Bridge hosts for Avian Influenza viruses at the wildlife/domestic interface: an eco-epidemiological framework implemented in southern Africa

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Abstract:

Wild terrestrial birds can act as potential local spreaders or bridge hosts for avian influenza viruses (AIV) between waterfowl (the maintenance hosts of AIV) and domestic avian populations in which AIV may cause disease Few studies have investigated this hypothesis, although it is an important knowledge gap in our understanding of AIV spread within socio-ecosystems. We designed a simple and reproducible approach in an agro-ecosystem in Zimbabwe based on: 1) bird counts at key target sites (i.e. wetlands, villages, intensive poultry production buildings and ostrich farms) to identify which wild birds species co-occur in these different sites and seasons when the risk of AIV transmission through these potential bridge hosts is maximal; and 2) targeted sampling and testing for AIV infection in the identified potential bridge hosts. We found that 12 wild bird species represented the vast majority (79%) of co-occurrences in the different sites whereas 230 bird species were recorded in this ecosystem. Specifically, three species, barn swallow, *Hirunda rustics,* red-billed quelea, *Quelea quelea* and cattle egret, *Bulbucus ibis* represented the main potential bridge host species (65% of co-occurrences). In 2 out of these 3 species (i.e. barn swallow and red-billed quelea) we detected AIV infections, confirming that they can play a bridge function between waterfowl and domestic species in the ecosystem. Our approach can be easily implemented in other ecosystems to identify potential bridge hosts, and our results have implications in terms of surveillance, risk management and control of AIV spread in socioecosystems

Keywords: avian influenza, wild/domestic interface, bridge host, waterfowl

Introduction

Anseriformes and Charadriiformes are known to maintain Low Pathogenic Avian Influenza viruses (LPAI) strains, whereas the role of other wild bird species is unclear (Olsen et al., 2006). Although the pathogenic effect of AIV infections on migrating bird is still debated (Arsnoe et al., 2011), the study of individual bird movements or waves of migrating populations in relation to the epidemiology of AIV in wild birds (Gaidet et al., 2010) indicates that these hosts play a role in medium and large scale spread of LPAI and possibly (highly pathogenic) HPAI (Wang et al., 2008; Reperant et al., 2010). In addition, phylogenetic analyses of LPAI indicated intercontinental movements of strains across waterfowl populations (Koehler et al., 2008). Therefore, understanding the role of wild birds in AIV epidemiology is of paramount importance to understand AIV strains' spread and emergence between wild and domestic avian populations (including the emergence of HPAI)(Caron et al., 2009).

At finer spatial scales, wild ducks and shorebirds (the main AIV hosts) are rarely in contact with domestic birds (including free-ranging poultry) due to their distinct ecological requirements. The potential epidemiological role of terrestrial birds that share waterbirds habitats and visit poultry farm for opportunistic foraging, has been proposed as a source of virus transmission between waterfowl and poultry populations (Burns et al., 2012) but little investigated so far (Veen et al., 2007). Risk-based approaches for the local spread of HPAI have identified wild birds as a risk factor for HPAI transmission (Tiensin et al., 2009). More recently, the isolation in a tree sparrow *(Passer montanus)* of the newly emerged H7N9 virus in China where it has caused severe human disease, and the evidence of replication of this H7N9 virus in several terrestrial birds, has raised concerns about the potential role of some passerines in the transmission of AIV with a pandemic potential (Jones et al., 2014; Zhao et al., 2014). Investigating this role is important to better protect poultry populations worldwide as they represent an increasing source of protein for human populations.

In most agro-ecosystems where domestic avian populations are exploited, hundreds of wild bird species cohabit and interact through direct contacts or indirectly by sharing habitat and

resources, offering a complex multi-host system for the transmission of AIV (Caron et al., 2012). In order to identify which wild bird species can spread AIV between waterfowl and domestic populations in a specific ecosystem, a framework is therefore necessary to identify which species amongst the avian diversity can act as bridge hosts. The requirements to qualify as a bridge host concern the physiology and the ecology of the species' population. First, it links with the capacity for the species to get infected, to replicate and excrete AIV, later called host competence for AIV. Little is known about the host competence of most wild bird species to AIV. Specific experimental infection trials have evaluated the competence of only a few species (e.g. (Fujimoto et al., 2010) and opportunistic field sampling targeting waterfowl species have provided some additional data, although usually with small sample size (e.g. (Caron et al., 2012). Overall, information on host competence is available for a maximum of a few hundreds of species whereas about ten thousands of bird species occur worldwide. However, based on available information, most avian orders seem to be susceptible to AIV, which needs to be confirmed by further experimental studies (Olsen et al., 2006).

The second requirement is that the potential bridge host is in contact with the maintenance population, i.e. the waterfowl community and the target population that one wants to protect, i.e. domestic population (Haydon et al., 2002). By contact, we mean the rate of infectious contacts that lead to pathogen transmission. This parameter is important for epidemiological models but has rarely been investigated empirically and is usually estimated through the contact rate between hosts, a proxy which often overstates the infectious contact parameter as most contact do not result into transmission (Richomme et al., 2006). However, the observation of an infectious contact is almost impossible *in situ* and contacts between hosts remain the best proxy available. At a local scale, an infected bird species undertaking local movements (from a few hundred meters to a few kilometres) while able to excrete AIV for a few days can potentially spread AIV between avian populations. Under these circumstances (i.e. lack of information on host competence for AIV and little constraint for virus local spread), the range of eligible bridge host is wide.

We developed an eco-epidemiological framework to identify bridge hosts in an agro-ecosystem. We focused our study on the risk of AIV spread by direct or indirect contact between wild and domestic birds, although we acknowledge the fact that other transmission pathways could be eligible (e.g. poultry trade, human vector). First we used bird counts to identify wild bird species potentially playing a bridge role in the ecosystem, allowing us to quantify the relative proportion of potential contacts between maintenance, bridge and target hosts and reducing the multi-host complexity by ranking species the most at risk of playing a bridge role. Then, we conducted targeted sampling on the species identified to investigate their exposure to AIV when present in the ecosystem. Therefore, instead of sampling “blindly” within the wild bird community, this prioritization process can help guiding AIV surveillance efforts towards the most likely bridge hosts.

Material and Methods

The epidemiological functions under study are identical to those described in Caron et al. (2012): 1) “reservoirs” or "maintenance hosts" of AIV are Anseriformes and Charadriiformes, as generally accepted for AIV epidemiology (Olsen et al., 2006); 2) “target species” according to Haydon (Haydon et al., 2002) are the host population to be protected from AIV infection; 3) "bridge host" has been defined as a species, non-maintenance for AIV as defined in 1), competent for AIV and with the potential to spread the pathogen from an infected population to a naive one. A compartment is defined here as “a set of avian populations under similar environmental conditions” (Caron et al., 2009) such as “waterfowl”, “intensive poultry production farm”, “extensive ostrich farms” or “backyard poultry”.

Study site

Lakes Chivero and Manyame in the Manyame river catchment in Zimbabwe are two artificial dams built in the 50's (respective centroid GPS coordinates for both lakes: 30°33'57'E, 17°49'11'S, 30° 47'51 "E, 17° 53'54" S). The Chivero and Manyame lakes cover an area of 65 and 185km^2 respectively. The study sites encompass the land areas within 12 km of the lakes' shores (Figure 1). Waterfowl living on lakes shores were defined as one bird compartment, considering that natural conditions define a common selective environment. In the direct periphery of the lakes, ostrich farms, intensive poultry farms and traditional backyard poultry systems define three additional domestic compartments. Each production system defines different environmental conditions for the domestic avian population produced: feeding, containment and management differ between each compartment (Sup. Data 1). We consider wild terrestrial birds (referred as wild birds subsequently and to be differentiated from waterfowl) surrounding the four compartments as potential bridge host (later called "bridge candidate") able to spread the virus from one compartment to the other.

