EcoHealth
DOI: 10.1007/s10393-011-0684-z

EcoHealth

Original Contribution

The Ecology of Influenza A Viruses in Wild Birds in Southern Africa

Graeme S. Cumming,[1] Alexandre Caron,[2,3,4] Celia Abolnik,[5,6] Giovanni Cattoli,[7] Leo W. Bruinzeel,[1,8] Christina E. Burger,[5] Krizia Cecchettin,[7] Ngoni Chiweshe,[2] Bontsi Mochotlhoane,[5] Gregory L. Mutumi,[1] and Mduduzi Ndlovu[1]

[1]*Percy FitzPatrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Rondebosch, Cape Town, 7701, South Africa*
[2]*UPR AGIRs, Department ES, Cirad, Harare, Zimbabwe*
[3]*UPR AGIRs, Department ES, Cirad, Montpellier, France*
[4]*Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa*
[5]*ARC-Pretoria Veterinary Institute, Old Soutpan Road, Onderstepoort 0110, South Africa*
[6]*Department of Animal Production, Faculty of Veterinary Sciences, University of Pretoria, Old Soutpan Road, Pretoria 0110, South Africa*
[7]*OIE/FAO Reference Laboratory for Avian Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, viale dell'Università 10, 35020 Legnaro, PD, Italy*
[8]*Altenburg & Wymenga Ecological Consultants, P.O. Box 32, 9269 ZR Feanwalden, The Netherlands*

Abstract: Avian influenza viruses (AIVs) are pathogens of global concern, but there has been little previous research on avian influenza in southern Africa and almost nothing is known about the dynamics of AIVs in the region. We counted, captured and sampled birds regularly at five sites, two in South Africa (Barberspan and Strandfontein) and one in each of Botswana (Lake Ngami), Mozambique (Lake Chuali) and Zimbabwe (Lakes Manyame and Chivero) between March 2007 and May 2009. The South African and Zimbabwean sites were visited every 2 months and the sites in Botswana and Mozambique every 4 months. During each visit we undertook 5–7 days of standardised bird counts followed by 5–10 days of capturing and sampling water-associated birds. We sampled 4,977 birds of 165 different species and completed 2,503 half-hour point counts. We found 125 positive rRT-PCR cases of avian influenza across all sites. Two viruses (H1N8 and H3N8) were isolated and additional H5, H6 and H7 strains were identified. We did not positively identify any highly pathogenic H5N1. Overall viral prevalence (2.51%) was similar to the lower range of European values, considerable spatial and temporal variation occurred in viral prevalence, and there was no detectable influence of the annual influx of Palearctic migrants. Although waterbirds appear to be the primary viral carriers, passerines may link wild birds and poultry. While influenza cycles are probably driven by the bird movements that result from rainfall patterns, the epidemiology of avian influenza in wild birds in the subregion is complex and there appears to be the possibility for viral transmission throughout the year.

Electronic supplementary material: The online version of this article (doi:10.1007/s10393-011-0684-z) contains supplementary material, which is available to authorized users.

Correspondence to: Graeme S. Cumming, e-mail: Graeme.Cumming@uct.ac.za

Published online: 23 April 2011

Keywords: avian influenza, pathogen, epidemiology, Anatidae, South Africa, Zimbabwe

Introduction

Influenza A viruses have long been acknowledged as pathogens of global concern. In recent years, outbreaks of highly pathogenic avian influenza (HPAI) in populations of domestic and wild birds, and the related deaths of nearly 300 people (WHO, 2010), have heightened fears of a new influenza pandemic in the human population (e.g. Pickles, 2006; Enserink, 2006). Assessments of the risks that are posed by avian influenza, and the development of appropriate response strategies in the event of an epidemic or pandemic, rely heavily on a fundamental scientific understanding of avian influenza virus (AIV) dynamics in populations of domestic and wild birds (Dudley, 2008).

Although low pathogenic avian influenza (LPAI) viral prevalence in western European and North American wild bird populations has been well documented (Olsen et al., 2006), it is unclear how the long-distance movements of migratory and nomadic bird species relate to larger-scale spatial and temporal variation in AIV genotypes, maintenance, and epizootics/epidemics (Krauss & Webster, 2010; Kilpatrick et al., 2006). One of the largest single gaps in the geographical coverage of AIV sampling has been southern Africa (Olsen et al., 2006; Kilpatrick et al., 2006; Gaidet et al., 2007), a region that is at risk following the detection of highly pathogenic strains in sub-Saharan Africa north of the Zambezi (Gaidet et al., 2008; Fasina et al., 2009). Although some intriguing data exist from South Africa (such as the finding that precursors to pathogenic AIV strains are introduced to and possibly moved between ostrich farms by Egyptian Geese *Alopochen aegyptiacus*; e.g. see Abolnik et al., 2010; Abolnik et al., 2009; Sinclair et al., 2005), little relevant research has been carried out in most southern African countries.

By comparison to western Europe, southern Africa has a mild winter; highly variable and often scarce rainfall; a higher diversity of bird species; no true geese or swans; and many nomadic waterbirds but no truly migratory afrotropical *Anas* ducks (Cumming et al., 2008; Underhill et al., 1999). We tested the predictions that (1) due to its more arid environment and absence of migratory Palearctic ducks, LPAI prevalence in wild waterbirds should be lower in southern Africa than in Europe; (2) due to the presence of many opportunistic, colonial and nomadic waterbird species, and the lack of migratory corridors (Hockey, 2000), LPAI prevalence in wild birds in southern Africa should show relatively little spatial variation along longitudinal and latitudinal gradients; and (3) the arrival of Palearctic migrants in September (see Appendix 1 in Supporting information for details), including charadriids known as potential LPAI reservoirs, should create a pulse in influenza occurrences in Afrotropical species.

