Chapter Six: Exploring the relation between avian communities and AIV ecology in Southern Africa using the concept of epidemiological functional groups

(Chapter reference: Caron, A., de Garine-Wichatitsky, M., Ndlovu, M., Cumming, G. S. (In Prep) Exploring the relation between avian communities and AIV ecology in Southern Africa using the epidemiological functional group concept)

Introduction

The ecology of pathogens and the emergence of disease in multi-host systems is complex (Woolhouse et al. 2001, Haydon et al. 2002), and understanding it often requires the incorporation of a wide variety of different kinds of evidence and different disciplinary approaches (Plowright et al. 2008). Traditional surveillance and control approaches have often focused on humans, domestic animals, and known vectors. However, an increasing body of information indicates that effective disease surveillance and control may be heavily dependent on understanding the epidemiology of pathogens in wild hosts and the ecology of these hosts (e.g. Chevalier et al. 2004, Olsen et al. 2006, Woodroffe et al. 2008, Leroy et al. 2009).

As more hosts are considered in an epidemiological system, understanding the specific relationship between each host and the pathogen (e.g. susceptibility, pathogenicity) in order to assign each hosts to a specific role in the epidemiological cycle (e.g. reservoir, dead end-host, spreader) quickly becomes challenging. There is therefore a need to summarize this complexity without oversimplifying it. A good starting model for system simplification comes from the field of community ecology, in which researchers have attempted for some decades to deconstruct the complexity of food webs (May 2006). Concepts such as trophic levels and foraging guilds have played an important role in the development of ecological theory, and successful approaches should in theory be readily modifiable to facilitate the analysis of the ecology of pathogen transmission in multi-host systems. The concept of epidemiological functional groups (EFGs) uses epidemiological roles instead of foraging guilds to classify hosts into groups that capture their role in the epidemiology of a pathogen or a group of pathogens (Chapter Seven - Caron et al. Submitted). Hosts in a group share a common function in the epidemiology of the pathogen(s) of interest. In this study we further develop

the concept of EFGs by using them as a lens through which to investigate the ecology of avian influenza viruses (AIV) in wild avian communities in Southern Africa.

AIVs in wild birds have recently received much attention due to the Highly Pathogenic AIV H5N1 strain epizooty and its potential threat to human health (Capua and Alexander 2002). Although numerous studies of low pathogenic AIV strains (LPAI) in waterfowl and wild birds have been published, encompassing tens of thousands of sampled wild birds, there is still little information on the susceptibility of individual bird species to AIV in relation to the global number of bird species (Olsen et al. 2006). Avian communities in a given ecosystem can span hundreds of interacting species. The use of EFGs in this context, based on known or hypothesised facts, should provide a powerful tool for exploring global patterns of AIV ecology. Most studies of AIV have concentrated on Anseriformes and Charadriiformes, which are known to be reservoirs for LPAI (Webster et al. 1992, Olsen et al. 2006). Little information on AI prevalence in the rest of the avian community has been published, and much of what has been published has been obtained as "by-catch" of capture protocols that have been focused on ducks. The minimum sample sizes that would be necessary to confidently estimate prevalence for most non-target bird species are thus often not reached.

In addition, lack of information regarding the composition of the wild bird community from which the sample is taken makes conclusions from AIV studies difficult to interpret. A total of 100 positive samples from species A, for example, carries a different epidemiological weight if species A represents 0.1% versus 90% of the number of wild birds present in the ecosystem; and similarly, the relevance of 100 positive samples from one species differs if the system contains 10 or 100 other species. Interpretation of the role for pathogen maintenance of species A cannot be made rigorously without considering the potential role of the rest of the community. Experimental infection trials indicate that it is impossible to predict the

susceptibility of species according to its ecology or phylogeny (Ellis et al. 2004, Werner et al. 2007, Brown et al. 2009). Determining the role of a particular species in AIV ecology is therefore heavily contingent on direct experimental or field results. In this article we use two years of regular and consistent bird census and epidemiological data on AIV in wild birds to explore AIV epidemiology in the context of the avian community in three different ecosystems across Southern Africa.

Our analysis followed three main steps: (1) comparison of the waterfowl communities' characteristics across the 3 sites; (2) comparison of the representativity of the epidemiological data for each site; and (3), bringing these two strands together, analysis of two Epidemiological Functional Groups that are based on known characteristics of AIV ecology in wild birds. The first ecological function (EF) relates to maintenance and non-maintenance functional groups (Figure 6.1). The target population (according to (Haydon et al. 2002) definition) is at risk of AIV transmission from the maintenance population directly or indirectly through the non-maintenance population. The second EF concerns the patterns of movements of wild bird species relative to the ecosystem (Figure 6.2). As birds move or migrate further from a given ecosystem, they will be exposed to more diverse AIV strains and could introduce those strains in the ecosystem. Depending on the circulation of AIV in the ecosystem under study, the introduction of exogenous strains could trigger epizooties if no immunity against this strain exists. These introductions could also play a role in the reassortment processes and the emergence of new strains (Webster and Hulse 2004, Chapter Two - Caron et al. 2009).