In this study site, AIV have been shown to circulate yearlong in wild birds and are present in all 4 compartments and the risk of AIV spread from wild to domestic birds is therefore realistic (Caron et al., 2011; Caron, pers. comm.; Cumming et al., 2011a).

Bird counts

Bird counts in each counting site were implemented as described previously (Caron et al., 2010; Cumming et al., 2011b). Focal counts were undertaken to estimate species diversity and the abundance of waterfowl and domestic communities. Count locations were selected based on expert knowledge for lakeshore's sites and randomly chosen amongst available potential sites for the three domestic compartments. Four 30-minute counts, each at a different time of the day (06:00-09:00; 09:00-12:00; 12:00-15:00; and 15:00-18:00) were carried out in a random sequence at each site for each recording session. Ten minutes after arrival on the counting site, counts of thirty minutes

within a 150m radius of the stationary observer were implemented, identifying and counting all birds present in the counting site. Each recording session encompassed the four counts per site during a week,

every two months during one year (May 2008 - May 2009) in 15 waterfowl sites, and in 6, 6 and 7 counting sites in Backyard poultry, Ostrich farms and Intensive poultry compartments respectively. This first protocol will be referred as “the intensive protocol” (part of this data has already been presented in Caron et al. (2010). Seven waterfowl counting sites, 2 backyard poultry and 2 intensive poultry counting sites from the intensive protocol were counted also from September 2009 to November 2010, during 8 additional sessions at two months interval (ostrich farms in the area were closed down in 2009-2010 for economic reasons). This longitudinal protocol encompassed 14 sessions from May 2008 to November 2010 and included only sites that were counted from May 2008 to November 2010 (7, 2 and 2 waterfowl, backyard and intensive poultry sites respectively). Sessions in waterfowl compartments were paired with the nearest sessions in domestic compartment in time to compare wild bird communities between compartments. Seasons were defined as “rainy" season from December to March, “dry-cold” between April and July and "dry-hot" between August to November. In order to minimize bias in the detection and counting of birds: 1) the vast majority of the counts have been done by the same two experience observers (one ornithologist by profession and one veterinarian specialised in ornithology); 2) Identification and counting procedures were standardized (initial counts were implemented with both observers together); 3) a complete randomization of the counting site sequence for each session and of observers across counting sites was implemented.

Estimation of the rate of host contact between compartments

In this study, a wild bird counted in the defined counting area (i.e. 150m radius) around a bird compartment (e.g. on the lake shore or around a poultry production building) is assumed to be in potential contact with the bird population in the compartment. This assumption is made on the

basis of the various transmission modes of AIV ranging from direct transmission through contacts to indirect transmission through water or fomites contaminated with faecal material (Webster et al., 1992). According to the counting protocol, the spatial window of co-occurrence between birds is 150m and the temporal window is a maximum of a week. This spatio-temporal window is enough for AIV transmission to occur in the ecosystem given the AIV capacity to survive in the environment (Brown et al., 2009; Nazir et al., 2011). In addition, for each domestic compartment, potential direct and indirect contacts between wild and domestic birds have been observed (Sup. Data 1). Between each pair of compartments and for each session, the count data for each species observed at the same time in both compartments constituted a shared community of bridge candidates co-occurring between compartments.

Inter-species contact through a bridge candidate from one compartment to another is dependent on the density of the bridge individuals in the socio-ecosystem, its attraction/repulsion towards specific compartments and its mobility. Our count data of bridge candidates around compartments provide a composite proxy of the density and of its attractiveness to that compartment (counts are implemented within a given surface). At the local scale considered in our study, the ability of a potential “bridge” individual to link any pair of compartments is assumed not to vary greatly among bridge candidates. This assumption may be challenged for some species but: 1) capture-recapture of wild birds in the study site demonstrated that individuals of multiple species could be at opposite locations of the study site along the lake shores (Cumming et al., 2011a; Chiweshe and Caron, 2012); 2) many bird species in southern Africa adopt a seasonal nomadic behaviour leading to a scale of movements far superior to the scale of the study site considered here (hundreds of kilometres vs. a few kilometres respectively).

We then considered that the product of a count (n_b) for a given bridge candidate in a compartment during a session by the count (n_b) for the same bridge candidate in another compartment during the same session could be used as a proxy of the risk of contact between

these two compartments by that bridge candidate. The product was preferred to the sum as any "0" values (species not seen around the compartment) *de facto* disqualify species as bridge candidates for the corresponding session. The sum was also tested but results did not differ from the method chosen. The Interaction Sum *(IS)* of inter-compartment contact was then computed as the sum of the products of $(n_b * n_b)$ across identified bridge candidates (Figure 2). The variable *IS* is therefore a proxy of the overall contact rate between two compartments and characterises the shared community of bridge candidates for each pair of compartments. In addition, the species richness of the shared community of bridge candidates was also computed by summing the number of species identified as bridge candidates for each pair of compartments. Pairs of compartment B/I, B/W, I/W, O/W, O/I and O/B (B = Backyard poultry, I = Intensive poultry, O = Ostrich farms and W = Waterfowl communities) will be later referred as “interfaces” (Figure 3).

To explore the variability of the shared community of bridge candidates between pairs of compartments, variations of *IS* and species richness were analysed for the intensive and longitudinal protocols. One statistical unit per interface and counting session was considered. For the analysis of data generated by the longitudinal protocol there were 14 counting sessions * 3 interfaces (B/I, B/W; I/W) = 42 statistical units. For the analysis of the data generated by the intensive protocol there were 6 counting sessions * 6 interfaces (B/I, B/W; I/W, O/B, O/I, O/) = 36 statistical units. *IS* and species richness values for each statistical unit were obtained by merging bird count data over sites belonging to a same compartment. The dependent variables were checked for normality and homogeneity of variance using the Fligner-Killeen test. Then ANOVA analyses were performed for both protocols using alternatively *IS* and species richness as dependent variables. *IS* was log transformed in order to comply with the assumption of normality. For the intensive protocol, a two-way ANOVA was run with "interface" and "season" (using the three seasons "rainy", "dry-cold" and "dry-hot" as described above) as factors. For the longitudinal protocol, a three-way ANOVA was run with "interface", "season" and "year" (using the three years that the study encompassed, 2008, 2009 and 2010) as factors. The “aov” R function was used (R

Development Core Team, 2011).

To explore the relative importance of contacts between bridge candidates and target domestic populations compared to contacts between reservoir/maintenance populations and domestic target hosts, we also calculated for the intensive protocol the proportion of Anseriformes and Charadriiformes (i.e. the maintenance hosts) observed in relation to the total number of wild birds counted for each compartment and for each session.

In order to further explore the qualitative and quantitative characteristics of *IS,* we identified dominant bridge candidates as the most represented bird species (with a threshold arbitrarily set at more than 20% of IS) in the shared community of bridge candidates.