While exploring these fundamental assumptions for the first time, we also provide a wealth of new and useful information on AIV and wild birds in southern Africa. Our results suggest that none of our starting assumptions can be strongly supported. Some re-thinking of prevailing assumptions about influenza A viruses in southern African bird populations thus appears necessary in planning health care and risk management strategies.

Methods

Project Design and Field Sites

Data were collected in Botswana, Mozambique, South Africa and Zimbabwe from March 2007 to May 2009. We worked on a regular basis at five different sites (Fig. 1) and 12–15 sampling locations per site. We counted and sampled birds at daily, bimonthly and annual time scales. Our three core sites [Barberspan and Strandfontein in South Africa, and the Manyame catchment in Zimbabwe (including Lakes Chivero and Manyame)] were sampled every 2 months and our Botswana site (Lake Ngami) and Mozambique site (Lake Chuali) every 4 months. We also sampled a small number of birds during a single 'test' sampling mission to Massingir Dam in Mozambique. Exact sampling dates and coordinates of capture sites are given in Appendix 2 (Supporting information) and additional details on study sites in Appendix 3 (Supporting information).

Counting Protocols

Each site visit included 5–7 days of standardised bird counts followed by 5–10 days of bird captures in the same locations (Fig. 2). Counts consisted of a 10-min habituation period followed by a 30-min counting period, during

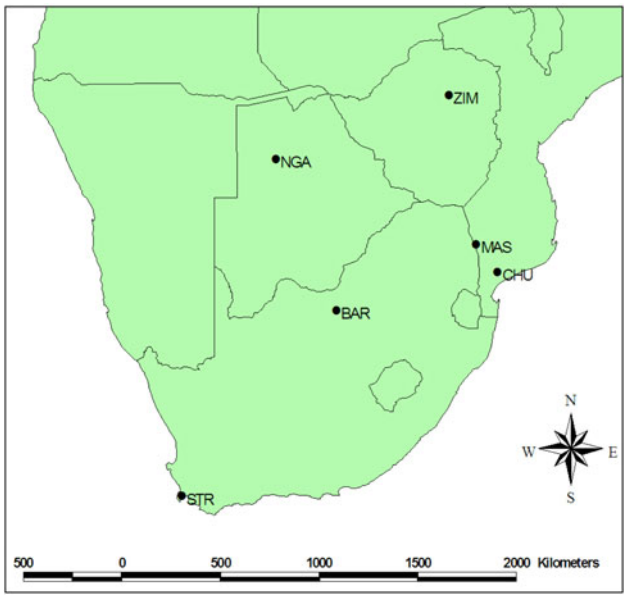

Figure 1. Map of southern Africa showing sampling sites mentioned in this article. Site codes: *ZIM* Lakes Chivero and Manyame, *NGA* Lake Ngami, *MAS* Massingir Dam, *CHU* Lake Chuali, *BAR* Barberspan, *STR* Strandfontein. Our three core sites were STR, BAR and ZIM, which fall in different biomes along a north–south latitudinal gradient.

which the number and species of all birds within a 150 m radius of the (stationary) observers were recorded. Each location was counted at four different times of day over a 5–7 day period prior to captures [additional details in Appendix 3 (Supporting information)]. Over the 2 years of the study we completed 2,503 half-hour point counts. For each of our three core sites (Barberspan, Manyame/Chivero and Strandfontein) the count data also provide estimates from 13 different points in time (i.e. every 2 months for 2 years), giving us a spatiotemporally balanced sampling design for exploring both spatial and temporal variation in the bird community.

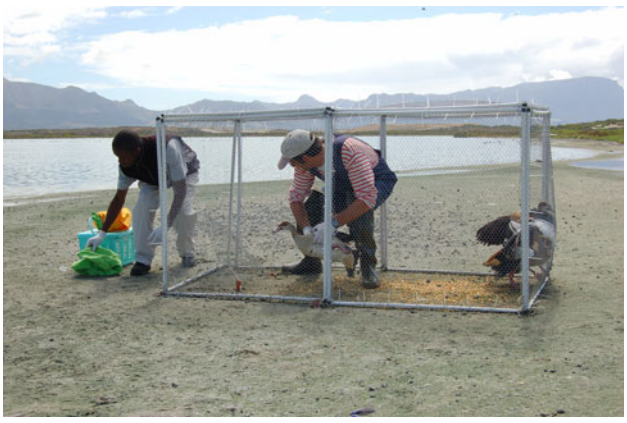

Figure 2. Example of a walk-in trap used to catch ducks. In this picture, Mduduzi Ndlovu (L) and Leo Bruinzeel (R) capture Egyptian geese at Strandfontein.

Capture and Sampling Protocols

Captures used standard procedures as detailed in Appendix 3 (Supporting information). We targeted ducks because they are considered reservoir hosts of some type A AIVs in Europe and Asia. The other sampled species were by-catch (i.e. they were captured during the process of catching ducks). In addition to ancillary data (morphometry, photographs, blood, feathers) we collected two cloacal and two tracheal swabs per bird. Birds recaptured in the same week were not resampled. All swabs were placed in cryovials in viral transport medium (Hank's salt solution with antibiotics and fungicides) and frozen in liquid nitrogen within half an hour of collection.

