Chapter Two: Evolutionary Biology, Community Ecology and Avian Influenza Research

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Background and context

The emergence in 1997 of the HPAI H5N1 strain (Highly Pathogenic Avian Influenza, Asian lineage) followed by its rapid spread and evolution across three continents challenges the previous understanding of host ecology and evolutionary processes of Avian Influenza Viruses (AIV). Before this emergence, the current understanding of the virus ecology in its hosts was that LPAI (Low Pathogenic) strains circulating in wild birds were acquiring their HP properties once introduced into gallinaceous domestic poultry without spill over to wild birds (with rare exceptions) (Alexander 2000, Webster et al. 2007). Therefore the interaction of AIV between wild and domestic birds was considered unidirectional. One of the specificities of the HPAI H5N1 strains is its potential to infect a wide range of both wild and domestic bird species (Webster et al. 2007). The coexistence of this HP strain in these two groups of hosts provides the potential for bilateral interactions between domestic and wild birds for the maintenance and evolution of the pathogen.

Avian systems, which can be classified broadly as “natural” versus “domestic” systems, have been studied through two radically different approaches by distinct bodies of academics (e.g. veterinarians and ornithologists). The integration of these fields of study in a common framework research should benefit the understanding of the HPAI H5N1 ecology and epidemiology. These multiple host – multiple pathogen interactions are complex and difficult to tackle within classical veterinary science, and increasingly multidisciplinary research flourishes.

We here use the theory of evolutionary biology and community ecology to investigate the processes of emergence, maintenance and evolution of AIV with HP characteristics in the avian hosts. Evolutionary biology aims at describing the interaction of organisms through the underlying mechanism of natural selection (Morin 1999). Natural selection can explain why,

for example, microorganisms such as viruses, with a short life cycle, evolve rapidly and pose a threat to other organisms with longer life cycles such as vertebrates. A community is an assemblage of species populations that coexist spatially and temporally and potentially interact (Begon et al. 2006). The processes of viral evolution, with genes as the unit of selection, are dependent on the structure of the host and pathogen communities: if different selection pressures occur in different avian populations, virus strains spreading from one population to another will experience a different evolutionary pathway. As community structures are dependent on biotic and abiotic factors, virus evolution will vary between ecosystems and regions.

An organism with its genes is the product of past adaptations to its environment (evolution) and the on-going interactions with the present biotic and abiotic surroundings (ecology). This dynamic view of an epidemiological case is essential to describe, predict and control the HPAI H5N1 epizooty.

Current understanding of AIV Epidemiology

Two major types of AIV are distinguished by their pathogenicity. They are classified as LPAI and HPAI viruses based on either their clinical lethality (measured on experimentally infected 10-day old chickens) or on molecular characteristics (sequencing of the hemagglutinin cleavage site) (OIE 2005). It is increasingly recognised that these definitions do not completely overlap. The clinical definition of pathogenicity is determined for domestic poultry only (Lee et al. 2007). A vast majority of AIV with high lethality in gallinaceous poultry fit the molecular definition; however the acquisition of high pathogenicity is recognised to be multigenic and cannot rely only on the HA molecular characteristic. The major transmission route for AIV strains is through faecal–oral transmission.

Wild waterbirds, particularly species of Anseriformes (ducks, swans and geese) and Charadriiformes (gulls, terns and waders), are the natural hosts for LPAI strains with endemicity and seasonal high prevalence in some species (Alexander 2000, Olsen et al. 2006). Data is not available from all waterbirds populations, but given worldwide surveillance, they seem to play the role of an AI strain reservoir and host a viral genetic pool in complex evolution but with low pathogenic impact (Chen and Holmes 2006, Webster et al. 2007). In gallinaceous poultry, LPAI strains appear to be occasionally found and do not seem to be maintained though this could have been overlooked (Alexander 2007).

HPAI strains were detected mainly in gallinaceous poultry and occasionally in terrestrial birds around these outbreaks. In waterbirds, no HPAI have been found with few exceptions: a H5N3 lethal in terns (Becker 1966) and a H5N2 with a HP genotype in healthy wild ducks (Gaidet et al. 2008). Currently, only H5 and H7 (and to a lesser extent H9) subtypes have been found to be highly pathogenic. These HPAI found in poultry outbreaks have been phylogenetically linked to LPAI maintained in wild waterbirds reservoir (Munster et al. 2005, Campitelli et al. 2008). The understanding is that the available viral gene pool in waterfowl populations feeds the gene pool in domestic poultry (Alexander and Brown 2000).

