Appendix One: Avian influenza A viruses in waterbirds in Africa

(Appendix reference: Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., Cattoli, G., Hagemeijer, W., Monicat, F. 2007. Influenza A viruses in waterbirds in Africa. Emerging Infectious Diseases, 13 (6): 626-629)

Introduction

Wild waterbirds are considered to be the major natural reservoir for influenza A virus (IAV) (Webster et al. 1992). Large numbers of Eurasian breeding waterbirds over winter in the sub-Saharan region of the African continent (Del Hovo et al. 1996), where the survival of IAV is considered to be restricted by tropical environmental constraints (Stallknecht et al. 1990). There is however a knowledge gap in the ecology of IAV in tropical regions (Webster et al. 1992, Olsen et al. 2006): it is not known if IAVs circulate in waterbird communities in Africa and if tropical ecosystems can have a role in the perpetuation of IAV among waterfowl. In this study, we report about results from a large-scale surveillance in waterbirds conducted in 12 countries over the African continent (Figure A1.1).

The Study

This surveillance programme was implemented in early 2006 within the framework of FAO's Technical Cooperation Programmes of Emergency Assistance for Early Detection and Prevention of Avian Influenza. We conducted field sampling operations in partnership with national experts from wildlife and veterinary services, and in collaboration with international conservation and research organisations (AFRING, OMPO, ONCFS, SOVON, WWT), local ornithological NGOs, as well as national hunting associations and safari operators. Study species were selected among bird families recognised as major IAV reservoirs (notably Anseriformes and Charadriiformes), in both Eurasian and Afro-tropical bird communities. Study sites were selected in key sites for congregatory waterbirds, including those where Palearctic and Afro-tropical birds mix, in accordance with national surveillance programmes and field logistic constraints.

Figure A1.1: *Locations of sampling sites (or cluster of sites) in surveyed countries (dark grey) initially participating in the FAO's Technical Cooperation Programmes (light and dark grey).*