Figure 6.1: *Epidemiological functional group 1: maintenance and non-maintenance community in relation to target population (here the domestic bird population). The maintenance community host the virus and maintain it. The non-maintenance community can transmit the viruses to the target population but cannot maintain it.*

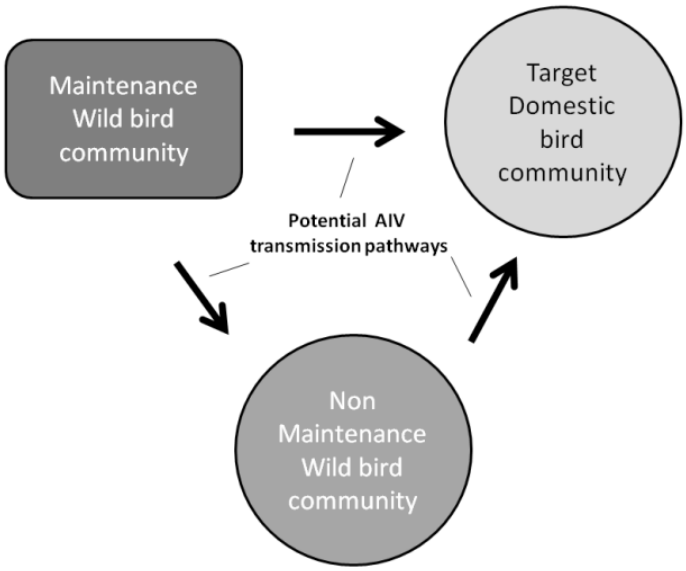
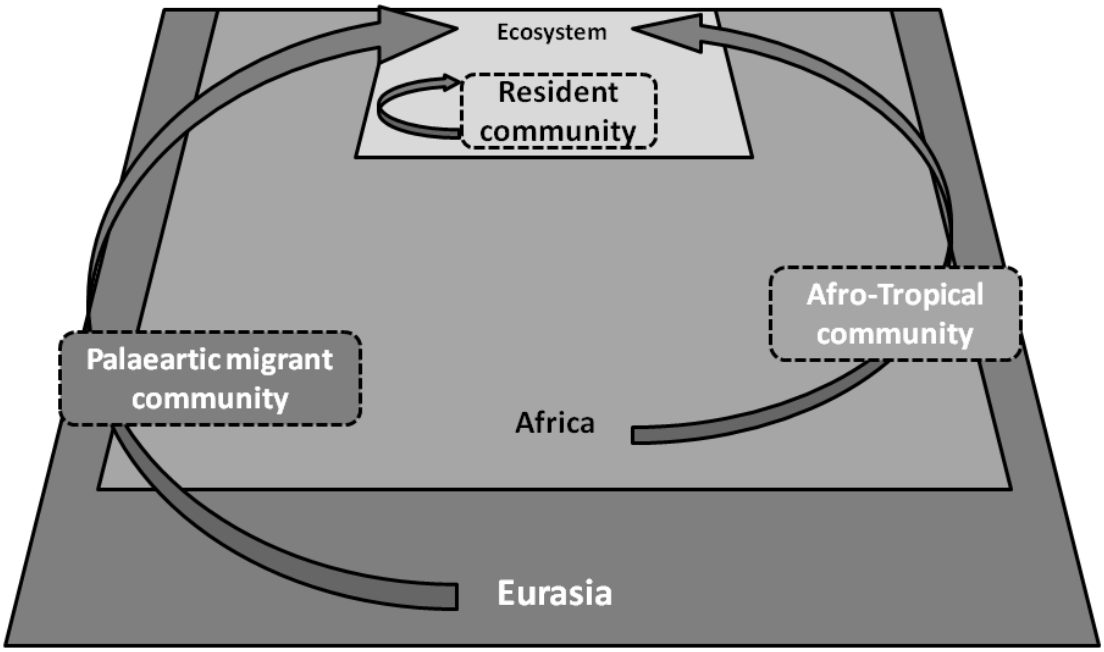


Figure 6.2: *Epidemiological Functional Group 2: bird species are allocated to groups according to their movement behaviour. Resident species do not leave the ecosystem; Afro-tropical species move within Southern Africa and/or African regions on both sides of the equator; Palaearctic migrants move seasonally between Eurasia and Africa. Arrows represent the potential for AIV strain introduction inthe focal ecosystem from different origins for each EFG.*



Methods

Study sites

Three sites in Southern Africa were investigated: (1) Barberspan (BAR) in Gauteng province, South Africa, is a RAMSAR wetland of total area varying between 1000 and 1700 ha; (2) Strandfontein wastewater treatment works (STR) in the Western Cape, South Africa, is a 319 ha water body located in Muizenberg on the immediate periphery of the city of Cape Town; and (3) the Manyame-Chivero dams (MAN) in Zimbabwe, which are man-made impoundments that are linked by the Manyame river and were built in the 1950s to supply the city of Harare with water. They cover areas of 6500 and 18500 ha respectively. More information on these sites is available as supplementary material in Appendix Five - Cumming et al. (2011).