HPAI H5N1 epidemiology

HPAI H5N1 epidemiological patterns do not conform to those of other HPAI. It is renowned for: a) a broader host range, with new bird and mammal species infected: particularly, domestic ducks and a vast range of wild birds (Chen et al. 2005, Webster et al. 2006); b) its heterogeneous pathogenicity for wild (Brown et al. 2006, Keawcharoen et al. 2008) and domestic (Sturm-Ramirez et al. 2005) waterfowl ; c) its increasing environmental

stability (Brown et al. 2007, Lowen et al. 2007, Park and Glass 2007); d) tracheal excretion more important than faecal excretion (Sturm-Ramirez et al. 2005, Antarasena et al. 2006).

The striking difference in the epidemiology of HPAI H5N1 is the co-occurrence of this pathogen in both wild and domestic birds. Dead wild birds have been found infected with the virus in Asia (Chen et al. 2004), Europe (Komar and Olsen 2008) and Africa (Ducatez et al. 2007a). Experimental inoculation studies of HPAI H5N1 have resulted in susceptibility in all bird species tested: the response is highly variable at the inter- and intra-specific level, with some individuals developing symptoms and dying while others remain asymptomatic (Brown et al. 2006, Pantin-Jackwood et al. 2007, Pasick et al. 2007, Keawcharoen et al. 2008). However, no HPAI H5N1 have been detected in waterbirds despite a massive international surveillance effort in recent years (Appendix One - Gaidet et al. 2007, Krauss et al. 2007, Wallensten et al. 2007) apart from few reported cases (Kou et al. 2005, Chen et al. 2006, FAO 2008a). This results needs to be interpreted according to the millions of waterbirds estimated but suggest that HPAI H5N1 is not circulating intensively in wild birds.

The intensive poultry production unit could play the role of reservoir despite the high mortality experienced: a metapopulation model can explain this phenomenon. Waves of infection leave behind recovered (resistant), or dead, individuals and re-infection occurs when the virus is introduced into a naïve population (new or re-stocked). However, the field data from South-East Asia do not support this hypothesis: despite large stamping-out and additional control measures, the HPAI H5N1 seems to be endemic (Chen et al. 2004, Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Gilbert et al. 2006a). The farming of free-ranging domestic ducks in paddy fields provides an epidemiological key explaining the making of an artificial reservoir (Songserm et al. 2006, Buranathai et al. 2007) although the mechanisms are unclear. However, this type of farming is not present in Europe and far less extensive in Africa where the virus nevertheless persists. In Europe, it is not known if the virus has

become endemic in an avian population. Reoccurrence of outbreaks with spatial spread including mortality in sentinel species such as swans raise the hypotheses of a reservoir to be discovered.

In summary, it seems unlikely that either wild birds or the poultry production units independently act as reservoir hosts. Therefore the community interactions between compartments need to be investigated as a mechanism by which the disease could be maintained. Considering the common origin of AIV strains and the exchanges of strains between wild and domestic birds, what makes a particular virus becoming HP or LP? Differences in host ecology need to be delineated to start understanding variables at stake.

Avian host ecology

Knowledge on population dynamics of waterfowl communities is based on decades of intensive ornithological studies. This data has provided support for research investigating the possible roles of wild birds in the spread of AIV, but is still lacking sufficient precision - the measurement of movement parameters - to answer epidemiological questions (Olsen et al., 2006). Wild waterfowl represent a large part of avian biodiversity and a broad genetic heterogeneity compared to the few domesticated species. They have relatively long life cycles and live under variable densities, presumably with multiple exposures to pathogens and a well-developed immunity. These hosts share an evolutionary history with their pathogens. Some waterbirds are also characterised by their gregariousness, including interspecific mixing in different environments, and they have different movement patterns (nomadic, resident or migratory).

Conversely, the domestic poultry production system is characterised by low diversity and high productivity in artificial systems, particularly in the last few decades. In 2006, gallinaceous poultry (92.5% chicken, 1.2% turkey) represented the bulk of domestic production with ducks only reaching 5% (FAO, 2008b). Over 50 billions of poultry heads produced in 2006 presented in this database are to be compared to the millions of waterfowls estimated. Intensive poultry are raised under very high densities (more than 20 birds per square meters for broilers) and for short life cycle rarely exceeding 36 days (for broilers) with very little chance of immunity development (enhanced by antibiotics use). Most birds in poultry population are not breeders and do not participate to the next batch. In addition, the current globalisation of the poultry trade tends to homogenise the breeds used resulting in homogeneity of existing genomes and day-old chicks globally available from any origin.