The swabs were stored in a −70°C freezer and transported in dry ice or liquid nitrogen to an FAO reference laboratory, either the Agricultural Research Council-Onderstepoort Veterinary Institute, Pretoria, South Africa (ARC-OVI) or the Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy (IZSVe) for analysis (see Appendix 3 in Supporting information for details). Sets of swabs were randomised by laboratory; each received the first cloacal and second tracheal swab from one bird and the second cloacal and first tracheal swab from the next bird. All samples from Botswana and Mozambique were analysed at IZSVe.

Sources of error included (1) failure to obtain a full complement of swabs, due to bird escapes or shortages of vials; (2) labelling errors; (3) loss or destruction of vials in transit and (4) mistakes in allocation of vials to laboratories. Most of these errors were random and hence unbiased. We had fewer than four swabs per bird in just under 4% of cases. Samples were only sent to IZSVe on completion of the project, giving a delay between sampling and analysis of 2–24 months that may have affected the probability of AIV detection (Forster et al., 2008).

Data Analysis

Virus prevalence was too low to determine the influence of the number of swabs on virus detection probability. Since missing swabs were $<4\%$ and randomly distributed by species, we assumed that each sampled bird (rather than

each swab) had an equal chance of viral detection. Virus prevalence was calculated as the ratio of the number of influenza viruses detected to the number of birds sampled. Since recaptures were not re-sampled during the same capture mission, and since each sampling effort was at least 2 months apart, we treated samples from recaptures (including birds that we had ringed and those ringed by others) as independent.

Having quantified virus prevalence for each species by site, we calculated overall prevalence for all bird species and all sites. Bird count summaries by site used the average number of birds counted across all point counts.

For the Palearctic migrant analysis we included all birds found in our study sites that were both listed in class 6 (i.e. intercontinental and marine migrants) of the Roberts' database (Hockey et al., 2004) and associated with wetland and estuarine habitats. A full list of Palearctic migrant species and their abundances is presented in the Supporting information in Appendix 1. The total number of foraging and non-foraging Palearctic migrants for each sampling mission was converted to a mean abundance by dividing the total count for a single mission by four, since each point count location was counted four times. Since the total numbers of birds are more relevant than their relative abundance to the role of Palearctic migrants in influenza transmission, we did not divide these data by the number of locations per site. We then used Spearman's rank-order correlations to test for a significant relationship between the number of Palearctic migrants, the abundance of anatids, and virus prevalence.

Results

We sampled a total of 4,977 birds of 165 different species, including 158 recaptures. Captures were distributed unevenly across sites (Table 1) despite comparable sampling effort, with the Zimbabwean site yielding the most birds (n = 1916), followed by Barberspan (n = 1418) and Strandfontein (n = 888). Differences in the composition of species caught resulted primarily from differences in local species composition and catchability. A full listing of the number of individuals of the 165 sampled species is provided in Appendix 1 (Supporting information). Some of the data from Zimbabwe have been presented previously by Caron et al. (2010a).

From 4,977 sampled birds, 125 were influenza A positive, giving a prevalence across all species and sites of 2.51%. The probability of an influenza-positive sample being from a cloacal or a tracheal swab was almost identical (n = 125, P = 0.48 vs. P = 0.52 for cloacal and tracheal swabs, respectively; 5 birds were positive on both cloacal and tracheal swabs, one on both tracheal swabs, and none on both cloacal swabs). Infuenza A virus prevalence across different bird families was uneven (Table 1), with four families (Anatidae, Jacanidae, Charadriidae and Dendrocygnidae) together contributing 72.8% of positive samples; the same four families represented 67.5% of birds captured.

Reliable conclusions cannot be drawn from small sample sizes. We sampled over 20 individuals (i.e. the influence of an outlier was 5% or less) for 18 different bird families. From these families the highest mean prevalence values across all sites occurred in the Alaudidae (larks; 24 birds, 3 positives, prevalence 12.5%) and the Dendrocygnidae (whistling ducks; 234 birds, 12 positives, prevalence 5.15%). Also of note were the Scolopacidae (sandpipers and snipes, 180 birds, 6 positives, prevalence 3.33%), Jacanidae (jacanas, 492 birds, 15 positives, prevalence = 3.05%), Ploceidae (weavers, 165 birds, 5 positives, prevalence = 3.03%), Charadriidae (plovers and lapwings; 458 birds, 12 positives, prevalence = 2.62%) and Anatidae (ducks; 2168 birds, 52 positives, prevalence = 2.4%). Conversely, despite reasonably large sample sizes, no AIV RNA was found in the Columbidae (pigeons and doves; n = 122), Glareolidae (pratincoles and coursers; n = 116) or Ardeidae (herons, egrets and bitterns; n = 88).

There was no spatial synchrony in influenza occurrences, with the prevalence of influenza viruses in any 2-month sampling period not being significantly correlated between any pair of sites (n = 12 or 13, Spearman's rho $<$ 0.43, P not significant to the 0.05 or 0.1 levels in all cases).