Baseline data

Bird census data was collected using point counts for two years in each site from February 2007 to May 2009. Each point count consisted of a 10-minute habituation period followed by a 30 minute focal count of all birds in a semi-circle of 150m radius, facing the waterbody. Point counts were undertaken at 12 to 15 points at each of our three sites (BAR, STR, and MAN) and were repeated four times at each location over five days during each counting and sampling session. Sessions were repeated every two months.

AIV prevalence was estimated by sampling captured birds every two months during two years in each site from February 2007 to March 2009. The capture sessions were undertaken during a week immediately following each 5-day counting session. Walk-in traps and mist nets were used to capture wild birds on the water body shores, with occasional use of

spring- or canon-nets. Additional details on the protocols have been given in Appendix Five - Cumming et al. (2011).

Data analysis

First step: comparison of bird communities between sites

Four complementary metrics were calculated to describe the waterfowl community in the three sites: species richness (total number of species), Shannon's diversity index (Shannon and Weaver 1949), species evenness, and the concentration ratio (proportion of the most represented species; e.g. Concentration Ratio 4 gives the proportions of the four most represented species). All metrics were calculated across the two years of counting. The bird species of the 3 sites were allocated to EFGs using available regional knowledge (Hockey et al. 2005) and the composition of these groups was compared across ecosystems (see below).

Second step: comparison of birds sampled and birds counted

We estimated the bias in terms of waterfowl community representativity in the sample induced by the bird capture techniques and the "catchability" of waterfowl species by comparing the proportion of each bird group captured and observed across the two years of capture.

Third step: prevalence & EFGs

The possibility of an endemic cycle has been raised by previous studies in Southern Africa (Chapter Four – Caron et al. 2011, Appendix Five - Cumming et al. 2011). The allocation of bird species into EFs is made on the basis of available knowledge and when no information is available for a set of species, they are grouped together by default. This approach allows the exploration of the possible relevance for AIV dynamics of a group of

species for which so little information is available that we are unable to say anything definite about their role in AIV epidemiology.

Epidemiological function 1, EF1, is related to the known and unknown role of bird orders in the maintenance of AIV in an ecosystem. Anseriformes and Charadriiformes are bird orders considered globally as reservoirs for AIV and many studies consider only these two orders for epidemiological investigations (e.g. Ito et al. 1995, Hansbro 2010). If there is an endemic AIV cycle in Southern Africa, we hypothesized that Anseriformes (and potentially Charadriiformes) would constitute the maintenance community. We allocated Anseriformes and Charadriiformes into two different maintenance EFGs because they do not always share the same viral pool and do not always share transmission pathways (Olsen et al. 2006). In Africa, a role as a reservoir for both groups has been suggested by recent studies (Appendix One - Gaidet et al. 2007, Chapter Four - Caron et al. 2011, Appendix Five - Cumming et al. 2011, Appendix Four - Gaidet et al. 2011). The other bird orders have not been investigated enough to allocate different groups to different roles in viral maintenance. We have therefore defined the three following groups: *Ans* (Anseriformes), *Cha* (Charadriiformes) and *RoC* group (Rest of Community), the later regrouping all non Anseriformes and non Charadriiformes bird species. If Anseriformes and Charadriiformes represent the main reservoir of AIV in Southern Africa, the *RoC* group should play a minor role in the ecology of AIV with occasional spillover of AIV strains triggering infections; and the estimated prevalence in this group should be lower than in the two other groups across the two years of study.

EF2 focuses on the capacity of a bird species to introduce AIV strains from different ecosystems across regions or continents. Southern Africa has never experienced any HPAI H5N1 outbreak but experiences recurrent outbreaks of HPAI H5N2 in ostriches (Sinclair et al. 2009, OIE 2011). It is therefore important when considering epidemiological functions to

differentiate bird species according to their movement patterns. We allocated birds in our study communities to the following groups: a) Long range spreader or Palaearctic (*Pal*) migrant, migrating from Eurasia where high prevalence of AIV including HPAI strains occurs (Wallensten et al. 2007); b) Middle range spreader or Afrotropical migrant, migrating North of the equator in Africa where HPAI H5N1 has become endemic in some regions; c) Small-scale spreader or nomad, moving regionally to follow resources and/or undertake moult or breeding-related local migrations; and d) Non spreader or Resident (*Res*) bird with limited local movements.

Despite the availability of detailed information about wild bird in Southern Africa (Hockey et al. 2005), the behaviour of some species remains unclear, particularly where two or more populations of the same species can behave differently. We therefore decided to regroup medium and local-scale spreader species into a single *Afr* (mobile Afro-tropical) group. A role for Palaearctic birds in the introduction of Eurasian AIV strain in Africa has been suggested (Abolnik et al. 2006, Cattoli et al. 2009). If there is no endemicity of AIV in Africa, we hypothesized that Palaearctic migrants should introduce AIV regularly in these ecosystems. By contrast, a community dominated by the "Resident" EFG should experience little AIV circulation. For each EF and for each site, we calculated the prevalence of AIV across all members of each EFG.