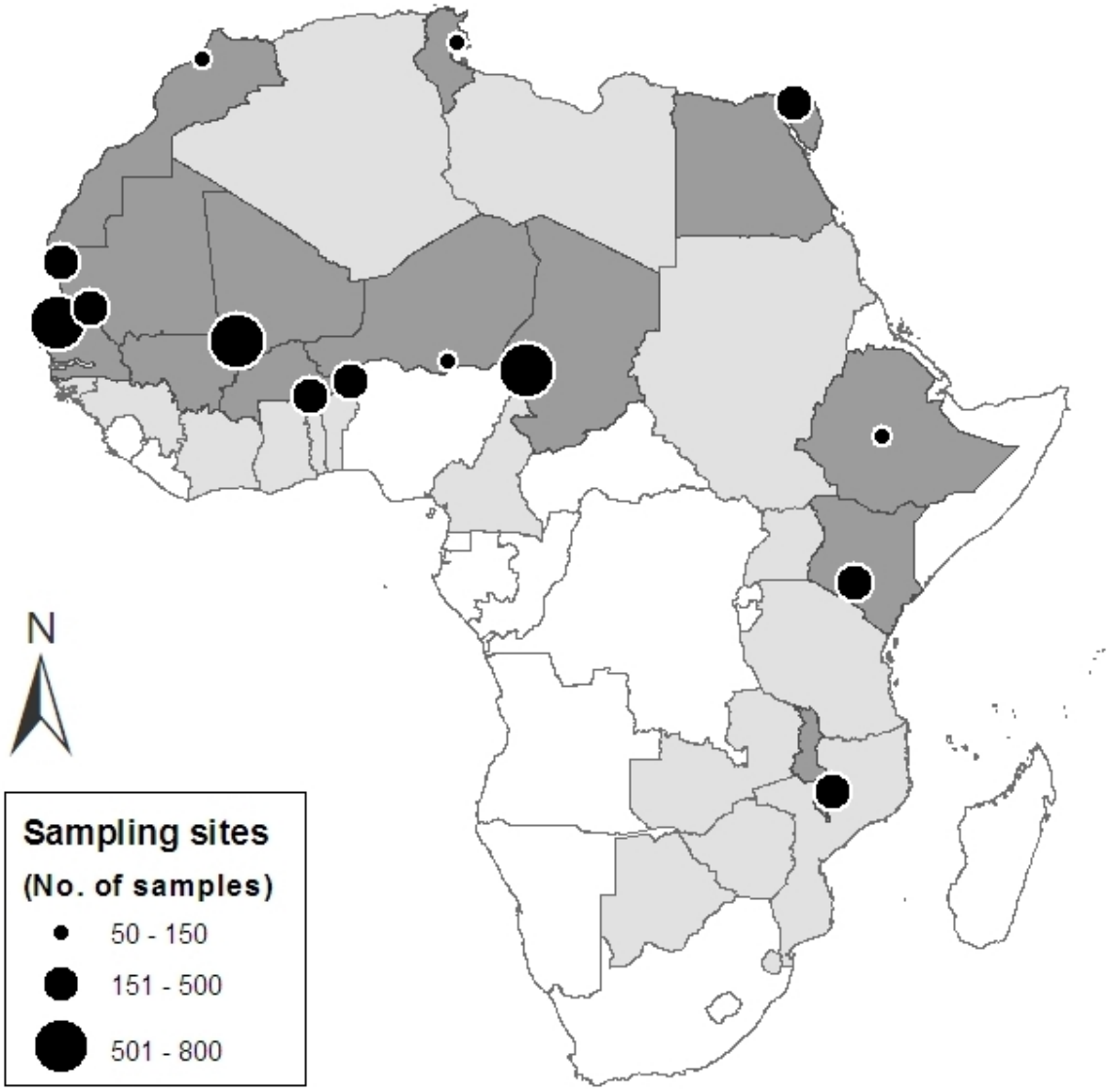

***Table A1.1**: Prevalence of influenza A virus in wild birds detected by RT-PCR.*

Bird group (No. species tested)	**No. samples tested**	**No. PCR-Positive (%)**	**RT-PCR positive bird species**	**Originating countries of positive samples**
African ducks (9)	1455	41 (2.8)	*Dendrocygna viduata* *Sarkidiornis melanotos*	Chad, Mali, Ethiopia, Mauritania, Niger, Senegal
Eurasian ducks (6)	1371	89 (6.5)	*Anas acuta* *Anas querquedula*	Chad, Mali, Mauritania, Morocco, Niger, Senegal
Gulls (3)	366	14 (3.8)	*Larus fuscus* *Larus genei*	Mauritania, Senegal
Terns (7)	159	2 (1.3)	*Sterna sp.***	Mauritania
Eurasian waders * (13)	379	6 (1.6)	*Calidris ferruginea* *Philomachus pugnax* *Tringa erythropus*	Mali, Tunisia
Rails (7)	416	3 (0.7)	*Gallinula chloropus* *Porphyrio porphyrio*	Mali
Cormorants (2)	148	0 (0)		
Others	259	0 (0)		
Total	4553	155 ***		

* Scolopacidae
** Unidentified fresh dropping samples from a multi-species flock of *Sterna caspia*, *S. maxima* and *S. sandvicensis*.
*** excluding 4 PCR positive samples from non specified wild birds.

From mid January to mid March 2006 (and May in Tunisia), we collected cloacal swabs from captured birds and from freshly killed birds provided by hunters. Fresh dropping samples were also collected at roosting areas for gulls, terns and some ducks. In Ethiopia, where there were hunting restrictions, and in countries where emergency surveillance operations were implemented following notification of H5N1 outbreaks in Nigeria (Burkina Faso, Niger), birds were shot through special permits for sample collection (n= 732).

The transport medium consisted of an isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing antibiotics (penicillin 10,000 units/ml, streptomycin 10 mg/ml, amphotericin B 25 µg/ml and gentamycin 250 µg/ml) supplemented with 10% glycerol. Samples were stored in liquid nitrogen containers in the field, or in a classic freezer before storage in a deep freezer (-80°C), and were shipped to laboratories in dry liquid containers or cryopacks.