Targeted AIV sampling on bird species most at risk of bridge role

Targeted sampling was implemented on the three most dominant bridge candidates to test for their potential infection with AIV. The detection of an infection in these species reveals exposure to an infectious host or environment as well as its receptivity to AIV infection and its capacity to replicate and shed the virus, thus confirming its potential role as a bridge host. The specific ecology of each species and the count data were used to implement the sampling when the potential bridge hosts where present in both wild and domestic compartments and . was representing a high risk of contact between compartments. Barn swallows are Palearctic migrants and arrive in our study area in September and leave in March-April. We conducted mist-netting and cloacal and tracheal sampling on a barn swallow roosting site in February 2010. Red-billed queleas are nomadic species and usually frequent the study area between May and November. We collected cloacal swabs on queleas in September 2010 during a culling operation conducted by the Park and Wildlife Management Authority (queleas are considered as a pest in southern Africa). Cattle egrets are believed to be resident in the ecosystem and we collected faecal samples shortly after deposition of the ground from identified individuals in April 2010 (cattle egrets are difficult to capture in traps or

nets). Faecal (and tracheal whenever possible) swabs were collected on birds randomly and were tested using the method described by Fuller et al. (2010). All real-time reverse transcription polymerase chain reactions (rRT-PCRs) were run on an Applied Biosystems StepOnePlus platform (Life Technologies, Carlsbad, CA, United States of America).

Results

During the intensive protocol, 165 bird species were observed including 15 reservoir species (i.e. anseriforms and charadriiforms) and during the longitudinal protocol, 230 bird species were observed including 33 reservoir species.

The results of the two-way ANOVA for the intensive protocol indicated that interfaces or pairs of compartments had significantly different species richness but not *IS.* No effect of season or of the interaction component was detected on *IS* and species richness. The three-way ANOVA for the longitudinal protocol indicated that interfaces had significantly different species richness and *IS.* No significant effects of season, of year or of the interaction terms were detected on *IS* or species richness (Table 1). The interfaces with the hiahest *IS* were B/I (for both protocols) then O/I and with the highest species richness were B/I or the intensive protocol and B/W for the longitudinal protocol-- (Table Sup1 and Sup2).

The percentage of maintenance hosts observed within the wild birds community around the three domestic compartments was 5.1,4.8 and 4.0% for the backyard, intensive poultry and ostrich compartments respectively with a peak in September or March depending on the compartment (Figure 4). For comparison, in he waterfowl compartment, this figure was 47.6%. In terms of number of birds observed, there were 20 times more potential bridge hosts in contact with domestic populations than maintenance hosts..

During the intensive protocol, seven dominant species (i.e. representing more han 20%

of the *IS* for that particular session and interface) were identified for the six interfaces and the 6 sessions encompassing 34 counting sites, ncluding five Passeriformes species, one Columbiformes and one Ciconiiformes Table 2). Three dominant species were more abundant than others and identified at all interfaces: barn swallow *(Hirunda rustics),* red-billed quelea *(Quelea quelea)* and cattle egret *(Bubulcus ibis).* During the longitudinal protocol, 9 dominant species were identified for the 3 interfaces for the 14 sessions, including 7 Passeriformes species and two Ciconiiformes (Table 3, Figure 5). The three same species were overabundant (red-billed quelea, barn swallow and cattle egret). In Table 2 and 3, for the highest *IS* values (grey shade), the three dominant species highlighted previously dominate again the interaction sum with only one other dominant species identified for these highest *IS* values (i.e. grey-umped swallow for the B/W interface in May 2010). Overall, dominant species n=12) represented the vast majority (79%) of the *IS* and the three most dominant pecies represented 65% of *IS.*

AIV was detected in two or the three dominant species sampled. Red-billed queleas and barn swallows had an AIV prevalence of 0.96 % (n = 208, Confidence interval at 95% [0-2.29]) and 3.00% (n = 133, Confidence Interval at 95% [0-5.92]) respectively, indicating that both species have been exposed to the virus and are competent to excrete the virus. None of the 166 faecal samples of cattle egret were positive for AIV (Figure 6).

Discussion

The need to explore the role of wild birds in the epidemiology of AIV at the wild/domestic interface has been emphasised (Veen et al., 2007), although it has not been investigated extensively so far (but see -(Burns et al., 2012). Following a two steps eco-epidemiological framework (Figure 6), we estimated contacts between maintenance hosts for AIV and target

avian populations (e.g. domestic species) and determined host competence for AIV for the most probable bridge candidates. Our two and half-year study demonstrated that: 1) the shared community of bridge candidates varies significantly according to the interfaces or pairs of bird compartments considered; 2) no seasonal nor inter-annual variability trends of *IS* (e.g a proxy of inter-compartment contacts through wild birds) have been detected in this shared community; 3) potential indirect contacts between reservoir maintenance and target species through bridge hosts are 20-fold more frequent than potential direct contacts between maintenance and target populations; 4) a few dominant bridge candidates represent most of the risk of contact between pairs of compartments despite hundreds of species observed; 5) Two out of three of the most co-occurring bridge candidates did harbour AIV genetic material in the agroecosystem studied, proving their bridge role in this ecosystem.

Bridge candidates were not distributed equally between compartments. Ecological, environmental and anthropological factors can influence the distribution of these species. The distance between compartments could also be a confounding factor. For example, all counting sites were located within a 120 kilometres radius from the lakes' shores, but ostrich farms tended to be on the outskirts of the study sites, while some backyard poultry sites were in direct contact with the lakeshores. However, the ostrich compartment interfaces with other compartments did not have a systematically lower *IS* value indicating that distance was not an important factor at our scale. Variability between sites belonging to the same compartment has not been taken into account in the analyses as it was assumed that sites of the same compartment (i.e. same production or natural systems) shared characteristics such as availability of resources or roosting potential. In a previous study, this assumption was not challenged (i.e. marked differences between compartments were observed). Domestic production systems can also provide attractors for wild birds. Ostrich are fed and watered outdoor in large drums that wild birds can easily access. Intensive poultry are fed indoor but

resources to wild birds. On the contrary, backyard poultry are left foraging within and outside villages and compete with wild birds for “natural food resources”. Buildings can also provide roosting site for passerine species such as swallow species as was often observed during this study. Therefore, we believe that compartment-specific characteristics tend to define the community of wild bird species using these compartments.

No significant difference of *IS* or species richness was observed between seasons or between years. This result is particularly counter intuitive as wild bird experience seasonal population dynamics due to movements (e.g. migration) or reproduction (Mundava et al., 2012). For example, months of May and November display high *IS* values for the three interfaces and for the length of the study (Figure 5). Rainfall patterns in sub-tropical Africa are more variable in time and space than in the northern hemisphere and wild birds adapt their behaviour to this variability through complex behaviour such as nomadism (Verschuren et al., 2000; Dodman and Diagana, 2007). For example, red-billed queleas are nomadic birds responding to resource availability determined by rainfall and the timing of their arrival in a specific ecosystem can vary (e.g. May 2008 & 2009 but not in May 2010 in Figure 4a)(Dallimer and Jones, 2002). Palaearctic migrants such as barn swallow leave Eurasia to arrive in Zimbabwe in September to depart again around March and April. If no seasonal or inter-annual trends were observed at the *IS* level, at the (dominant) species level, wild bird ecology induces periodic trends in potential contacts. The low variability of species richness across season and years supports the hypothesis that domestic compartment “artificially” attract wild bird species, as the species richness varied on the lake shores in a previous study (Caron et al., 2010).