Results

First step: comparison of waterfowl communities between sites

Comparing diversity indices across the three study sites (Table 6.1), MAN has higher species richness, a higher evenness and therefore a higher Shannon index than STR and BAR.

By contrast, the bird density (represented by the total number of birds observed divided by the total number of counts, given that all counts were undertaken within a 150m semicircle) in MAN is inferior to the bird densities in STR & BAR. The concentration ratio (CR) at four and eight species was also inferior to the CRs in STR and BAR. Differences between BAR and STR were smaller: STR is less diverse (139 against 199 species recorded) and the values of the Shannon and evenness indices were smaller in BAR. Concentration ratios for the three sites were quite high, meaning that the first 4 and 8 species represented a high proportion of the global community.

The community composition relative to EF1 and EF2 across the three sites differed (Table 6.1). BAR and MAN were dominated by the *RoC* group. STR had a higher proportion of *Cha*, slightly higher than *RoC* (40.8% compared to 39.7%). Densities of *Ans* were similar across the three sites but their proportion was higher in MAN compared to the two other sites. *Afr* dominated all three bird communities. For proportions of *Res* species, MAN > STR> BAR. *Pal* species represented a higher proportion in MAN compared to BAR then to STR. When combining EFG 1 and 2 (Figure 6.3), the community composition varied even more between the three communities. BAR & STR were dominated by *RoC-Afr*, whereas MAN was dominated by *Ans-Afr*. STR had more *Cha-Res* compared to BAR & MAN. Densities were smaller in MAN compared to STR & BAR (as already mentioned for Table 6.1).

Second step: comparison of bird sampled and bird observed

The proportions of bird groups observed and sampled differed between sites (Figure 6.3). In all three sites the Anseriformes family was overrepresented in the sampled birds, primarily reflecting the use of specific capture techniques (e.g. walk-in traps) to target this bird family. In addition, some observed dominant bird families at the community level are

poorly represented or absent from the samples. This was the case for the *RoC-Afr* group for all three sites and for *Char-Afr* in STR. No Palaearctic waders were sampled at STR.

Third step: Prevalence & EFGs

Ans-Afr represented the only Anseriformes present in the three sites and their AIV prevalence was 1.1, 1.2, and 5.0% respectively for BAR, STR & MAN (Figure 6.4). *Cha* in the three categories of EF2 had zero prevalence at both BAR and STR, albeit with small sample sizes. At MAN, *Char-Afr* had a relatively high AIV prevalence (as for *Char-Pal*) but with a large confidence interval. The *RoC* group has non-zero prevalence in the three sites for *Res* and *Afr* for BAR, *Afr* for STR and all three groups for MAN. Bars in the background of Figure 6.4 represent the proportion of birds observed for each group. Except for *Cha-Afr* (but only 38 individuals sampled), any bird groups representing more than 15% of the community had a non-zero AIV prevalence.

Table 6.1: *Indicators of waterfowl community diversity:"Birds Obs/Count": average number of birds observed per count and standard error displayed; "Species richness": number of species observed across the two years; "Shannon's index" & "Evenness": both diversity index; "CR4" & "CR8"concentration ratios of the first four and eight species respectively. Proportion of each combined groups of EF 1 & 2 are displayed in each ecosystem (Ans=Anseriformes, Cha=Charadriiformes, RoC=Rest of Community, Res=Resident, Afr=Afro-tropical, Pal=Palaearctic).*

	BAR	**STR**	**MAN**
Bird Obs/Count	246±537	234±216	144±171
Species richness	198	138	249
Shannon's index	2.72	2.95	3.54
Evenness	0.514	0.598	0.641
CR4	64.9%	53.3%	43.6%
CR8	76.1%	70.7%	56.7%
Ans	17.0%	19.5%	34.0%
Char	10.8%	40.8%	22.6%
RoC	72.2%	39.7%	43.4%
Res	7.0%	12.7%	14.5%
Afr	88.4%	84.0%	78.4%
Pal	4.6%	3.3%	7.1%

Figure 6.3: *Community observed (left) and captured (right) in the three sites according to EF 1 & 2 groups. Bird density ("Observed") is calculated by the number of birds observed divided by the number of counts (counts implemented in a given area). Bird abundance ("Captured") is the number of birds captured. Dark grey = Anseriformes, Medium grey=RoC and Light Grey=Charadriiformes.*