Samples were analysed at the Istituto Zooprofilattico Sperimentale delle Venezie (Italy), except for samples from Egypt analysed at the US Naval Medical Research Unit-3 (Egypt), from Kenya and Malawi at the Agricultural Research Council Onderstepoort Veterinary Institute (RSA) and from Tunisia at the Southeast Poultry Research Laboratory USDA/ARS (USA). The samples were all screened by real-time RT-PCR specific for type A influenza viruses (Spackman et al. 2002), and positive samples were tested by RT-PCR specific for H5 subtype. All type A positive samples were subsequently processed for virus isolation using standard methods. Briefly, 100 µl of the original sample were inoculated into the allantoic cavity of 9-10 day-old embryonated specific pathogen free eggs for virus isolation attempts according to EU Directive 92/40. Haemagglutinating isolates were characterized by haemagglutination-inhibition test and neuraminidase inhibition test using specific hyperimmune chicken antisera to the reference strains of influenza virus (Alexander

et al. 1979). Molecular pathogenicity of H5 subtype positive samples was determined by sequencing the haemagglutinin gene segment. Sequences were performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) in a 3100-Avant Genetic Analyzer (Applied Biosystems).

A total of 4553 birds were tested (Table A1.1), consisting of a majority of Afro-tropical and Eurasian ducks (32% and 30% of samples respectively). Other samples originated mostly from gulls and terns (11%), rails (9%), waders (8%) and cormorants (3%). The overall detection of IAV was 3.5 % (n=159 RT-PCR positive samples, including both cloacal swabs and fresh droppings). Low pathogenic IAVs were detected in 12 bird species of ducks, waders, gulls, terns and rails, including both Eurasian and Afro-tropical bird species (Table A1.1). Positive samples were obtained from birds collected in 8 distinct countries (Chad, Ethiopia, Mali, Mauritania, Morocco, Niger, Senegal and Tunisia). In the two most frequently sampled species, a Eurasian duck (Garganey *Anas querquedula*, n=1329) and an Afro-tropical duck (White-faced Whistling Duck *Dendrocygna viduata*, n=1157), IAVs were detected from most surveyed countries, but with a highly variable prevalence (Table A1.2). No H5N1 virus was detected, nor any highly pathogenic IAV. A total of 11 samples were positive for H5 subtype, mostly from Garganey (H5 prevalence of 0.7%). Finally, five low pathogenic IAVs could be isolated: three distinct isolates originated from Garganey sampled in the Inner Niger Delta in Mali (H5N3, H11N9, H12N5), and two isolates originated from White-faced Whistling Duck sampled in Ethiopia (H8N4) and in Senegal (H1N1).

***Table A1.2**: RT-PCR based detection of influenza A virus in two wild ducks sampled in various surveyed countries.*

Species	Country	No. samples tested	No. PCR-Positive (%)
Anas querquedula	Chad	381	11 (2.9)
	Kenya	104	0 (0)
	Mali	411	22 (5.4)
	Mauritania	225	33 (14.7)
	Niger	87	4 (4.6)
	Senegal	121	17 (14.0)
Dendrocygna viduata	Burkina Faso	167	0 (0)
	Chad	232	1 (0.4)
	Ethiopia	76	10 (13.2)
	Malawi	59	0 (0)
	Mali	36	1 (2.8)
	Mauritania	183	7 (3.8)
	Niger	232	8 (3.4)
	Senegal	172	11 (6.4)

Conclusions

The African continent, and in particular its sub-Saharan region, constitutes a seasonal shelter for a large number of Eurasian waterbirds, including an estimated 5.4 million ducks that gather in Western and Eastern Africa during the northern winter (Dodman In Press). In their over-wintering sites, these birds congregate and mix with a wide variety of Afro-tropical waterbirds.

Results from this surveillance programme established that IAVs are present in wild birds in Africa during the northern winter. Low pathogenic IAVs were detected and isolated in wild birds in several major wetlands of Northern, Western and Eastern Africa, indicating that environmental conditions in Afro-tropical ecosystems are favourable to the persistence and transmission of IAV.