Two influenza viruses were isolated and several different strains identified (Table 2). An H1N8 influenza virus was isolated from an Egpytian Goose *Alopochen aegyptiacus* caught at Barberspan (see Abolnik et al., 2010) and an H3N8 influenza virus from a Red-billed Teal *Anas erythrorhyncha* caught at Strandfontein. Type-related information was obtained via rRT-PCR for an additional 22 viruses, which included 10 H5-positive and 10 H7-positive samples as well as two H6-positives. Amplicons from the reactions were insufficient for obtaining DNA sequences, and thus the amino acid sequence at the HA0 cleavage sites could not be determined; it is therefore unknown whether the H5 and H7 viruses were of high or low pathogenicity. H7 strains were only identified from Zimbabwe but were found in five different species.

Table 1. Numbers of Birds Sampled for Avian Influenza, by Family and by Site, and Prevalence of Avian Influenza

Family	BAR	CHU	MAS	NGA	STR	ZIM	Totals	Total prevalence %
Accipitridae	0	0	0	0	2	1	3	0
Alaudidae	6	0	0	1	0	17(3)	24(3)	12.5
Alcedinidae	0	3	1	0	0	9(1)	13(1)	7.7
Anatidae	696(8)	27	0	69(1)	680(8)	698(35)	2170(52)	2.4
Apodidae	1	0	0	0	0	0	1	0
Ardeidae	6	14	0	3	27	35	85	0
Burhinidae	1	1	0	0	0	1	3	0
Caprimulgidae	0	0	1	1	0	3	5	0
Cerylidae	1	10	0	4	0	25(1)	40(1)	2.5
Charadriidae	99	31	10	79	17	225(12)	461(12)	2.6
Ciconiidae	0	0	0	1	0	0	1	0
Cisticolidae	0	0	0	0	0	5	5	0
Columbidae	4	0	0	48	26	44	122	0
Coraciidae	0	0	0	0	0	4	4	0
Dacelonidae	0	0	0	0	0	8	8	0
Dendrocygnidae	5	13	1	9(2)	0	206(10)	234(12)	5.1
Estrildidae	0	0	0	2	0	5	7	0
Fringillidae	0	0	0	0	0	1	1	0
Glareolidae	0	13	3	79	0	21	116	0
Haematopodidae	0	0	0	0	4	0	4	0
Hirundinidae	0	2	0	1	3	7(1)	13(1)	7.7
Indicatoridae	0	0	0	0	0	1	1	0
Jacanidae	1	116	0	39	0	337(15)	493(15)	3
Laniidae	1	0	0	0	0	2	3	0
Laridae	3	0	0	2	42	16	63	0
Lybiidae	1	0	0	0	0	2	3	0
Malaconotidae	0	0	0	0	0	2	2	0
Meropidae	0	0	0	1	0	0	1	0
Motacillidae	2	1	0	0	10	30(2)	43(2)	4.7
Muscicapidae	1	0	0	1	1	1	4	0
Numididae	10(1)	0	0	0	8	5	23(1)	4.3
Passeridae	2(1)	0	0	1	3	2	8(1)	12.5
Phalacrocoracidae	0	1	0	0	5	2	8	0
Phasianidae	2	0	0	8	7	3	20	0
Phoenicopteridae	7	0	0	0	0	0	7	0
Ploceidae	25	17	0	32	10	81(5)	165(5)	3
Podicipedidae	0	1	0	0	0	1	2	0
Pycnonotidae	2(1)	0	0	0	3	3	8(1)	12.5
Rallidae	491(7)	2	0	1	13	7	514(7)	1.4
Recurvirostridae	4	0	0	4	8	0	16	0
Rostratulidae	1	6	0	25	0	0	32	0
Scolopacidae	36	11	0	48	0	86(7)	181(7)	3.9
Sturnidae	0	0	1	4	2	9	16	0
Sylviidae	3	2	0	1	0	7(2)	13(2)	15.4
Threskiornithidae	1	0	0	3	15(1)	1	20(1)	5
Tytonidae	4	0	0	0	1	2	7	0
Upupidae	2(1)	0	0	0	0	1	3(1)	33.3

Table 1. continued

Family	BAR	CHU	MAS	NGA	STR	ZIM	Totals	Total prevalence %
Zosteropidae	0	0	0	0	1	0	1	0
TOTALS	1418(19)	271	17	467(3)	888(9)	1916(94)	4977(125)	0

Numbers in brackets indicate the number of birds that tested positive for avian influenza (these are included only when $n > 0$). BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZIM, Zimbabwe (Chivero and Manyame). Sample sizes for Anatidae were similar across our three core sites (BAR, ZIM, STR). Some of the most obvious differences in bird species composition between sites occurred in the Jacanidae (jacanas; mostly ZIM and CHU), Dendrocygnidae (whistling ducks; mostly ZIM) and Rallidae (coots and rails; mostly BAR).

Influenza viruses are in circulation across the subregion throughout the year (Fig. 3), with no obvious pattern in relation to temperature or rainfall. Patterns between years also appear to be inconsistent, with peaks in viral prevalence in December 2007 and January 2008 in Zimbabwe and Barberspan not present in 2008–2009.

These data should be interpreted within the context of the sampled bird communities. We had relatively high numbers of influenza-positive birds from each of four avian families: Anatidae, Charadriidae, Dendrocygnidae and Jacanidae. The birds in each of these families show differing seasonal trends in abundance as well as considerable spatial variation between our three core sites (see examples in Appendix 4 in Supporting information).

