Chapter Five: Risk of diffusion of a Highly Pathogenic Avian Influenza virus between wild and domestic avian compartments through bridge species in Zimbabwe

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Introduction

The panzooty of Highly Pathogenic Avian Influenza (HPAI) H5N1 and its threat to poultry and human health (Webster et al. 1992, Olsen et al. 2006, Webster et al. 2006) have raised concerns about the role of wild birds in the ecology of Avian Influenza viruses (AIV). Waterfowl (Anseriformes and Charadriiformes) are considered reservoir for Low Pathogenic AIV (LPAI) strains but the massive HPAI H5N1outbreaks in waterfowl tend to dismiss these species as reservoir for HPAI (Wang et al. 2008) although this role cannot be confidently ruled out. Wild birds could also be involved in the epidemiology of AIV through their potential to spread these viruses. Phylogenetic analyses of isolated strains indicated inter-continental movements of strains across wild bird populations (Koehler et al. 2008). Gaidet et al. (2008) followed the movements of a HPAI H5N2 infected healthy waterfowl across international borders. If the consequences of AIV infections on migrating bird is still discussed (van Gils et al. 2007, 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 HPAI (Wang et al. 2008, Reperant et al. 2010).

At a finer spatial scale, little is known about the role of wild birds as potential spreaders of AIV virus (Veen et al. 2007). This role is however crucial in linking the AIV natural reservoir to the avian production sector. Risk-based approaches for the local spread of HPAI have identified wild birds as a risk factor for HPAI transmission (Gilbert et al. 2006, Tiensin et al. 2009). As the spatial scale changes, the criteria for eligibility of wild bird species as spreaders of AIV also differ. A bird excreting the virus for a few hours and involved in small-scale movements could spread the pathogen without having to consider the ecological fitness issue at this spatio-temporal scale. Under these circumstances, the range of eligible spreader species increases vastly and approaches the size of the local avian species richness.

Once the virus is introduced in an ecosystem, it can spread through the movements of wild birds and infect naive bird populations through contacts with infected populations. Species spreading a pathogen from an infected to a naive population are termed "bridge species". Throughout this article, the term "bridge species" will be used for any bird species with the potential to spread AI strains from an infected bird compartment, defined here as "a set of avian populations under similar environmental drivers" (Figure 5.1, Chapter Two - Caron et al. 2009) to a naive bird compartment; as a consequence, any waterfowl species can in principle be eligible as bridge species when they are in contact with birds of a naive compartment. Despite experimental infection trials (Brown et al. 2009, Fujimoto et al. 2010, Nemeth et al. 2010) and field sampling (opportunistic sampling when targeting waterfowl species), the capacity of potential bridge species to locally spread HPAI between bird populations, particularly at the wild/domestic interface is still largely unexplored.

The selection of potential bridge species out of the available avian diversity in an ecosystem is difficult. Caron et al. (2010) (Chapter Three) presented a framework for this selection process. In order to adequately study the role of bridge species in transmitting AIV in-between bird populations of interest, the epidemiological interaction (EI) concept is used (Chapter Three - Caron et al. 2010). Here the EIs are estimated using bird census data. The co-occurrence of bird species at different counting points during the same period are used to estimate the potential EIs within and between these counting points. At the ecosystem level, the co-occurrence of birds from the same species at different focal points and at the same time suggests potential contacts. This approach assumes that all bird species are potential bridge species, since there is limited data available on the susceptibility for AIV for most bird species.

In this article, ecological and epidemiological data are integrated to provide a risk analysis of the spread of a HPAI virus from an infected bird population to naive bird populations. In an

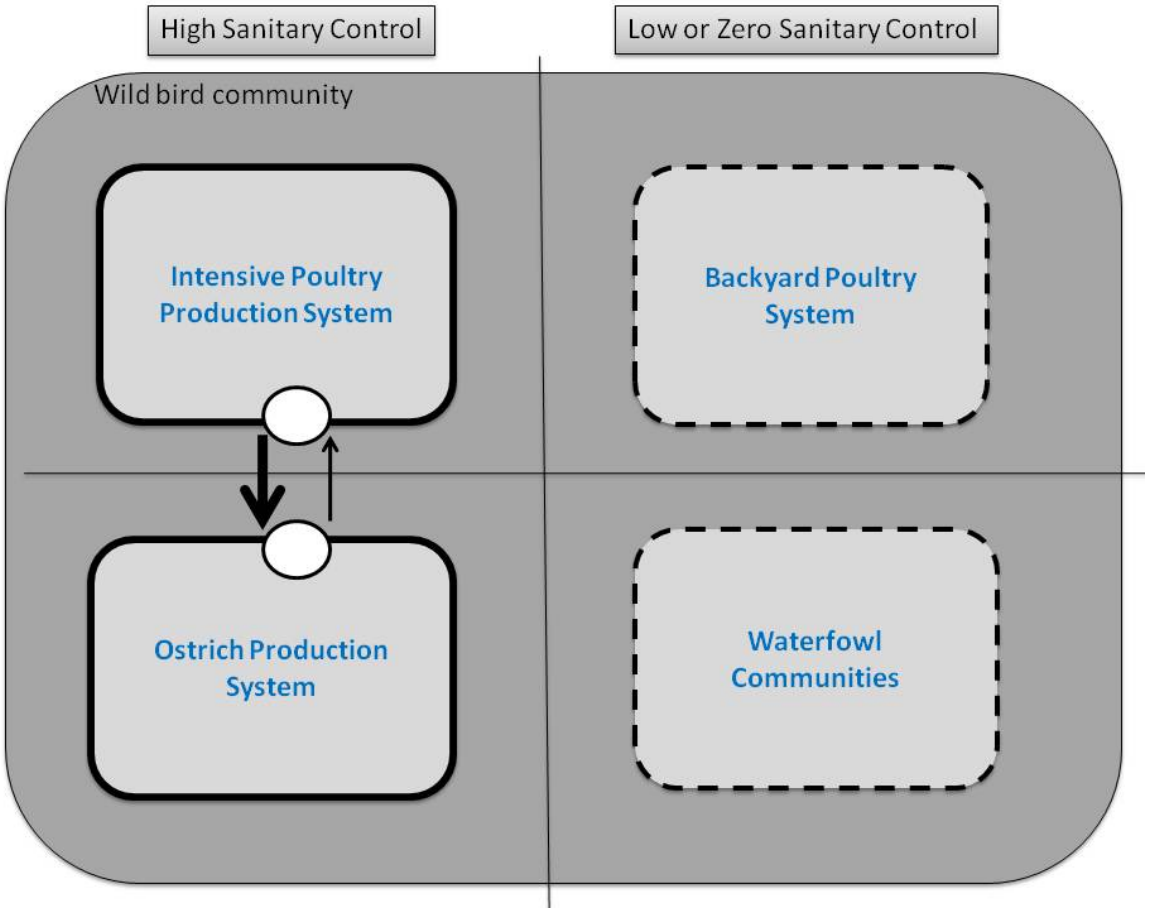
African ecosystem with four bird "compartments", we use bird census to construct the most likely spread pathways and identify most probable bridge species for spreading a HPAI to naive avian compartments.