We detected and isolated IAV in both Eurasian and Afro-tropical species. This finding reveals that IAVs circulate in the migratory waterbirds originating from Eurasia, but also in the African species that remain in the continent all year long. Moreover, the detection of viruses in some Eurasian wader species, during both wintering (in January in Mali) and migration (in May in Tunisia), contrasts with the apparent absence of IAVs reported in previous studies of waders (Fouchier et al. 2003) in Europe. Waders being widely the most abundant African-Eurasian migratory waterbird group (Stroud et al. 2004), this result suggests that these shorebirds might play a significant role in the perpetuation and transmission of IAV in waterbird communities across continents.

The presence of IAV detected in Eurasian ducks in several of their major over-wintering sites in West Africa (e.g. the Inner Niger Delta, the Senegal River Delta and Lake

Chad) supports the former hypothesis that IAVs can persist in wild duck populations all year round through a continuous circulation in a proportion of birds (Webster et al. 1992). The different isolates obtained from Garganey in the Inner Niger Delta in Mali also indicate that various subtypes are circulating at the same time in a population, in agreement with patterns observed in Europe and North America (Fouchier et al. 2003, Krauss et al. 2004).

Various IAV subtypes were isolated from apparently healthy Garganey and White-faced Whistling Ducks indicating that both Eurasian and Afro-tropical ducks can serve as reservoirs of IAV. These results suggest that some Eurasian ducks are likely to be carriers of IAV on their northwards spring migration, but also raise the possibility for a potential persistence of IAV in the tropical region and dissemination over Africa through intra-African migratory ducks. The presence of IAV in African wintering and stop-over sites where birds from various geographical origins congregate and mix provide the opportunity for IAV to be transmitted between different bird populations and to be spread over extensive areas in both Eurasia and Africa

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Appendix Two: Influenza Surveillance in Wild Birds in Eastern Europe, the Middle East, and Africa: Preliminary Results from an Ongoing FAO-led Survey

(Appendix reference: Gaidet, N., Dodman, T., Caron, A., Balança, G., Desvaux, S., Goutard, F., Cattoli, G., Martin, V., Tripoli, A., Lamaruqe, F., Hagemeijer, W., Monicat, F. 2007. Influenza Surveillance in Wild Birds in Eastern Europe, the Middle East, and Africa: Preliminary Results from an Ongoing FAO-led Survey. Journal of Wildlife Diseases, 43: S22-S28)

Introduction

Migratory waterfowl generally are considered the natural reservoir of avian influenza (AI) virus (Olsen et al. 2006). Large numbers of waterbirds that breed in the Palearctic over-winter on the African continent. In the context of the spread of the highly pathogenic avian influenza (HPAI) Asian lineage H5N1 virus through Eurasia during summer 2005, concerns arose that this virus could be spread southward toward Africa in wild birds during fall migration. In November 2005, the Food and Agriculture Organization (FAO) set up five regional Technical Cooperation Programmes (TCP) of Emergency Assistance for Early Detection and Prevention of AI, in five regions of Eastern Europe, the Middle East, and Africa. These programmes were developed to provide on a country basis support for strengthening emergency preparedness against the potential introduction and progressive spread of HPAI H5N1 virus within these regions, specifically in relation to migration of and trade in wild birds, and the interface between wild birds and domestic poultry.

The FAO has been collaborating with national veterinary services, national wildlife institutions and international collaborating centres **(Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD);** Istituto Zooprofilattico Sperimentale delle Venezie; Royal Veterinary College, University of London; and Wetlands International) to strengthen field surveillance and laboratory diagnostic capacities through training and capacity building. A risk analysis procedure was implemented for the development of contingency action plans to strengthen early warning of and early reaction to HPAI introduction. These TCPs also aimed to promote the development of capacity for sharing HPAI disease intelligence through the establishment of information and technology network linkages within and between the regions, in relation to the development of a global system for HPAI surveillance.

Within the framework of these TCPs, a surveillance study was launched in early 2006 to evaluate if HPAI H5N1 virus could be perpetuated in wild bird populations in countries where HPAI H5N1 outbreaks occurred or may occur considering the movement of wild birds. The objective also was to provide technical support to national surveillance programmes through capacity building, and to standardise field procedures.