During counts we recorded 32,153 individuals belonging to 32 different Palearctic migrant bird species from 12 avian families (Supporting Table S2). The abundance of Palearctic migrants showed a strong peak in the southern African summer (Fig. 4), although the exact timing and magnitude of the peak varied between sites and years. Comparison of the abundance of Palearctic migrants and the prevalence of viruses from the same site and time, treating each site as an independent sample at each time step, found no dependency of viral prevalence on numbers of migrants (Spearman's $r = 0.039$, $P < 0.8$, $n = 42$). Viral prevalence was also independent of the numbers of anatid ducks (Spearman's $r = -0.1$, $P < 0.5$, $n = 42$). At time lags of 2 and 4 months, and excluding the Lake Ngami data, the relationship remained insignificant (2 months, Spearman's $r = 0.2$, $P < 0.22$, $n = 35$; and at 4 months, $r = 0.1$, $P < 0.57$, $n = 33$).

Discussion

The overall prevalence of LPAI influenza viruses that we found in anatid ducks across southern Africa is 2.4%. The range in PCR prevalence in anatids reported from Northern Europe is between 2.1% and 3.8% (Munster et al., 2006; Munster & Fouchier, 2009); and an extended survey in EU member states documented an overall LPAI prevalence in Europe of 1.87% (Breed et al., 2007). Some studies have found higher prevalences, ranging from 4% in Switzerland (Baumer et al., 2010) through 6.1% for European dabbling ducks (Munster et al., 2007) to as high as 12–15% (Wallensten et al., 2007; Terregino et al., 2007; Olsen et al., 2006). Estimates depend on the time of year when sampling occurred and the species that were tested (Olsen et al., 2006); our results are within the range of northern hemisphere estimates rather than notably lower.

One of our most interesting results is the lack of a predictable annual spike in prevalence. In Canada, for example, AIV prevalence in anatids may be as high as 60% on breeding grounds in early fall (Olsen et al., 2006). Our highest prevalence across all birds for any one sampling event was 21.43%, in summer in Zimbabwe; but in the same month in the following year, albeit with a relatively small sample size, prevalence was zero (Fig. 3). We attribute this unpredictability to the relatively stochastic nature of southern African seasonality and the flexible movement strategies of nomadic southern African ducks (Hockey, 2000; Hockey et al., 2004).

The prevalence of influenza A viruses in southern Africa appears to be twice as high in dendrocygnid (whistling) ducks (5.2%) as in anatid ducks (2.4%), although this result may be partly an artefact of whistling ducks having been sampled in largest numbers at the site with the highest overall virus prevalence. Most of our dendrocygnid samples were from White-faced Whistling Duck *Dendrocygna viduata*, but Fulvous Duck *Dendrocygna bicolor* are common (if almost uncatchable) at Lake Chuali and Lake Ngami. Whistling ducks are less abundant in South Africa but we observed both Fulvous and White-backed Duck *Thalassornis leuconotus* as far south as Strandfontein, and

Table 2. Information on Viral Strains and Types

Common name	Latin name	Family	Total +ves	H1 +ve	H3 +ve	H5 +ve	H6 +ve	H7 +ve	Typed
African Hoopoe	*Upupa africana*	Upupidae	1						
African Jacana	*Actophilornis africanus*	Jacanidae	15			2			
African Pipit	*Anthus cinnamomeus*	Motacillidae	1						
African Red-eyed Bulbul	*Pycnonotus nigricans*	Pycnonotidae	1			1			
African Snipe	*Gallinago nigripennis*	Scolopacidae	1						
African Wattled Lapwing	*Vanellus senegallus*	Charadriidae	3					1	
Barn Swallow	*Hirundo rustica*	Hirundinidae	1						
BlackSmith Lapwing	*Vanellus armatus*	Charadriidae	8			1		1	
Cape Teal	*Anas capensis*	Anatidae	1						
Chestnut-backed Sparrowlark	*Eremopterix leucotis*	Alaudidae	3			1			
Common Ringed Plover	*Charadrius hiaticula*	Charadriidae	1						
Common Sandpiper	*Actitis hypoleucos*	Scolopacidae	2						
Egyptian Goose	*Alopochen aegyptiaca*	Anatidae	7	1					H1N8
Fulvous Duck	*Dendrocygna bicolor*	Dendrocygnidae	2				1		
Glossy Ibis	*Plegadis falcinellus*	Threskiornithidae	1						
Helmeted Guineafowl	*Numida meleagris*	Numididae	1						
Hottentot Teal	*Anas hottentota*	Anatidae	3			1			
Little Rush-warbler	*Bradypterus baboecala*	Sylviidae	1						
Little Stint	*Calidris minuta*	Scolopacidae	3					1	
Malachite Kingfisher	*Alcedo cristata*	Alcedinidae	1			1			
Pied Kingfisher	*Ceryle rudis*	Cerylidae	1						
Red-billed Quelea	*Quelea quelea*	Ploceidae	1						
Red-billed Teal	*Anas erythrorhyncha*	Anatidae	35		1			2	H3N8
Red-knobbed Coot	*Fulica cristata*	Rallidae	7						
South African Shelduck	*Tadorna cana*	Anatidae	2			1	1		
Southern Grey-headed Sparrow	*Passer diffusus*	Passeridae	1						
Southern Masked-weaver	*Ploceus velatus*	Ploceidae	1						
Spur-winged Goose	*Plectropterus gambensis*	Anatidae	2						
Village Weaver	*Ploceus cucullatus*	Ploceidae	2						
White-faced Duck	*Dendrocygna viduata*	Dendrocygnidae	10			1		5	
Willow Warbler	*Phylloscopus trochilus*	Sylviidae	1						
Wood Sandpiper	*Tringa glareola*	Scolopacidae	1			1			
Yellow Bishop	*Euplectes capensis*	Ploceidae	1						
Yellow-billed Duck	*Anas undulata*	Anatidae	2						
Yellow-throated Longclaw	*Macronyx croceus*	Motacillidae	1						
Totals			125	1	1	10	2	10	(2)

This table describes birds that tested positive, rather than positive samples; the 6 birds that tested positive for the same type on two different swabs provide 6 entries rather than 12. Note that blank cells are zeros rather than unknown values.