Materials and Methods

Study site

Lake Chivero and Manyame in the Manayme river catchment in Zimbabwe are two artificial dams built in the 50's to supply water for Harare, capital of Zimbabwe (approximate GPS coordinates: E30°30'30'', S17°45'45''). Chivero and Manyame lakes cover an area of 65 and 185km^2 respectively, although they experience substantial variation in their water surface areas due to the seasonality of rainfall and artificial water management by humans. The study site encompassed the area in a 10km radius around the lakes. Waterfowl living on lakes shore were defined as a 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 existed and were defined as three additional compartments. We considered wild bird communities (distinct from the waterfowl compartment, which is restricted to water-dependent species) surrounding the four compartments as the source for potential bridge species able to spread the virus from one compartment to another (Figure 5.1).

***Figure 5.1**: Conceptual representation of the study site using compartments (light grey rectangles with border line representing the intensity of sanitary control in the compartment) and the wild bird community (large dark grey rectangle encompassing all compartments) in which each compartment is embedded. In order to illustrate the method, two counting sites are represented as circles and potential epidemiological interactions estimated from these counting sessions are shown as arrows between compartments.*



Counting protocols

Wild bird counts in each site were implemented according to a protocol previously described (Chapter Three - Caron et al. 2010, Appendix Five - Cumming et al. 2011). Focal count points of thirty minutes were implemented four times every two months during one year (May 2008 – May 2009, n=6 sessions) in 15 waterfowl sites, and in 6, 6 and 7 sites in respectively backyard poultry, Ostrich farms and Intensive poultry compartments. This first protocol will be later called the "intensive protocol". A protocol restricted to 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites was repeated from September 2009 to November 2010, during 8 counting sessions at two months interval. This "longitudinal protocol" encompassed 14 sessions from May 2008 to November 2010. Counting sessions in waterfowl compartments were associated with the closest domestic counting sessions in time to calculate variables related to the shared community of wild birds.

Questionnaires in the three domestic production systems were conducted with managers in order to understand the population dynamics in each of these three compartments and to calculate in each compartment the number of infected (n_i) or susceptible (n_s) hosts (questionnaire data not shown).

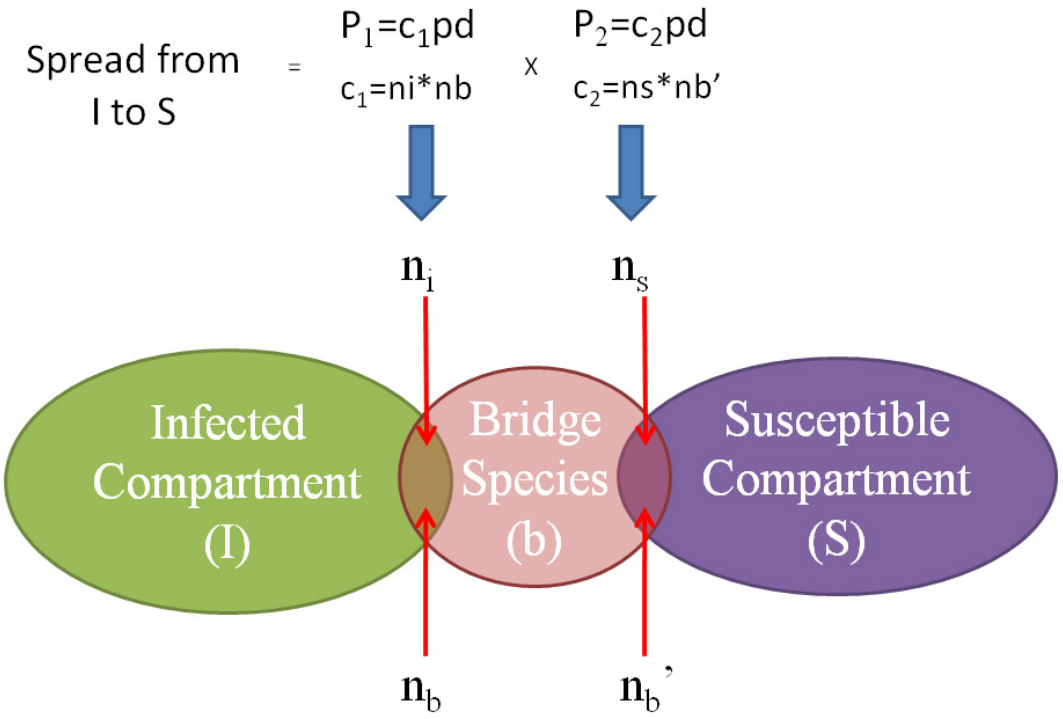
Estimation of epidemiological interactions (EIs)

EIs were estimated by using the shared community of wild birds (potential bridge species) between two counting sites during one counting session (same season). Each bird species observed in both compartments at the same time was selected as potentially participating in the epidemiological interaction between the two compartments. The estimation of EIs is based on: 1) the assumption that all individuals of the same species belong to the same population which is, given the size of our study site (10km radius around

lake's shores) and the ecology of wild birds, quite reasonable; 2) all individuals in a population have the same health status regarding the HPAI: this cannot be true in practice but given the efficiency of the HPAI virus transmission, it stands as a limitation of this model that will be discussed later.

In the context of a SIR model in multi-host species (Dobson 2004), EIs were estimated using bird counts data and as previously discussed no information about the species-specific susceptibility of wild birds could be incorporated in the model. In infectious disease epidemiology, the effective contact rate (i.e. a contacts that result in the transmission of the pathogen) is the product between the contact rate, the probability of transmission between infected and naive individuals and the pathogen infectious period (Dohoo et al. 2009). The probability of transmission and the infectious period will be assumed constant across species because of the lack of information available and the relative good and homogeneous efficiency of the HPAI to be transmitted between birds. The contact rate between an infected and a susceptible compartment will be dependent on two events (Figure 5.2). The first one is the transmission from an infected compartment to a bridge species and will be dependent on n_i, the number of infected individuals in the infected compartment and the sum of n_b the number of susceptible individuals in each of the bridge species in contact and shared with the susceptible compartment. The second event is the transmission from an infected individual of a bridge species to a susceptible individual of a susceptible compartment and will be dependent of the number of infected individual in the potential bridge species identified (sum of n_b' for each shared bridge species) and the number of susceptible, n_s, of individuals in the susceptible compartment. The probability of transmission will therefore be proportional to the products ($n_i * n_b$) and ($n_b' * n_s$) for each bridge species.