We measured the potential direct contacts between the maintenance and target domestic hosts: they represented between 4.0 and 5.1% during the intensive protocol of the potential indirect contacts through bridge candidates. Some of the domestic compartments we have monitored during this study were close to or on the lakeshores: direct contacts between reservoir and target species were more likely to occur under these circumstances. Maintenance

hosts should experience a higher prevalence and a better propensity to transmit AIV to target populations and could therefore trigger less but more efficient infectious contacts. However, as shown recently in the same ecosystem (Caron et al., 2012), non-maintenance species can harbour AIV and their abundance justifies the need to investigate more in depth their potential role as bridge hosts.

Only 12 bridge candidate species (9 passeriforms species, two ciconiiforms and one columbiform) were identified as dominant (i.e. representing more than 20% of *IS* during at least one session) during the intensive and longitudinal protocol in which 165 and 230 bird species were observed including 15 and 33 maintenance species respectively. Firstly, it indicates that the majority of potential contacts between compartments could be managed by concentrating efforts on a limited number of candidate bridge hosts. Of interest is the presence of 6 swallow-type species, which are probably attracted to farming systems by the same resources (e.g. insects, roosting) and may therefore be managed by the same measures. Interestingly, proofs of AIV susceptibility for the three species dominating the potential contacts between compartments exist in the literature (Gronesova et al., 2008; Mizakova et al., 2008; Breithaupt et al., 2010; Phuong et al., 2011). Barn swallow was also identified as potential bridge host in a very different ecosystem (Burns et al., 2012). Our PCR results confirmed the role of bridge host in our agro-ecosystem for barn swallow and quelea, but not for cattle egret. These results seem to support that the proposed framework based on count data is an efficient approach to identify potential bridge host. However, these results could result from the fact that most wild bird species are competent for AIV. In which case, the framework presented here would still be valid, as it identifies the hosts that have the highest intensity/frequency of contacts with maintenance and target population and therefore highlights high-risk bridge hosts for AIV transmission.

More precise estimations of contact rates between hosts may be obtained using other methods such as individual telemetry or direct observation of contacts. However, not all contacts between hosts result in the transmission of pathogens (i.e. infectious contact**)** (McCallum et al.,

2001**)**, and some contacts may have a higher probability of pathogen transmission than others. The susceptibility of a bird in contact with an infected individual is highly variable and depends on its individual history (immunity) and on the susceptibility of the species to the particular pathogen and/or strain. Patterns of pathogen transmission between bridge hosts and their influence on the risk of transmission from infected to susceptible compartments can be complex and extremely difficult to assess. As our objective was to design an easily reproducible protocol to identify bridge hosts, this aspect was not taken into account.

Our results have direct implication for the management of the AIV risk in the agro-ecosystem. For example, red-billed queleas are considered as a crop pest in southern Africa and a variety of control options are currently used to avoid those visiting crops, which could also be used to limit contacts with domestic poultry. Barn swallows are probably mainly visiting production units to feed on insects. Insect control options may for example reduce these visits. Modification of the habitat could also reduce roosting sites at proximity of production buildings. Cattle egrets are often following cattle in the proximity of farms. Avoiding cattle visiting to those farms (as it has been often observed during field visits) would reduce the interface. A few control options could therefore significantly reduce the risk of spread of AIV between compartments. However the inter-compartments variability would require adapted management depending on production systems.

Conclusion

This eco-epidemiological framework should be used to investigate the neglected role of bridge host in AIV epidemiology, a role that could be determinant in the local spread of the disease between avian populations and even towards humans (Jones et al., 2014). The bird count protocol presented here is time consuming (i.e. 584 hours of bird counts) but requires only ornithological skills which may be obtained at low/no cost in many areas through volunteer

participation of ornithological associations. In addition, available local ornithological datasets could be used to prioritise bridge candidate amongst the avian diversity. Instead of sampling blindly the avian community, this framework can structure surveillance by targeting the bird species with the highest contact rates between pairs of compartments as these species are the most likely to spread diseases. From a theoretical point of view, it would be interesting to investigate the shared communities of bridge hosts in other ecosystems to assess if our findings (i.e. that most of the potential contacts are made by a few species) are site specific or not. Finally, as this approach is based primarily on potential contact and co-occurrence pattern, it could be used for other pathogens and different animal models, by simply adapting the definition of “maintenance” and “target” populations to the pathogen considered.

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Table 1: Two-way (pairs of compartment "poc", "season" as factors) and three-way ("poc", "season" and "year" as factors) ANOVA for the intensive and longitudinal protocols respectively. *IS* is the interaction sum as defined in the text and Sp.Rich. is the species richness.

				IS		**Sp.Rich.**	
Protocol	**ANOVA**	**Source**	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Intensive	Two-way	poc	5	1.98	0.13	8.94	<0.001*
		season	2	1.11	0.35	0.03	0.97
		poc*season	10	0.27	0.98	1.82	0.13
		residuals	18	-	-	-	-
Longitudinal	Three-way	poc	2	11.66	<0.001*	15.39	<0.001*
		season	2	1.05	0.37	0.46	0.64
		year	1	2.19	0.15	0.02	0.89
		poc*year	2	0.40	0.67	1.94	0.16
		poc*season	4	0.22	0.93	2.10	0.11
		season*year	2	1.55	0.23	0.75	0.48
		residuals	25	-	-	-	-

Table 2: Proportion of IS value represented by each dominant species for each interaction sum for each session of the intensive protocol for each pair of compartments. Scientific names not specified in the text: bronze mannikin *(Lonchura cucullata)*, southern red bishop *(Euplectes orix)*, dark-capped bulbul *(Pycnonotus tricolor)* and cape-turtle dove *(Streptopella capicola)*. B/O corresponds to the Backyard poultry and Ostrich farm compartment interface (W stands for the Waterfowl compartment and I for the Intensive poultry compartment).

	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
B/W						
Red-billed quelea	24%		47%			26%
Barn swallow			64%	47%		
Cattle Egret	55%	64%			38%	56%
I/W						
Red-billed quelea	36%	33%	81%	65%		31%
Barn swallow			22%			
Cattle Egret	40%	39%			75%	34%
O/W						
Red-billed quelea	33%					68%
Barn swallow			64%	80%	44%	
Cattle Egret	28%	39%			50%	
B/I						
Red-billed quelea	88%	34%	56%			70%
Barn swallow			26%			
Cattle Egret		20%			25%	
Bronze mannikin			31%	20%		
Passerine sp.		23%				
Southern red bishop					21%	
B/O						
Red-billed quelea	82%					93%
Barn swallow			68%	60%		
Cattle Egret						
Bronze mannikin		30%	24%	25%		
Cape-turtle dove		35%				
Dark-capped bulbul			21%			
I/O						
Red-billed quelea	89%			27%		94%
Barn swallow				45%	24%	
Cattle Egret					51%	
Bronze mannikin		63%	33%	23%		

Dominant species are defined as participating in more than 20% in the total interaction sum. In grey, sessions with highest interaction sum for each pair of compartments. (For B/I. “Passerine sp.” refers to unidentified small passerines such as red-billed quelea or bronze manikin).

Table 3: Proportion of IS value represented by each dominant species for each interaction sum for each session of the longitudinal protocol for each pair of compartments. Scientific names not specified in the text: bronze mannikin (*Lonchura cucullata),* African palm swift (*Cypsiurus parvus),* little swift *(Apus affinis),* grey-rumped swallow *(Pseudhirundo griseopyga),* banded martin *(Riparia cincta)* and reed cormorant *(Microcarbo africanus).* B/W corresponds to the Backyard poultry and Waterfowl compartment interface (I for the Intensive poultry compartment).