Barberspan periodically hosts flocks of >20 White-faced Whistling Duck. Analyses of the movements of White-faced Whistling Duck in southern Africa suggest displacement on the scale of around 100 km/year, although ringing records suggest displacements of up to 1125 km and seasonally nomadic movements to ephemeral wetlands (Petrie and Rogers, 1997; Hockey et al., 2004; Underhill et al., 1999). White-faced Whistling Duck and Fulvous Duck have an extensive pan-African range and individuals from populations north of the equator may mix with Palearctic species, such as Garganey *Anas querquedula*, that migrate annually to western Europe. Gaidet et al. (2007) reported an AI prevalence of 3% in West African dendrocygnids and found HPAI H5 genomes in White-faced Whistling Duck in West

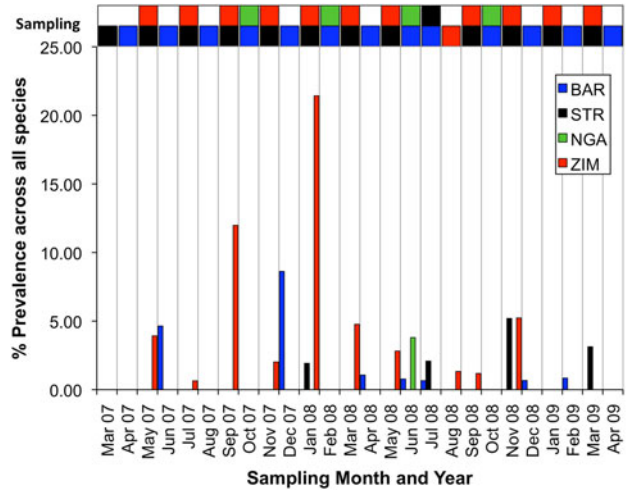

Figure 3. Prevalence of avian influenza by site and month across all captured birds. Sites are *BAR* Barberspan, *STR* Strandfontein, *NGA* Ngami and *ZIM* Zimbabwe (Manyame and Chivero). Note that (1) another 294 birds were sampled in Mozambique over the same period, with no AIV positives found; and (2) BAR, STR and ZIM were sampled every 2 months and NGA every 4 months, so birds were not sampled in some months. The *shaded squares at the top* of the chart indicate when a given site was sampled, using the same colour codes as the *bars*.

Africa. Given their high abundance and mobility (Cumming et al., 2008), whistling ducks may play an important regional role in the dynamics of AIV.

Sequencing and phylogenetic analysis of the H1N8 virus that was typed from Barberspan, together with other AIVs found in South Africa (Abolnik et al., 2010), yielded no evidence of internal genes associated with Asian HPAI H5N1 strains. The H1N8 virus from our study was the first isolate of an H1 AIV in southern Africa; its hemagglutanin gene grouped closely (96.4–97.4%) with homologous genes of Italian H1 N1 samples, suggesting a possible link to Europe (Abolnik et al., 2010).

Although no HPAI viruses were positively identified, potentially virulent H5 and H7 strains are in circulation in southern Africa in resident wild bird populations. Both viral abundance and the presence of potentially virulent strains appeared to be higher in the Manyame catchment, our northernmost site. There is some hint of a latitudinal gradient in prevalence, with Manyame > Barberspan > Strandfontein; but data from Mozambique and Botswana do not fit this pattern, although the sample sizes (n = 271 and 467 birds, respectively) are too small to draw strong inferences.

Most studies of avian influenza have focused on Anseriformes and Charadriiformes (ducks and waders), but other waterbirds may play a role in maintaining AIV in southern Africa. Rallids and jacanids (e.g. Red-knobbed Coot *Fulica cristata* and African Jacana *Actophilornis africanus*) occur year-round in high abundances in many wetlands and were frequently observed foraging near to dabbling and diving ducks. Cormorants and darters (Phalacrocoridae) are common in our study sites, mobile and frequently seen roosting with ducks. Risks of transmission to humans are increased by their capture in fishing