Figure 5.2*: Schematic representation of the transmission of a HPAI virus from an infected to a naive bird compartment through bridges species.* n_b *and* n_b' *are the sum of the number of observed individuals from each bridge species b in the infected and susceptible compartments respectively;* n_i *and* n_s *are the number of individuals from the infected and susceptible compartment respectively exposed to the shared community of bridge species. The spread of a HPAI H5N1from compartment* I *to* S *is equal to the product of probabilities (*P_1**P*$_2$*),* c_1 *and* c_2 *being the contact rates,* p *the probability of transmission and* d *the infectious period.*



In order to explore on one side the variability of the bridge species component and on the other side the relative exposure of infected and naive compartments to the transmission of the pathogen, the sum of the products (n_b * n_b') later named the Interaction Sum (IS) will be explored first and the variation of n_i and n_s will be discussed later.

The differences between pairs of compartment of the sum of products IS and the species richness of the shared community were tested using a One-way ANOVA (after checking for normality and homogeneity of variance; when heterogeneity of variance was observed, a log transformation was performed): a) between pairs of compartments (across seasons and years), b) between seasons (across pairs of compartments and years) and c) between years (across pairs of compartments and seasons). Differences between pairs of compartments and seasons were tested for both the longitudinal and intensive protocol and difference between years only for the longitudinal protocol.

In order to further explore the composition of the EIs, we use the dominant species (more than 10% of the abundance of the shared community) for each EIs for each counting session for each pair of compartments for the longitudinal protocol and the intensive protocol.

Risk analysis approach

A risk analysis approach as defined by OIE recommendations (OIE 2009) was used to define the risk and the spread pathways in the ecosystem. The standard steps of risk analysis are presented: hazard identification, risk assessment (composed of release, exposure and consequence assessment) and risk management (in discussion section).

Hazard Identification

The hazard is identified as a HPAI strain, which by definition can kill chicken but also potentially other avian species such as ostriches and wild birds.

Framing the risk question

The risk question is defined as the risk of spread of a HPAI strain by bridge species in the four avian compartments once it has been introduced in one of the compartments.

Release assessment

Release assessment concerns the release of the hazard. Here it refers to the release of a HPAI from an infected compartment to a susceptible compartment using bridge species. The release assessment is therefore dependent on n_i, the number of infected individuals in the infected compartment. This number will depend on the host dynamics in each of the compartment. We considered the release possibilities in each of the four compartments by using population dynamics of hosts inferred by questionnaire or ornithological data (Chapter Four - Caron et al. 2011).

Exposure assessment

EIs between compartment are dependent on the number of individuals potentially in contact with bridge species in each compartment n_i (considered in the release assessment) and n_s, the number of naive individuals in the naive compartment, and on the number of individuals n_b and n_b' of each bridge species. Risk exposure was therefore estimated using the IS parameter, as defined above.

Consequence assessment

For each risk estimated a qualitative approach was used by transforming each quantitative or qualitative probability into a three-value classification: low, medium, high. Quantitative probabilities were transformed according to their values and expert assessment. Qualitative probabilities were allocated to one of the three categories using available knowledge and/or expert opinion. The product of probabilities was determined using Table 5.1.

The global risk of spread from one infected compartment to a susceptible compartment was defined as the product of the risk of contact between the infected compartment and bridge species (dependent on n_i), the risk of interaction between compartment (dependent on IS) and the risk of contact between a bridge species and the susceptible compartment (dependent on n_s).

Table 5.1: *Table of correspondence for the products of qualitative probability*

Probability	**Low**	**Medium**	**High**
Low	Low	Low	Low
Medium	Low	Medium	Medium
High	Low	Medium	High

Results

Risk assessment

Release assessment

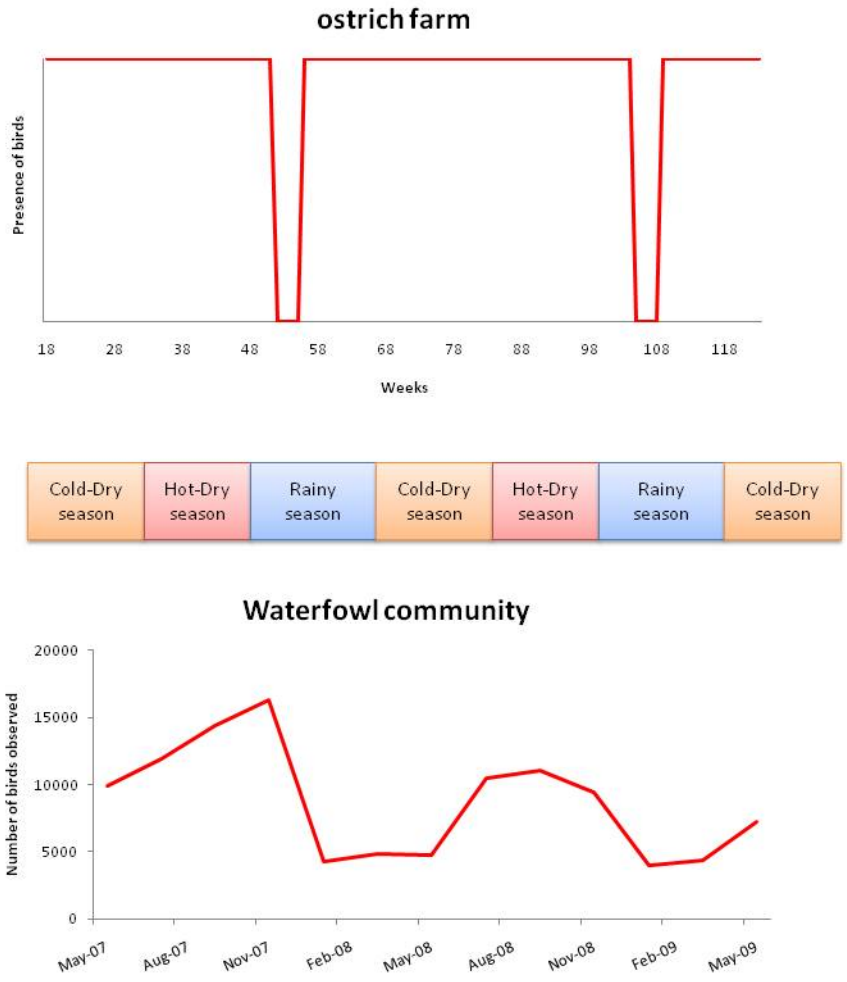
Two possibilities of HPAI release were identified: a) risk of release from the ostrich compartment infected through importation of ostriches or movements of staff; b) risk of release from the waterfowl compartment infected through wild bird movements patterns or local maintenance (Chapter Four - Caron et al. 2011) (Table 5.2). We therefore considered these two entry points for predicting pathogen spread to other compartments in the ecosystem. Importation of ostriches from outside the ecosystem (Bulawayo region, South of Zimbabwe or South Africa) is likely to occur at the beginning of the year when farms are re-stocking meat animals. Therefore ostrich numbers in farms is stable throughout the year from the importation of youngsters in January until their slaughtering in November-December (Figure 5.3). A quarantine period is respected in-between December and January. However staff movements between farms from the Bulawayo area and importation of reproductive birds could in principle take place any time during the year.

