EcoHealth
DOI: 10.1007/s10393-010-0356-4

EcoHealth

Short Communication

Persistence of Low Pathogenic Avian Influenza Virus in Waterfowl in a Southern African Ecosystem

Alexandre Caron,[1,2,3] Celia Abolnik,[4] Josephine Mundava,[5] Nicolas Gaidet,[2] Christina E. Burger,[4] Bontsi Mochotlhoane,[4] Leo Bruinzeel,[6,7] Ngoni Chiweshe,[1] Michel de Garine-Wichatitsky,[1,2] and Graeme S. Cumming[6]

[1]*UPR AGIRs, Department ES, Cirad, 37 Arcturus Road, Highlands, P.O. Box 1378, Harare, Zimbabwe*
[2]*UPR AGIRs, Department ES, Cirad, Montpellier, France*
[3]*Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa*
[4]*ARC-Onderstepoort Veterinary Institute, Pretoria, South Africa*
[5]*Forestry Resources and Wildlife Management, National University of Science and Technology, Bulawayo, Zimbabwe*
[6]*Percy FitzPatrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Rondebosh, South Africa*
[7]*Altenburg & Wymenga Ecological Consultants, Veenwouden, The Netherlands*

Abstract: Waterfowl were counted and sampled in a Zimbabwean wetland over 24 months. LPAI strains were detected during 20 consecutive months, providing evidence of regional yearly persistence of LPAI. We discuss the role of Afro-tropical ducks in viral maintenance and transmission, and attempt to explain the observed patterns.

Keywords: avian influenza, waterfowl, Africa

Low pathogenic avian influenza (LPAI) viruses in the Northern Hemisphere are maintained by their waterfowl reservoirs—mainly Anseriformes—and the environment (Webster et al., 1992; Olsen et al., 2006). Waterfowl can provide a source of LPAI strains for domestic avian populations, in which they can evolve into highly pathogenic avian influenza (HPAI) (Abolnik et al., 2007, 2009; Caron et al., 2009). Previous studies have established the presence of LPAI in paleartic migrants and Afro-tropical birds in Africa, but no maintenance mechanism has been described (Abolnik et al., 2006; Gaidet et al., 2006). A priori, the high temperatures experienced by Afro-tropical regions should decrease the potential survival of the virus in the environment, and hence prevent the persistence of LPAI throughout the year (Brown et al., 2009). In this study, we present results from a longitudinal survey of LPAI in waterfowl conducted in Zimbabwe in 2007–2009 to test their potential persistence in an African ecosystem.

The study was undertaken in the Manyame catchment (30°30′30″, 17°45′00″), 35 kilometers West of Harare, on two adjacent lakes (Lakes Chivero and Manyame, 65 and 185 km^2, respectively) (Fig. 1). Both lakes are important waterfowl habitats in Zimbabwe. Although paleartic Anseriformes seldom reach this area during their migration, it hosts a range of other paleartic waterbirds as well as passerines and raptors during September–April (Fig. 2a). In addition, Afro-tropical nomadic and resident species inhabit these lakes. Waterfowl were counted and captured every 2 months between May 2007 and March 2009, resulting in 12 count and capture sessions encompassing 2 years. Fifteen representative sites were selected using

