Wild bird surveillance around outbreaks of highly pathogenic avian influenza A(H5N8) virus in the Netherlands, 2014, within the context of global flyways

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Highly pathogenic avian influenza (HPAI) A(H5N8) viruses that emerged in poultry in east Asia since 2010 spread to Europe and North America by late 2014. Despite detections in migrating birds, the role of free-living wild birds in the global dispersal of H5N8 virus is unclear. Here, wild bird sampling activities in response to the H5N8 virus outbreaks in poultry in the Netherlands are summarised along with a review on ring recoveries. HPAI H5N8 virus was detected exclusively in two samples from ducks of the Eurasian wigeon species, among 4,018 birds sampled within a three months period from mid-November 2014. The H5N8 viruses isolated from wild birds in the Netherlands were genetically closely related to and had the same gene constellation as H5N8 viruses detected elsewhere in Europe, in Asia and in North America, suggesting a common origin. Ring recoveries of migratory duck species from which H5N8 viruses have been isolated overall provide evidence for indirect migratory connections between East Asia and Western Europe and between East Asia and North America. This study is useful for better understanding the role of wild birds in the global epidemiology of H5N8 viruses. The need for sampling large numbers of wild birds for the detection of H5N8 virus and H5N8-virus-specific antibodies in a variety of species globally is highlighted, with specific emphasis in north-eastern Europe, Russia and northern China.

Introduction

Wild aquatic birds are the natural reservoir for low pathogenic avian influenza A (LPAI) viruses, which are classified based on their surface proteins haemagglutinin (HA, H1–H16) and neuraminidase (NA, N1–N9) [1,2]. These viruses can be carried over long distances along migratory flyways [3-5]. LPAI viruses of the H5 and H7 subtype can evolve into highly pathogenic avian influenza (HPAI) viruses upon introduction into poultry. HPAI H5N8 viruses, such as A/duck/Jiangsu/k1203/2010, were first detected in birds on live bird markets in China in 2010 [6]. These H5N8 viruses contain genes derived from HPAI H5N1 viruses of the so-called A/Goose/Guangdong/1/1996 (GsGd) lineage [7] that have caused outbreaks in numerous countries of the eastern hemisphere since 1997.

In January 2014, HPAI H5N8 viruses were detected in South Korea, where they infected birds of 161 poultry farms and resulted in the culling of 14 million poultry by September 2014 [8]. In April 2014, HPAI H5N8 virus was detected on a chicken farm in Japan. Over the summer of 2014, no new cases were reported outside South Korea. In September, HPAI H5N8 virus was detected in China in a domestic duck and an environmental sample. During the same month, H5N8 virus was also detected in north-eastern Russia in a Eurasian wigeon (*Anas penelope*). From November 2014 to February 2015, HPAI H5N8 virus has been found in poultry and/or free-living wild birds in Asia (Japan and Taiwan), Europe (Germany, Hungary, Italy, the Netherlands and the United Kingdom (UK)), and North America (US) [9,10]. HPAI H5N8 virus was also detected in captive wild birds: dead gyrfalcons (*Falco rusticolus*) in the north west of the United States (US) and white storks (*Ciconia ciconia*) in a zoo in Germany (Table 1) [11]. The HA of HPAI H5N8 viruses detected in domestic and wild birds in Asia, Europe and North America belonged to the GsGd H5 clade 2.3.4.4 [12]. Genetic closely related H5N8 viruses belonging to the same GsGd H5 clade 2.3.4.4 were detected in China since 2010.

So far, HPAI H5N8 virus has been isolated from free-living wild birds of the orders *Accipitriformes*, *Anseriformes*, *Charadriiformes*, *Falconiformes* and *Gruiformes* in several countries including Germany, Japan, Russia, South Korea, Taiwan, the Netherlands,

Table 1

Global detection of highly pathogenic avian influenza A(H5N8) virus and other viruses belonging to the H5 clade 2.3.4.4 in wild birds and poultry, 2014

Host type	Order	Family	Poultry type or bird species	AIV subtype	Geographical area
Captive	*Anseriformes*	Ducks	Domestic duck	H5N2; H5N3; H5N8	Canada (H5N2); China (H5N8; H5N2); Hungary (H5N8); Netherlands (H5N8); South Korea (H5N8); Taiwan (H5N2, H5N3, H5N8); UK (H5N8); US (H5N2, H5N8)
		Geese	Domestic Goose	H5N2; H5N3; H5N8	Canada (H5N2); South Korea (H5N8); Taiwan (H5N2, H5N3, H5N8); US (H5N2, H5N8)
	Galliformes	Chickens	Chicken	H5N1; H5N2; H5N3; H5N8	Canada (H5N1, H5N2); China (H5N2); Japan (H5N8); Netherlands (H5N8); South Korea (H5N8); Taiwan (H5N2, H5N3, H5N8); US (H5N2, H5N8)
		Turkeys	Domestic turkey	H5N2; H5N3; H5N8	Canada (H5N2); Germany (H5N8); Italy (H5N8); Taiwan (H5N2, H5N8); US (H5N2, H5N8)
	Ciconiiformes	Storks	White stork (*Ciconia ciconia*)	H5N8	Germany
	Falconiformes	Falcons	Gyrfalcon (*Falco rusticolus*)	H5N8	US
			Peregrine falcon (*Falco peregrinus*)	H5N2	US
	Strigiformes	Owls	Great horned owl (*Bubo virginianus*)	H5N2	US
Wild	*Accipitriformes*	Eagles	Bald eagle (*Haliaeetus leucocephalus*)	H5N8	US
		Hawks	Cooper's hawk (*Accipiter cooperii*)	H5N2	US
			Red-tailed hawk (*Buteo jamaicensis*)	H5N2	US
	Anseriformes	Ducks	Baikal teal (*Anas formosa*)	H5N8	South Korea
			Mallard (*Anas platyrhynchos*)	H5N2; H5N8	Germany (H5N8); Japan (H5N8); South Korea (H5N8); US (H5N2, H5N8)
			Common teal (*Anas crecca*)	H5N8	Germany; South Korea
			Green-winged teal (*Anas carolinensis*)	H5N1; H5N8	US
			Spot-billed duck (*Anas poecilorhyncha*)	H5N8	South Korea
			Eurasian wigeon (*Anas penelope*)	H5N8	Netherlands; Russia
			Northern pintail (*Anas acuta*)	H5N2	US
			Mandarin duck (*Aix galericulata*)	H5N8	Japan
			Gadwall (*Anas strepera*)	H5N8	US
			American wigeon (*Anas americana*)	H5N8	US
			Wood duck (*Aix sponsa*)	H5N2	US
			Northern shoveler (*Anas clypeata*)	H5N2	US
		Geese	Bean goose (*Anser fabalis*)	H5N8	South Korea
			White-fronted goose (*Anser albifrons*)	H5N8	South Korea
		Swans	Bewick's swan (*Cygnus columbianus bewickii*)	H5N8	Japan; South Korea
	Charadriiformes	Gulls	Great black-backed gull (*Larus marinus*)	H5N8	Germany
	Falconiformes	Falcons	Peregrine falcon (*Falco peregrinus*)	H5N8	US
	Gruiformes	Cranes	White-naped crane (*Grus vipio*)	H5N8	Japan
			Hooded crane (*Grus monacha*)	H5N8	Japan
		Coots	Common coot (*Fulica atra*)	H5N8	South Korea
	Passeriformes	Bulbuls	Light-vented bulbul (*Pycnonotus sinensis*)	H5N3	Taiwan
	Pelecaniformes	Herons	Black-crowned night-heron (*Nycticorax nycticorax*)	H5N2	Taiwan