In the Waterfowl compartment, based on the emergence risk calculated in Choater Three - Caron et al. (2010), the risk of release of a HPAI strain was higher during the hot-dry season when the highest number of birds was entering the system (mainly Palaearctic and Afro-tropical migrants) (Figure 5.3). However this risk is relative and birds are entering the ecosystem any time during the year.

Table 5.2: *Emergence pathways identified in the four avian compartments of the ecosystem*

Compartment	Risk of primary emergence in the ecosystem	Justifications
Backyard Poultry	No	- No poultry market - Village autoproduction - Village auto-consomption
Intensive Poultry	No	- Eggs produced in Harare and delivered at 1 day-old to farms - No exchange between farms - Staff belonging to one farm only
Farmed Ostrich	Yes	- Occasional importation of birds from South Africa - Possibility of staff moving from farms outside the ecosystem - Past occurrence of HPAI H5N2 outbreaks
Waterfowl	Yes	- Worldwide reservoir of LPAI - Potential spreader of HPAI strains - Regional, continental and international migration of waterfowl

Figure 5.3: *Population dynamics in the ostrich and waterfowl compartments on a bi-annual basis (for ostrich, the x axis uses "weeks" as unit when for waterfowl it uses "month"; however, the scale is similar for both graphics). Seasons are represented for a better interpretation of the dynamics. Rainy Season= November to March; Cold-dry season= April to July; Hot-dry season= August to October.*



The mean number of ostriches and waterfowl observed in the ostrich and waterfowl compartment respectively during the counting protocols was 189 ostriches and 145 wild birds respectively (in a given area). These numbers reflected the quantity n_i. The number of ostrich in contact with potential bridge species throughout the year was considered constant (as ostrich chicks enter the farm in January and leave the farm for slaughtering 11 months later). The variation of potential n_i from the waterfowl (Figure 5.3) can triple between the rainy season and the end of the dry season. Release from the ostrich compartment was considered as "Medium" when chicks are introduced in the system (January) and 'Low" for the rest of the year (based on the fact that young animals are more susceptible to disease compared to adult). Based on the epidemiological data presented in Chapter Four - Caron et al. (2011), risk of release from the waterfowl compartment was considered as: "Medium" from September to November and "Low" for the rest of the year. We concluded that for both emergence pathways, there are higher risk periods and no period during the year when the emergence risk is null.

Exposure assessment

Overall, exposure from the Intensive poultry compartment is difficult to estimate but is the lowest of the four compartments ("Low" risk)(Box 5.1). The Backyard compartment, Waterfowl compartment were both considered as "Medium" risk and the Ostrich farm compartment as "High" risk due to the number of birds exposed.

***Box 5.1**: Additional information on the exposure assessment of intensive & backyard chicken, waterfowl and ostriches.*

The mean number of target birds observed during the counting protocol per compartment was 22 chickens, 145 waterfowl, 189 ostriches and 5240 chickens respectively for Backyard, Waterfowl, Ostrich farm and Intensive poultry compartment (counted from the intensive protocol). These numbers reflected the quantity of individual birds n_s, 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, production 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 of chicken escaped from production building, 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 bridge species on several occasions. During sanitary quarantine (with no chicken in the building) between two production cycles in the intensive compartment, 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.

- End of the Box -

The mean of IS varied between pair of compartments as presented in Table 5.3 and between years and seasons (Table 5.4). None of the one-way ANOVA tests for differences between compartments, seasons and years for both protocols were significant. However, in the longitudinal protocol, the mean of IS increased from the interface between B/W, I/W and B/I (B, I and W for Backyard poultry, Intensive poultry and Waterfowl compartment respectively). In the intensive protocol, the results were consistent with the longitudinal protocol with O/W<B/W<I/W<B/O<I/O<B/I (O for Ostrich farm compartment).

The species richness varied between compartments for both protocols but varied little between years and seasons. Two one-way ANOVA tests were significant: the difference of species richness between the compartments' interface for both longitudinal ($df=2$, $F=13.1$, $p<0.01$) and intensive protocols ($df=5$, $F=7.4$, $p<0.01$). There were also some consistency in the ranking of the species richness' mean between the two protocols (I/W<B/I<B/W).

A high variability of IS was observed intra- and inter-pairs of compartment (Figure 5.4). Dominant species (here more than 25% of IS for a better visualisation) are indicated in Figure 5.4. For the B/I interface, May and November were peak periods almost every year with dominant species being respectively red-billed quelea (*Quelea quelea*) and barn swallow (*Hirunda rustica*). In the B/W interface, no peak season was observed and more species were involved in the dominant species. Barn swallow and cattle egret (*Bubulcus ibis*) were the most common dominant species. Two species of ducks were present as dominant species in two peak periods. In the I/W interface, barn swallow was the dominant species in all four peak counting sessions.

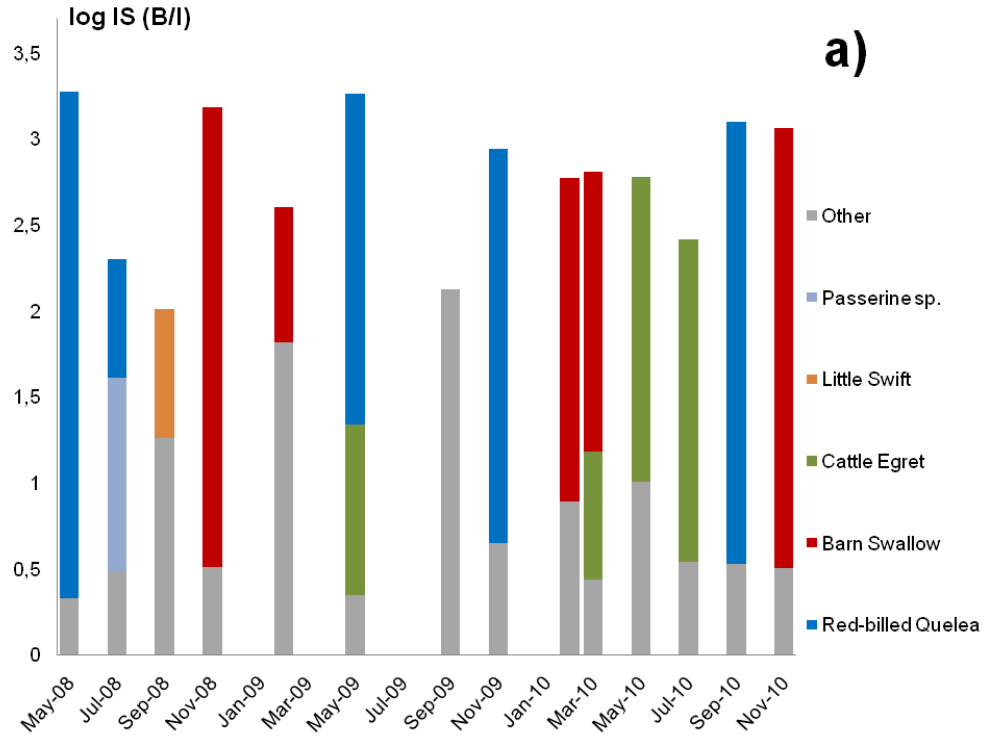
Table 5.3: *Means and standard error (Std. Error) of IS (i.e. Σ [n_b * n_b']) and of species richness (Sp. Rich.) between each pair of compartment (B=Backyard compartment, I=Intensive poultry compartment, W=Waterfowl compartment, O=Ostrich compartment). Each value is calculated by calculating the IS (Mean & Std Error) and species richness indices for each count, for each site for each session. The intensive protocol encompassed 6 sessions, with 15 waterfowl sites, and 6, 6 and 7 sites in respectively backyard poultry, ostrich farm and intensive poultry, with 4 counts per site per session. The longitudinal protocol en compasses 14 sessions, with 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites, with 4 counts per site per session.*