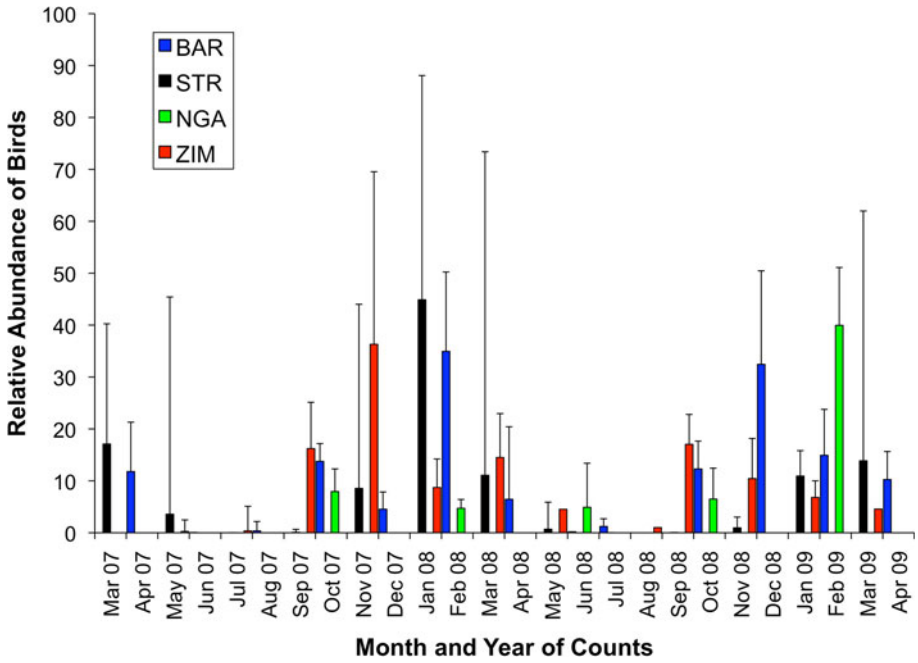

Figure 4. Relative abundance of Palearctic migrants per half-hour point count by site. *Bars* represent a mean number of migrants per point count; *error bars* represent an average standard deviation across point counts for all species for a given mission. A breakdown by species is given in Appendix 1 (Supporting information). Birds arrive earlier at more northern sites (ZIM and NGA); seasonal peaks coincide with the austral summer and the boreal winter.

nets (and indeed, we were able to sample a White-breasted Cormorant that survived an encounter with a fishing net on Lake Manyame). A variety of other species such as Sacred Ibises (*Threskiornis aethiopicus*; Threskiornithidae) also share foraging habitats with grazing and dabbling ducks (Hockey et al., 2004); Sacred Ibises in particular may feed on carcasses, making them potentially vulnerable during AIV epizootics in locations (e.g. unmonitored lakes) where carcass removal is not rapid.

For the Passeriformes, a prevalence of 4.5% (14 positives out of 308 birds) suggests a potential role in influenza epidemiology. Most of the AIV positive species that we found in this order are residents (Yellow-throated Longclaw, Chestnut-backed Sparrowlark, Red-billed Quelea and Village Weaver; Appendix 1 in Supporting information) but Barn Swallows and Willow Warblers are Palearctic migrants. Our data and those from other studies (e.g. Caron et al., 2010b) suggest that some passeriform families (e.g. Alaudidae and Ploceidae) may contribute to the persistence and spread of AIV in southern African ecosystems. Our results are unusual from a European perspective, suggesting higher prevalence than expected in southern African passerine populations, but agree with recent findings from the United States (e.g. Fuller et al., 2010) that imply a larger role for passerines in avian influenza dynamics than has been previously proposed.

In practical terms, our results preclude the assumptions of an annual cycle of virus circulation and strong seasonal variation in wild bird-related risks that hold in many northern hemisphere regions. From the perspective of both humans and poultry, AIV transmission by wild birds appears to be possible at any time of the year. The opportunistic behavioural responses of waterbird populations to environmental drivers, and the lag between rainfall and bird and pathogen responses, may nonetheless make it possible to obtain short-term predictions of AIV risks using information on rainfall.

Acknowledgments

We thank the many people who helped us during the course of this study. Logistics and permits were facilitated by Deon Hignett (Cape Nature), Dalton Gibbes (City of Cape Town), Daan Buijs (NorthWest Parks Board), Ongai Musemburi (Zimbabwe Parks and Wildlife Authority), Dr. Pious Makaya and Dr. Chris Foggin (Zimbabwe Veterinary Services), Felix Monggae (Kalahari Conservation Society), Dr. Neo Mapitse (Government of Botswana) and Raimundo Matisse (Government of Mozambique). Sampie van Der Merwe provided accommodation and field support at Barberspan. We are grateful to our >80 field assistants, especially those who helped with three or more sampling missions: Jonathan Aaronson, Joel Avni, Tertius Gous, Dominic Henry, Rhinos Kambanje, Mmapula Kgagodi, Mike Kock, Amos Koloti, Innocent Magunje, Josphine Mundava, Admire Muzeziwa, Andrew Mvundle, David Nkosi, Khumbulani Nyathi and Sydwell Setuki. This research was funded by a USAID-sponsored Global Avian Influenza Network for Surveillance subcontract from the Wildlife Society to GSC, with additional contributions from the DST/NRF Centre of Excellence at the Percy FitzPatrick Institute. Steve Osofsky and Scott Newman facilitated parts of the funding process. Analyses by ARC-OVI were funded by the South African National Department of Agriculture, Forestry and Fisheries; and by IZSVe, by the Italian Ministry of Health and a grant from the Food and Agriculture Organization of the United Nations (FAO). In Zimbabwe we benefited from the "Mesures d'Urgence" and GRIPAVI projects funded by the French Ministry of Foreign Affairs and the scientific and logistical support of the Research Platform Produce and Conserve in Partnership (RP-PCP).

