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Research, part of a Special Feature on Risk mapping for avian influenza: a social-ecological problem

Estimating Dynamic Risk Factors for Pathogen Transmission Using Community-Level Bird Census Data at the Wildlife/Domestic Interface

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ABSTRACT. The ecology of host species is crucial for understanding the mechanisms of pathogen transmission and spread in complex multi-host systems. In this article, we use detailed observations of the host community to develop and apply a new approach to mapping temporal variation in risk for avian influenza. Working in an extensive wetland system near Harare, Zimbabwe, we use the overlap in space and time of highly variable bird communities, combined with ecological risk factors, to assess the risk of Avian Influenza viruses (AIV) maintenance and transmission between bird populations. The estimated introduction and maintenance risks associated with waterfowl populations at a given time are then multiplied by the level of interactions with neighboring domestic production systems during the same period. This approach is used to develop hypotheses for the dynamics of the introduction and circulation of AIV strains in waterfowl populations and as a way of understanding the potential role of "bridge" species at the wild/domestic interface. The novel approach presented here offers a potentially useful way to explore AIV risk, identify which wild bird species may be acting as reservoirs or vectors of pathogens at a local scale, and improve local surveillance.

Key Words: *Avian influenza; bridge species; community ecology; risk factor; wild/domestic interface*

INTRODUCTION

The success of multi-host infectious pathogens in ecosystems is heavily dependent on the composition of the community of organisms in which they occur (Ostfeld 2009). The species composition of the host community and the temporal dynamics of its constituent populations will influence pathogen success through variation in such parameters as host susceptibility, host abundance, host population turn-over, the presence and absence of reservoir species, and encounter rates between hosts and pathogens (Dwyer et al. 1997, Childs et al. 2007, Borer et al. 2009).

For pathogens that are transmissible either by direct contact or via the shared use of the same habitat at different times, transmission parameters often cannot be directly measured in the field. Doing so is particularly difficult for multi-host pathogens. Transmission is usually evaluated through host-pathogen models (Breban et al. 2009, Rohani et al. 2009) that lack direct measurements of actual interspecies contact. Epidemiological interactions (i.e., ecological interactions that may result in the transmission of a pathogen) between susceptible, infected, and recovered hosts can be used to define a network from which to explore transmission pathways and assess spatial and temporal variation in transmission risks (Takeuchi and Yamamoto 2006, Duerr et al. 2007, Kenah and Robins 2007). While graph theoretic methods for creating and analyzing networks from direct data on species interactions are fairly well established (Williams et al. 2002, Lafferty et al. 2008), the application of standard network methods in cases where interactions and mechanisms must be inferred from higher-level data on co-occurrences is poorly

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developed and computationally challenging (Bascompte and Melian 2005, Rabbat et al. 2008).

Here we consider the use of co-occurrence data to infer avian influenza virus (AIV) potential transmission pathways in communities of birds in Zimbabwe. The recent HPAI H5N1 (Highly Pathogenic AI) panzootic has spread across the world, exploiting avian communities and sporadically infecting humans (Webster et al. 2007). The mechanisms of AI spread across ecosystems are still unclear. The international poultry trade and waterfowl migration are the two most intensively tested hypotheses that have been proposed to explain patterns of HPAI spread (Olsen et al. 2006). However, existing information implies different roles for different modes of dispersal across regions, indicating a need for regional or subregional research frameworks (Kilpatrick et al. 2006). The epidemiology of LPAI (Low Pathogenic AI) is better understood than that of HPAI: waterfowl are considered to be the primary reservoirs of LPAI with spill-over to domestic poultry occurring periodically (Webster et al. 1992). These cross-species transmission events can lead to HPAI selection in domestic populations (Caron et al. 2009). We use AIV as a complex multi-host pathogen model with a potentially high impact on the socio-economic level for Africa and the world.

The importance of the ecology of wild birds in the epidemiology of AIV strains has been underlined by numerous studies (Olsen et al. 2006, Stallknecht and Brown 2007, McCallum et al. 2008, Munster and Fouchier 2009), but the high diversity of potential host species and a lack of information on their susceptibilities to LPAI and HPAI makes the overall picture unclear (Perkins and Swayne 2002, 2003, Brown et al. 2006, Brown et al. 2007a, Pasick et al. 2007). Some key features of waterbird ecology are thought to strongly facilitate virus maintenance or spread. These features include: their relatively high degree of inter- and intra-specific mixing; their tendency to move long distances during annual migrations and/or broad-scale nomadic movements; their colonial feeding and roosting habits; and their use of water, which improves viral survival outside the host. Some studies have already used these criteria to estimate hotspots of potential virus infection, regional spread, or inter-continental contamination (Kilpatrick et al. 2006, Veen et al. 2007, Cumming et al. 2008). However, at a local level, most AI risk factors show seasonal variation as species breed and as they respond to variations in resource availability, rainfall, the presence or absence of other species (including pathogens), and seasonal changes in human behavior. The corresponding variation in AI risk has not been thoroughly analyzed in wild bird communities.

In addition to the many uncertainties regarding spatiotemporal variation in transmission pathways, it is worth noting that most current field research still follows traditional distinctions: veterinarians investigate the health of domestic species and ornithologists focus on wild birds, but the gap between these two approaches is poorly filled.

The classical one-pathogen approach aims at detecting (directly or not) the pathogen in different hosts and inferring transmission pathways that are specific to this pathogen (Plowright et al. 2008). In this article we present a novel approach to assessing transmission risks in a complex epidemiological network that consists of spatiotemporally variable bird communities (i.e., waterbirds, domestic birds, and bridge species that interact with both wild and domestic communities). Rather than attempting to develop a formal network-based model, we integrate data on the frequency and intensity of inter- and intraspecific co-occurrences, together with information about relevant aspects of species ecology and behavior, to obtain a risk score for each species in the community and to build an adapted risk assessment model. In conceptual terms, this approach offers a mid-point between data-intensive, mechanistic network analysis (Takeuchi and Yamamoto 2006) and looser, more subjective assessments of risk (Cumming et al. 2008, Peterson and Williams 2008). Our approach has the advantage that it incorporates aspects of fine-scale transmission mechanisms while not being excessively data-demanding; the analysis is undertaken using the kinds of survey data that standard ornithological censusing procedures typically yield. In addition to presenting a useful picture of seasonal variation in AI risk, our analysis demonstrates how dynamic aspects of risk can still be included into epidemiological risk assessment in the absence of detailed pair-by-pair interaction data.