AIV: avian influenza virus; UK: United Kingdom; US: United States.
Data from [8,9].

and the US (Table 1). In live wild birds, H5N8 virus detections were limited to ducks (order: *Anseriformes*) of the species common teal (*Anas crecca*), mallard (*Anas platyrhynchos*), spot-billed duck (*Anas poecilorhyncha*), Eurasian wigeon, American wigeon (*Anas americana*) and gadwall (*Anas strepera*) [8,9] (Table 1). In addition, H5N8-virus-specific antibodies were detected in 10 to 53% of ducks of the species Baikal teal (*Anas formosa*), common teal, mallard, Eurasian wigeon and spot-billed duck in South Korea [8], suggesting that this virus had been circulating in these species for some time and that these individual birds had survived infection and thus may have played a role in the dispersal of H5N8. Wild ducks of some species (e.g. *Anas* spp.) may be less likely to exhibit clinical signs when infected with HPAI H5N8 than e.g. geese, swans and cranes; alternatively, ducks are more intensively hunted and sampled, potentially explaining a higher detection rate of H5N8 in live wild ducks than in other wild bird species. Despite H5N8 virus detections in a range of wild bird species globally, it is unknown to what extent these viruses circulate in wild bird populations in Europe.

This study presents data on wild bird surveillance activities in the Netherlands that were intensified in the country, in response to the HPAI H5N8 virus outbreaks on poultry farms at the end of 2014. We present our findings in the perspective of the distribution and migratory flyways of H5N8-virus-positive bird species.

Methods

Sampling wild birds

After detection of HPAI H5N8 virus on a chicken farm in the Netherlands on 14 November 2014, sampling of live wild birds of various species was intensified in the country in an attempt to detect H5N8 virus. Birds were captured using duck decoys, clap nets, mist nets, noose or by hand. Capturing of wild birds was approved by the Dutch Ministry of Economic Affairs based on the Flora and Fauna Act (permit number FF/75A/2009/067 and FF/75A/2014/054). Handling and sampling of wild birds were approved by the Animal Experiment Committee of the Erasmus MC (permit number 122–11–31). Sampling activities targeted long-distance migratory bird species and/or bird species that had been found infected with HPAI H5N8 virus earlier in 2014, e.g. Bewick's swan (*Cygnus columbianus bewickii*) in Japan. Sample locations were both within and outside a 10 km radius of Dutch poultry farms where H5N8-virus-infections had been detected and varied in function of the distribution of wild bird species of interest combined with capture opportunities. Disposable gloves and disinfectants for boots and equipment (Virkon S) were used. Birds were sampled for virus detection by collecting samples from cloaca, from both cloaca and oropharynx, or from fresh faeces as described by Munster et al. [13]. For cloaca and oropharynx samples, the number of tested birds depended on the bird species, capture method and capture success. For fresh faeces, swab samples were collected from flocks of single species. The number of faeces droppings sampled per flock was on average less than 40% of the total number of birds in the flock with at least one metre in between each dropping (to limit sampling the same individual twice).

Virus detection, isolation and characterisation

Samples for virus detection were analysed for presence of H5N8 virus using a matrix-specific and H5-specific polymerase chain reaction (PCR) followed by H5 sequencing. Samples that tested positive in matrix-specific PCR were used for virus isolation in embryonated chicken eggs as described previously [13].

Virus sequencing and phylogeny

Of the HPAI H5N8 viruses isolated within this study, the sequences of the complete genome were obtained and deposited in a public database (http://www.gisaid.com). Sequencing was performed using specific primers as described previously [14]. Nucleotide (nt) sequences were supplemented with sequences of HPAI H5 viruses of clade 2.3.4.4 detected globally in 2014 and with sequences of HPAI H5N8 viruses detected in China before 2014. These additional sequences were obtained from public databases as of 3 March 2015, which included the Global Initiative on Sharing Avian Influenza Data database (http://www.gisaid.com) (Table 2) and Genbank (www.ncbi.nlm.nih.gov). Sequences retrieved from GenBank had the following accession numbers: AJE30335; AJE30344; AJE30360; AJM70554; AJE30333; AJM70565; AJM70567; AJM70576; AJM70578; AJM70587; AJM70598; AJM70609. Maximum Likelihood (ML) phylogenetic trees were constructed based on the HA gene of 1,545 nt in length (position: 108–1,652) and the NA gene of 1,377 nt in length (position: 1–1,377). ML trees were generated using the PhyML package version 3.1 using the general time-reversible model with the proportion of invariant sites (GTR+I model) of nt substitution, performing a full heuristic search and subtree pruning and regrafting (SPR) searches. The best-fit model of nt substitution was determined with jModelTest [15]. The reliability of the phylogenetic grouping was assessed with 1,000 bootstrap replicates. Trees were visualised using Figtree version 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).