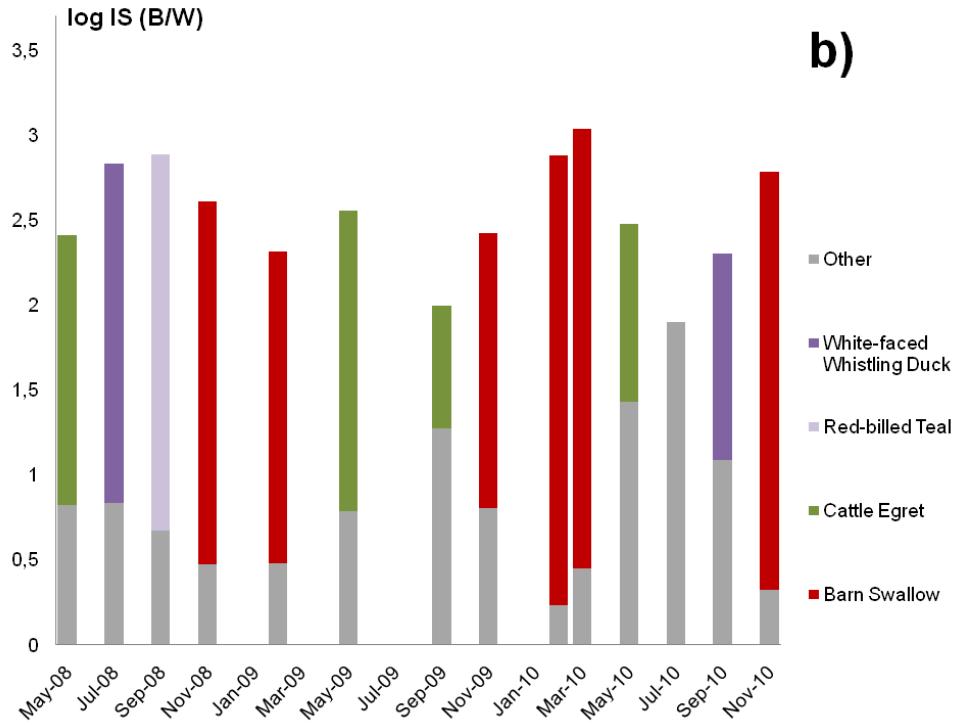
		IS or Σ(n_b * n_b')		**Sp. Rich.**	
	Interaction	**Mean**	**Std. Error**	**Mean**	**Std. Error**
Longitudinal protocol	B/I	819	617	24.6	4.0
	B/W	433	298	28.1	4.2
	I/W	649	1408	20.6	3.4
Intensive protocol	B/I	537	558	53.3	8.3
	B/W	144	74	55.0	9.3
	I/W	212	212	36.0	8.2
	O/W	75	86	63.0	7.6
	I/O	453	560	45.8	7.4
	B/O	334	470	49.8	8.4

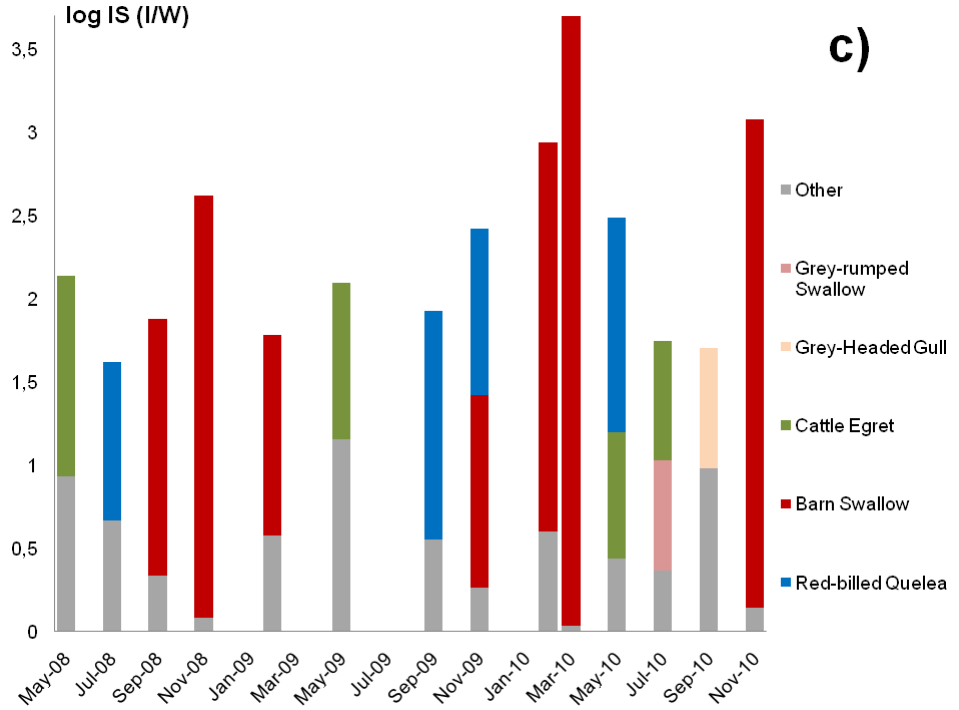
Table 5.4: *Means and standard error of IS (Σ [n_b * n_b']), and of species richness (Sp. Rich.) for seasons and years (B=Backyard compartment, I=Intensive poultry compartment, W=Waterfowl compartment, O=Ostrich compartment). The intensive protocol encompassed 6 sessions, with 15 waterfowl sites, and 6, 6 and 7 sites in respectively backyard poultry, ostrich farm and intensive poultry, with 4 counts per site per session. The longitudinal protocol en compasses 14 sessions, with 7 waterfowl sites, 2 backyard poultry and 2 intensive poultry sites, with 4 counts per site per session.*

		IS or Σ (nb*nb')		Sp. Rich.	
		Mean	**Std. Error**	**Mean**	**Std. Error**
Longitudinal protocol	2008	618	655	25.9	6.6
	2009	449	575	26.1	3.6
	2010	632	465	26.1	4.2
	Dry-Cold	475	591	24.0	4.7
	Dry-Hot	527	483	24.9	5.7
	Rainy	1111	1636	24.0	4.0
Intensive protocol	Dry-Cold	408	510	50.8	10.8
	Dry-Hot	222	179	50.2	12.2
	Rainy	87	39	50.3	13.6