In between these two domestic and wild systems, a significant proportion of avian populations is raised under a mix of these life history traits, such as backyard chickens production and farmed ostriches. How can we associate the current knowledge in the virus epidemiology with this variability in host ecology?

Role of interactions of hosts between compartments in AIV evolution

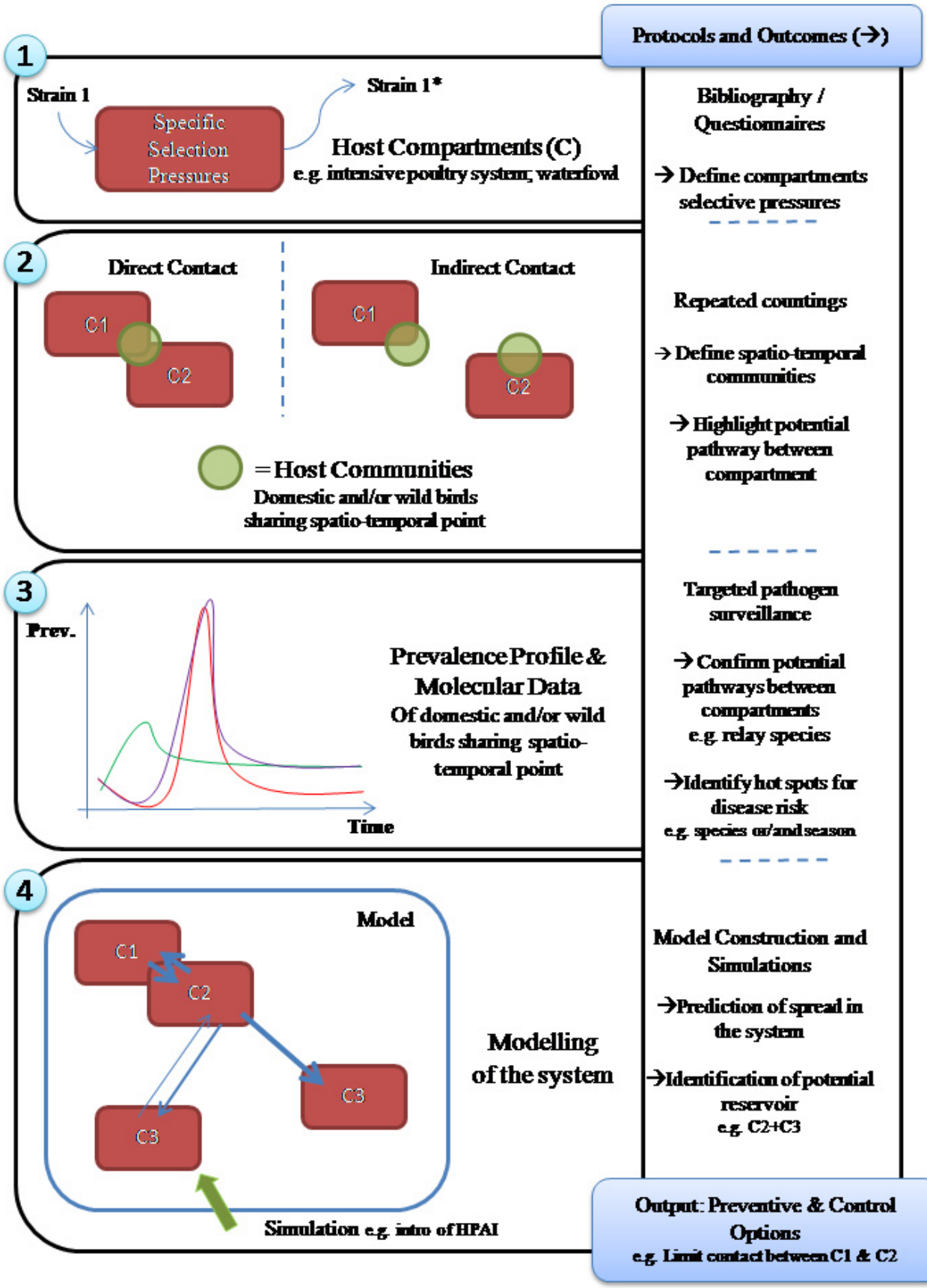
These wild and domestic populations can be clustered into a network of "compartments" (Figure 2.1) each one defined as a set of avian populations under similar environmental drivers: chicken in an intensive production building would be part of the intensive poultry production compartment for example; whereas waterfowl form another compartment. The key to understanding AIV epidemiology and particularly the current HPAI H5N1 threat may be in the interaction between compartments, and the different evolutionary processes in these systems. Interactions between these compartments can occur through direct

contacts– i.e. mixing of waterfowls and domestic birds - or management practices – i.e. free-ranging farming -. Indirect contacts, poorly studied yet, can also occur when wild "bridge" species share space and/or time with birds in two different compartments.