Results

Wild bird surveillance activities to detect H5N8 virus in the Netherlands: newly acquired and historical data

Surveillance of avian influenza virus in wild birds in the Netherlands has been in place in the country since 1998. After the first HPAI H5N8 detection in poultry on 14 November 2014, activities to detect the virus were increased and a total of 4,018 wild birds of 25 different species belonging to five orders were sampled (Table 3). Of those, 623 birds (16%) were sampled within 10 km of farms previously affected by HPAI H5N8-virus. In the six months before the first detection of HPAI H5N8 in poultry, a total of 2,745 wild birds of nine different

Table 2A

Information on influenza A virus sequences obtained from the Global Initiative on Sharing Avian Influenza Data used for the study

Segment ID	Segment	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI552760	HA	NL	2014-Nov-24	A/eurasian wigeon/Netherlands/emc-1/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI552762	NA	NL	2014-Nov-24	A/eurasian wigeon/Netherlands/emc-1/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI552768	HA	NL	2014-Nov-24	A/eurasian wigeon/Netherlands/emc-2/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI552770	NA	NL	2014-Nov-24	A/eurasian wigeon/Netherlands/emc-2/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI552776	HA	NL	2014-Nov-21	A/chicken/Netherlands/emc-3/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI552778	NA	NL	2014-Nov-21	A/chicken/Netherlands/emc-3/2014 (H5N8)	Erasmus MC	Erasmus MC	Fouchier et al.
EPI547678	HA	NL	2014-Nov-14	A/Chicken/Netherlands/14015526/2014 (H5N8)	Central Veterinary Institute	Central Veterinary Institute	Heutink et al.
EPI547683	NA	NL	2014-Nov-14	A/Chicken/Netherlands/14015526/2014 (H5N8)	Central Veterinary Institute	Central Veterinary Institute	Heutink et al.
EPI548623	HA	NL	2014-Nov-15	A/chicken/Netherlands/14015531/2014 (H5N8)	Central Veterinary Institute	Central Veterinary Institute	Heutink et al.
EPI548626	NA	NL	2014-Nov-15	A/chicken/Netherlands/14015531/2014 (H5N8)	Central Veterinary Institute	Central Veterinary Institute	Heutink et al.
EPI544756	HA	DE	2014-Nov-04	A/turkey/Germany-MV/R2472/2014 (H5N8)	Friedrich-Loeffler-Institut	Friedrich-Loeffler-Institut	NA
EPI544759	NA	DE	2014-Nov-04	A/turkey/Germany-MV/R2472/2014 (H5N8)	Friedrich-Loeffler-Institut	Friedrich-Loeffler-Institut	NA
EPI552746	HA	DE	2014-Nov-04	A/turkey/Germany/R2474-Lo0899/2014 (H5N8)	Friedrich-Loeffler-Institut	Friedrich-Loeffler-Institut	NA
EPI552748	NA	DE	2014-Nov-04	A/turkey/Germany/R2474-Lo0899/2014 (H5N8)	Friedrich-Loeffler-Institut	Friedrich-Loeffler-Institut	NA
EPI547673	HA	UK	2014-Nov-14	A/duck/England/36254/14 (H5N8)	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Hanna et al.
EPI547675	NA	UK	2014-Nov-14	A/duck/England/36254/14 (H5N8)	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Hanna et al.
EPI553144	HA	IT	2014-Dec-15	A/turkey/Italy/14VIR7898-10/2014 (H5N8)	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Luca et al.
EPI555068	NA	IT	2014-Dec-15	A/turkey/Italy/14VIR7898-10/2014 (H5N8)	Istituto Zooprofilattico Sperimentale Delle Venezie	Istituto Zooprofilattico Sperimentale Delle Venezie	Luca et al.
EPI553349	HA	RU	2014-Sep-25	A/wigeon/Sakha/1/2014 (H5N8)	State Research Center of Virology and Biotechnology Vector	State Research Center of Virology and Biotechnology Vector	Susloparov et al.
EPI553350	NA	RU	2014-Sep-25	A/wigeon/Sakha/1/2014 (H5N8)	State Research Center of Virology and Biotechnology Vector	State Research Center of Virology and Biotechnology Vector	Susloparov et al.
EPI548485	HA	JP	2014-Nov-18	A/duck/Chiba/26-372-48/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI548487	NA	JP	2014-Nov-18	A/duck/Chiba/26-372-48/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI548493	HA	JP	2014-Nov-18	A/duck/Chiba/26-372-61/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI548495	NA	JP	2014-Nov-18	A/duck/Chiba/26-372-61/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI553208	HA	JP	2014-Nov-23	A/crane/Kagoshima/KU1/2014 (H5N8)	Kagoshima University	Kagoshima University	NA
EPI553210	NA	JP	2014-Nov-23	A/crane/Kagoshima/KU1/2014 (H5N8)	Kagoshima University	Kagoshima University	NA
EPI553343	HA	JP	2014-Dec-16	A/chicken/Miyazaki/7/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI553345	NA	JP	2014-Dec-16	A/chicken/Miyazaki/7/2014 (H5N8)	National Institute of Animal Health	National Institute of Animal Health	NA
EPI553362	HA	JP	2014-Dec-01	A/environment/Kagoshima/KU-ngr-H/2014 (H5N8)	Kagoshima University	Kagoshima University	NA
EPI553364	NA	JP	2014-Dec-01	A/environment/Kagoshima/KU-ngr-H/2014 (H5N8)	Kagoshima University	Kagoshima University	NA
KJ476669	HA	CN	2013-Nov-14	A/duck/Zhejiang/W24/2013 (H5N8)	NA	Other database import	Wu et al.

CA: Canada; CN: China; DE: Germany; IT: Italy; JP: Japan; KR: South Korea; NL: Netherlands; RU: Russia; UK: United Kingdom.