Figure 5.4: *Variation of the log of the interaction sum (IS) in the longitudinal protocol across the fourteen counting sessions; species representing more than 25% of the interaction sum are indicated; a) Interface between Backyard chicken and Intensive poultry compartments ; b) Interface Backyard chicken and Waterfowl compartments; c) Interface between Intensive poultry and Waterfowl compartments. "Passerine sp." refers to unidentified small passerines such as red-billed quelea or bronze manikin.*




log IS (B/W)
b)
3,5
3
2,5
2
1,5
1
0,5
0
May-08
Jul-08
Sep-08
Nov-08
Jan-09
Mar-09
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10
Other
White-faced Whistling Duck
Red-billed Teal
Cattle Egret
Barn Swallow


log IS (I/W)
c)
3,5
3
2,5
2
1,5
1
0,5
0
May-08
Jul-08
Sep-08
Nov-08
Jan-09
Mar-09
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10
Other
Grey-rumped Swallow
Grey-Headed Gull
Cattle Egret
Barn Swallow
Red-billed Quelea

During the longitudinal protocol, all the dominant species (here as >10% of IS) were represented by only 14 species for the three pairs of compartments for the fourteen sessions (eight per pair of compartments) (Table 5.5). Three species were overrepresented: red-billed quelea, barn swallow and cattle egret. Four other species of hirundidae (e.g. swallows, swift and martin) were observed, making this family the most represented bird family in terms of number of species.

During the intensive protocol, all the dominant species for the six pairs of compartments for the 6 sessions encompassing 34 counting sites were represented by only 12 species (Table 5.6). The three same species were also overrepresented (red-billed quelea, barn swallow and cattle egret). In Table 5.5 & 5.6, during high IS period (in grey), the overrepresentation of red-billed quelea, barn swallow and cattle egret was even greater.

In Tables 5.7 & 5.8, qualitative risks already discussed are summarised. In addition, each IS was allocated to “Low”, “Medium” or “High” by dividing each vector of values into three categories. For Table 5.7, classes are: “Low”<150; 150<“Medium”<300; “High”>300. For Table 5.8, classes are: “Low”<210; 210<“Medium”<700; “High”>700.

Table 5.5: *Dominant species for each interaction sum for each session of the longitudinal protocol for each pair of compartments. Dominant species are defined as participating in more than 10% in the total interaction sum. In grey, sessions with highest interaction sum for each pair of compartment. (For B/I, "Passerine sp." refers to unidentified small passerines such as red-billed quelea or bronze manikin).*

B/I	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
Red-billed quelea	90%	30%				59%	24%	78%			14%		83%	
Barn Swallow			12%	84%	30%			23%	68%	58%				83%
Cattle Egret					24%	30%				27%	64%	78%		
Bronze Mannikin					21%		18%		11%					
African Palm Swift			20%									12%		
Little Swift			37%				20%							
Passerine Sp.		49%												
Rock Dove							11%							

B/W	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
Red-billed quelea						17%	20%	24%			18%	15%		
Barn Swallow				82%	79%			67%	92%	85%				88%
Cattle Egret	66%	18%				69%	36%			12%	42%	22%		
Red-billed Teal			77%											
White-faced Whistling Duck	22%	71%	11%										53%	
African Palm Swift												11%		
African Jacana					12%									
Grey Rumped Swallow							17%				32%	30%		

I/W	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
Red-billed quelea		59%				22%	71%	41%			52%			
Barn Swallow			82%	97%	68%			48%	80%	99%				95%
Cattle Egret	56%	20%			19%	45%			14%		31%	41%		
Bronze Mannikin						20%								
African Palm Swift												14%	16%	
Grey-headed Gull	23%												42%	
Grey Rumped Swallow							10%				12%	38%		
Banded Martin													18%	

Table 5.6: *Dominant species for each interaction sum for each session of the intensive protocol for each pair of compartments. Dominant species are defined as participating in more than 10% in the total interaction sum. In grey, sessions with highest interaction sum for each pair of compartment. (For B/I, "Passerine sp." refers to unidentified small passerines such as red-billed quelea or bronze manikin).*

B/W	May-08	Jul-08	Sep-08	Nov-08	Jan-09	Mar-09
Red-billed quelea	19%					25%
Barn swallow				55%	39%	
Cattle Egret	44%	25%			32%	55%
White-faced duck	19%	59%	22%			
Red-billed teal			56%			
African jacana					16%	
I/W	**May-08**	**Jul-08**	**Sep-08**	**Nov-08**	**Jan-09**	**Mar-09**
Red-billed quelea	30%	22%	71%	39%		23%
Barn swallow				14%		
Cattle Egret	34%	26%			58%	26%
White-faced duck	11%	29%			14%	18%
Red-billed teal				29%		
O/W	**May-08**	**Jul-08**	**Sep-08**	**Nov-08**	**Jan-09**	**Mar-09**
Red-billed quelea	33%			16%		68%
Barn swallow			64%	80%	44%	
Cattle Egret	28%	39%	12%		50%	11%
Bronze mannikin		13%				
Cape-turtle dove		10%				
B/I	**May-08**	**Jul-08**	**Sep-08**	**Nov-08**	**Jan-09**	**Mar-09**
Red-billed quelea	88%	33%	54%	11%		70%
Barn swallow				26%	16%	
Cattle Egret		20%			25%	18%
Bronze mannikin				31%	20%	
Passerine sp.		23%				
Dark-capped bulbul			10%			
Common waxbill				15%		
Southern red bishop					21%	
B/O	**May-08**	**Jul-08**	**Sep-08**	**Nov-08**	**Jan-09**	**Mar-09**

Red-billed quelea	82%					93%
Barn swallow				68%	60%	
Cattle Egret		14%			17%	
Bronze mannikin		30%	24%	25%		
Cape-turtle dove		35%				
Dark-capped bulbul			21%			
European bee-eater			12%			
I/O	**May-08**	**Jul-08**	**Sep-08**	**Nov-08**	**Jan-09**	**Mar-09**
Red-billed quelea	88%			27%		94%
Barn swallow			13%	45%	24%	
Cattle Egret		13%	13%		51%	
Bronze mannikin		63%	33%	23%		
Dark-capped bulbul			15%			

***Table 5.7**: Risk Table combining the risk of spread from the ostrich compartment. The risk of interaction is based on the interaction value in the intensive protocol. The Risk of spread is a combination of the three risks presented before (calculation based on -1 for Low, 0 for Medium and +1 for High). In the last column, the main dominant species for the particular session is given.*