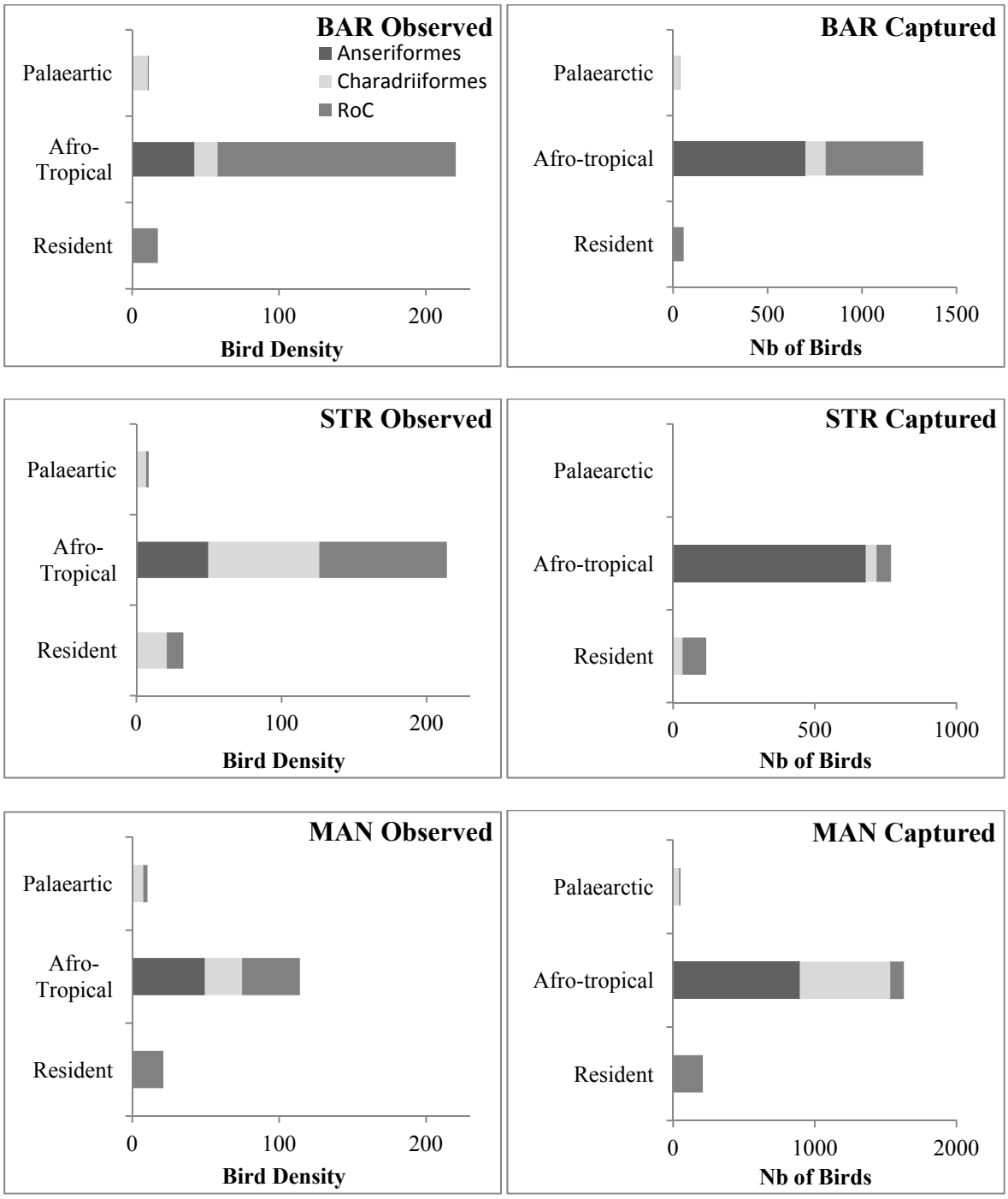
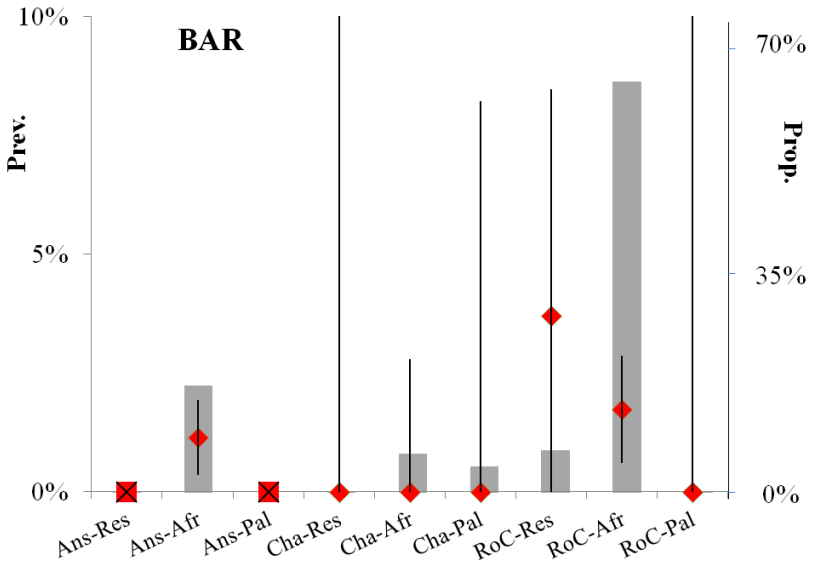