Table 2B

Information on influenza A virus sequences obtained from the Global Initiative on Sharing Avian Influenza Data used for the study

Segment ID	Segment	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
KJ476673	NA	CN	2013-Nov-14	A/duck/Zhejiang/W24/2013 (H5N8)	NA	Other database import	Wu et al.
EPI507673	HA	CN	2013-Nov-18	A/mallard_duck/Shanghai/SH-9/2013 (H5N8)	Institute of Military Veterinary, Academy of Military Medical Sciences	Institute of Laboratory Animal Sciences, Chinese Academy	Fan et al.
EPI507675	NA	CN	2013-Nov-18	A/mallard_duck/Shanghai/SH-9/2013 (H5N8)	Institute of Military Veterinary, Academy of Military Medical Sciences	Institute of Laboratory Animal Sciences, Chinese Academy	Fan et al.
JQ973694	HA	CN	2010-Dec-05	A/duck/Jiangsu/k1203/2010 (H5N8)	NA	Other database import	Zhao et al.
JQ973696	NA	CN	2010-Dec-05	A/duck/Jiangsu/k1203/2010 (H5N8)	NA	Other database import	Zhao et al.
KJ413842	HA	KR	2014-Jan-17	A/broiler_duck/Korea/Buan2/2014 (H5N8)	NA	Other database import	Lee et al.
KJ413844	NA	KR	2014-Jan-17	A/broiler_duck/Korea/Buan2/2014 (H5N8)	NA	Other database import	Lee et al.
KJ413850	HA	KR	2014-Jan-17	A/baikal_teal/Korea/Donglim3/2014 (H5N8)	NA	Other database import	Lee et al.
KJ413852	NA	KR	2014-Jan-17	A/baikal_teal/Korea/Donglim3/2014 (H5N8)	NA	Other database import	Lee et al.
KJ746111	HA	KR	2014-Feb-05	A/mallard/Korea/W452/2014 (H5N8)	NA	Other database import	Choi et al.
KJ746113	NA	KR	2014-Feb-05	A/mallard/Korea/W452/2014 (H5N8)	NA	Other database import	Choi et al.

CA: Canada; CN: China; DE: Germany; IT: Italy; JP: Japan; KR: South Korea; NL: Netherlands; RU: Russia; UK: United Kingdom.

species belonging to three orders had also been sampled for HPAI H5 virus detection (Table 3). Results of the surveillance before and after mid-November 2014 are presented, covering a period from 14 May 2014 to 20 February 2015.

Taking into consideration the whole sampling period (May 2014 to February 2015), most avian influenza viruses were detected in ducks (719 of 4,495; 16%), swans (23 of 183; 13%) and gulls (254 of 1,185; 21%). Avian influenza viruses of the H5 subtype were detected in common teal, Eurasian wigeon and mallard, whereby most H5 viruses were LPAI viruses (27 of 29; 93%). On 24 November 2014, HPAI H5N8 virus was isolated from two of 52 faecal samples collected from 150 Eurasian wigeons foraging on grassland between Kamerik and Kockengen (52 °08'35.5"N, 4°55'22.7"E). The birds were located ca 15 to 28 km away from three of five H5N8-virus-infected poultry farms; the remaining two H5N8-virus-infected farms were located ca 80 km away. In the Netherlands, the affected poultry farms were located in wild-bird-rich areas where water is abundant and with low to medium poultry densities. The distribution in time of sampled birds is shown per age, location, sample type and species in Figure 1.

Genetic analyses of H5N8 viruses

Genetic analyses of the HA and NA gene showed that H5N8 viruses from Europe and Russia were genetically most closely related to H5N8 viruses detected in Japan in November and December of 2014 followed by viruses detected in South Korea in 2014 (Figure 2). Also, genetic analyses of the HA gene showed that H5N8 viruses from North America were genetically most closely related to HPAI H5N2 and H5N1 viruses detected in North America followed by H5N8 virus detected in South Korea and Japan. The NA of North American H5N8 viruses was genetically most closely related to H5N8 viruses from South Korea and Japan (i.e. A/crane/Kagoshima/KU1/2014, Figure 2).

Genetic analyses of all gene segments showed that the gene constellation of H5N8 viruses from domestic and wild birds in Europe and from birds in North America was very similar to H5N8 viruses from domestic and wild birds in South Korea and Japan (data not shown). Of these viruses, four of eight gene segments (i.e. basic polymerase 2 (PB2), HA, nucleoprotein (NP) and NA) were derived from viruses similar to A/Duck/Jiangsu/k1203/2010 (H5N8). Of those, PB2 and HA genes were derived from viruses of the HPAI H5 GsGd lineage. The remaining four gene segments (i.e. basic polymerase 1 (PB1), acidic polymerase (PA), matrix protein (MP) and non-structural protein (NS)) were derived from common LPAI viruses [6,7]. Nucleotide sequence identity per segment between European, North American and the genetically closest Asian relatives was high (i.e. 99 to 100% identical). Two genetic lineages (A and B) of H5N8 virus were identified in both domestic and wild birds from South Korea in January 2014, of which lineage A was more frequently detected in both domestic

Table 3A

Wild bird species sampled for highly pathogenic avian influenza (HPAI) H5N8 virus before and after the first detection of HPAI H5N8 virus in poultry on 14 November 2014, the Netherlands, May 2014–February 2015 (n=6,763)