To Backyard	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction =R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	High	Medium	Medium	**Medium**	**Red-billed quelea**
Jul-08	Low	High	Low	Medium	**Low**	
Sep-08	Low	High	Low	Medium	**Low**	
Nov-08	Low	High	High	Medium	**Medium**	**Barn swallow**
Jan-09	Medium	High	Low	Medium	**Low**	
Mar-09	Low	High	High	Medium	**Medium**	**Red-billed quelea**
To Intensive	**Risk of introduction**	**Risk of contact ni/nb=R1**	**Risk of interaction =R2**	**Risk of contact nb'/ns=R3**	**Risk of spread =R1*R2*R3**	
May-08	Low	High	High	Low	**Low**	
Jul-08	Low	High	Low	Low	**Low**	
Sep-08	Low	High	Low	Low	**Low**	
Nov-08	Low	High	High	Low	**Low**	
Jan-09	Medium	High	Low	Low	**Low**	
Mar-09	Low	High	High	Low	**Low**	
To Waterfowl	**Risk of introduction**	**Risk of contact ni/nb=R1**	**Risk of interaction =R2**	**Risk of contact nb'/ns=R3**	**Risk of spread =R1*R2*R3**	
May-08	Low	High	Low	Medium	**Low**	
Jul-08	Low	High	Low	Medium	**Low**	
Sep-08	Low	High	Low	Medium	**Low**	
Nov-08	Low	High	Medium	Medium	**Medium**	**Barn swallow**
Jan-09	Medium	High	Low	Medium	**Low**	
Mar-09	Low	High	Low	Medium	**Low**	

Table 5.8: *Risk Table combining the risk of spread from the waterfowl compartment to the remaining two compartments (the ostrich compartment was not included in the longitudinal protocol) discussed in the text. The risk of interaction is based on the interaction value in the longitudinal protocol. The Risk of spread is a combination of the three risks presented before. In the last column, the main dominant species for the particular session is given.*

To Backyard	Risk of introduction	Risk of contact Σ ni/ Σ nb=R1	Risk of interaction=R2	Risk of contact Σ nb'/ Σ ns=R3	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	Medium	Medium	Medium	**Medium**	**Cattle egret**
Jul-08	Low	Medium	High	Medium	**Medium**	**White-faced duck**
Sep-08	Medium	Medium	High	Medium	**Medium**	**Red-billed teal**
Nov-08	Medium	Medium	Medium	Medium	**Medium**	**Barn swallow**
Feb-09	Low	Medium	Low	Medium	**Low**	
May-09	Low	Medium	Medium	Medium	**Medium**	**Cattle egret**
Sep-09	Medium	Medium	Low	Medium	**Low**	
Nov-09	Medium	Medium	Medium	Medium	**Medium**	**Barn swallow**
Feb-10	Low	Medium	High	Medium	**Medium**	**Barn swallow**
Mar-10	Low	Medium	High	Medium	**Medium**	**Barn swallow**
May-10	Low	Medium	Medium	Medium	**Low**	
Jul-10	Low	Medium	Low	Medium	**Low**	
Sep-10	Medium	Medium	Low	Medium	**Low**	
Nov-10	Medium	Medium	High	Medium	**Medium**	**Barn swallow**

To Intensive	Risk of introduction	Risk of contact ni/nb=R1	Risk of interaction=R2	Risk of contact nb'/ns=R3	Risk of spread =R1*R2*R3	Dominant species
May-08	Low	Medium	Low	Low	**Low**	
Jul-08	Low	Medium	Low	Low	**Low**	
Sep-08	Medium	Medium	Low	Low	**Low**	
Nov-08	Medium	Medium	Medium	Low	**Low**	
Feb-09	Low	Medium	Low	Low	**Low**	
May-09	Low	Medium	Low	Low	**Low**	
Sep-09	Medium	Medium	Low	Low	**Low**	
Nov-09	Medium	Medium	Medium	Low	**Low**	
Feb-10	Low	Medium	High	Low	**Low**	
Mar-10	Low	Medium	High	Low	**Low**	
May-10	Low	Medium	Medium	Low	**Low**	
Jul-10	Low	Medium	Low	Low	**Low**	
Sep-10	Medium	Medium	Low	Low	**Low**	
Nov-10	Medium	Medium	High	Low	**Medium**	**Barn swallow**

Discussion

Exposure assessment

The variability of IS and species richness can be summarised as follow: a) there was a (non significant) variability of IS and (significant) variability of species richness between pairs of compartments, indicating that the potential bridge species were not distributed equally between compartments; b) there was a variability of IS between seasons, not significant and not consistent across both protocols but little variability of species diversity; c) there was a (non significant) variability of IS across years but no variability of the species diversity emphasising that the variability of the IS was not only dependent on seasons but also on inter-annual variability.

The variability of the IS and species diversity across pairs of compartment indicated that factors influenced the distribution of potential bridge species between compartments. The habitat surrounding compartments was different in terms of vegetation. Resource availability could attract different species, roosting and reproduction sites also. Additionally, the distance of compartments from each other could also be a confounding factor. For example, all sites were in a radius of 10 kilometres from the lakes' shores, but ostrich farms tended to be on the outskirts of the study sites, while some backyard sites were in direct contact with the lakes' shores. However, the ostrich compartment interface with other compartments did not have a systematically lower IS value compared to other interfaces (Table 5.3). Another factor influencing the difference in IS was the type of farming practices in each compartment which could influence artificial food resource availability: ostriches were fed and watered in pens with open access for potential bridge species. Backyard poultry were not fed in most circumstances and searched for their food. Therefore, the combination of

attractors and environmental variability made the distribution of potential bridge species variable across compartments.

The variability of IS across seasons is a consequence of wild bird ecology (e.g. reproduction, migration and other behavioural adaptation). For example, red-billed queleas are nomadic birds responding to resource availability linked to rainfall. They are known to move in huge numbers (roosting sites of millions of birds; Dallimer and Jones 2002, Hockey et al. 2005). Palaearctic migrants are living Eurasia during autumn and arrive in Zimbabwe in September to depart again around March and April. This seasonality of nomadic and Palaearctic birds is quite evident in Table 5.5 & 5.6 (see red-billed quelea and barn swallow). The variability of IS observed across years can be interpreted by the variability in climatic patterns in the region. Southern Africa is known to have inconsistent and highly variable rainfall patterns (Verschuren et al. 2000). Wild birds respond to this variability through a nomadic behaviour (Dodman and Diagana 2007). As our study focused on one ecosystem, the observed variability can be explained by the differential use of this ecosystem by different wild bird populations in response to rainfall variability.

The lack of variability of species richness across season and years is more complex to explain. The ecological niche concept proposes that one ecosystem can be subdivided into ecological niches for specific host species (Begon et al. 2006). The compartments offered a limited number of niches to bird species and they were constantly occupied during the course of the study. This result could be interesting to explore as the impact of changes in biodiversity on epidemiological processes (given that only an –unknown- fraction of the avian diversity is susceptible to AIV) could be significant. For example, a dilution effect has been proposed for some epidemiological multi-host systems (Keesing et al. 2006).