METHODS

Study site

We undertook this study in the Manyame catchment (30°30'30'', 17°45'45''), located 35 km West of Harare, the capital city of Zimbabwe. Our primary study sites were two impoundments, Lake Chivero and Lake Manyame, both of which were created in 1952. Together they form a linked wetland system (connected by the Manyame River) that harbors a community of waterfowl species. Part of the shoreline of Lake Chivero is a protected area. In addition, several commercial farms are located in the Manyame catchment, including industrial poultry farms and semi-extensive ostrich farms. Farm employees living in compounds located on the farm estates also raise backyard chickens for domestic consumption.

We considered the different avian communities in the study area to be four 'compartments' as defined in Caron et al. 2009: (1) the waterfowl compartment, consisting of the community of wild waterbird species sharing the lake habitat through the year; (2) the industrial compartment, being the population of domestic chickens raised in buildings at high densities for a period of about 40 days; (3) the backyard chicken compartment, in which chicken populations are free-ranging during the day, using fields and human-modified natural habitats in the vicinity of compounds, and resting in chicken pens at night; and (4) the ostrich farm compartment, consisting of a few hundred birds kept in open paddocks (usually around a hundred birds per paddock) surrounded by wooden fences. These different management practices result in variable contacts between domestic poultry and the surrounding wild bird communities, and biosecurity measures are implemented in intensive poultry and ostrich farms.

It is important to note that the status of AIV in this ecosystem is unknown. No H5N1 outbreaks have been recorded south of the equator in Africa. Outbreaks of H5N2 in the southern part of Zimbabwe in ostrich farms occurred in 2005, which had a possible link with outbreaks of the same strain in South Africa in 2004 (Sinclair et al. 2005, Abolnik et al. 2006).

The methodology followed a six-step process: (1) identify the hazard in relation to the objective; (2) describe the waterfowl and domestic bird communities; (3) define dynamic and non-dynamic ecological risk factors (RFs) for the presence of AIV infection in the waterfowl community; (4) combine RFs for both the release assessment (introduction risk - IR) and the exposure assessment (maintenance risk - MR) in the waterfowl community; (5) identify epidemiological interactions between waterfowl and domestic compartments through direct contact, indirect contact via shared habitat or potential bridge species; and (6) estimate the release assessment for each of the domestic compartments (transmission from waterfowl to the domestic compartments) through a dynamic Domestic Risk variable (DR).

Hazard identification

The risk of AIV introduction from the waterfowl community to the three domestic compartments is dependent on the introduction of strains in the waterfowl community, the ability of this community to maintain such strains and the potential for spill-over from the waterfowl compartment to the domestic compartments. This risk increases from non-H5 and H7 LPAI (which can still recombine with other strains to produce HP strains) to H5 and H7 LPAI (which are the most likely strains to evolve into HPAI) to already high pathogenicity (HP) strains (including HPAI H5N1). Because of the little epidemiological information available for African bird species and because any AIV strain could be involved in the creation of HP strains, we identified all AIV strains as hazardous for this risk assessment.

Community composition

Focal counts were undertaken to estimate species diversity and the abundance of waterfowl and domestic communities. Based on local knowledge of the field site, 15 shoreline sites were selected for their high diversity of waterfowl species and abundance of birds. From May 2007 to March 2009, bird community counts were carried out every two months at each of these sites. 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. Prior to each count, the counter waited for 10 minutes to habituate the birds to the presence of the counter. During each

count, the counter stood or sat at a distance of 30-50 m from the lake shore and recorded all birds in a 150-m-radius semicircle.

In a radius of ten kilometers from the lake shoreline, we selected 19 domestic compartment sites, located in (or in direct proximity to) production units (buildings, paddocks, or villages). Six sites in three different ostrich farms, seven sites in intensive poultry farms and six sites in villages with backyard poultry were selected. At each of these domestic sites, the same counting protocol (10-minute wait plus 30-minute count) was applied from June 2008 to April 2009 with both wild and domestic birds being counted.

Ecological Risk Factors (RFs)

The use of variables that capture ecologically relevant variation to build epidemiological RFs has been applied in different studies related to AIV maintenance and spread at regional or continental scales (Kilpatrick et al. 2006, Veen et al. 2007, Cumming et al. 2008). We were interested in describing, at the community level, the risk of introduction and maintenance of AIV within and between bird communities across seasons in response to variability in host ecology. We thus developed dynamic RFs based on seven ecological variables that were likely to influence the epidemiology of AIV, including two variables for the introduction risk (estimated local immigration and risk related to AIV strain in relation to the origin of the birds) and five variables for the maintenance risk (the overall abundance of birds, the gregariousness of the species, interspecific aggregation, percentage of juveniles and feeding habits) (Stallknecht et al. 1990b, Olsen et al. 2006). These risk factors were characterized as RFs 1 to 7 (Table 1). Susceptibility to AIV infection and the immunological status of the birds were not considered in this model because of a lack of information for African bird species.

Introduction and maintenance risk (IR and MR) in waterfowl community

For each bird community count, the species values of each RF were multiplied by 1 for species recorded at least once, and by 0 when the species was absent. IR and MR were calculated as described in Table 2. IR was calculated for any AIV strain and specifically for HPAI H5N1 in order to display the proportion of the relative risk for exposing the community to HPAI H5N1 introduction.

The standard deviation of each RF of the MR was calculated. A Spearman Rank Correlation test was performed for each RF in relation to the MR in order to assess their relative contributions.

Quantifying epidemiological interactions (domestic risk - DR) and their risks

Calculating the degree of ecological interaction between wild and domestic compartments

Each waterfowl count session was paired with count sessions carried out in domestic compartments (separated by a maximum of three weeks). The community composition of each domestic compartment was calculated using the same method as for the waterfowl compartment. For each domestic compartment, all species seen during the same session in the waterfowl compartment were identified as the shared community. We calculated the proportion of the shared community for each domestic compartment potentially in contact with the waterfowl compartment during the same period.

Calculating the interaction risk (DR) of the shared community

For each species recorded in the waterfowl and domestic compartments during the same period, the DR was calculated as described in Table 2. For each session, we estimated the DR (i.e., of AIV spreading from the waterfowl to the domestic compartments) by summing the DR of all species in the shared community.

RESULTS

Dynamics of waterfowl community

Variation in waterbird numbers observed across the two years was characterized by a peak during the end of the cold-dry season and running into the hot-dry season (July-September-November; Figure 1).

Table 1. Risk factors (RFs) used in this study, their derivation, and the motivation for including them.