Order	Family	Species[a]	Sampling period							
			14 May–13 November 2014				14 November 2014–20 February 2015			
			No. birds sampled	No. birds AIV positive	No. birds H5 positive	Pathotype	No. birds sampled	No. birds AIV positive	No. birds H5 positive	Pathotype
Anseriformes	Ducks	**Duck species**	**2,071**	**455**	**19**	**LPAI**	**2,424**	**264**	**10**	**2 HPAI; 8 LPAI**
		Common teal (*Anas crecca*)	19	3	1	LPAI	85	19	1	LPAI
		Egyptian goose (*Alopochen aegyptiaca*)	0	0	0	–	40	0	0	–
		Eurasian wigeon (*Anas penelope*)	140	26	8	LPAI	1,185	33	2	HPAI
		Gadwall (*Anas strepera*)	18	2	0	–	127	1	0	–
		Mallard (*Anas platyrhynchos*)	1,876	422	10	LPAI	979	208	7	LPAI
		Northern pintail (*Anas acuta*)	2	0	0	–	0	0	0	–
		Northern shoveler (*Anas clypeata*)	16	2	0	–	4	2	0	–
		Red-breasted merganser (*Mergus serrator*)	0	0	0	–	1	1	0	–
		Tufted duck (*Aythya fuligula*)	0	0	0	–	3	0	0	–
	Geese	**Goose species**	**0**	**0**	**0**	**0**	**340**	**3**	**0**	**–**
		Barnacle goose (*Branta leucopsis*)	0	0	0	–	38	2	0	–
		Brent goose (*Branta bernicla*)	0	0	0	–	39	1	0	–
		Greylag goose (*Anser anser*)	0	0	0	–	17	0	0	–
		White-fronted goose (*Anser albifrons*)	0	0	0	–	246	0	0	–
	Swans	**Swan species**	**0**	**0**	**0**	**–**	**183**	**23**	**0**	**–**
		Bewick's swan (*Cygnus columbianus bewickii*)	0	0	0	–	72	4	0	–
		Mute swan (*Cygnus olor*)	0	0	0	–	109	18	0	–
		Whooper swan (*Cygnus cygnus*)	0	0	0	–	2	1	0	–
Charadriiformes	Gulls	**Gull species**	**434**	**219**	**0**	**–**	**751**	**35**	**0**	**–**
		Black-headed gull (*Chroicocephalus ridibundus*)	434	219	0	–	611	22	0	–
		Caspian gull (*Larus cachinnans*)	0	0	0	–	3	0	0	–
		Common gull (*Larus canus*)	0	0	0	–	35	2	0	–
		Great black-backed gull (*Larus marinus*)	0	0	0	–	10	0	0	–
		Herring gull (*Larus argentatus*)	0	0	0	–	85	10	0	–
		Lesser black-backed gull (*Larus fuscus*)	0	0	0	–	7	1	0	–
	Terns	**Tern species**	**240**	**1**	**0**	**–**	**0**	**0**	**0**	**–**
		Black tern (*Chlidonias niger*)	176	1	0	–	0	0	0	–
		Common tern (*Sterna hirundo*)	64	0	0	–	0	0	0	–

AIV: avian influenza virus; HPAI: highly pathogenic avian influenza; LPAI: low pathogenic avian influenza; No: number.

[a] Unless otherwise specified.

Table 3B

Wild bird species sampled for highly pathogenic avian influenza (HPAI) H5N8 virus before and after the first detection of HPAI H5N8 virus in poultry on 14 November 2014, the Netherlands, May 2014–February 2015 (n=6,763)

Order	Family	Species[a]	Sampling period						
			14 May–13 November 2014				14 November 2014–20 February 2015		
			No. birds sampled	No. birds AIV positive	No. birds H5 positive	Pathotype	No. birds sampled	No. birds AIV positive	No. birds H5 positive	Pathotype
Gruiformes	Coots	**Coot species**	**0**	**0**	**0**	–	**289**	**9**	**0**	–
		Common coot (*Fulica atra*)	0	0	0	–	289	9	0	–
	Rails	**Rail species**	**0**	**0**	**0**	–	**20**	**0**	**0**	–
		Common moorhen (*Gallinula chloropus*)	0	0	0	–	20	0	0	–
Passeriformes	Crows	**Crow species**	**0**	**0**	**0**	–	**1**	**0**	**0**	–
		Carrion crow (*Corvus corone*)	0	0	0	–	1	0	0	–
Strigiformes	Owls	**Owl species**	**0**	**0**	**0**	–	**1**	**0**	**0**	–
		Barn owl (*Tyto alba*)	0	0	0	–	1	0	0	–
Total	–	–	**2,745**	**675**	**19**	–	**4,018**	**334**	**10**	–

AIV: avian influenza virus; HPAI: highly pathogenic avian influenza; LPAI: low pathogenic avian influenza; No: number.

[a] Unless otherwise specified.

and wild birds [7,8,16]. H5N8 viruses detected in Europe (Germany, Italy, the Netherlands, and the UK), Russia and in North America belonged to lineage A based on analyses of the HA gene [8]. The close genetic relationship between European, Asian and North American isolates suggested that these H5N8 viruses have a common origin.