References

Abolnik C, Gerdes GH, Sinclair M, Ganzevoort BW, Kitching JP, Burger CE, Romito M, Dreyer M, Swanepoel S, Cumming GS, Olivier AJ (2010) Phylogenetic analysis of influenza A viruses (H6N8, H1N8, H4N2, H9N2, H10N7) isolated from wild birds, ducks, and ostriches in South Africa from 2007 to 2009. *Avian Diseases* 54:313–322

Abolnik C, Londt BZ, Manvell RJ, Shell W, Banks J, Gerdes GH, Akol G, Brown IH (2009) 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

Baumer A, Feldmann J, Renzullo S, Müller M, Thür B, Hofmann MA (2010) Epidemiology of avian influenza virus in wild birds in Switzerland between 2006 and 2009. *Avian Diseases* 54:875–884

Breed AC, Harris K, Hesterberg U, Gould G, Londt BZ, Brown IH, Cook AJ (2007) Surveillance for avian influenza in wild birds in the European Union in 2007. *Avian Diseases* 54:399–404

Caron A, Abolnik C, Mundava J, Gaidet N, Burger CE, Mochotlhoane B, Bruinzeel L, Chiweshe N, De Garine-Wichatitsky M, Cumming GS (2010a) Persistence of low pathogenic avian influenza virus in waterfowl in a Southern African ecosystem. *EcoHealth*. doi:10.1007/s10393-010-0356-4

Caron A, De Garine-Wichatitsky M, Gaidet N, Chiweshe N, Cumming GS (2010b) Estimating dynamic risk factors for pathogen transmission using community-level bird census data at the wildlife/domestic interface. *Ecology and Society* 15(3):25. http://www.ecologyandsociety.org/vol15/iss3/art25/

Cumming GS, Hockey PAR, Bruinzeel LW, Du Plessis MA (2008) Wild bird movements and avian influenza risk mapping in southern Africa. *Ecology and Society* 13:26

Dudley JP (2008) Public health and epidemiological considerations for avian influenza risk mapping and risk assessment. *Ecology and Society* 13:21

Enserink M (2006) Avian influenza—H5N1 moves into Africa, European Union, deepening global crisis. *Science* 311:932

Fasina FO, Bisschop SP, Joannis TM, Lombin LH, Abolnik C (2009) Molecular characterization and epidemiology of the highly pathogenic avian influenza H5N1 in Nigeria. *Epidemiology and Infection* 137:456–463

Forster JL, Harkin VB, Graham DA, Mccullough SJ (2008) The effect of sample type, temperature and RNA*later*^{TM} on the stability of avian influenza virus RNA. *Journal of Virological Methods* 149:190–194

Fuller TL, Saatchi SS, Curd EE, Toffelmier E, Thomassen HA, Buermann W, Desante DF, Nott MP, Saracco JF, Ralph CJ, Alexander JD, Pollinger JP, Smith TB (2010) Mapping the risk of avian influenza in wild birds in the US. *BMC Infectious Diseases* 10:187

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

Gaidet N, Dodman T, Caron A, Balanca 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

Hockey PAR (2000) Patterns and correlates of bird migrations in sub-Saharan Africa. *Emu* 100:401–417

Hockey PAR, Dean WRJ, Ryan PG (2004) *Roberts' Birds of Southern Africa*, Cape Town: Russell Friedman Books CC, pp 1296

Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P (2006) 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, Webster RG (2010) Avian influenza virus surveillance and wild birds: past and present. *Avian Diseases* 54:394–398

Munster VJ, Baas C, Lexmond P, Waldenström J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WE, Schutten M, Olsen B, Osterhaus AD, Fouchier RA (2007) Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathogens* 3:e61

Munster VJ, Fouchier RAM (2009) Avian influenza virus: of virus and bird ecology. *Vaccine* 27:6340–6344

Munster VJ, Veen J, Olsen B, Vogel R, Osterhaus AD, Fouchier RA (2006) Towards improved influenza A virus surveillance in migrating birds. *Vaccine* 24:6729–6733

Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus A, Fouchier RAM (2006) Global patterns of influenza A virus in wild birds. *Science* 312:384–388

Petrie SA, Rogers KH (1997) *Ecology, nutrient reserve dynamics and movements of white-faced ducks in South Africa*, Pretoria: Department of Environmental Affairs and Tourism

Pickles H (2006) Avian influenza—preparing for the pandemic—using lessons from the past to plan for pandemic flu. *British Medical Journal* 332:783–786

Sinclair M, Bruckner GK, Kotze JJ (2005) Avian influenza in ostriches: epidemiological investigation in the Western Cape Province of South Africa. *Elsenburg Joernaal* 2:2–4

Terregino C, De Nardi R, Guberti V, Scremin M, Raffini E, Martin AM, Cattoli G, Bonfanti L, Capua I (2007) Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. *Avian Pathology* 36:337–344

Underhill LG, Tree AJ, Oschadleus HD, Parker V (1999) *Review of ring recoveries of waterbirds in southern Africa*, Cape Town: Avian Demography Unit, University of Cape Town

Wallensten A, Munster VJ, Latorre-Margalef N, Brytting M, Elmberg J, Fouchier RA, Fransson T, Haemig PD, Karlsson M, Lundkvist A, Osterhaus AD, Stervander M, Waldenström J, Olsen B (2007) Surveillance of influenza A virus in migratory waterfowl in northern Europe. *Emerging Infectious Diseases* 13:404–411

WHO (2010) Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to the World Health Organization. World Health Organization on-line fact sheet, available at http://www.who.int/csr/disease/avian_influenza/country/cases_table_2010_08_12/en/print.html