Methods

Implementation of the field surveillance campaign was coordinated by CIRAD and Wetlands International, in partnership with national wildlife and veterinary services. The investigations targeted natural sites where waterbirds from various breeding grounds congregate and mix, hence providing the opportunity for AI virus to be transmitted among various host populations and spread over extensive geographical ranges. Study sites were selected in accordance with national surveillance programmes and field logistic constrains. Operations were conducted during 7 to 10-day sampling periods. With the spread of HPAI H5N1 over the TCP region in the course of the survey period, complementary sampling sites were identified in the proximity of recent notified outbreaks (in particular Egypt, Niger, and Burkina Faso; Figure A2.1;Table A2.1).

Target species were selected among bird families recognised as major AI reservoirs (notably Anseriformes and Charadriiformes), in both Eurasian and Afro-tropical bird communities. A restricted number of species were targeted in each study site to maximize the number of samples collected per species.

***Table A2.1**: Avian influenza surveillance campaign results in Eastern Europe, the Middle East, and Africa in early 2006. (Bird group is indicated only for sample number > 20% total number collected at each sampling site: ED Eurasian Ducks, AD African Ducks, WD Waders, RL Rails, GT Gulls and Terns, CM Cormorants, HS Herons and Storks).*

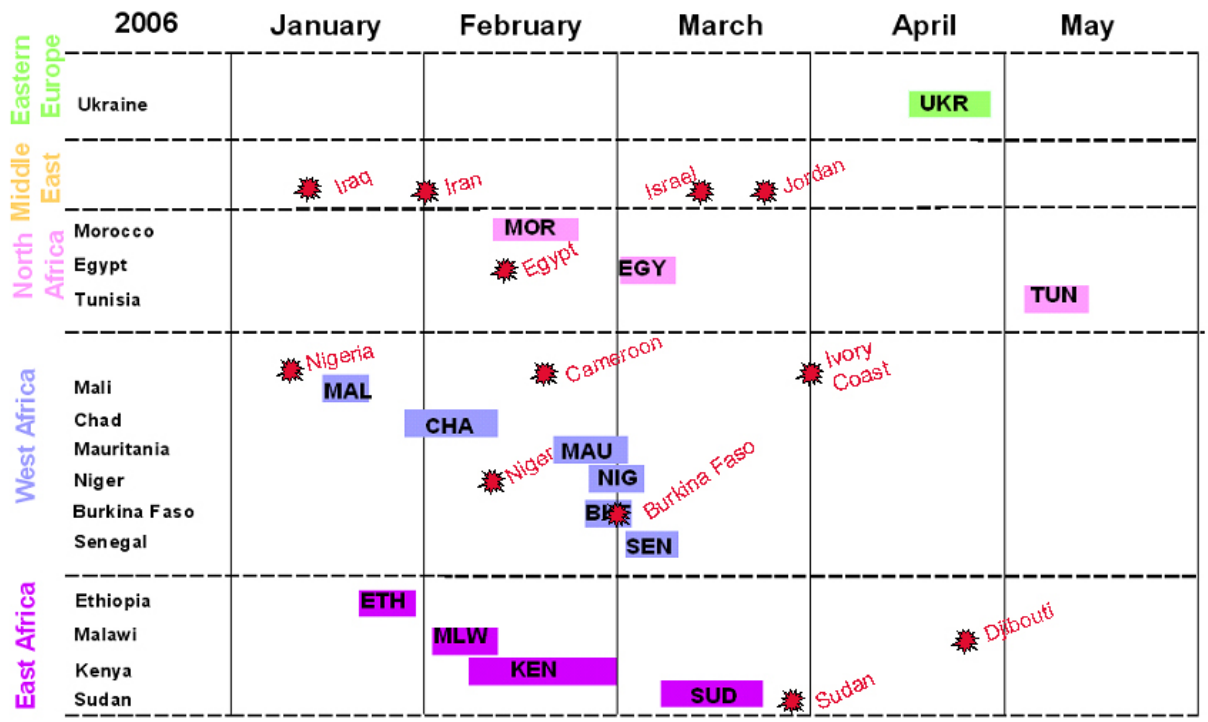
TCP	Area	Country	Sampling site	Period	Samples	Bird Group	Laboratory
Eastern Europe	Black Sea	Ukraine	Sivash / Askania-Nova Biospheric Reserve	April	344	WD	CRL
North Africa	Nile Delta	Egypt	Lake Manzala	March	244	GT, CM	NAMRU3
	Atlantic coast	Morocco	Bas Loukkos marshland	February	91	ED, WD, RL	IZS
	Mediterranean coast	Tunisia	Thyna salt-pans, Sfax	May	51	WD	SEPRL
Western Africa	Lake Chad Basin	Chad	Douguia	February	740	ED, AD	IZS
		Niger	Maradi and Zinder regions	February	98	ED, AD	IZS
	Southern Niger	Niger	Gaya region	March	276	ED, AD	IZS
	South East Burkina Faso	Burkina Faso	Lake Kompienga	February	349	AD, WD	IZS
	Inner Niger Delta	Mali	Mopti region	January	692	ED, WD	IZS
	Senegal Delta	Mauritania	Diawling NP, Lake Aleg	February	462	ED, AD	IZS
		Senegal	Djoudj NP, Langue de Barbarie NP	March	460	ED, AD, GT	IZS
	Atlantic coast	Mauritania	Banc d'Arguin NP	February	279	GT	IZS