Correspondence to: Alexandre Caron, e-mail: alexandre.caron@cirad.fr

Published online: 02 December 2010

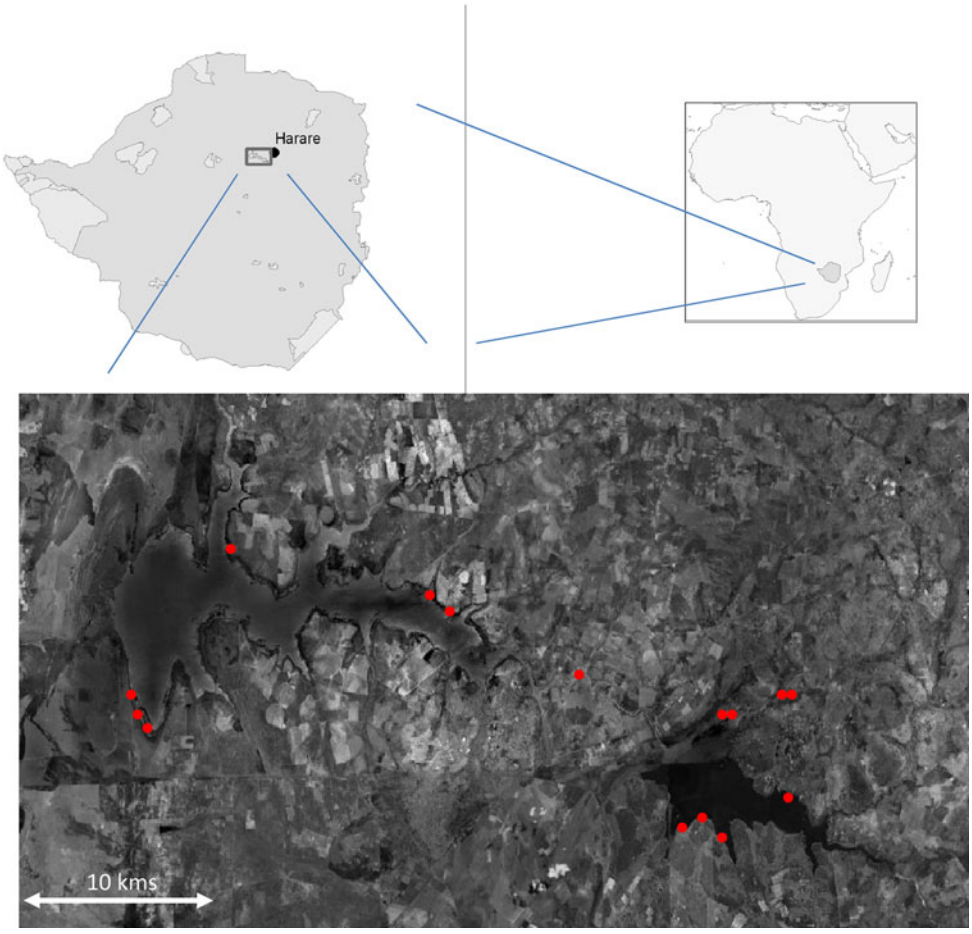

Figure 1. Counting and capture sites (*red dots*) in Manyame (*left*) and Chivero (*right*) Lakes, with location of the ecosystem in Zimbabwe (light gray areas represent national parks of Zimbabwe), and location of Zimbabwe in Africa. Lakes' satellite pictures obtained through Google Earth® database.

local ornithological expertise (Fig. 1). For each session, four point counts were done in each site at different times of day. Counts lasted half an hour and included all birds within a 150-m radius. After a week of site preparation (baiting), captures were performed during a week in suitable counting sites using baited walk-in traps and mist-nets. For each bird captured, cloacal and tracheal swabs were collected, placed in viral transport medium (phosphate-buffered saline/glycerol/antibiotics), and transported in liquid nitrogen prior to testing. RNA was extracted using the MagNaPure LC total nucleic acid isolation kit-high performance (Roche, Mannheim, Germany) on a MagNAPure robot (Roche). Real-time reverse transcription PCR was performed using the VLA TaqMan® Influenza A/H5/H7 Detection Kits (Applied Biosystems) on either a StepOnePlus (Applied Biosystems, Carlsbad, CA, USA) or a Light Cycler 480 (Roche) platform. Positive samples were inoculated into embryonating fowls' eggs for virus isolation according to standard procedures (OIE, 2008).

A total of 1601 waterbirds were captured. The number of birds captured averaged 133 per session (with a minimum of 61 and a maximum of 247) (Fig. 2b). These captures represented 96 species. Anseriformes constituted 46.8% of captures, and were dominated by two species, viz. *Anas erythrorhyncha* (red-billed teal, 66.5%) and *Dendrocygna viduata* (white-faced whistling duck, 26.9%). The composition of birds sampled per order is reported in Table 1.