Table 6.2: *Prevalence (Prev) of AIV and confidence interval (CI, Lower and Upper boundaries) at 95% calculated for each site across the 12 sampling sessions at the community level and for each group of both EFs. n = number of birds sampled; Prev. = Estimated Prevalence (based on results presented in Appendix Five - Cumming et al. (2011).*

	BAR				STR				MAN			
	n	Prev (%)	CI (%) Lower	CI (%) Upper	n	Prev (%)	CI (%) Lower	CI (%) Upper	n	Prev (%)	CI (%) Lower	CI (%) Upper
Global Community	1418	**1.3**	0.7	1.9	887	**1.0**	0.4	1.7	1891	**5.0**	4.0	5.9
Ans-Res	0	**na**	na	na	0	**na**	na	na	0	**na**	na	na
Ans-Afr	701	**1.1**	0.4	1.9	680	**1.2**	0.4	2.0	894	**5.0**	3.6	6.5
Ans-Pal	0	**na**	na	na	0	**na**	na	na	0	**na**	na	na
Cha-Res	2	**0.0**	0.0	98.0	33	**0.0**	0.0	9.0	0	**na**	na	na
Cha-Afr	106	**0.0**	0.0	2.8	38	**0.0**	0.0	7.8	639	**4.4**	2.8	6.0
Cha-Pal	36	**0.0**	0.0	8.2	0	**na**	na	na	41	**14.6**	3.7	25.6
RoC-Res	54	**3.7**	0.0	8.5	84	**0.0**	0.0	3.5	210	**5.2**	2.2	8.3
RoC-Afr	517	**1.7**	0.6	2.9	51	**2.0**	0.0	5.8	96	**2.1**	0.0	5.0
RoC-Pal	2	**0.0**	0.0	98.0	1	**0.0**	0.0	100.0	11	**18.2**	0.0	42.1

Figure 6.4: *For each site (BAR, STR, MAN): a) prevalence and confidence interval (left axis) for each combination between EF1 & EF2 (Ans=Anseriformes, Cha=Charadriiformes, RoC=Rest of Community, Res=Resident, Afr =Afro-tropical, Pal=Palaearctic migrant); b) Proportion of each bird group in the bird community observed (or counted) during the 2 years of the project (right axis).*


BAR
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5%
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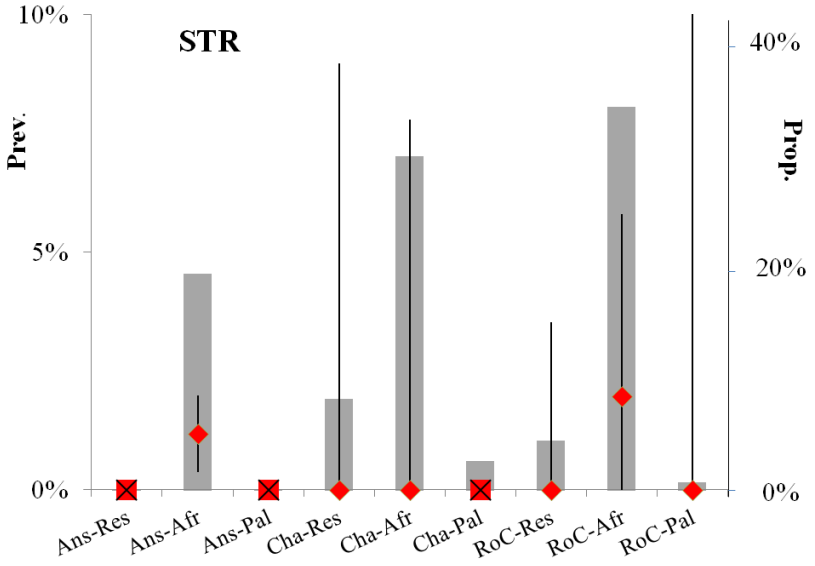
35%
0%
Ans-Res
Ans-Afr
Ans-Pal
Cha-Res
Cha-Afr
Cha-Pal
RoC-Res
RoC-Afr
RoC-Pal


STR
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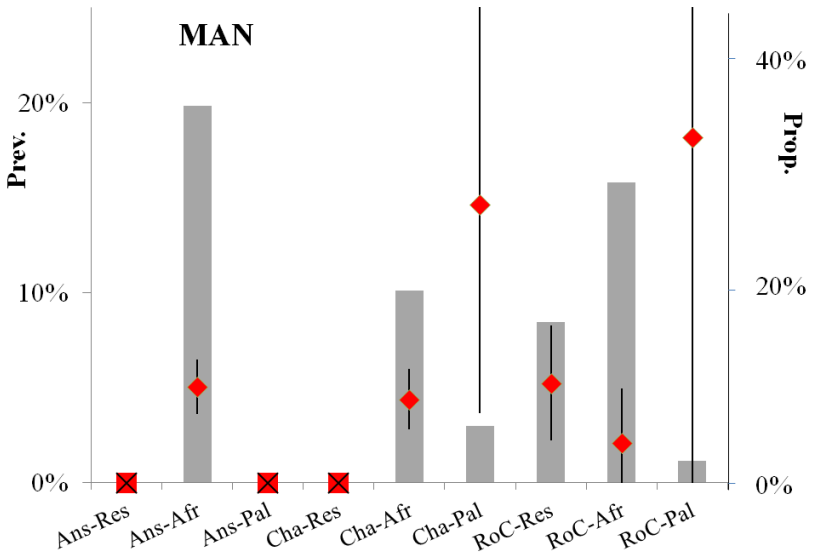
MAN
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Ans-Pal
Cha-Res
Cha-Afr
Cha-Pal
RoC-Res
RoC-Afr
RoC-Pal