Risk RF	Name	Properties	Description
Introduction			
1	*Immigration*	Dynamic	Any bird arriving in the ecosystem can potentially carry a strain of influenza from another ecosystem. We quantified immigration conservatively, as the difference between the number of birds observed in a count at time *t* and a count in the same location at time *t-1*. Negative changes (emigration) were entered as zeros. No value for this *RF* exists for the first count session by definition.
2	*Related AIV Risk*	Non-dynamic	Birds can introduce different pathogens from different areas. The local risk (notably for farmers) is therefore related to the type of strains that are likely to be introduced. We defined four movement patterns and ranked them according to the associated risk of introducing different strains of AIV in Chivero-Manyame ecosystem: a) resident species, associated with risk value of 0; b) species nomadic in Southern Africa, associated with a risk value of 1 (HPAI H5N1 has not been recorded in African South of the equator -OIE 2009- but other HP strains have been recorded) or 0 for H5N1 risk; c) Trans-equatorial migrants, with a risk value of 2 as HPAI H5N1 is now endemic in some African countries and outbreaks occurred in 11 countries (OIE 2009); and d) paleartic migrants, associated with a risk value of 3 because of the high number of HPAI H5N1 outbreaks and reported prevalence of LPAI is higher than in Africa(Olsen et al. 2006). For species that evidence several different strategies, as with the wood sandpiper *Tringa glareola* which has both migratory and resident populations (Underhill et al. 1999, Hockey et al. 2005), a mean between the two relevant coefficients was taken.
Maintenance			
3	*Abundance*	Dynamic	Total number of bird observed per species, obtained by summing numbers seen during the 60 counts. Note that since only 56 counts were done during the first count session (May 2007), we multiplied the numbers of birds recorded during this session by 60/56 for full comparability.
4	*Gregariousness*	Dynamic	The degree of intra-species aggregation. Aggregation facilitates pathogen transmission and maintenance in the species. For each species we calculated the average group size observed across all study sites.
5	*Mixing*	Dynamic	The degree of inter-specific aggregation, which facilitates pathogen transmission from one species to another. We estimated the degree of mixing for each species and for each count session as the ratio of the number of species observed on the same sites and at the same time, divided by the total number of species counted during the 60 counts of the count session (total species diversity measured during a count session).
6	*Percentage of juveniles in the population*	Dynamic	Juveniles are considered to play a role in the epidemiology of AIV once they have joined the adult population (i.e., after fledging). Juveniles are also thought to remain epidemiologically naïve in the population for about 2 months (Stallknecht et al. 1990b). To capture this risk, we used Roberts' Birds of Southern Africa (Hockey et al. 2005) to provide data on: a) clutch size; b) breeding success; and c) laying dates for the 254 species in the data set. Using a simple population model assuming constant mortality in adults (4,5% per month) and a decreasing mortality in juveniles (starting at 40% in month 1 and reaching 4,5% at 6 months), and integrating the reproductive information, the percentage of juveniles in the population was estimated by month. Incubation and fledging periods were added to determine the delay between egg laying and the entry of juveniles into the population. We considered juveniles for each species to be susceptible to AIV infection based on their naïve immunological status but despite lack of information on susceptibility for most African species.
7	*Feeding habits*	Non-dynamic	Transmission of AIV strains in surface water is possible (Stallknecht et al. 1990a, Brown et al. 2007b), and we identified four feeding behaviors that were ranked according to the risk of birds being infected with AI during their feeding activities. They include: (0) feeding on insects on flight, seeds, nectar or fruits; (1) feeding on birds, small vertebrates, or insects close to water; (2) diving or feeding on insects gleaned from open water; and (3) dabbling, gleaning on or near surface and subsurface vegetation, or probing.

Table 2. Justification and equations for introduction, maintenance and domestic risk (all RFs have previously been multiplied by the presence-absence matrix)

	*RF*s used and equation	Transformation	Justification
Introduction Risk (*IR*)	*RF*1**RF*2 For each count, for each species (no value for may 2007 due to *RF*1 calculus)	None	Each bird entering the community is associated with a AIV risk related
Maintenance Risk (*MR*)	*RF*3+*RF*4+*RF*5+*RF*6+*RF*7 For each count, for each species	Standardized	Each *RF* is additive to the others
Domestic Risk (*DR*)	*RF*1+*RF*2+*RF*3+*RF*4+*RF*5+*RF*6+*RF*7 For each species observed simultaneously in waterfowl and domestic compartment	Standardized	Each *RF* is additive to the other and *RF*1 & 2 represent also a risk of introduction for the domestic compartment

This peak resulted from two general trends: (1) the concentration of nomadic sub-Saharan waterfowl (Dendrocygnidae and Anatidae) on larger bodies of water as seasonal wetlands within the subregion dried down; and (2) the return of paleartic migrants from Europe during the (European) fall migration. The paleartic migrants leave the ecosystem between March and April, at the end of the Zimbabwean rainy season. Species diversity was highest during the dry season and lowest during the rainy season in both years. Note that there are no paleartic migrant duck species in southern Africa (Cumming et al. 2008).