This is the first report, to our knowledge, of the persistence of LPAI strains over a year in waterfowl in an African wetland. LPAI strains were detected in the waterfowl community during 10 consecutive sessions, over a period of 20 months, with a prevalence ranging from 1.3 to 22.3% (Fig. 2c). Of 2791 (51.1% cloacal, 48.9% tracheal) test results, 100 samples were positive for the presence of RNA of the influenza A virus group (95 birds, 5.9% of total of birds), 9 (0.6%) and 10 (0.6%) were positive for the H5 and H7 subtype, respectively (Table 2); 49.5% of positive birds were ducks, of which 93.7% were *Anas erythrorhyncha* (Anatidae) or *Dendrocygna viduata* (Dendrocygnidae). Global prevalence per session varied between 0.0 and 22.3% across species, and between 0.0 and 20.0% for Anseriformes (Fig. 2c). No viruses could be isolated.

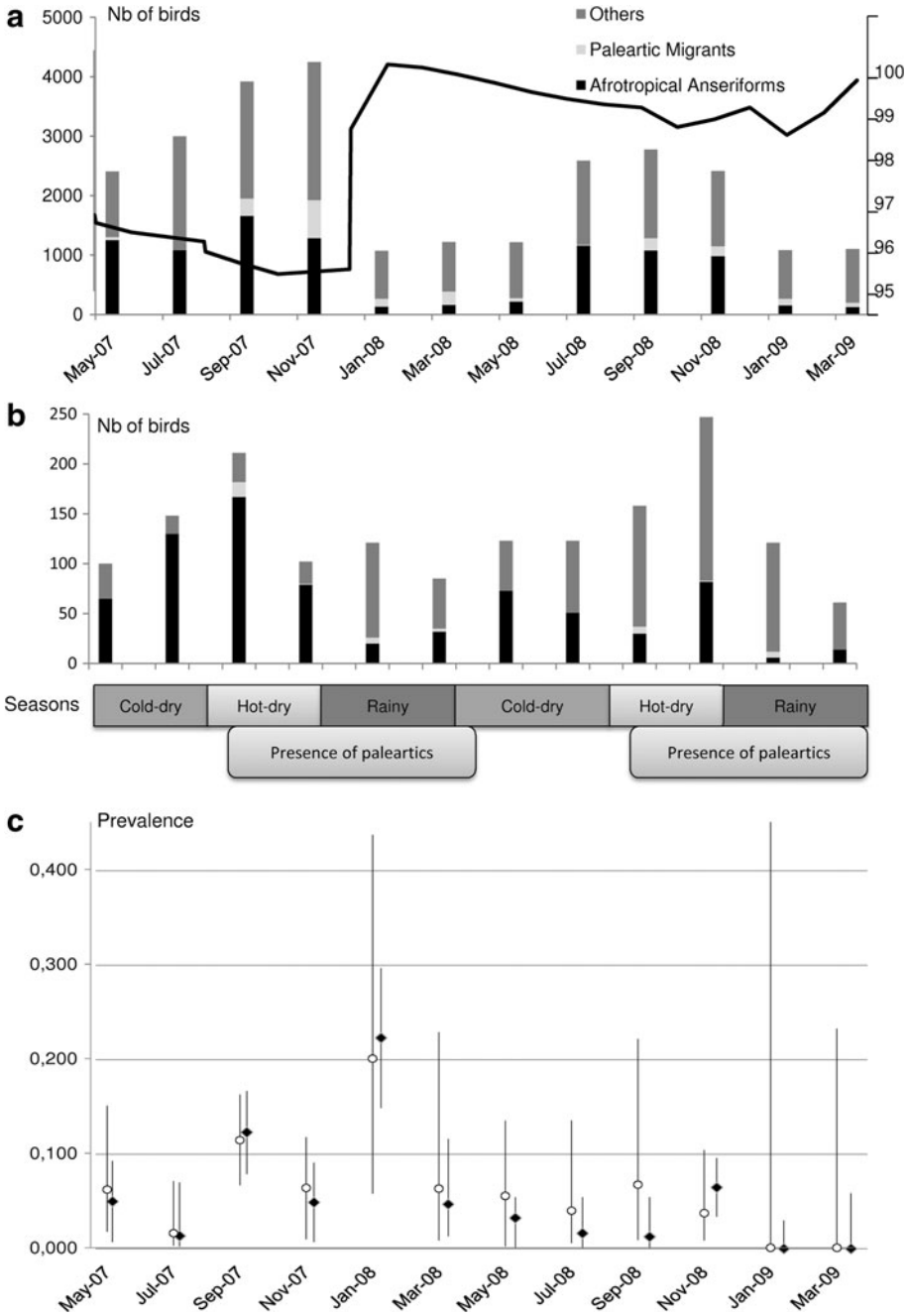

Figure 2. **a** Birds counted per session: in black duck species, in light gray paleartic migrants, and in dark gray other species. The *solid line* (linked to the right vertical axis) represents the variations of the lake level. **b** Birds captured per session: in black duck species, in light gray paleartic migrants, and in dark gray other species. **c** Global (*black dots*) and duck (*white dots*) prevalence per session with confidence interval. An indication of the seasons in this ecosystem is given in blocks; the period when paleartic migrants are present in the system is also presented.

In both years, viruses were detected in the waterbird community during the period when paleartic birds are absent or rare (May–July sessions). This result suggests the yearly persistence of LPAI in Afro-tropical waterfowl, and raises the hypothesis of an endemic cycle in Zimbabwe, or at least in Southern Africa. However, Table 2 indicates higher prevalence when the