Discussion

The data that are needed to compare the estimated AIV prevalence from a non-random host sample with the global host community available across a two-year period have not previously been assembled. *A priori*, we expected to find that different host community compositions in different ecosystems should lead to different epidemiological patterns.

First step: comparison of bird communities between sites

The bird communities differ between the three sites. MAN differs from BAR and STR in almost every index of community (Table 6.1). To summarize, MAN is more diverse, more even in terms of species composition and bird density is lower compared to BAR and STR. The larger size of the MAN ecosystem compared to the two other wetlands could explain this difference in density. It is important to note that if MAN, STR and BAR do not differ in *Ans-Afr* density, they differ in the proportion in the total community (Figure 6.3). Based on available information about AIV ecology in waterfowl, the community composition in MAN is more favourable to AIV maintenance because the community is dominated by Anseriformes. In STR, the important presence of Charadriiformes suggests the possibility of AIV maintenance. In BAR, the *RoC* group dominates: as little information is available on the numerous species composing this group, inferences on AIV circulation are difficult to make.

There are no *Ans-Pal* reaching Southern Africa and only a few Anseriformes belonging to the *RoC* group. *Pal* are present in small proportion in all three ecosystems, most of them being Charadriiformes. There is potential for AIV introduction through seasonal movements of these *Pal species*, when they arrive in the region from Eurasia, in late September-early October. Most birds in the three communities are *Afr* (88.4, 84.0 and 78.4% for respectively BAR, STR and MAN). This *Afr* group encompasses African migratory and nomadic species using local resource availability as a driver for regional movements

(Dodman and Diagana 2007). For some species in this group, it is not known what proportion of the population undertake nomadic movements and trans-equatorial migration (e.g. red-billed teal, *Anas erythroryncha*). These gaps in knowledge prevent the separation of the *Afr* group into two.

The representativity of the bird communities observed in each site of this study is not perfect: focal counts from the shore line of water bodies cannot census all bird species. The role of water bodies considered in relation to other water bodies in the direct vicinity (non-perennial and perennial) is also important to consider. Birds moving from one water body to the next can spread pathogens and resource availability attracting birds at different seasons can create a meta-population system for AIV with extinction and introduction events in the local network of water bodies. Sampling in a single water body will therefore be biased by the role of this water body in a broader network. However, in the three ecosystem studied, the water bodies sampled constitute the main ones in the vicinity.

Second step: comparison bird sampled and bird observed

Sampling in wild populations is biased in several ways (Morgan et al. 2004, Yasue et al. 2006). As for many studies investigating the relation between wild birds and AIV, this study initially aimed at waterfowl and in particular Anseriformes as no information was available about AIV ecology in these ecosystems. Our initial objective was to test the most common hypothesis of Anseriformes as the main reservoir for AIV in waterfowl. Secondly, the bias observed is also a consequence of the catchability of wild birds in general. Most Anseriformes are easy to catch using baited walk-in traps. Charadriiformes can be difficult to catch as you need expertise to set mist nets at the appropriate location and time of the day. Our sampling composition reflects these issues and highlights the bias in prevalence that can be introduced by waterfowl sampling. All the non-target species captured as by-catch have

been sampled. As a result the representativity of the sample size in relation to the group composition is not well respected as on Figure 4, in STR, the *Cha-Afr* & *Roc-Afr* groups. The juxtaposition of community composition and sampling allow identifying future groups to be targeted in this community in order to complete the epidemiological picture.

Third step: AIV prevalence estimation using the EFG approach

The EFG approach considers groups of avian hosts according to their functional role relative to AIV epidemiology. Such approach decreases the complexity of multi-host systems but includes inevitable approximations. In EF1, if the first two groups, *Ans* and *Cha* are well defined taxonomic groups, the third group, *RoC*, brings together more than a hundred species for each site with little information about their respective role in AIV epidemiology. Similarly in EF2, the grouping of bird species according to movement patterns is approximate. The complexity and flexibility of animal behaviour lead some species to behave differently according to the population they belong to and to their environment. The nomadic behaviour of many species in Southern Africa complicates the picture as bird movements are driven by local patterns of rainfall known to be unpredictable from one year to the next (Dodman and Diagana 2007).