	May-08	Jul-08	Sep-08	Nov-08	Feb-09	May-09	Sep-09	Nov-09	Feb-10	Mar-10	May-10	Jul-10	Sep-10	Nov-10
B/I														
Red-billed quelea	90%	30%				59%	24%	90%					83%	
Barn Swallow		57%							34 %	27 %				56 %
Cattle Egret					33 %	30%				50%	64 %	78 %		
Bronze Mannikin		27 %	30%						23 %					
African Palm Swift			22 %											
Little Swift			41%				20 %							
Passerine Sp.		49 %												
B/W														
Red-billed quelea							22%	49%						
Barn Swallow		67 %	70 %					34%	83%	59%				70 %
Cattle Egret	86%	65 %				73 %	39 %			28%	42%	23 %	22 %	
Reed cormorant		28 %												
Grey Rumped Swallow											32%	32 %		
I/W														
Red-billed quelea		62%				23%	72%	65%			52%			
Barn Swallow		55 %	89 %	34 %					49%	96%				84%
Cattle Egret	82%	21 %			39%	48 %			36%		31 %	41 %		
Bronze Mannikin						20%								
African Palm Swift													30 %	
Grey Rumped Swallow												38 %		
Banded Martin													33%	

Dominant species are defined as participating in more than 20% in the total interaction sum. In grey, the 4 sessions with highest interaction sum for each pair of compartments. (For B/I. “Passerine sp.” refers to unidentified small passerines).

Figure 1 : A view of the study site. Top right is a map of Africa indicating the location of Zimbabwe. Top left is a map of Zimbabwe indicating the location of the study site (in the rectangle); the bottom section is a map of the study site with Lake Manyame on the left and Lake Chivero on the right with the main town Norton indicated on the Manyame shores. Red dots indicate waterfowl counting sites, blue dots indicate intensive poultry counting sites, green dots indicate backyard poultry counting sites and yellow

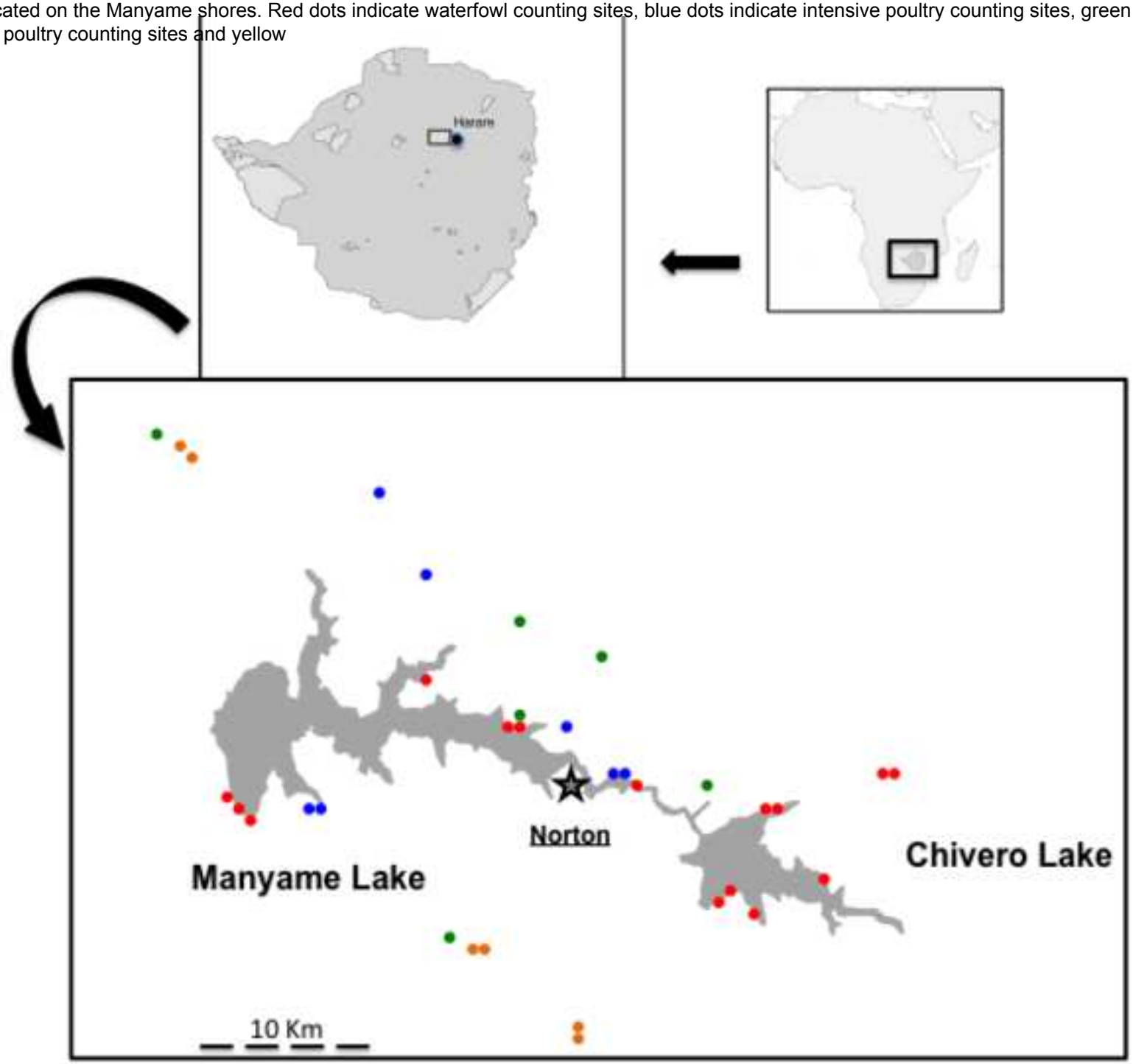
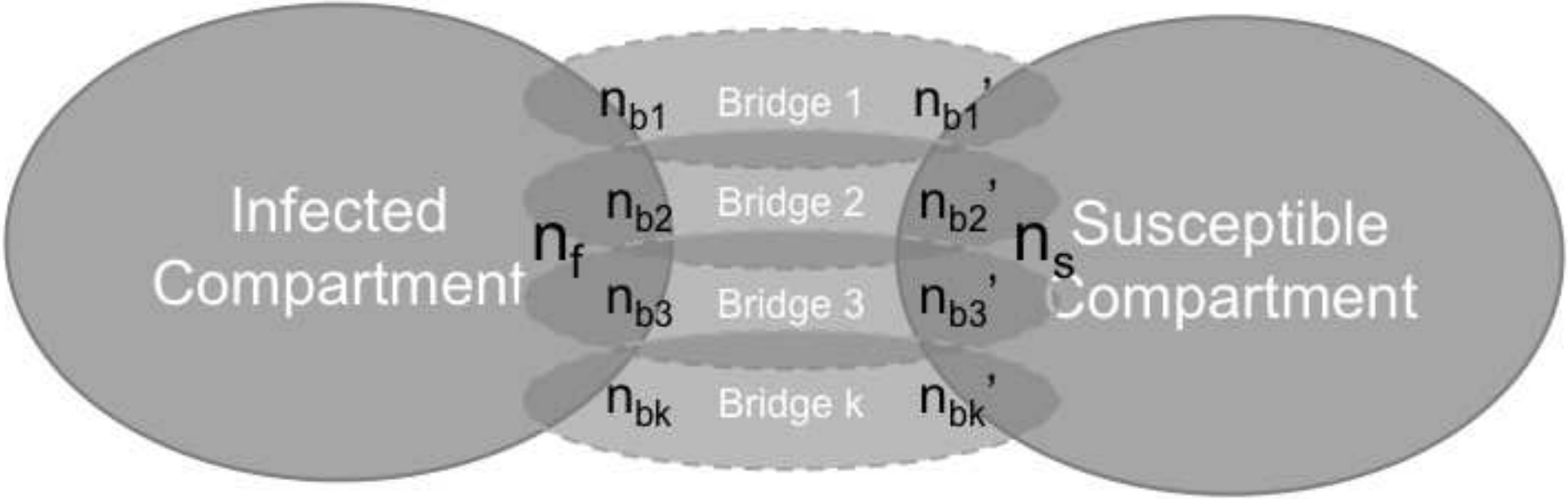