paleartic birds are present in the ecosystem (mainly from September to January), compared to when they are absent (May–July). Therefore, avian influenza viruses (AIV) appear to be present all year long, although we cannot exclude the necessity of seasonal introduction of AIV by paleartic birds in September to maintain the cycle. Observing viral persistence, we cannot prove maintenance of LPAI in this ecosystem.

The detection of subtypes H5, H7, and other influenza A viruses (subtypes undetermined) in ducks indicates the simultaneous circulation of multiple subtypes. The H7 subtype was detected during 14 consecutive months, although not for each session (4 out of 12 sessions), during both dry and wet season and on 2 consecutive years. From these data, one cannot deduce that the H7 or H5 subtypes detected in subsequent years were related. However, the

Table 1. Sample size per session and proportion of swabs sampled per order[a]

Swab results (c + t) :	May-07 $n = 200$	Jul-07 $n = 279$	Sep-07 $n = 414$	Nov-07 $n = 161$	Jan-08 $n = 227$	Mar-08 $n = 163$	May-08 $n = 216$	Jul-08 $n = 218$	Sep-08 $n = 271$	Nov-08 $n = 356$	Jan-09 $n = 194$	Mar-09 $n = 92$	Total $n = 2791$
Anseriformes	66.0	87.8	79.0	79.5	16.3	37.4	59.3	41.3	16.6	33.7	5.7	22.8	48.2
Charadriiformes	29.0	10.8	19.1	17.4	60.4	27.6	16.7	43.6	52.0	48.3	67.5	33.7	35.2
Passeriformes	1.0	0.7	1.4	0.6	16.3	26.4	10.2	9.2	17.7	12.4	7.2	8.7	8.8
Columbiformes	0.0	0.7	0.0	0.0	0.0	2.5	4.6	3.2	3.0	1.4	9.8	10.9	2.3
Coraciiformes	1.0	0.0	0.0	1.2	6.6	3.7	3.7	0.0	5.2	1.1	1.0	10.9	2.3
Ciconiiformes	1.0	0.0	0.0	0.0	0.4	1.2	0.9	0.0	4.8	2.2	6.7	8.7	1.8
Divers	2.0	0.0	0.5	1.2	0.0	1.2	4.6	2.8	0.7	0.8	2.1	4.3	1.4

[a]Swab results (c + t) indicates the number of swabs (cloacal or tracheal) tested per session. The following rows indicate percentage of swabs belonging to different bird orders.