Eastern & Southern Africa	Eastern Africa	Ethiopia	Lake Awasa, Debre Zeit, Longano, Ziway	January	115	AD	IZS
	Eastern Africa	Kenya	Dandora Sewage Works, Nairobi	February	286	ED, RL, WD	OVI
	Eastern Africa	Sudan	Am Gar	March	356	WD, HS	Not analysed
	Southern Africa	Malawi	Lake Chilwa	February	413	RL, AD	OVI
Total		**14 countries**				**5,256 samples**	

CRL (Community Reference Laboratory, Weybridge, UK); IZS (Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italia); NAMRU3 (US Naval Medical Research Unit-3, Cairo, Egypt); OVI (Agricultural Research Council Onderstepoort Veterinary Institute, South Africa); SEPRL (Southeast Poultry Research Laboratory, USDA/ARS, USA).

NP National Park

Figure A2.1: *Concomitance in timing of field campaigns in surveyed countries (sampling periods distributed along a temporal axis) and first HPAI H5N1 reported outbreaks (OIE-World organisation for animal health notification reports) in the surveyed regions between January and May 2006.*



Samples were collected by teams of national and international experts, in collaboration with international conservation and research organizations (AFRING, OMPO-Oiseaux Migrateurs du Paléarctique Occidental, ONCFS-Office National de la Chasse et de la Faune Sauvage, SOVON-Dutch centre for Field Ornithology, WWT-Wildfowl & Wetlands Trust), local ornithological organizations, and national park departments, as well as national hunting associations and safari operators. Cloacal swabs were collected from recently killed birds provided by hunters, and from live-caught birds. In countries with hunting restrictions (Ethiopia) and in countries where emergency surveillance operations were implemented following notification of HPAI H5N1 outbreaks in Nigeria (Burkina Faso, Niger), birds were shot through special permits for sample collection (n= 732). Fresh faecal samples were collected on some occasions at roosting areas for gulls and terns (Laridae) and ducks (Anatidae). Duplicate sampling was performed in the field in order to submit samples to both national and international reference laboratories. The transport medium consisted of an isotonic phosphate buffered saline (PBS), pH 7.0-7.4, containing the antibiotics penicillin (10,000 units/ml), streptomycin (10 mg/ml), amphotericin B (25 µg/ml), and gentamycin (250 µg/ml) supplemented with 10% glycerol. Samples were stored in the field in liquid nitrogen containers or on ice and then stored at < -70°C after a few hours (generally <4h, maximum 24h). They were shipped in dry ice in cryopacks until processed.