Figure 2 : Schematic representation of the role of multiple bridge candidates between an infected and a susceptible avian compartment and showing how the interaction sum (IS) variable was calculated. n i = the number of birds in the infected compartment; n s = the number of birds in the susceptible compartment; n b1 = the number of birds in bridge species population 1 in contact with the infected compartment; and n′b1=then′b1=the number of birds in bridge species population 1 in contact with the susceptible compartment.



$$IS_{bridge} = \sum_{(i:1 \rightarrow k)} (n_{bi} * n_{bi}')$$

Figure 3 : Conceptual framework of the study: four avian compartments (intensive poultry, backyard poultry, ostrich farm and waterfowl community) occur in the system and are surrounded by a wild bird community (wild bird being defined as non-waterfowl species). Bird counts in each compartment (white circle) are implemented and for each species n and n′ individuals are observed around each compartment. These counts from the same session (same time) are compared to identify which wild bird species can act as a potential bridge candidate between the pair of compartments.

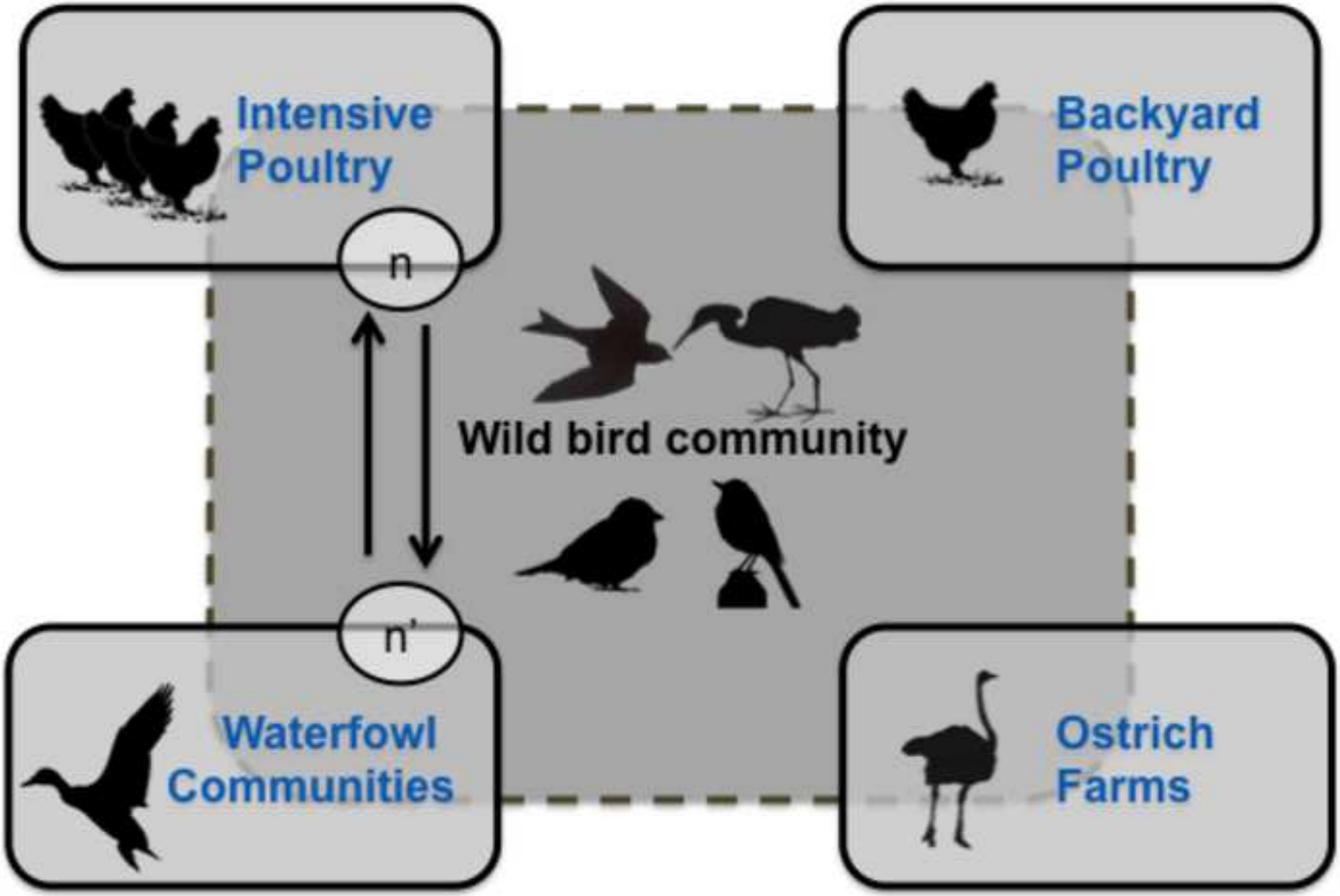


Figure 4 : Variation during the intensive protocol of the proportion of maintenance hosts (i.e., Anseriformes and Charadriiformes) in the counted birds around the three domestic compartments: backyard, intensive poultry and ostrich farm compartment.