The result on a smaller dataset that a limited number of species constitutes the majority of IS, was confirmed here with a larger dataset (Chapter Three - Caron et al. 2010). A maximum of four species was above the 10% threshold for all counting sessions per pair of compartment. In the intensive protocol, only twelve species of wild birds were dominant species across the all year of study (6 sessions in 34 sites for 408 hours of counts during one year) and in the longitudinal protocol, thirteen species were found to be dominant in at least one count (out of 14 sessions in 11 sites for 308 hours of counts in three years and half). This number has to be compared with the 249 bird species observed in the waterfowl compartment in the same ecosystem (Chapter Three - Caron et al. 2010). The risk of spread related to bridge species is therefore concentrated in a few bird species. In the 24 counts with the highest IS (out of 78), only barn swallow was involved 14 times, red-billed quelea 12, cattle egret 6 and three other species involved seven times combined (grey column in Table 5.5 & 5.6).

Therefore, we observed much variability in the quantitative estimation of IS with little predictability between compartments, seasons and years. However, we showed that from a qualitative point of view, the IS can be summarised with a few dominant bird species, which, by their ecology, bring some predictability in the interaction between compartments. What are the consequences for the risk pathways identified?

Consequence assessment

The spread of a hypothetical HPAI strain in the ecosystem is considered according to the two introduction routes identified in the release assessment.

- Spread from the ostrich compartment (intensive protocol)

Overall, the risk of spread from the ostrich compartment is low (Table 5.7). The risk of spread to the Backyard compartment is higher than to the Waterfowl compartment and both are higher than the risk of spread to the Intensive compartment. The risk of diffusion from the Ostrich compartment is low when the highest risk of introduction in this compartment is the highest (January) and is Medium a couple of month later in March and May when red-billed queleas are in the ecosystem and in November when barn swallows are present in numbers. The risk of spread from the Ostrich compartment is therefore higher when red-billed queleas and barn swallows are in the ecosystem. For the former, the March and May period is therefore at risk, for the later mainly September-November. Inter-annual variability can shift these periods.

- Spread from the Waterfowl compartment (longitudinal protocol)

The overall risk of diffusion from the Waterfowl compartment was Low towards the Intensive compartment and Medium towards the Backyard compartment (Table 5.8). The high risk period occurred during the dry-hot season and to a lesser extent in February-March, just before Palaearctic birds such as barn swallows left the ecosystem. In May 2008 and 2009, the risk of spread from the Waterfowl to the Backyard compartments was considered as Medium and cattle egret were the dominant species for each period. In May 2010, cattle egret was again the dominant species although the risk of spread was from the Waterfowl to the Backyard compartments considered Low. The fact that the peak season for IS coincided with the highest risk for HPAI strain introduction in the Waterfowl compartment increased the risk of diffusion of the pathogenic strain the ecosystem.

Risk Management

The decision to use this level of analysis instead of finer estimators of contact such as individual telemetry or direct observation of contacts was based on its repeatability using cost-effective counting protocols or already available ornithological databases. The risk estimation presented here is not absolute. In practice, parameters of the transmission event assumed to be constant in Figure 5.2 are not. First, the prevalence in the infected compartments is not constant across species. The definition of a HPAI strain is based on the mortality induced in poultry chicks (Alexander 2000). This does not mean that this strain produces mortality in ostrich or in waterfowl. The contrary has been shown in various studies on the pathogenicity of HPAI strain in ostriches (Capua et al. 2000). This variability in pathogenicity can have consequences on the number of birds infected in the compartment and the time lag between introduction and detection (if birds do not die, the outbreak could go undetected). We focus on the contact rate between hosts, but not all contacts between hosts are followed by the transmission of the pathogen (McCallum et al. 2001), and some contacts could have a higher probability of pathogen transmission compared to others, adding heterogeneity in the model. The susceptibility of the bird in contact with an infected individual is highly variable and dependent on its personal history (immunity) and on the susceptibility of the species to the particular pathogen and strain (some host species may be more susceptible to some strains than others). However, this study had the objective to investigate the potential bridge species dynamics between compartments, seasons and years. Its results have implication for disease management. There are other transmission pathways for a HPAI strain in this ecosystem, but they have not been addressed here (e.g. human movements between compartments).

There has been so far little work on bridge species and the reason could be that the complexity of this multi-host system was not possible to address. Our findings suggest that in

this Zimbabwean ecosystem, there are only a few key species visiting the ecosystem at specific time of the year which constitutes the majority of the epidemiological interactions between compartments. Furthermore, the risk of spread varies in relation to bird species ecology (e.g. annual palearctic migration of barn swallows, nomadic movements of red-billed queleas, reproductive behaviour of cattle egret). This has direct implication for the management of the HPAI risk if it was introduced in this ecosystem. Management options to limit contact between production stock and these key species during high risk seasons are possible. For example, red-billed quelea is a pest in Southern Africa and a variety of control options exist to avoid their feeding on crops. Barn swallows are mainly visiting production unit to feed on insects. Insect control could reduce these visits. Modification of the habitat could also reduce roosting sites at proximity of production buildings. Cattle egrets are usually following cattle in the proximity of farms. Avoiding cattle visiting those farms would reduce the interface. A few control options could therefore drastically reduce the risk of spread of the pathogen between compartments. However the inter-annual variability observed would require a monitoring system to make sure the control options are put in place when the risk is present. In 2010, our field team looked for red-billed quelea roosting sites around the lakes as they have been observed at this season for the past two years. The main roosting colonies arrived only in the ecosystem in September 2010. This change in behaviour could be an adaptation to the late rains which occurred that year in April. As can be seen in Figure 5.4, the red-billed quelea risk has shifted from May to September-November this year.

The protocol presented here is time consuming but cost-effective. Instead of sampling blindly the avian community, our protocol structures the approach and targets the bird species with the highest impact on disease transmission. We can present to level of validation of our model; First, barn swallows, red-billed queleas and cattle egrets have been shown to be susceptible to AIV in other ecosystems (Gronesova et al. 2008, Mizakova et al. 2008, Squires

et al. 2008, Breithaupt et al. 2010) indicating that they should be susceptible also in our ecosystem. Second, each of these potential bridge species has been sampled during high risk period highlighted by this model: barn swallows and red-billed queleas have been found to be positive to AIV by RT-PCR technique when cattle egrets have not. Therefore, if one of these species is infected by a HPAI strain, the risk of its diffusion in the ecosystem would be possible and controllable. As presented in Chapter Four - Caron et al. (2011), the Waterfowl compartment is hosting LPAI strain all year long. In the three domestic compartments, positives RT-PCR detection of LPAI strain were found (Caron, unpublished data). Therefore the risk of a HPAI diffusing in this ecosystem represents a reality and the bridge species pathways a good candidate to explain these results.

From a more theoretical point of view, it would be interesting to investigate bridge species communities in other ecosystems to assess if our findings are site specific or not. If not, in addition to offer a framework to identify potential bridge species at the ecosystem level, our approach could help exploring potential rules of bridge species communities between avian compartments. If similar findings are found elsewhere, this would mean that the risk of spread of pathogens by bridge species in an ecosystem is the result of the epidemiological interaction of a few key species, offering a limited number of control options with potential high impact on the sanitary risk.

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