Table 2. Prevalence and sample size for all birds sampled and ducks only sampled compared between sessions when palearctic birds are present in the ecosystem (September–March) and absent (May–July)[a]

	May–July		Sept–Mar		χ^2	P
	Prev	n	Prev	n		
All birds	2.63	494	7.23	1106	13.19	<0.001
Ducks only	3.76	319	8.14	430	5.94	<0.01

Prev, prevalence (in percent); *n,* sample size.
[a]Results for χ^2 test and level of significance, testing the null hypothesis H0: Prev (May–July) = Prev (Sept–Mar).

results do suggest that, at least for some subtypes, some strains may be maintained throughout the year and between years. The H5 subtype was detected only during the January 2008 session (Table 3), but in a relatively high prevalence; H5 does not appear to be a common H subtype in waterbird communities (Krauss et al., 2004; Wallensten et al., 2007).

The high prevalence that we observed during the 2007 hot-dry season (September–November), and beginning of the rainy season (January 2008), is comparable to the prevalence level reported in sites in the Northern hemisphere during fall migration (September) (Krauss et al., 2004; Wallensten et al., 2007). The seasonal variations in prevalence have to be interpreted with caution because the sample composition in species and abundance varied between sessions, due to capture bias (Fig. 2b). For example, January 2008 and 2009 report the highest and lowest densities, both with small sample size. However, the profile of Anseriformes' prevalence (controlling for part of this bias) is similar in trends and intensity to global prevalence. A small sample size of ducks was always associated with small numbers of ducks counted (Fig. 2a, b). During the hot-dry season, ducks tend to concentrate in lakes, and these flocks provide ample opportunity for disease transmission. During the rainy season (December–March), ducks tend to disperse out of the study lakes to breed; our sample size reflects these movements. During the first rainy season (January 2008), viruses were circulating despite few ducks in the system (four ducks out of 27 positive birds), suggesting a complementary role of local and palearctic Charadriiformes and Passeriformes in the persistence process (Tables 1, 3).

Environmental conditions in this ecosystem (1500m altitude and an average annual temperature of 17.9°C) are

Table 3. PCR-positive swabs for avian influenza and hemagglutinase type when available[a]

Swab positive :	May-07 p = 5	Jul-07 p = 2	Sep-07 p = 26	Nov-07 p = 5	Jan-08 p = 34	Mar-08 p = 4	May-08 p = 4	Jul-08 p = 2	Sep-08 p = 2	Nov-08 p = 16	Jan-09 p = 0	Mar-09 p = 0	Total p = 100
Anseriformes	4*na 1*H7	2*na	17*na 2*H7	5*na	3*na 3*H5	2*na	4*H7	2*na	1*na	3*na	0	0	39*na 3*H5 7*H7
Charadriiformes	0	0	5*na 1*H7	0	14*na 4*H5 2*H7	2*na	0	0	0	8*na	0	0	29*na 4*H5 3*H7
Passeriformes	0	0	1*na	0	5*na 1*H5	0	0	0	1*na	5*na	0	0	12*na 1*H5
Coraciiformes	0	0	0	0	1*na 1*H5	0	0	0	0	0	0	0	1*na 1*H5

[a]Swab positive indicates the number of swabs positive (p) per session. The following rows indicate the number of swabs tested PCR-positive for avian influenza; the hemagglutinase type is determined (H5 or H7) or not available (na).