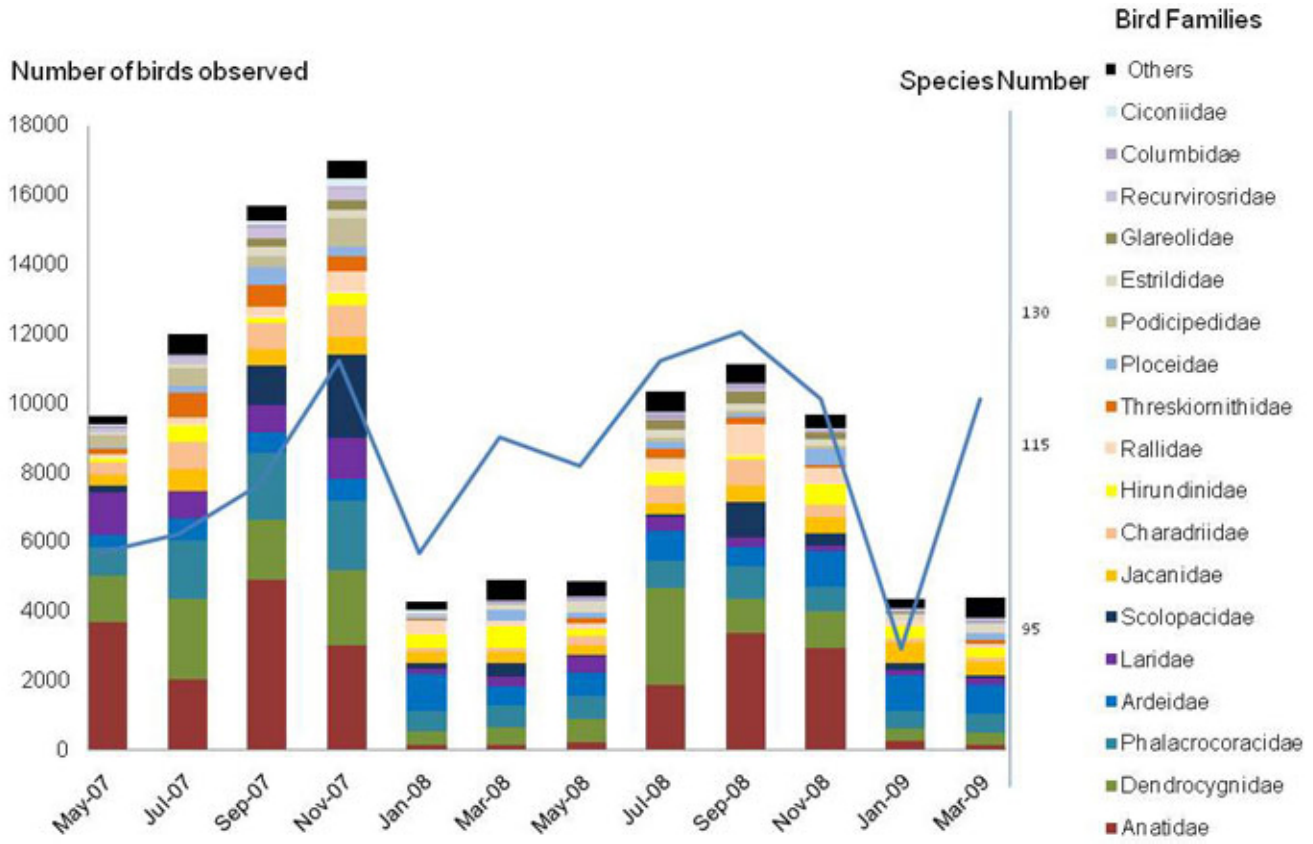
Dynamics of domestic communities

The birds observed per family and the species diversity of the three domestic compartments are shown in Figure 2. For each of the domestic compartments, domestic species dominated the counts. Intensive poultry represented 98% of all birds observed in the intensive poultry compartment, backyard chickens represented 25% of the backyard compartment, and ostrich represented 79% of the ostrich compartment. The remaining bird community in each of the domestic compartments was quite homogenous across the compartments, dominated by birds from the Ploceidae, Estrildidae, Ardeidae, Columbidae and Hirundinidae families, which represent between 59 and 67% of the birds observed. The maximum number of birds observed in these three communities was in April, mainly due to an increase in Ploceidae, particularly the red-billed queleas (*Quelea quelea*). This species is considered a pest species by local farmers and exhibits high variability in population dynamics. Species diversity varied between the three compartments as well as seasonally, particularly in the ostrich compartment.

Patterns of IR and MR in the waterfowl compartment and relation to the RFs

IR peaked in September 2008 and July 2009 (Figure 3). MR peaked in November in both years (Figure 4). For both risks, there was a difference in the intensity of the peak between the two years, which correlated with the variability in waterfowl abundance (Figure 1). The trends of all five RFs followed the MR with one major peak per year during or slightly before the dry season (Figure 4).

Fig. 1. Waterfowl community abundance per family (bars) and species diversity (blue line) across the 12 missions (encompassing 2 years).