Distribution and migratory flyways of H5N8-virus-positive bird species

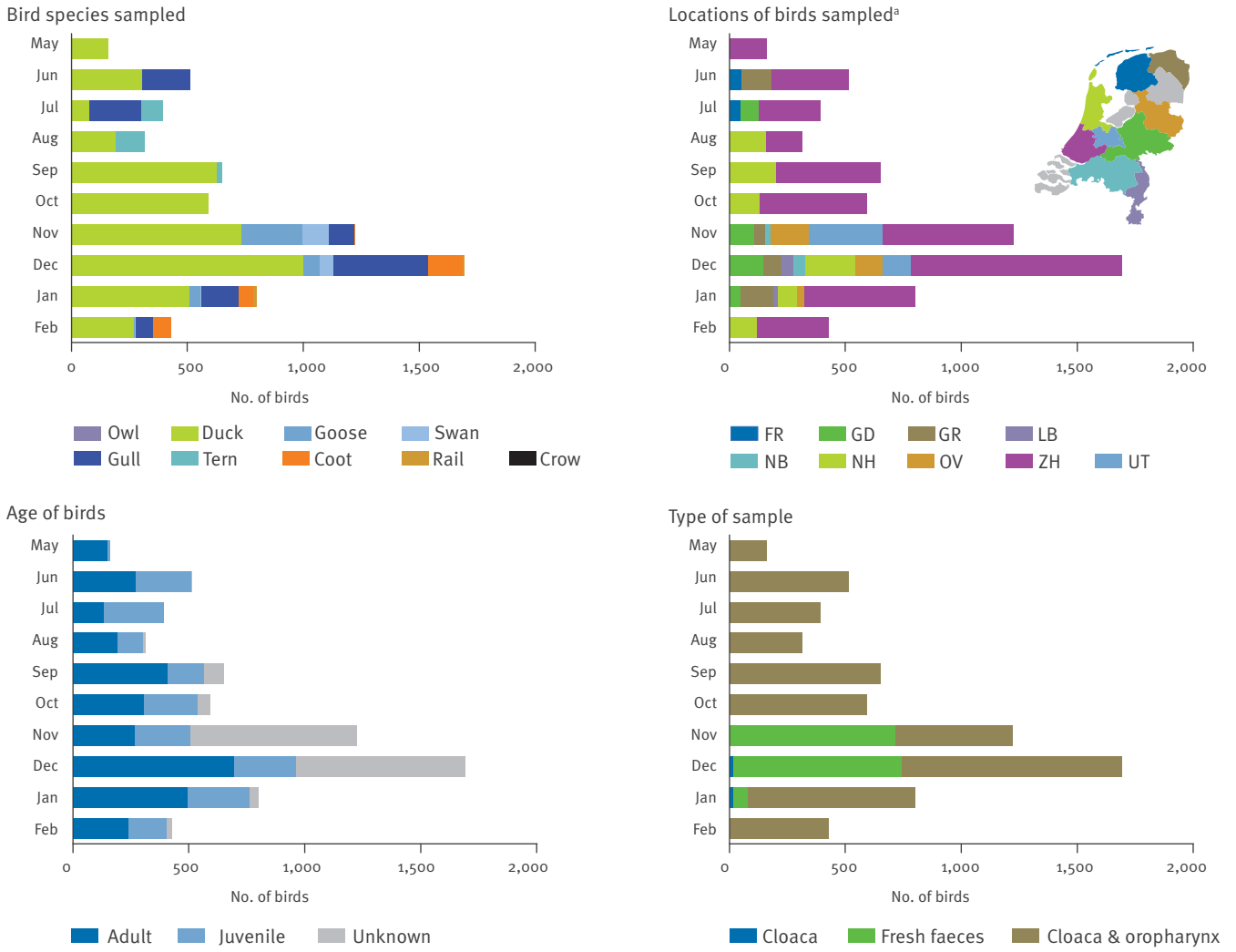
Migrating birds from which H5N8 viruses have been isolated (Table 1) and that have circumpolar breeding grounds (e.g. northern pintail, *Anas acuta*) or that cover multiple major migratory flyways (e.g. Eurasian wigeon) are of specific interest with respect to global H5N8 virus epidemiology (Figure 3). Most of those species can be divided into distinct populations based on their geographically separate wintering areas. However, less is known about the degree of mixing among these populations in their breeding areas in Russia, and to which degree birds are loyal to their wintering areas.

Ring recoveries suggest that some waterfowl species (including ducks and geese) with populations wintering in East Asia and populations wintering in western Europe may have overlapping breeding grounds. For instance, ring recoveries of Eurasian wigeon and northern pintail ringed in Japan indicate that they migrate mostly north to north-east to the Russian Far East during spring migration, but a minority strays more north-west, some as far as the Western Siberian Lowlands [17] (Figure 3A and 3B). Here, ring recoveries indicate that some conspecifics originating from western Europe also may be found [18] (Figure 3A and 3B). Hence, although the probability of an actual meeting between east and west seems low, ring recoveries suggest it is not impossible. Furthermore, ring recoveries of Eurasian wigeon and northern pintail indicated a direct migratory connection between north Russia and north India (Figure 3A and 3B). Baikal teal and spot-billed duck, from which H5N8 viruses have also been isolated, have more restricted ranges, but could be involved in transport of virus from wintering grounds to breeding grounds in north-eastern Russia (Figure 3C and 3D). Mallards and teals have extensive ranges, and potentially can also be involved in transport of virus, but ring-recovery data from Russia were not available (Figure 3E and 3F).

Ring recoveries and satellite tracking have shown various waterfowl species from East Asia to be in indirect and sometimes even direct migratory connection with North America. Satellite tracking and colour banding of various waterfowl species, including emperor goose (*Chen canagica*) [19], black brant (*Branta bernicla nigricans*) [20], lesser snow goose (*Chen caerulescens caerulescens*) [21] and northern pintail have shown them to cross the Bering Strait [22]. Ring recoveries of northern pintail in particular show that the connection between East Asia and North America is quite strong, albeit most likely still indirect with contact zones in the

Figure 1

Monthly sampling of wild birds for H5N8 virus detection, by species, location, age, and sample type, the Netherlands, 14 May 2014–20 February 2015 (n=6,763)



FR: Friesland; GD: Gelderland; GR: Groningen; LB: Limburg; NB: Noord-Brabant; NH: Noord-Holland; OV: Overijssel; UT: Utrecht; ZH: Zuid-Holland.

[a] Locations were categorised according to Dutch provinces.

Russian Far East and Wrangel Island [17,23]. The same is true for some other species than waterfowl, which have not been identified as H5N8 virus hosts, but may play a role in the epidemiology of influenza, such as waders [24,25].

