, R.A. (2010) Dynamics and ecological consequences of avian influenza virus infection in greater white-fronted geese in their winter staging areas. *Proceedings of the Royal Society, Series B*, **277**, 2041–2048.

Koehler, A.V., Pearce, J.M., Flint, P.L., Franson, J.C. & Ip, H.S. (2008) Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). *Molecular Ecology*, **7**, 4754–4762.

Kvist, A., Lindström, A., Green, M., Piersma, T. & Visser, G.H. (2001) Carrying large fuel loads during sustained bird flight is cheaper than expected. *Nature*, **413**, 730–732.

Kwon, Y.K., Thomas, C. & Swayne, D.E. (2010) Variability in pathobiology of South Korean H5N1 high-pathogenicity avian influenza virus infection for 5 species of migratory waterfowl. *Veterinary Pathology*, **3**, 495–506.

Latorre-Margalef, N., Gunnarsson, G., Munster, V.J., Fouchier, R.A.M., Osterhaus, A.D.M.E., Elmberg, J., Olsen, B., Wallensten, A., Fransson, T., Brudin, L. & Waldenstrom, J. (2009) Effects of influenza A virus infection on migrating mallard ducks. *Proceedings of the Royal Society, Series B*, **276**, 1029–1036.

Liu, J., Xiao, H., Lei, F., Zhu, Q., Qin, K., Zhang, X., Zhang, X., Zhao, D., Wang, G., Feng, Y., Ma, J., Liu, W., Wang, J. & Gao, G. (2005) Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science*, **309**, 1206.

Matson, K., Cohen, A., Klasing, K., Ricklefs, R. & Scheuerlein, A. (2006) No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. *Proceedings of the Royal Society, Series B*, **273**, 815–822.

Miyabayashi, Y. & Mundkur, T. (1999) *Atlas of Key Sites for Anatidae in the East Asian Flyway*. Wetlands International, Tokyo, Japan.

Møller, A.P. & Erritzøe, J. (1998) Host immune defence and migration in birds. *Evolutionary Ecology*, **12**, 945–953.

Nathan, R. (2008) An emerging movement ecology paradigm. *Proceedings of the National Academy of Sciences, USA*, **105**, 19050–19051.

Nathan, R., Perry, G., Cronin, J.T., Strand, A.E. & Cain, M.L. (2003) Methods for estimating long-distance dispersal. *Oikos*, **103**, 261–273.

OIE (2009) *Highly Pathogenic Avian Influenza, Germany*. World Organization for Animal Health. http://www.oie.int/wahis/public.php?page = single_report&pop = 1&reportid = 7874.

Olsen, B., Munster, V.J., Wallensten, A., Waldenstrom, J., Osterhaus, A.D. & Fouchier, R.A. (2006) Global patterns of influenza a virus in wild birds. *Science*, **312**, 384–388.

Pasick, J., Berhane, Y., Embury-Hyatt, C., Copps, J., Kehler, H., Handel, K., Babiuk, S., Hooper-McGrevy, K., Yan, L., Le, Q.M. & Phuong, S.L. (2007) Susceptibility of Canada geese (*Branta canadensis*) to highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases*, **13**, 1821–1827.

Reed, K.D., Meece, J.K., Henkel, J.S. & Shukla, S.K. (2003) Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. *Clinical Medicine and Research*, **1**, 5–12.

Roshier, D.A. & Asmus, M.W. (2009) Use of satellite telemetry on small-bodied waterfowl in Australia. *Marine and Freshwater Research*, **60**, 299–305.

Saad, M.D., Ahmed, L.S., Gamal-Eldein, M.A., Fouda, M.K., Khalil, F.M. & Yingst, S.L. (2007) Possible avian influenza (H5N1) from migratory bird, Egypt. *Emerging Infectious Diseases*, **13**, 1120–1121.

Salzberg, S.L., Kingsford, C., Cattoli, G., Spiro, D.J., Janies, D.A., Smith, G.J.D., Fan, X.H., Wang, J., Li, K.S., Qin, K., Zhang, J.X., Vijaykrishna, D., Cheung, C.L., Huang, K., Rayner, J.M., Peiris, J.S.M., Chen, H., Webster, R.G. & Guan, Y. (2007) Genome analysis linking recent European and African influenza (H5N1) viruses. *Emerging Infectious Diseases*, **13**, 713–718.

Scott, D.A. & Rose, P.M. (1996) *Atlas of Anatidae Populations in Africa and Western Eurasia*. Wetlands International, Wageningen, The Netherlands.

Uchida, Y., Mase, M., Yoneda, K., Kimura, A., Obara, T., Kumagai, S., Saito, T., Yamamoto, Y., Nakamura, K., Tsukamoto, K. & Yamaguchi, S. (2008) Highly pathogenic avian influenza virus (H5N1) isolated from Whooper Swans, Japan. *Emerging Infectious Diseases*, **14**, 1427–1429.

USGS. (2008) *List of Species Affected by H5N1 (Avian Influenza)*. U S Geological Survey. http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp.

Webster, R.G., Yakhno, M., Hinshaw, V.S., Bean, W.J. & Murti, K.G. (1978) Intestinal influenza: replication and characterization of influenza viruses in ducks. *Virology*, **84**, 268–278.

Wetlands International. (2006) *Waterbird Population Estimates*, 4th edn. Wetlands International, Wageningen, The Netherlands.

WHO/OIE/FAO H5N1 Evolution Working Group. (2008) Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases*, http://www.cdc.gov/EID/content/14/7/e1.htm.

Received 9 December 2009; accepted 21 June 2010
Handling Editor: Jenny Gill

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. List of participants in field operations, coordination, and data management of this project.

Table S1. Summary of satellite-tracked birds equipped and considered in our study.

Table S2. Estimated maximum individual values of the maximum potential dispersal distance – Dmax.1, Dmax.4 and Dmax.8 (km) of HPAI H5N1 viruses for each species of satellite-tracked birds (n) for a min., mean and max. AID.

Table S3. Estimated mean (+/− SE) maximum potential dispersal distance – Dmax.1 and Dmax.8 (Km) of HPAI H5N1 viruses for each species of satellite-tracked birds (n) for a minimum and a maximum AID.

Table S4. Number of days per year of individual potential for dispersal of HPAI H5N1 virus over a distance > 100 and > 500 km estimated for each species of sedentary or nomadic (SN) birds for a mean AID.

Fig. S1. Maximum potential dispersal distance of HPAI H5N1 virus as a function of AID for long-distance migratory species during breeding–post breeding and non-breeding stages and for nomadic/sedentary species.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.