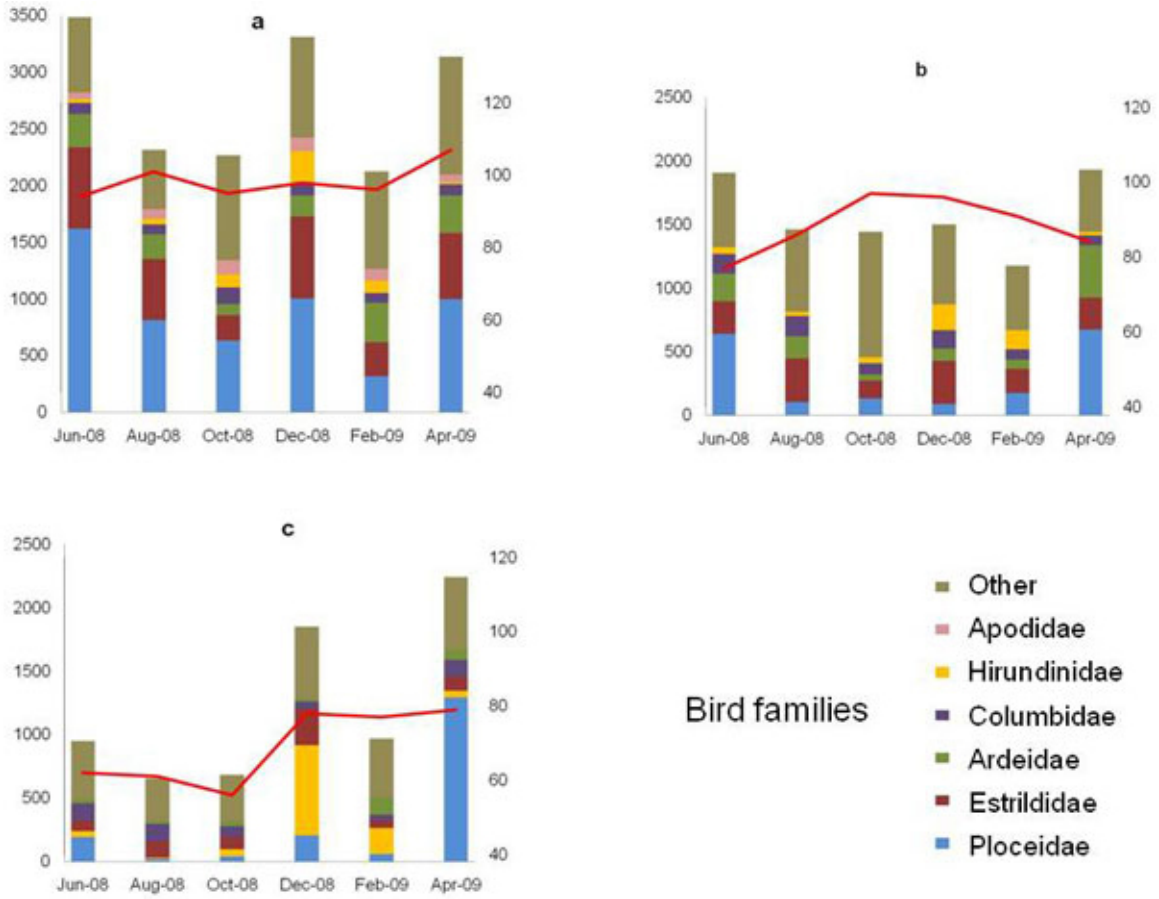


The "Feeding" RFs had a slightly advanced peak in July or September depending on the year. Some RFs, such as "Mixing" and "Juvenile," had a higher variability than others (Table 3). Three RFs had a significant correlation with the MR curve (Gregariousness, Abundance and Feeding, in decreasing order). The results are consistent with a higher risk of the presence of AIV strains in the waterfowl community at the end of the dry season. Waterfowl species contributing the most to IR and MR are presented respectively in Tables 4 and 5. IR for AIV and HPAI H5N1 was dominated by Charadriiformes (35.8% and 47.0%, respectively) and Anseriformes (37.1% and 33.7%, respectively). MR was largely dominated by Anseriformes.

Variation in DR for the three domestic compartments

The intensive poultry and ostrich farm DR curves were similar (Figure 5), with two peaks of similar amplitude: one in November, the other in March. For the backyard poultry curve, only the peak in March was evident. The DR curves for the intensive poultry and ostrich compartments, and the MR curve for the waterfowl, showed the highest risk during the month of November (end of the hot-dry season). In our model, this month had the highest risk for transmission of AIV strains from the waterfowl to the domestic compartment. The second peak observed in the intensive poultry and ostrich farm DR curves was not related to a peak in the waterfowl MR. There was consistency in the most represented families for each of the three domestic compartments (Table 6).

Fig. 2. Number of birds observed per family (left axis – bars) and species diversity (right axis, red line) in a) intensive poultry compartment (n=7 sites); b) backyard poultry compartment (n=6 sites); c) ostrich farm compartment (n=6 sites). This results are compiled after withdrawing the domestic species, always overrepresented in these communities (intensive poultry = 98%; backyard chicken = 25%; ostriches = 79%).



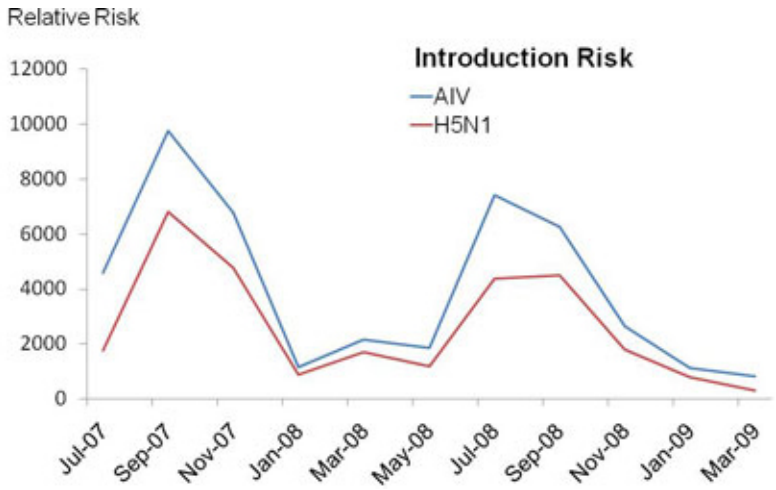
DISCUSSION

Our results provide a clear illustration of the ways in which community-level risk varies over time, both within and between years. IR peaked during the early hot-dry season, when regional waterbirds were concentrating on larger water bodies and migrants began to arrive from Europe. By contrast, MR peaked in November at the end of the dry season when the largest waterbird concentrations were observed. A number of bridge species were shared between different epidemiological compartments, suggesting a strong potential for interactions between domestic and wild birds in this system.

On the use of dynamic risk factors

We are not aware of any previous studies that have attempted to track variations in community-level risk factors through time. Although we worked primarily with indicators rather than with empirical proof of pathogen transmission, it is important to remember that community ecology and epidemiology have been used in combination for the last 25 years to explore and understand the behavior of multi-host and/or multi-pathogen systems (Holt and Pickering 1985, Hudson and Greenman 1998). A solid body of empirical evidence suggests that the availability of hosts, their movements, and their interactions

Fig. 3. Variation in the introduction risk (combined immigration and AIV risk related RFs) calculated for all AIV and for H5N1 in particular (birds potentially migrating from the northern hemisphere).