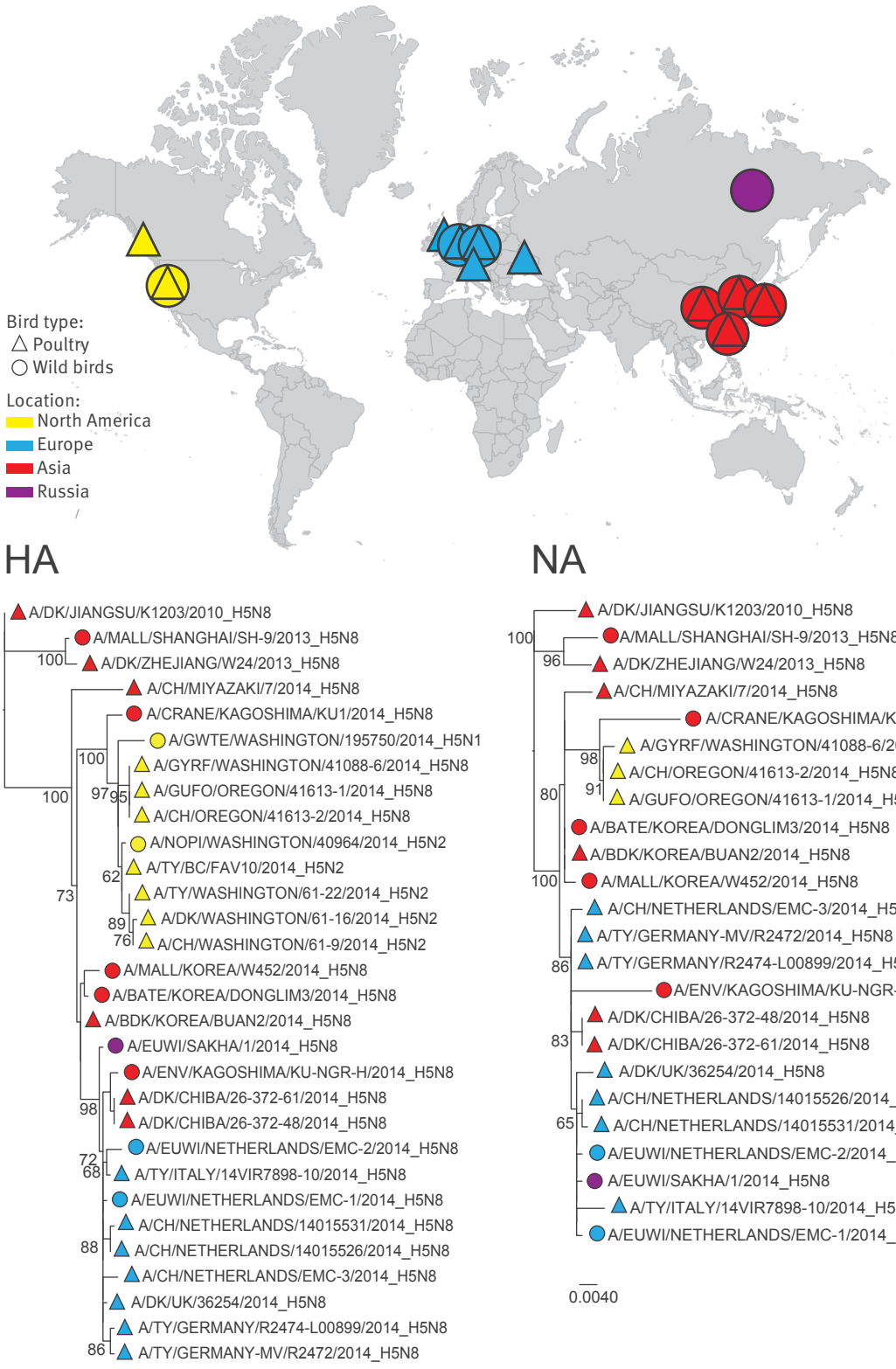
Discussion

The detection of the newly emerging HPAI H5N8 virus in at least 17 migratory bird species in Asia, Europe and North America, emphasises the need to study the role of migratory birds in the epidemiology of these H5N8 viruses. After the first detection of H5N8 virus in poultry in the Netherlands, wild bird sampling activities were intensified and HPAI H5N8 virus was detected in samples from two of 4,018 birds sampled within a three months period. The virus was isolated from Eurasian wigeons exclusively, whereas other bird species like mallards, white-fronted geese (*Anser albifrons*), black-headed gulls (*Chroicocephalus ridibundus*) and common coots (*Fulica atra*) also had been sampled intensively. The Eurasian wigeon is a long-distance migrant in which species H5N8-virus-specific antibodies had been detected in South Korea in 2014 [8]. As HPAI H5N8 virus, like other avian influenza viruses, causes an infection of short duration in birds [26], the chance of detection is low and large sample sizes are needed to determine its presence in the population. The chance of detection of H5N8-virus-specific antibodies in wild bird sera is much higher, and serology can be used as a tool to target surveillance and determine past exposure to H5N8 virus, as H5 viruses of the HPAI GsGd lineage differ antigenically from common LPAI H5 viruses [27].

The H5N8 viruses isolated from wild birds in the Netherlands were genetically closely related to and had

Figure 2

Phylogenetic analysis of haemagglutinin (HA) and neuraminidase (NA) genes from highly pathogenic avian influenza (HPAI) H5N8 viruses recovered in China in 2010–2013 together with respective HA and NA genes from HPAI H5N8 and other HPAI viruses belonging to the H5 clade 2.3.4.4, detected in poultry and wild birds in Asia, Europe, Russia and North America in 2014