These interactions will draw potential pathways for viruses spread between compartments. As compartments are defined by a set of selective pressures, can one predict viral evolution in their specific context?

The main evolutionary mechanisms for AIV are high mutation frequency, reassortment (both leading to antigenic drift) and recombination (Webster and Hulse 2004), making them rapidly evolving organisms (Boyce et al. 2008). The high rate of co-infection, notably in waterfowls (Sharp et al. 1997), enhances reassortment (Wang et al. 2008). The different avian compartments will drive viral evolution through these mechanisms, but the different environments will have different evolutionary consequences.

Figure 2.1: *Conceptual research framework using community ecology and evolutionary biology to explore AIV ecology within their host in ecosystems. Four steps are presented referring to numbers on top left of each box: 1) Definition of domestic and wild compartments in the ecosystem; 2) Definition of the communities (Begon et al., 2006) in the ecosystems (temporal and spatial variation); 3) Targeted surveillance to test hypotheses from (2); strains isolation will confirm or not prediction on host selective pressure on viral evolution per compartment; 4) Modelling of the system for predictive purpose and definition of new hypotheses – feedback to (2) and/or (3). On the right vertical box, protocols and outcomes (preceded by arrow) proposed for each box. As an example we could consider: C1= backyard poultry compartment; C2=waterfowl compartment; C3= Intensive poultry compartment.*


Protocols and Outcomes (→)
1
Strain 1
Specific Selection Pressures
Strain 1*
Host Compartments (C)
e.g. intensive poultry system; waterfowl
Bibliography / Questionnaires
→ Define compartments selective pressures
2
Direct Contact
C1
C2
Indirect Contact
C1
C2
= Host Communities
Domestic and/or wild birds sharing spatio-temporal point
Repeated countings
→ Define spatio-temporal communities
→ Highlight potential pathway between compartment
3
Prev.
Time
Prevalence Profile & Molecular Data
Of domestic and/or wild birds sharing spatio-temporal point
Targeted pathogen surveillance
→ Confirm potential pathways between compartments
e.g. relay species
→Identify hot spots for disease risk
e.g. species or/and season
4
Model
C1
C2
C3
C3
Simulation e.g. intro of HPAI
Modelling of the system
Model Construction and Simulations
→Prediction of spread in the system
→Identification of potential reservoir
e.g. C2+C3
Output: Preventive & Control Options
e.g. Limit contact between C1 & C2

Intensive poultry production units are an ideal viral breeding ground due to: a) Genetic homogeneity, decreasing the number and variability of resistance genes, little variation between individuals and therefore high infection potential for viruses; b) No reproduction for the majority of birds prevent any co-evolution with the pathogen providing the conditions for a highly lethal strain to be maintained; c) Artificial density of individual hosts increases spread to other individuals; d) Short life cycles prevent the host organism from developing immunity; e) In a compartment with high density and short life spans contagiousness is not under selective pressure and a selection criteria on strains, allowing strains to be selected mainly on virulence (harm to the host; linked to host exploitation; Read and Taylor 2001) ; f) Sometimes inadequate disinfection measures between batches allow the virus to survive in the environment. These suggest some direct inferences: the change in excretion of the H5N1 HPAI could be an adaptation of the virus to intensive production systems where infection would be enhanced by aerosol transmission, whereby a three dimensional space can be utilized for spread compared to faecal transmission which is constrained in a two dimensional space on the ground.

This combination of variables will select for viruses with high virulence, as other life history traits are released from selective pressure (Read and Harvey 1993). Therefore, a highly virulent mutation which would be selected against under natural conditions (since spread would be limited at low density) will thrive in these conditions. In intensive poultry production systems, HPAI strains, if introduced or created will be selected. HPAI H5N1 could be a product of such selective process.