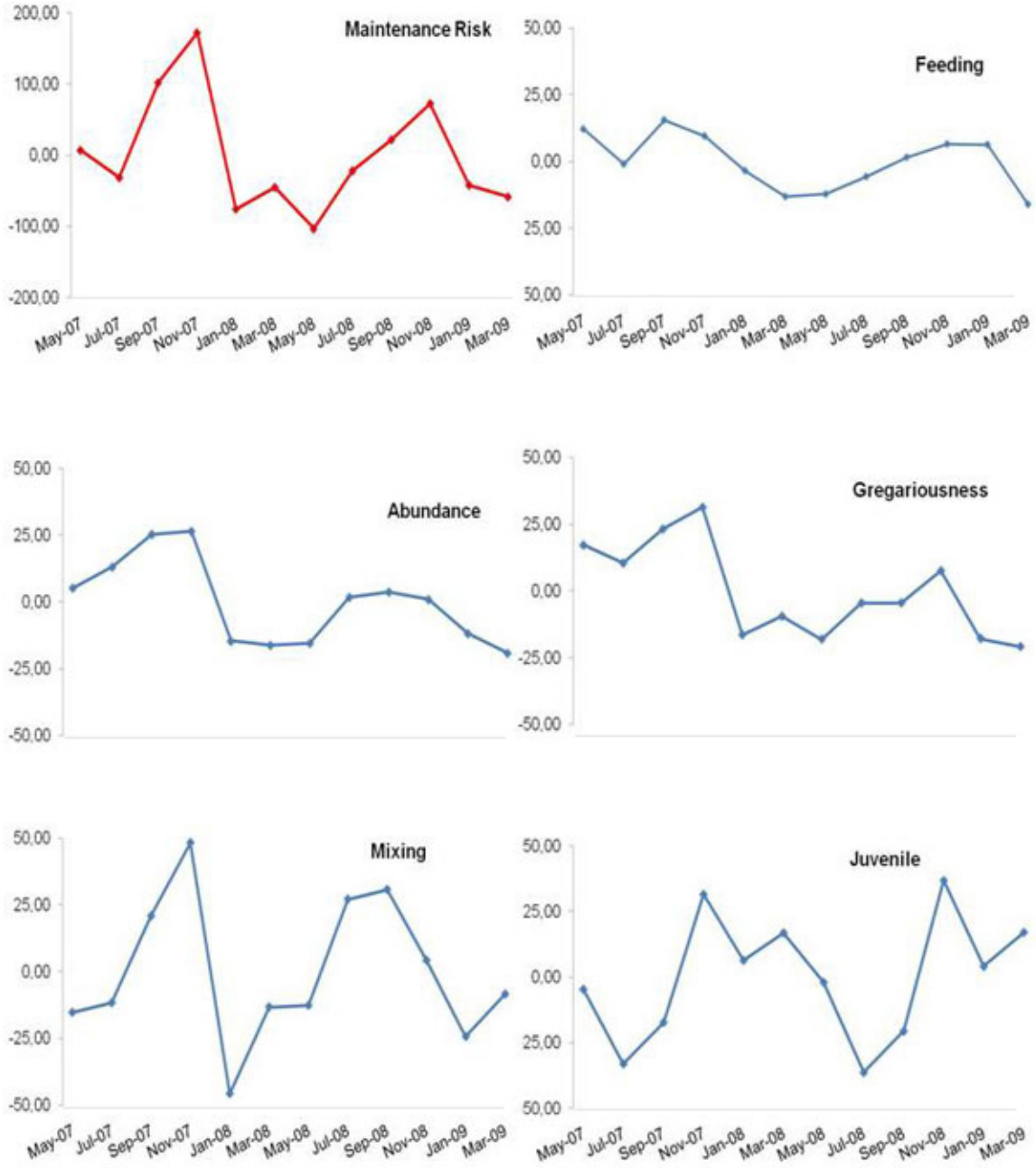


AIV - Avian influenza virus
RFs - Ecological risk factors

with other hosts will influence pathogen transmission (Morgan et al. 2006, Bordes et al. 2009). Intra- and inter-species mixing, the presence or absence of particular species, and the proportion of juveniles in the population vary seasonally for waterfowl and are important influences on the ecology of infectious diseases (Wallensten et al. 2007). There is therefore a lot of evidence-based support for the a priori definition of RFs that take into account the ecology of hosts and the ways in which host ecology may influence the behavior of pathogens in a system. At the same time, it is important to note that these RFs remain hypotheses until such time as further data on influenza occurrence within the system become available.

The development of dynamic RFs in previous studies has primarily focused on differences between summer and winter bird communities. Bimonthly risk mapping presents a finer-scale and considerably more informative pattern. Despite the high quality of our count data, however, a number of parameters used in this analysis remain difficult to estimate. For example, the immigration RF assumes that the arrival in the counts of new birds represents a risk for AIV introduction; in reality, numbers could stay constant while individuals change, and a proportion of the birds arriving in the system may be coming from nearby areas. For some bird species (e.g., red-billed teal *Anas erythroryncha* and white-faced duck *Dendrocygna viduata*), movement patterns are estimated from scarce ring recovery data. Often, the proportion of the population undertaking nomadic vs. trans-equatorial movements is unknown (Underhill et al. 1999). This information is important for estimating a risk of introduction (according to different AIV strains) but cannot be taken into account in our model (Cumming et al. 2008). Dispersal is particularly crucial for the two species mentioned above because they constitute some key species identified by the IR. Environmental RFs could have been taken into account in this model. In the Manyame catchment, measurements of water temperature at various seasons averaged 21.08°C (n=70; min 14.85°C; max 25.4°C, Caron, unpublished data); this supports the idea that the environment may be a potential reservoir throughout the year (with better conditions for virus survival during May-August) using data from recent studies (Brown et al. 2007b, Brown et al. 2008, Weber and Stilianakis 2008).

Fig. 4. Evolution of the maintenance risk (MR) and of each RFs included in the MR in the waterfowl compartment.



RFs - Ecological risk factors

Table 3. Standard deviation for each risk factors participating to the maintenance risk and the species diversity (across the values for the 12 sessions) and Spearman Rank Correlation Coefficient for each risk factors and the species diversity in relation to the global risk.

	Standard Deviation	Spearman Rank Correlation Coefficient	p value
Abundance	15,9	0,83	0,001
Gregariousness	17,7	0,87	<0,001
Mixing	26,8	0,69	0,13
Juvenile	23,6	-0,01	0,96
Feeding	10,4	0,76	0,04
Species diversity	10,2	0,35	0,258

MR is calculated without weighting the RFs because there is no empirical evidence from which to argue that one RF is more important than another. With suitable data collection and sampling for influenza viruses, it may eventually be possible to use linear models to weight different risk factors. Another important assumption used in this analysis is that birds seen within the counting area are potentially in contact. This assumption may not truly reflect fine-scale non-randomness in interaction networks.

Anseriformes and Charadriiformes represent the main families identified for IR, the first mainly as a function of their numbers and the second by their potential risk in introducing dangerous strains. Charadriiformes, mainly paleartic waders, but also Anseriformes crossing the equator are identified by the model as potential introducers of HPAI H5N1. Interestingly, when waterfowl are ranked for each of the five RFs and the ranks are summed across the two years, the species contributing the most to the MR (Table 5) belong to the bird orders known to be reservoirs for LPAI strains (Anseriformes and Charadriiformes) with the two most influential species in the model, the white-faced duck and the red-billed teal, being the most abundant ducks in the system. The only other orders present in the 20 most important species were Gruiformes (Coot sp.) and Ciconiiformes (Egret and Ibis spp.). These orders and families have been found with, or dead of, LPAI or HPAI strains (Gauthier-Clerc et al. 2007, Hars et al. 2008, Stoops et al. 2009). Additionally, the MR curve (Figure 4) was consistent across the two years and indicated a maximum risk of AIV presence in the waterfowl community during the hot-dry season, when migratory and paleartic waterfowl are present in the system, coming from areas where AIV strains circulate. This result is consistent with a basic epidemiological model for AIV in Africa (Gaidet et al. 2006) that assumes a strong likelihood of introduction of strains during the paleartic migration. The fact that most of the RFs follow the MR trends reflects some consistency in the model: the high risk season for AIV presence in the waterfowl community derives from a convergence of peaks of RFs during this season. The "Gregariousness" and "Abundance" RFs have a high correlation with MR and an increase in the weight of these factors would accentuate the current trend in MR (Table 3).