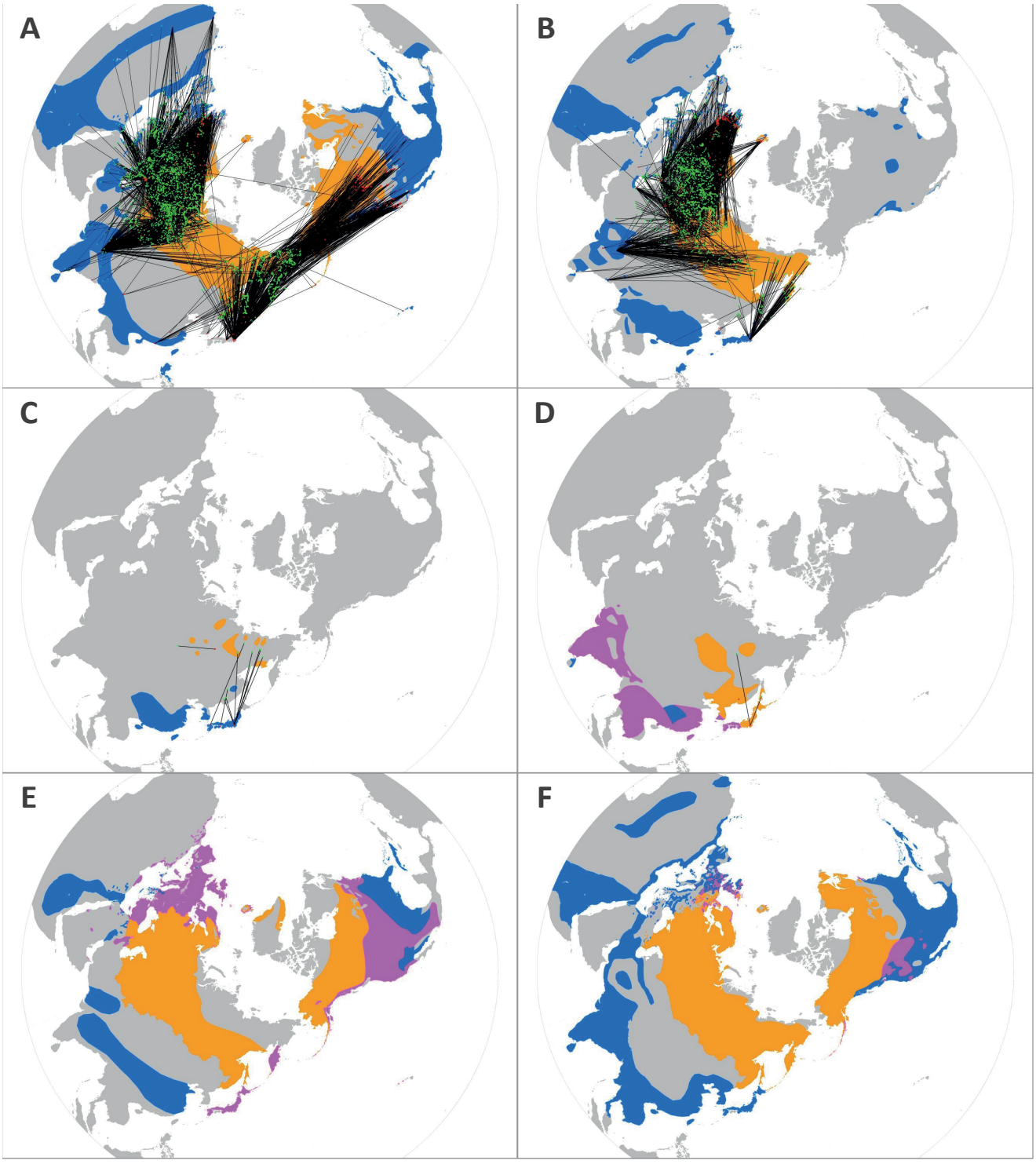


BATE: Baikal teal; BDK: broiler duck; CH: chicken; DK: duck; ENV: environment; EUWI: Eurasian wigeon; GUFO: guinea fowl; GWTE: green-winged teal; GYRF=gyrfalcon; HPAI: highly pathogenic avian influenza; MALL: mallard; NOPI: northern pintail; TY: turkey.

Maximum likelihood trees were based on the haemagglutinin gene (HA; 1,545 nucleotides) and neuraminidase gene (NA; 1,377 nucleotides). Bootstrap values are shown if >60%.

Figure 3

Breeding and wintering range and ring recoveries from 1940–2010[a] of wild duck species from which highly pathogenic avian influenza (HPAI) H5N8 viruses have been isolated



Top: wide range, long-distance migratory species northern pintail (*Anas acuta*) (A) and Eurasian wigeon (*Anas penelope*) (B); **Middle:** restricted range, short-distance migratory or resident species Baikal teal (*Anas formosa*) (C) and spot-billed duck (*Anas poecilorhyncha*) (D); **Bottom:** wide-range, long-distance migratory or resident species mallard (*Anas platyrhynchos*) (E), and teal (*Anas crecca / carolinensis*) (F).

Orange: summer (breeding) range, blue: wintering range, purple: all-year (resident) range. Lines in maps A, B, C and D connect ringing locations (red dots) and recovery locations (green dots).

[a] The majority of ring recoveries were conducted during 1960–1990.

Data source: Lines in maps A, B, C and D are based on ring-recovery data from the database of the Russian ringing scheme and are reprinted with permission from the Waterfowl Migration Atlas from the Bird Ringing Centre of Russia database and OMPO. Breeding and wintering ranges are reproduced from [30]. Breeding ranges of Baikal teal and spot-billed duck have been updated from [31].

the same gene constellation as H5N8 viruses detected elsewhere in Europe, in Asia and in North America, suggesting a common origin. In wild and domestic birds in North America, HPAI reassortant viruses of the subtypes H5N2 and H5N1 have been detected. These viruses contain genes originating from both HPAI H5N8 and LPAI viruses. Reassortant viruses of the subtypes H5N2 and H5N3 have been detected in domestic birds in Taiwan. In Europe, no reassortant viruses with HPAI H5N8 genes have been detected so far. Monitoring wild birds to detect H5N8 virus and derived reassortants is warranted given their potential to cause severe disease and mortality in poultry and some species of wild birds (e.g. eagles and hawks).

Ring recoveries of migratory duck species from which H5N8 viruses have been isolated provide evidence for indirect migratory connections between East Asia and western Europe and between East Asia and North America. In addition, ring recoveries of northern pintails and Eurasian wigeons demonstrated a direct migratory connection between north India and north Russia and between north India and Europe. If these species are involved in the global spread of H5N8 virus, we hypothesise that H5N8 viruses may also spread to north India as occurred previously with HPAI H5N1 virus of clade 2.2 [28]. During large-scale surveillance activities in north India from 2009 to 2011, no avian influenza viruses had been detected in 3,522 wild bird samples [29]. To which extent migrating bird populations of different flyways come in direct or indirect contact (e.g. using the same water source during stop over) with each other needs further study. To understand the role of wild birds in the epidemiology of H5N8 virus, sampling activities need to aim at detection of both the virus and specific antibodies with an emphasis on migrating birds in north-east Europe, Russia, and north China.

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Conflict of interest

None declared.

Authors' contributions

JH: compiling the data, drafting the manuscript; HJ: initiation of study, providing data, critical review the manuscript; BN: providing data, drafting the manuscript, critical review the manuscript; RS: initiation of study, providing data, critical review the manuscript; SK: providing data Russian ring recoveries; PV: collecting field data, working on figure; OV: analysing samples; FM: collecting field data; TK: collecting field data, critical review the manuscript; RF: initiation of study, providing data, critical review the manuscript.

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