Despite a similar *Ans-Afr* density in the three sites (Figure 6.1), the AIV estimated prevalence differs significantly between MAN (5.0%) and BAR (1.1%) and STR (1.2%) (Figure 6.4, both chi-square tests being highly significant, $p<0.001$). This observation can be explained by two hypotheses: a) as MAN is a much larger area than BAR and STR, the total *Ans-Afr* population is a better predictor of AIV prevalence, compared to their density; b) the composition of the rest of the host community has an influence on the level of AIV circulation in Anseriformes. The second hypothesis is supported by the estimated prevalence in the other EFGs. All groups in MAN have a non-zero mean prevalence. The MAN prevalence for *RoC-*

Res and *RoC-Afr* are significantly higher than BAR *RoC-Afr* (chi-square test, $p<0.01$ and $p<0.05$ respectively) and higher but not significantly (because the sample size for the two follwing groups is small) from BAR *RoC-Res* and STR *RoC-Afr*. The prebalence of *RoC* and *Cha* groups in MAN are not significantly different from the *Ans-Afr* group. In BAR and STR, the overall AIV prevalence is lower than in MAN and seems to be relatively similar in well-sampled groups. These observations cannot prove which of the above hypotheses are relevant but they support a role of the non-Anseriformes groups in the AIV prevalence in these ecosystems. The temporal examination of this data led to the same interpretation between duck and non-duck species (Chapter Four - Caron et al. 2011).

In terms of number of AIV infected birds in each of the three sites (by multiplying prevalence by community proportion in Figure 6.4), in BAR and STR, the number of *Ans-Afr* infected is lower than *RoC-Afr* in both sites. In MAN, *Ans-Afr* would represent the group with the highest number of infected birds but the sum of the 5 other groups with an estimated prevalence would be higher. Therefore, in the three sites, our results indicate that there are more non-Anseriformes infected birds than Anseriformes infected birds. These results point again at a role played by non-Anseriformes groups in the maintenance of AIV in these ecosystems (Stallknecht and Brown 2007). The *RoC* groups represent more than 100 species. Most of the species in these groups had zero positive individuals for a small sample size. However a few others are driving the prevalence at the group level and proper sampling should be implemented for these species in order to clarify their role. For some terrestrial species, experimental data suggests a potential role in virus shedding (e.g. Breithaupt et al. 2010, Forrest et al. 2010, Fujimoto et al. 2010, Phuong et al. 2011). Concerning Palaearctic species, too few samples have been obtained through this study in order to have a clear picture of their role (n=2, 1, and 44 respectively for BAR, STR and MAN with only 8 positives in

MAN). However, the 17% prevalence estimated for *Cha-Pal* in MAN (n=35) indicates the need for more information about this group in particular.

This study has been implemented to provide the first longitudinal AIV information in these ecosystems. Its design was similar to most wild bird AIV survey, focusing on most probable reservoir groups, namely Anseriformes and Charadriiformes. As a result and as most wild bird AIV studies, there is little information concerning the rest of the bird community. However, we could here combined our sampling and prevalence data with counting data in the same bird communities and used available ornithological knowledge to allocate the large number of bird species into two EFGs in order to simplify the multi-host complexity. Our results do not highlight *Ans-Afr* as the main reservoir compared to other groups. In addition, all groups but one representing more than 15% of the community have a non-zero prevalence. Therefore, our data support the hypothesis that other bird groups including groups not usually regarded as important for AIV epidemiology do play a role in AIV epidemiology in these ecosystems. Our analysis points at which bird groups should be targeted for additional sampling in order to investigate further the multi-host complexity mainly, *RoC-Afr, Roc-Res*, *Cha-Pal* and *RoC-Pal.* Therefore, the EFG approach intend to reduce the complexity of 100+ multi-host systems in order to generate iteratively more precise hypotheses on the role of bird groups or species in the epidemiology of AIV.

In conclusion, these results are unlikely to be specific to Southern Africa. Here we observe significant AIV prevalence in *Ans-Afr* between ecosystems and provide hypotheses to explain these differences. It serves to highlight the fact that for various reasons (and most of them were valid at the time), previous studies overlooked the role of most wild bird species by focusing on a few orders or families. Comparing prevalence results from multiple sites (even if the sampling was done at similar time) is compromised if environmental and ecological variability is not accounted for. If one wants to explore AIV epidemiology in wild birds to

understand key issues such as HPAI strain emergence and local maintenance, the role of the avian community as a whole must be considered. Others EFs for AIV such as a reproductive EF taking into account fecundity and seasonality of reproduction could be used. In the Northern hemisphere, the proportion of juvenile in the population is a good indicator of AIV prevalence (Stallknecht et al. 1990) when in Southern Africa there is less synchrony in breeding. We believe that the EFG approach is a way forward to start exploring community-level epidemiology. There is a vast amount of ornithological data available which can help in designing sampling protocols. Furthermore, bird census requires expertise and time but usually does not cost much. Knowledge about the susceptibility for AIV at the bird species level is not an achievable goal in the near future. The present approach is an iterative process to select the best candidates for experimental studies to focus on. We therefore advocate for an increased integration between ecological and epidemiological data and for the necessity to develop adequate tools to study multi-host systems.

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