The difference in MR between the two years reflects the differences in bird abundance. There is a relationship between lake level (determined by the rainfall in the previous year and human management) and bird abundance; the lakes dry down during the hot-dry season and exposed shorelines offer a muddy, vegetated, resource-rich habitat for dabbling ducks and waders. MR defined here could be predicted in advance with rainfall from the previous years, offering the potential for disease forecasting in this system. The use of environmental data to predict epidemiological patterns through an ecological (host or vector) link

Table 4. Twenty most important species influencing the introduction risk (IR) of AIV (and their relative introduction risk for H5N1) in the waterfowl compartment in our model (combined risk of RF 1 & 2)

Species	Order	Family	Relative IR for AIV	Relative IR for H5N1
Red-billed Teal	Anatidae	Anseriformes	8680	5787
Ruff	Scolopacidae	Charadriiformes	5922	5922
White-faced Duck	Dendrocygnidae	Anseriformes	5232	3488
Barn Swallow	Hirundinidae	Passeriformes	2841	2841
Unidentified wader sp.		Charadriiformes	1689	1689
Kittlitzs Plover	Charadriidae	Charadriiformes	1636	1091
Cattle Egret	Ardeidae	Ciconiiformes	1240	827
White-winged Tern	Laridae	Charadriiformes	1218	1218
Little Stint	Scolopacidae	Charadriiformes	1026	1026
Red-billed Quelea	Ploceidae	Passeriformes	964	0
Collared Pratincole	Glareolidae	Charadriiformes	865	577
Common Sandpiper	Scolopacidae	Charadriiformes	858	858
Grey-rumped Swallow	Hirundinidae	Passeriformes	757	0
White-backed Duck	Anatidae	Anseriformes	746	0
Reed Cormorant	Phalacrocoracidae	Ciconiiformes	666	0
Wood Sandpiper	Scolopacidae	Charadriiformes	648	324
Glossy Ibis	Threskiornithidae	Ciconiiformes	516	0
Red-knobbed Coot	Rallidae	Gruiformes	516	0
Egyptian Goose	Anatidae	Anseriformes	476	0
European Bee-eater	Meropidae	Corafiiformes	438	438

have already been demonstrated (Harvell et al. 2002).

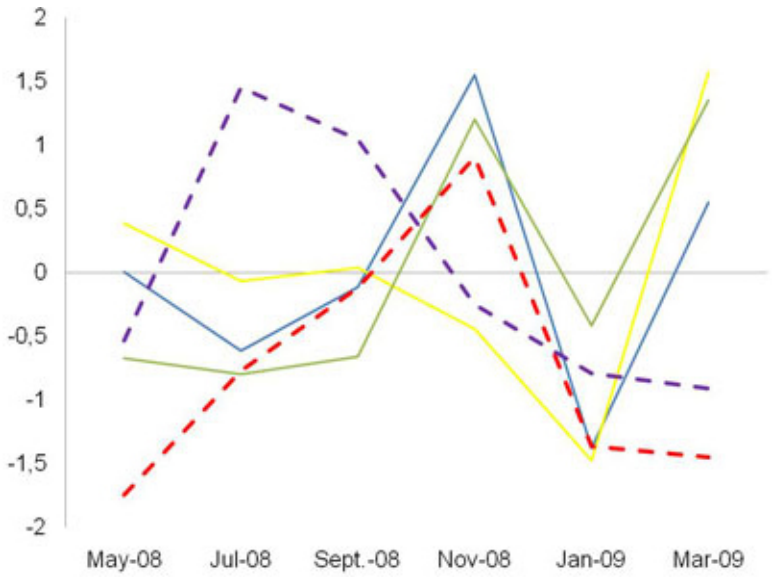
Domestic Risk (DR) between waterfowl and domestic compartments

The trends in the DR curves for the three domestic compartments were different. The 19 domestic sites chosen varied between zero and ten kilometers from the lake shore, and this distance could have influenced the observed wild bird community. However, although the ostrich farm sites were the farthest from the lake shore, their DR curve followed the intensive poultry DR curve. There may be other factors besides distance to the lake that influence the wild bird community, including variation in artificial resource availability in the production

Table 5. Twenty most important species influencing the maintenance risk in our model ranked per risk factor (decreasing ranking) and sum across the 5 RFs values for the last column (Maintenance Risk) ; A = Abundance dynamic RF; G = intraspecies mixing dynamic RF; M = interspecies mixing dynamic RF; J = proportion of juvenile in the population dynamic RF;; F = feeding non-dynamic RF; Maintenance Risk = sum of the ranks of the preceeding 5 RFs per species.

Species	Family	Order	A	G	M	J	F	Maintenance Risk
White-faced Duck	Dendrocygnidae	Anseriformes	2	3	4	1	1	11
Red-billed Teal	Anatidae	Anseriformes	1	1	9	5	1	17
African Jacana	Jacanidae	Charadriiformes	5	18	1	15	1	40
Reed Cormorant	Phalacrocoracidae	Ciconiiformes	3	7	2	6	28	46
White-breasted Cormorant	Phalacrocoracidae	Ciconiiformes	7	11	7	20	28	73
Grey-headed Gull	Laridae	Charadriiformes	4	9	5	57	1	76
Black Crake	Rallidae	Gruiformes	23	42	6	21	1	93
Red-knobbed Coot	Rallidae	Gruiformes	11	5	35	26	21	98
Egyptian Goose	Anatidae	Anseriformes	13	23	20	44	1	101
Grey Heron	Ardeidae	Ciconiiformes	17	53	3	27	1	101
Cattle Egret	Ardeidae	Ciconiiformes	9	10	13	51	21	104
Glossy Ibis	Threskiornithidae	Ciconiiformes	18	27	33	7	21	106
Black Heron	Ardeidae	Ciconiiformes	19	32	24	34	1	110
Kittlitzs Plover	Charadriidae	Charadriiformes	8	8	41	41	27	125
Common Moorhen	Rallidae	Gruiformes	31	45	18	36	1	131
Spur-winged Goose	Anatidae	Anseriformes	43	17	61	2	17	140
Yellow-billed Egret	Ardeidae	Ciconiiformes	32	63	17	30	1	143
African Sacred Ibis	Threskiornithidae	Ciconiiformes	21	25	36	33	38	153
Squacco Heron	Ardeidae	Ciconiiformes	27	62	8	56	1	154
Southern Pochard	Anatidae	Anseriformes	36	28	53	11	41	169

Fig. 5. Interaction Risk (DR) for each domestic compartment (plain lines, intensive poultry –blue, backyard poultry –yellow, ostric famrs-green) associated with introduction (IR) and maintenance risk (MR) for the waterfowl community (dashed lines, IR-purple, MR-red)



buildings, farms and villages; natural resource availability; breeding sites; predation; and so on. The most likely explanation for the similar trends between intensive poultry and ostrich farm DRs is that they both used artificial feed, attracting specific bird communities, while backyard chickens forage for their own food like wild birds.