Most samples were processed at IZS delle Venezie-Italia, while some samples were analysed in other laboratories (Table A2.1). The samples were all screened by real-time RT-PCR specific for type A influenza viruses (Spackman et al. 2002), and positive samples were then tested by RT-PCR specific for H5 subtype. The molecular pathogenicity of all H5 positive samples was determined by sequencing the haemagglutinin gene segment. Sequences were performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) in a 3100-Avant Genetic Analyzer (Applied Biosystems). On the other

hand, all type A positive samples were subsequently processed for virus isolation using standard methods. Briefly, 100 µl of the original sample were inoculated into the allantoic cavity of 9 to 10-day embryonated specific pathogen free (SPF) eggs for virus isolation attempts according to European Union Directive 92/40. Haemagglutinating isolates were characterized by haemagglutination-inhibition test (HI) and neuraminidase inhibition (NI) test using specific hyperimmune chicken antisera to the reference strains of influenza virus (Alexander and Spackman 1981).

Results

A total of 5,256 samples was collected in 14 countries, mostly on the African continent, and mostly between mid-January and mid-March (Table A2.1, Figure A2.1). Field surveillance operations were postponed in Romania due to severe weather conditions and in Iran and Turkey because of delayed official approval from national authorities.

Samples were collected from 87 bird species, with 17 species representing 90% of all samples collected. A majority of these samples originated from Anatidae, including both Afro-tropical ducks (30% of all samples collected, mostly white-faced whistling duck: *Dendrocygna viduata*) and Eurasian ducks (29%, mostly garganey: *Anas querquedula*). Other species consisted mostly of waders (16%), gulls and terns (11%), rails (9%) and cormorants (3%) (Table A2.2).

Table A2.2: *Prevalence of low pathogenic avian influenza virus detected by RT-PCR in wild birds.*

Bird group (No. species tested)	**No. species**	**No. tested**	**No. PCR-Positive (%)**	**PCR-positive bird species**
African ducks	9	1455	41 (2.8)	*Dendrocygna viduata* *Sarkidiornis melanotos*
Eurasian ducks	10	1409	93 (6.6)	*Anas acuta, A. querquedula, A. crecca, A. clypeata*
Eurasian waders	12	688	6 (0.9)	*Calidris ferruginea* *Philomachus pugnax* *Tringa erythropus*
Rails	7	416	3 (0.7)	*Gallinula chloropus* *Porphyrio porphyrio*
Gulls	3	366	14 (3.8)	*Larus fuscus* *Larus genei*
Terns	3	151	2 (1.3)	*Sterna sp.**

* Unidentified fresh faecal samples from a multi-species flock of *Sterna caspia*, *S. maxima,* and *S. sandvicensis*.

The overall prevalence for type A influenza viruses from samples tested by RT-PCR was 3.3% (n=159). No HPAI H5N1 virus was detected, nor any HPAI virus in the samples. Eleven samples were positive for H5 subtype, mostly from garganey (n=10), representing an H5 prevalence of 0.7% in this species.

Low pathogenic avian influenza (LPAI) viruses were detected in 14 species of 5 bird families (Anatidae, Rallidae, Scolopacidae, Laridae, Sternidae), including both Eurasian and Afro-tropical bird species (Table A2.2) from 8 countries (Chad, Ethiopia, Mali, Mauritania, Morocco, Niger, Senegal, and Tunisia). LPAI viruses were detected in Palearctic migratory waterbirds in their over-wintering sites in Africa, including ducks (garganey, northern pintail *Anas acuta*, green-winged teal *A. crecca,* and northern shoveler *A. clypeata*), waders (curlew sandpiper *Calidris ferruginea,* ruff *Philomachus pugnax*, spotted redshank *Tringa erythropus*), gulls (lesser black-backed gull *Larus fuscus*), as well as in some Afro-tropical waterbirds, including ducks (white-faced whistling duck, knob-billed duck *Sarkidiornis melanotos*), gulls (slender-billed gull *Larus genei*) and rails (purple swamphen *Porphyrio porphyrio*). In Anatidae, a higher prevalence was detected in Eurasian ducks (6.5%) than in Afro-tropical ducks (2.8%) (chi-square test, $p< 0.001$).

Five viruses were isolated in embryonated eggs from the 159 RT-PCR positive samples. Three distinct isolates were obtained from garganey in the Inner Niger Delta in Mali, and two isolates were recovered from white-faced whistling duck in Ethiopia and in Senegal (Table A2.3).