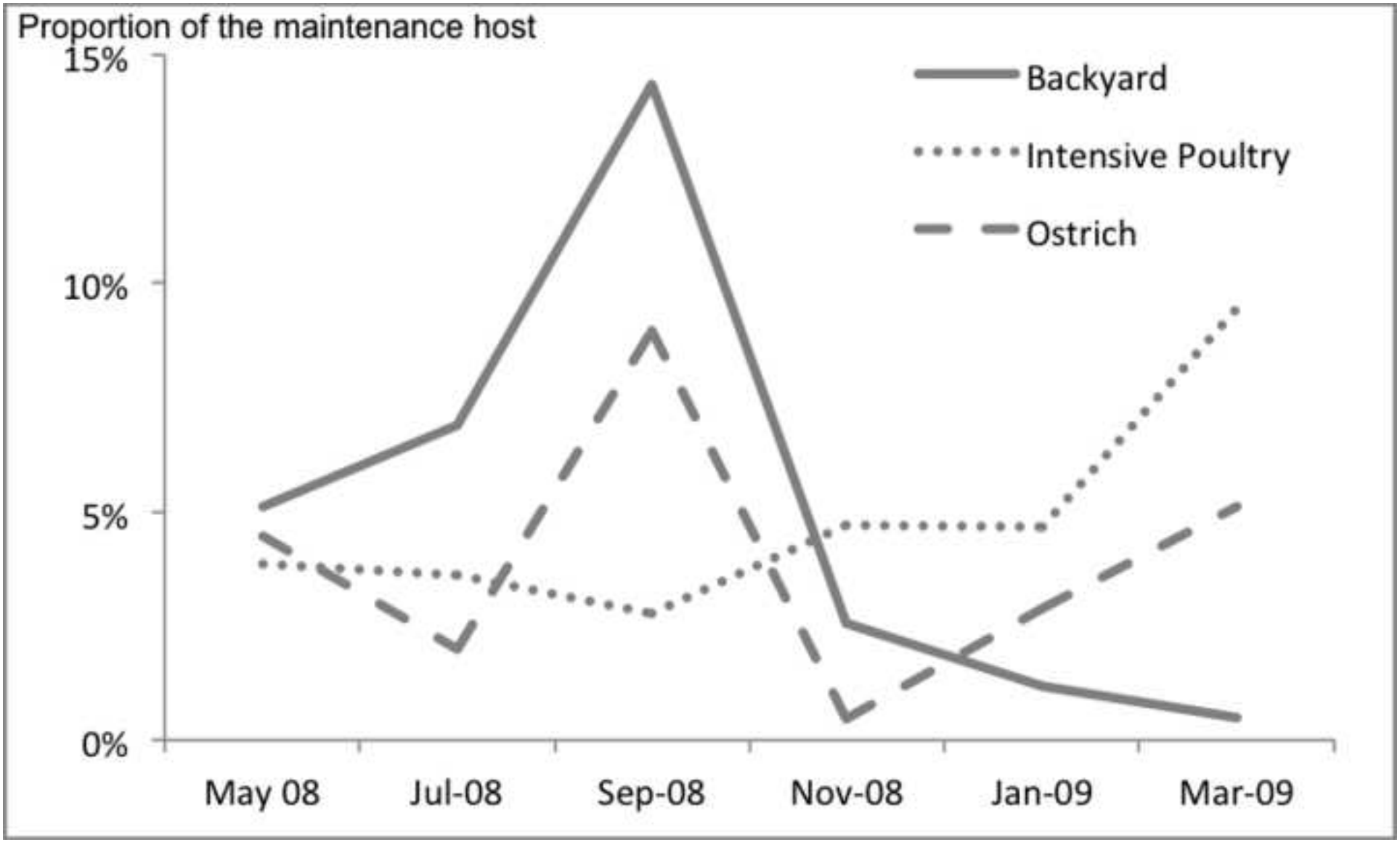


Figure 5 : Variation of the log of the interaction sum (IS) in the longitudinal protocol across the 14 sessions. The proportion of the three main dominant species is indicated: in dark grey, red-billed quelea; in medium grey, barn swallow; and in light grey, cattle egret. Dash bars indicate the rest of the bird community. In (a) the backyard/intensive poultry interface, (b) the backyard poultry/waterfowl interface and (c) the intensive poultry/waterfowl interface.

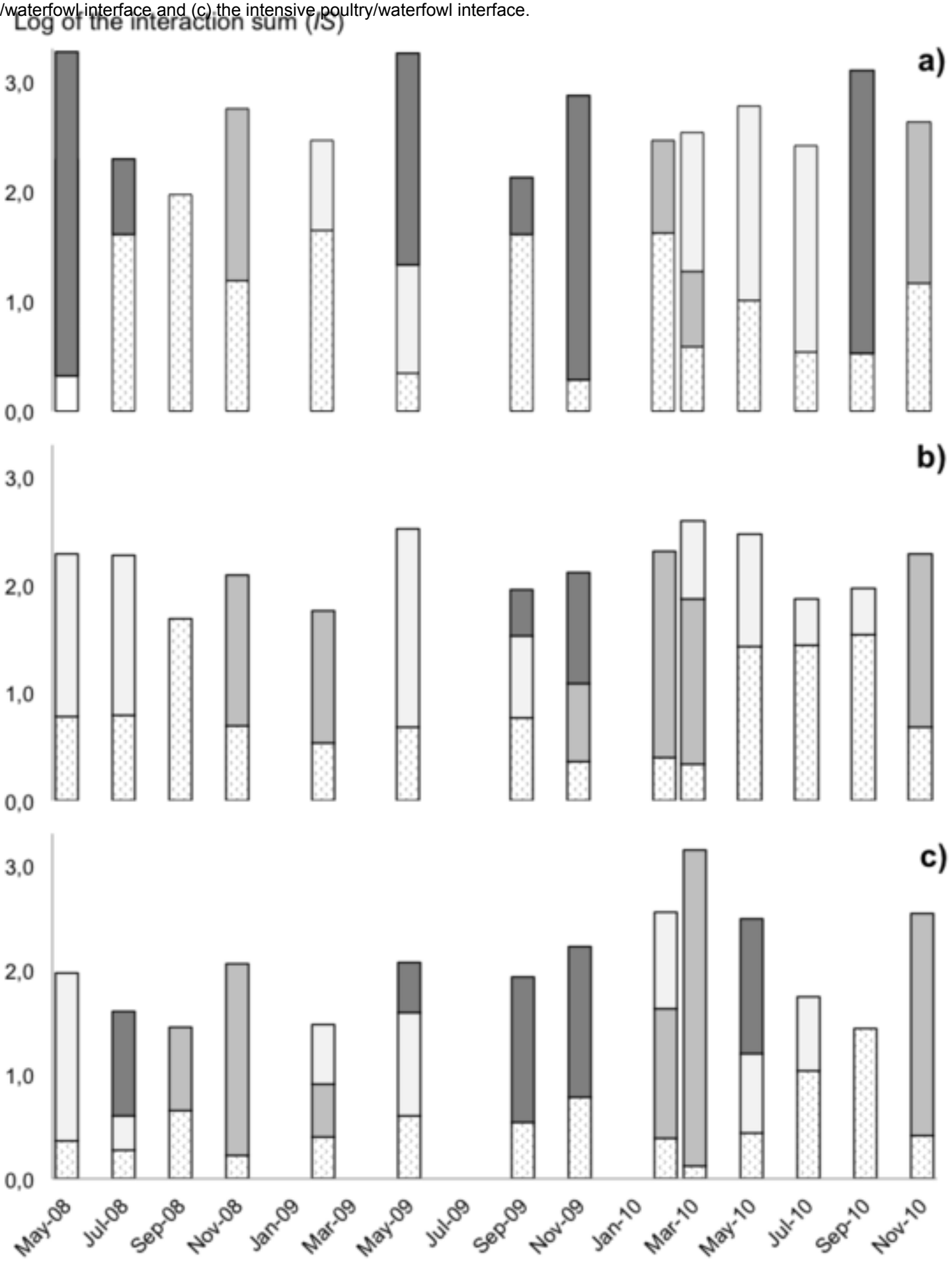
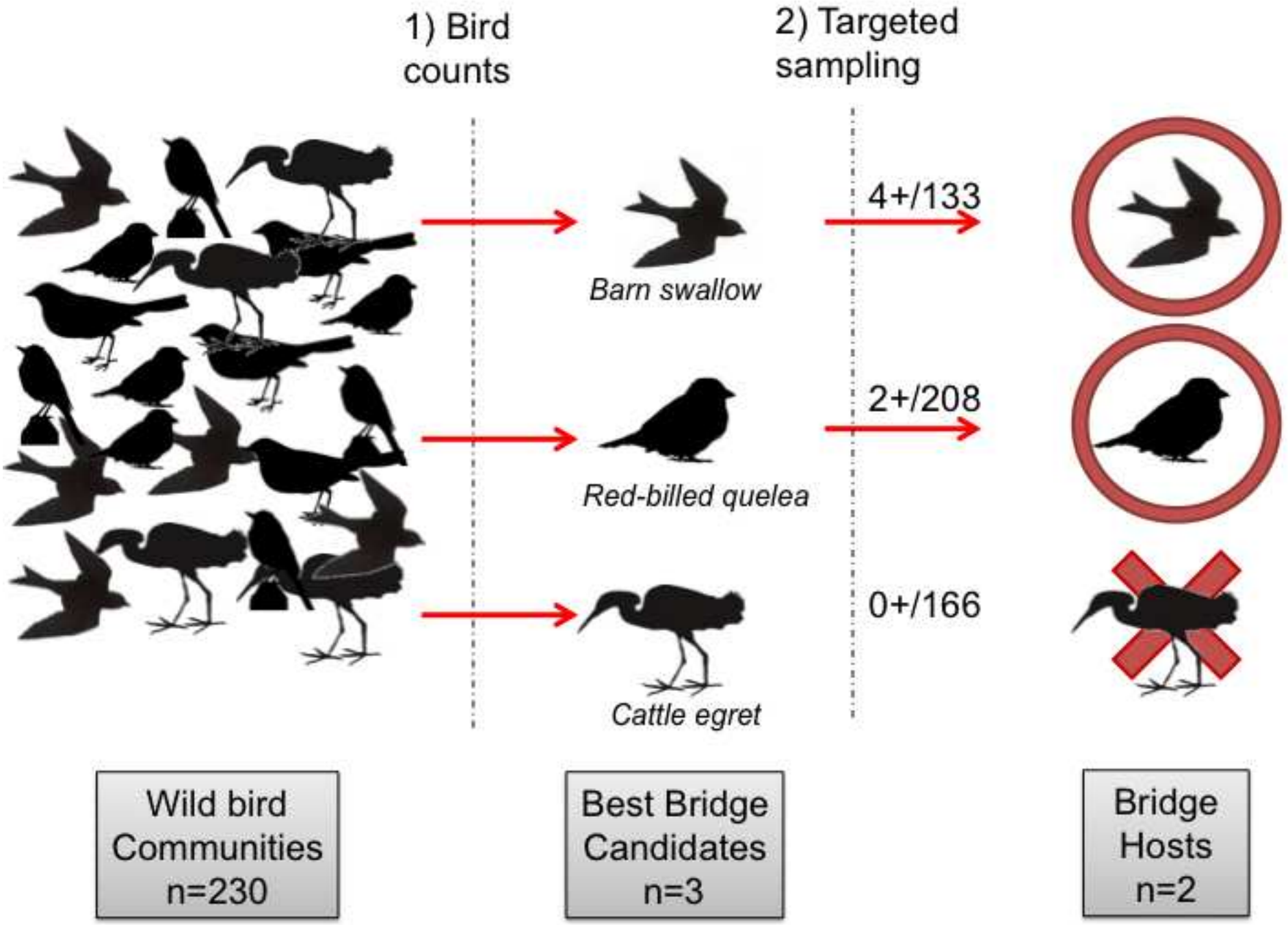