IR was not related to any peak of the DR. However, according to our model, there are always interactions between the waterfowl and domestic compartments. In a specific epidemiological situation (e.g., regional spread of a HP strain threatening the ecosystem), this IR could help to target surveillance and control measures during high interaction seasons. The fact that the highest DR curve for two domestic compartments coincided with the highest waterfowl MR is of interest (Figure 5). The end of the hot-dry season is a high risk period for these two domestic compartments, not only because the waterfowl community has the highest risk of harboring AIV strains but also because the epidemiological interactions between the compartments are at their highest. We can hypothesize that this period represents a hotspot for pathogen circulation and transmission between compartments (Jones et al. 2008). The second peak after the end of the rainy season (in March) was consistent for the three domestic compartments but was not linked with a peak in risk associated with the waterfowl community. However, the shared community of wild birds between the waterfowl community and the three domestic compartments was always high (Table 6) suggesting a year-long risk of pathogen transmission from the waterfowl compartment. The validity of the DR estimate is limited by its population-level approach; birds of the same species observed in two different compartments were assumed to belong to the same population. However, we cannot prove that they were indeed the same individuals beyond the fact that the study site is fairly small.

Validating the model and testing the bridge species hypothesis

In order to validate the global approach and the RFs used, long-term and intensive monitoring of waterfowl will be necessary. Community analyses

Table 6. Most important families (% of the total shared community between domestic and waterfowl compartment in birds observed)) participating to the epidemiological interaction defined as domestic risk (DR) between the waterfowl and each of the 3 domestic compartments during peak risk period; in the last column, the most representative species of these families (% of the number of birds observed for this family)

Intensive Poutlry	November Peak	Mars Peak	Representative Species
Ploceidae	30,30%	31,80%	Red-billed quelea (77%)
Estrilidae	21,80%	18,60%	Bronze mannikin (50%)
Hirundidae	8,50%	0,00%	Barn swallow (90%)
Ardeidae	0,00%	10,60%	Cattle egret (85%)
Total	60,60%	61,00%	
Backyard Poultry	**May Peak**	**Mars Peak**	**Representative Species**
Ploceidae	33,40%	34,80%	Red-billed quelea (89%)
Estrilidae	13,50%	13,10%	Bronze mannikin (52%)
Ardeidae	11,50%	21,10%	Cattle egret (97%)
Total	58,40%	69,00%	
Ostrich Farm	**November Peak**	**Mars Peak**	**Representative Species**
Hirundidae	38,30%	0,00%	Barn swallow (99%)
Estrilidae	15,90%	9,30%	Bronze mannikin (60%)
Ploceidae	11,20%	57,50%	Red-billed quelea (80%)
Columbidae	0,00%	5,80%	Cape Turtle Dove (76%)
Total	65,40%	72,60%	

based on bird census data, as presented here, can contribute to the development of specific hypotheses relating to AIV maintenance and spread in the system. The community level perspective is often missing in multi-host wild population studies (Yasue et al. 2006). Usually, access to wild individuals is difficult, technically biased or limiting, and for most capture protocols it is not possible to choose precisely the epidemiological sample composition and size (Wobeser 2002). By contrast, as this study demonstrates, bird count data can drive the sampling design and/or provide an indication of the representativeness of the samples obtained from the system.

To test hypotheses concerning the role of bridge species between waterfowl and the domestic compartments usually requires selective sampling among a broad range of avian diversity. More than 100 species in 25 families of birds have been detected dead or alive with AIV strains (Olsen et al. 2006). Some terrestrial birds have been found to harbor AIV strains and even HPAI H5N1 strains (Nestorowicz et al. 1987, Boon et al. 2007, Brown

et al. 2009). We thus assumed that any wild bird species could be capable of harboring and transmitting AIV strains. Consideration of the families and species contributing the most to the peak DR for each domestic compartment (Table 6) shows that the first four families represent between 58% and 72% of the total of birds involved in the DRs. For each of these families, there is one species that represents between 50 and 99% of the birds observed. This unexpected result means that only a few species represent the bulk of the DR and that a targeted sampling focusing on these species will achieve not only a surveillance of the species most at risk of transmitting AIV but also an extensive coverage of the overall DR. Sampling protocols targeting these species should cast light on the role of potential bridge species between the waterfowl and domestic compartments. To our knowledge, there has not been sufficient local-scale testing of potential bridge species to characterize a bridge species community, despite some published suggestions (Veen et al. 2007) and an obvious missing link in HPAI outbreaks that have involved spatially segregated poultry and waterfowl.

CONCLUSIONS

The ultimate goal of this study was to integrate ecological and epidemiological data in a risk-mapping context (as discussed by Caron et al. 2009). The main outputs are a set of hypotheses that describe the mechanisms that generate patterns of AIV circulation in the waterfowl community and the role of bridge species between the waterfowl and the domestic compartments. Although we have focused on a one-way analysis (from the waterfowl compartment to each of the domestic compartments), the same analysis could be conducted for transmission between the four compartments in both directions.

An important advantage of our sampling protocol is that it provides the information that is needed to assess the adequacy of epidemiological sampling. This step is often missing in wildlife surveillance and decreases the validity of results. The next step will be to add to this data set an AIV prevalence layer (i.e., of wild and domestic compartments) to test the model and the bridge species hypotheses. The protocol described here is intensive but feasible. Its approach could easily be simplified and reproduced. In the context of AIV surveillance, a series of counts by ornithologists during suspected high-risk seasons would prepare the ground for targeted sampling. In some countries, this type of data is regularly collected by ornithological organizations and is therefore already available.

The strength of this research relative to traditional epidemiological analyses lies in its ecological dimensions. Although our model was designed with the ecology of AIV in mind, most pathogens with direct transmission will be dependent on the ecological traits estimated by the RFs (with some adjustments; e.g., “Feeding” RF). Can this risk factor analysis be extended to other pathogens to develop more ‘ecological’ predictions of disease risk? Such approaches may ultimately provide useful guidelines for surveillance in hotspots of disease emergence at the wildlife/domestic interface (Jones et al. 2008).

Responses to this article can be read online at:
http://www.ecologyandsociety.org/vol15/iss3/art25/responses/

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