typical of Eastern and Southern African highland, and could be compatible with virus survival in the water (Stallknecht et al., 1990). We failed to detect any positive samples during the 2009 rainy season. This result may be explained by a small sample size or by the nonpersistence of LPAI strains in this ecosystem. The observed concentration of Afro-tropical ducks on the lake shores during the hot-dry season is driven by a combination of: increased resource availability on receding lake shores (Fig. 2a); the complete drying-up of nonperennial wetlands, leaving the ecosystem with few water bodies to be occupied by the same bird community; and the need for the birds to undergo flightless moult in a deepwater location. This influx of waterbirds into the ecosystem offers numerous opportunities for viral introductions from other African regions (Afro-tropical nomadic species) or from Eurasia (paleartic migrants) (Abolnik et al., 2006). In addition, this concentration of hosts during the hot-dry season, when the viruses should have the lowest survival in the environment, may contribute to viral maintenance; the high density of birds provides higher host availability and higher rate of contact between hosts, decreasing the time necessary for successful fecal–oral transmission. The seasonal aggregation of ducks in the Manyame catchment could explain the higher prevalence observed during the first hot-dry and early rainy season. The combination of favorable environmental conditions during particular seasons (rainy and dry-cold season) and the presence of paleartic birds and high duck density during harsh season (hot-dry) could support a persistence or maintenance hypothesis in this ecosystem, although we cannot resolve the effects of specific factors. Environmental transmission has been shown to play an important role in viral persistence in stochastic models of disease transmission (Breban et al., 2009; Rohani et al., 2009).

The observed interannual variation (including the non persistence in 2009) could be explained by variability in environmental factors. Rainfall determines lake levels and triggers a differential waterfowl concentration during the following hot-dry season. This hypothetical relation can be observed in Fig. 2a: a low lake level is associated with a high bird concentration in 2007, in contrast to 2008. Viral persistence (driven by host availability and susceptibility) could thus be dependent on waterfowl density. Our data could be interpreted as showing that, during the hot-dry season of 2008, the threshold density was not reached and the LPAI did not persist.

In conclusion, our data suggest the persistence of multiple AIV strains in the waterfowl community of a

Zimbabwean ecosystem for 20 consecutive months. The persistence of LPAI in an African ecosystem indicates that the role of Afro-tropical ducks in LPAI epidemiology requires further assessment. This study suggests that African ecosystems are not merely passive receptors of AIV from Eurasia. African waterfowl communities have the potential to harbor multiple viral strains for an extended period, indicating that they play a broadscale role in the epidemiology of AIV. The recent detection in Nigeria of HPAI H5N2 in apparently healthy waterfowl (including *Dendrocygna viduata*) reinforces this hypothesis (Gaidet et al., 2008), with phylogenetic data linking this strain to European and African waterfowl. A relation between environmental determinants, host community ecology, and virus ecology is presented with potential for a predictive approach. These observations are particularly relevant for animal and public health at the wildlife/domestic/human interface in two contexts: (a) in Africa, where HPAI H5N1 is recurring in domestic poultry production systems (Ducatez et al., 2007; Cattoli et al., 2009) (providing opportunities for recombination with local LPAI), veterinary/public health sectors may be weak, and the wild/domestic bird interface is rarely monitored or controlled despite a predicted risk (Kilpatrick et al., 2006); and (b) in Eurasia, because the spring migration of paleartic birds can theoretically expose Eurasian ecosystems to LPAI strains from Africa (although thus far, with little information available, no genes of African origin have been detected in the Eurasian viral pool) (Abolnik et al., 2006; Abolnik, 2007).

Acknowledgments

We are grateful to the many people who assisted with the bird counts and capture, particularly Fadzai Matzvimbo and Innocent Magunje. The Zimbabwe Parks and Wildlife Management Authority and the Zimbabwean Veterinary Services kindly granted permission to work in areas under their jurisdiction. This work was conducted within the framework of the ''Mesures d'Urgence'' and GRIPAVI projects, and the Research Platform ''Production and Conservation in Partnership'' (RP–PCP). It benefited from funds from the French Ministry of Foreign Affairs. Additional funding support was provided by the USAID through the Wildlife Conservation Society's GAINS (Global Avian Influenza Network for Surveillance) program, and the South African Department of Agriculture, Forestry and Fisheries.