***Table A2.3**: Virus subtypes isolated from the RT-PCR positive samples.*

Species	Country	Virus isolate
Anas querquedula	Mali	H5N3 LPAI
	Mali	H11N9
	Mali	H12N5
Dendrocygna viduata	Senegal	H1N1
	Ethiopia	H8N4

Discussion

Little information is available about circulation of influenza viruses in waterbirds on the African continent, and the potential for transmission of AI viruses between Eurasia and Africa is poorly understood (Olsen et al. 2006). Our results are the first large-scale AI surveillance in waterbirds over the African continent and beyond.

No evidence was found of HPAI H5N1 virus circulating in wild birds, including samples collected in countries that had experienced recent avian influenza outbreaks, some of which were ongoing at the time of the surveys. However, this absence of H5N1 viruses among thousands of samples investigated must be interpreted in relation to the millions of waterbirds gathering in African wetlands during the northern winter. This outcome is coherent with the absence of H5N1 virus reported from recent surveillance programmes in European countries (EFSA 2006, Pitman et al. 2007) and with the very limited detection rate of H5N1 virus so far from healthy wild bird populations (Chen et al. 2006). However, results from experimental infection in ducks indicate that, contrary to other AI viruses, HPAI H5N1 virus concentration may be higher in the trachea than in the cloaca (Sturm-Ramirez et al. 2004, Hulse-Post et al. 2005), suggesting that HPAI H5N1 virus could have potentially remained undetected in the cloacal and faecal samples we tested.

Avian influenza virus was detected from cloacal swabs and fresh faeces collected from white-faced whistling duck, including samples originating from the same study site (i.e. the Senegal delta). Similar to temperate regions, the collection of freshly deposited faecal droppings can provide a valid method for monitoring LPAI virus presence in tropical regions.

An unusually low virus isolation rate was however obtained from the type A RT-PCR positive samples. Major attention was given to appropriate storage of all samples at <-70°C, and to the preservation of the cold chain from the field to the lab. Nevertheless, logistic

constrains in some remote field sampling areas and unexpected international shipment delays may account for this low recovery rate.

The measurement of AI virus prevalence in wild birds in Africa provides new insights into the host ecology of AI virus in tropical regions. LPAI viruses were detected in both Palearctic and Afro-tropical waterbirds in several sampling sites, indicating that viruses were circulating in Africa during the northern winter (Appendix Two - Gaidet et al. 2007).

The detection of LPAI viruses in Eurasian ducks in several of their major over-wintering sites in West Africa (i.e. Lake Chad, Inner Niger and Senegal River deltas) supports the hypothesis that AI viruses persist in wild duck populations through a continuous circulation in a proportion of birds. The different viruses isolated from Garganey sampled in the Inner Niger Delta in Mali also indicate that various subtypes are circulating at the same time in a single wintering population, in agreement with patterns observed in Europe and North America (Fouchier et al. 2003, Krauss et al. 2004).

The detection of viruses in some Eurasian wader species contrasts with the apparent absence of AI viruses reported in previous studies of waders in Europe (Fouchier et al. 2003), but is consistent with results founds in surveillance in North America (Krauss et al. 2004). Several Afro-tropical bird species from various bird families also were found positive for LPAI viruses, raising the possibility of a potential persistence of AI viruses in the tropical environment all year round.

Results from this large-scale surveillance study provide evidence that LPAI viruses circulate in wild birds in sub-tropical environments during the northern winter, including in Eurasian waterbirds wintering in sub-Saharan Africa before their northwards spring migration. This suggests a potential role of tropical regions for the perpetuation of some AI viruses and in their potential intercontinental transmission. At the same time, no evidence was

found for the transmission of HPAI between Eurasia and Africa through bird migration. Such findings stress the need to improve our understanding of the host ecology of AI viruses, in particular in sub-tropical and tropical regions, which should contribute to the prevention and control of HPAI. During fall 2006 and in winter 2007, this surveillance programme implemented within the framework of the TCPs will be replicated and extended over Eastern Europe, the Middle East, and Southern Africa.

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