Figure 6 : Schematic representation of the eco-epidemiological approach. In order to reduce the complex multi-host system of the wild bird community, bird counts identify the bridge candidates with the highest risk of bridging the two compartments studied. Here, three bird species are identified as such, and targeted and adapted sampling and AIV testing on these species confirm (barn swallow and red-billed quelea) or not (cattle egret) their bridge role in the agro-ecosystem.



Supplementary Data 1:

The mean number of birds observed during the counting protocol per compartment was 22 chickens, 145 waterfowl, 189 ostriches and 5240 chickens respectively for Backyard poultry, Waterfowl, Ostrich farm and Intensive poultry compartment (counted from the intensive protocol). These numbers reflected the quantity of individual birds potentially exposed to bridge species for each compartment, except for the intensive poultry compartment. In the Intensive poultry compartment, security measures are put in place to minimise contacts between production chicken and their environment (e.g. confinement in buildings, mesh). However, despite some variability in the level of biosecurity between the seven intensive farms in the study, chickens roaming outside buildings have been observed in each of them. No systematic recording of this data was made during both protocols but on 19 records, an average of 12.5 birds per count was observed outside the production buildings (maximum 42 birds). These escapes were the results of holes in the mesh (or size of the mesh in some places not adapted to a few days-old chicks) or staff negligence (gate left open during feeding). No active recovery of the escaped birds was observed and these chickens were observed feeding in proximity to potential bridge species on several occasions. During sanitary quarantine (with no chicken in the building) between two production cycles in the intensive compartment, potential bridge species (particularly small passerines) have been observed feeding on food left-over in the production building. This type of behaviour could lead to indirect contacts with bridge species leaving infected material in the building.

The Ostrich compartment is characterised by open paddocks where ostrich from the same age groups are raised together. They are fed outdoor and remain in the open paddock night and day, exposed to potential contact with wild birds. At the beginning of the year (January), farms re-stock their pens with young ostrich chicks imported from South Africa or other parts

of the country. In November-December, the ostrich are slaughtered for meat exportation except for a few individuals kept for reproduction.

The backyard poultry compartment is a typical household production system where chicken are raised with very little input, gathered in sheds at night and left foraging in the village and its direct periphery during the day.

The waterfowl compartment is strongly linked to the two lakes of the study areas and can be considered as natural bird community dependent on water.

*Table S1: Mean estimates and associated standard error (Std. Error) of IS, I (n_b * n_b'), and of species richness (Sp. Rich.) for each pair of compartment.*

		IS or I (n_b * n_b')		Sp. Rich.	
	Interaction	**Mean**	**Std. Error**	**Mean**	**Std. Error**
Longitudinal protocol	B/I	638.1	595.7	24.6	4.0
	B/W	173.8	106.8	28.1	4.2
	I/W	223.6	350.6	20.6	3.4
Intensive protocol	B/I	534.5	558.0	59.8	8.1
	B/W	81.5	37.3	49.3	6.9
	I/W	153.4	66.5	50.0	8.3
	O/W	74.8	85.8	35.2	8.0
	I/O	454.8	557.3	49.3	8.6
	B/O	334.3	470.0	45.3	8.0

(B=Backyard compartment, I=Intensive poultry compartment, W=Waterfowl compartment, O=Ostrich compartment).

*Table S2: Mean estimates and associated standard error of IS, I (n_b * n_b'), and of species richness (Sp. Rich.) for seasons and years.*

		IS or I (nb*nb')		Sp. Rich.	
		Mean	**Std. Error**	**Mean**	**Std. Error**
Longitudinal protocol	2008	296.8	516.2	24.2	6.5
	2009	335.7	511.1	25.7	3.8
	2010	383.7	369.2	23.7	4.4
	Dry-Cold	431.8	572.0	24.0	4.7
	Dry-Hot	260.3	318.6	24.9	5.6
	Rainy	370.6	396.8	24.0	4.0
Intensive protocol	Dry-Cold	248.1	407.1	44.0	8.4
	Dry-Hot	188.7	172.1	47.8	10.6
	Rainy	379.9	528.7	52.7	11.1