References

Abolnik C) Detection of a North American lineage H5 avian influenza virus in a South African wild duck. *Onderstepoort Journal of Veterinary Research* 74:177–180

Abolnik C, Bisschop S, Gerdes T, Olivier A, Horner R) Outbreaks of avian influenza H6N2 viruses in chickens arose by a reassortment of H6N8 and H9N2 ostrich viruses. *Virus Genes* 34:37–45

Abolnik C, Cornelius E, Bisschop SPR, Romito M, Verwoerd D (2006) Phylogenetic analyses of genes from South Africa LPAI viruses isolated in 2004 from wild aquatic birds suggests introduction by Eurasian migrants. In: *OIE/FAO International Scientific Conference on Avian Influenza*, Basel, Switzerland: Karger

Abolnik C, Londt BZ, Manvell RJ, Shell W, Banks J, Gerdes GH, et al.) Characterisation of a highly pathogenic influenza A virus of subtype H5N2 isolated from ostriches in South Africa in 2004. *Influenza and Other Respiratory Viruses* 3:63–68

Breban R, Drake JM, Stallknecht DE, Rohani P) The role of environmental transmission in recurrent avian influenza epidemics. *PLoS Computational Biology* 5:e1000346

Brown JD, Goekjian G, Poulson R, Valeika S, Stallknecht DE) Avian influenza virus in water: infectivity is dependent on pH, salinity and temperature. *Veterinary Microbiology* 136:20–26

Caron A, Gaidet N, de Garine-Wichatitsky M, Morand S, Cameron EZ) Evolutionary biology, community ecology and avian influenza research. *Infection, Genetics and Evolution* 9:298–303

Cattoli G, Monne I, Fusaro A, Joannis TM, Lombin LH, Aly MM, et al.) Highly pathogenic avian influenza virus subtype H5N1 in Africa: a comprehensive phylogenetic analysis and molecular characterization of isolates. *PloS ONE* 4:e4842

Ducatez MF, Olinger CM, Owoade AA, Tarnagda Z, Tahita MC, Sow A, et al.) Molecular and antigenic evolution and geographical spread of H5N1 highly pathogenic avian influenza viruses in western Africa. *Journal of General Virology* 88:2297–2306

Gaidet N, Cattoli G, Hammoumi S, Newman SH, Hagemeijer W, Takekawa JY, et al.) Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. *PLoS Pathogens* 4:e1000127

Gaidet N, Dodman T, Caron A, Balança G, Desvaux S, Goutard F, et al.) Influenza A viruses in waterbirds in Africa. *Emerging Infectious Diseases* 13:626–629

Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P) Predicting the global spread of H5N1 avian influenza. *Proceedings of the National Academy of Sciences of the United States of America* 103:19368–19373

Krauss S, Walker D, Pryor SP, Niles L, Chenghong L, Hinshaw VS, et al.) Influenza A viruses of migrating wild aquatic birds in North America. *Vector Borne Zoonotic Diseases* 4:177–189

OIE (2008) Avian influenza. In: *Manual of diagnostic tests and vaccines for terrestrial animals,* Paris: OIE, pp 1–20

Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA) Global patterns of influenza A virus in wild birds. *Science* 312:384–388

Rohani P, Breban R, Stallknecht DE, Drake JM) Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion. *Proceedings of the National Academy of Sciences of the United States of America* 106:10365–10369

Stallknecht DE, Kearney MT, Shane SM, Zwank PJ) Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Diseases* 34:412–418

Wallensten A, Munster VJ, Latorre-Margalef N, Brytting M, Elmberg J, Fouchier RA, et al.) Surveillance of influenza A virus in migratory waterfowls in northern Europe. *Emerging Infectious Diseases* 13:404–411

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y) Evolution and ecology of influenza A viruses. *Microbiological Reviews* 56:152–179