One critical research area for the ecology of HPAI H5N1 in avian populations is interactions, both intra- and inter-strain, with other pathogens and with the host's immune system. Data on the variability of HPAI H5N1 strains is available due to the increased surveillance targeting this specific strain (de Jong and Hien 2006, Ducatez et al. 2007b,

Salzberg et al. 2007, Starick et al. 2007, Zohari et al. 2008) and new HPAI strains are identified (Monne et al. 2008). Since 2004, different strain types from the same clade have circulated in South-East Asia before spreading to other continents. This data could fit in the model of strains with increased virulence which would outcompete previous dominant strain types. As these strains types spread to other domestic or wild avian populations, they would compete with other LPAI strains. The result of intra-specific competition would be dependent on their environment, particularly the host population. The outcome could be co-infection of avirulent pathogens or the selection of a highly virulent pathogen (Van Baalen 1998): 1) the LPAI-waterfowl system illustrates the first alternative. Longitudinal studies in North America (Krauss et al. 2004) and Europe (Munster et al. 2007) have described two year cycles of LPAI strains in wild bird populations, indicating competition between strains, an effect of acquired host immunity and turnover in a population of susceptible host through recruitment; 2) the second alternative is represented by HPAI strains selected in intensive domestic populations with an environment enabling them to outcompete other less virulent strains. Consequently, the penetration of HPAI H5N1 strains (selected for their high virulence in intensive poultry) in the co-evolved systems between waterfowl and LPAI would be difficult: low density of host population leading to a selection on contagiousness, a developed host immunity inducing resistance and the presence of intra-strain competition with other AIV will all be factors against their maintenance and spread. The hypothesis that HPAI H5N1 could persist in some wild populations is not supported by this theoretical approach except through a meta-population mechanism with host population extinction and spread dynamics. The susceptibility of each wild species to HP strains is the missing variable to test this model. Finally, competition between different pathogens at both the population and individual levels are under-studied. For example, Newcastle disease is widespread and induces more than 50% of mortality in domestic avian populations infected. The role of wild birds in the

epidemiology is unclear. A HPAI strain infecting a population where Newcastle disease occurs will be in a direct competition for hosts. The outcome of this competition is difficult to predict but will be important for the maintenance of HPAI strains in wild populations.

Gene reassortment can occur when two strains co-infect one host individual. It combines the genetic history of two strains which have evolved in different environments. This could result in an increase of host range (Webster et al. 2006) and pandemic threats (Nelson et al. 2008). Co-infection is therefore important, and dependent on the evolutionary interactions between different strains (intraspecific competition) and the host immunity. Reassorting strains selected in different environments will create more variable new subtypes than strains selected under similar selection pressures. Reassortment between LPAI should occur often in wild bird populations with a low probability of making of a HPAI. However, HPAI, selected in intensive poultry units, mixing with a LPAI (event with a low probability) can acquire more variable life history traits, leading to hazardous mixing for animal and public health. However, these events will depend on the degree of interaction between domestic and wild bird populations. For example, the gene pool from the American waterfowl community is separated from the one of Eurasian origin with limited exchanges despite overlap in wild birds migrating flyways (Olsen et al. 2006; Krauss et al., 2007); practices such as the farming of domestic ducks, the raising of mallard ducks for hunting in Europe and the extensive farming of ostriches in Southern Africa artificially places a species in potential contact with different compartments under different selection pressures and hazardous mixing possibilities

Implication of evolutionary biology and community ecology for AIV research

The epidemiological dynamics of HPAI H5N1 in different regions seem to be variable. The global analysis of bird migrations has provided until now little information in understanding the pattern of geographical spread (Gilbert et al. 2006b): the lack of information for a large number of species, variation between individuals (age and gender) and populations impede the generalisation from the data used. Each region at risk will differ in species composition, spatio-temporal patterns of movement and contact between domestic and wild avian populations. Compartment specificities will drive strain evolution toward different pathways creating "selection mosaics" or a "geographical evolution of virulence" (Thompson 1999, Hochberg et al. 2000). Similarly, phylogenetic analysis is an important tool but has limitations: with the detection of related strains at two distinct spatio-temporal points one can only speculate on virus spread or origin. Recent studies have suggested that LPAI strains can influence migration success of a swan species (van Gils et al. 2007). If confirmed and generalised to other strains and hosts, there could be a negative relationship between strain virulence and the distance travelled. Furthermore, if birds infected with LPAI migrate less efficiently, the bird populations at departure will decrease in size but with a higher concentration of infected birds. The average migration departure time is not representative of the movement of infected birds and the research should be concentrated on the tail of this migration distribution profile.

Therefore we suggest that a local approach (Figure 2.1) may enhance our understanding of the virus epidemiology, using community ecology to evaluate contact patterns between compartments. Such an approach will provide a cross-cutting analysis of the compartment-specific selection pressures on viruses and the intra-community interactions that

define the potential spread pathway. For example, a rural village close to a wetland will host two avian compartments: a backyard flock and a waterfowl population. Regular bird counts at particular places will identify any direct or indirect contact between compartments. Targeted disease surveillance, relevant for each bird compartment, should be used to test hypotheses on the maintenance and spread of the virus using molecular techniques. A weakness of this approach lies in the low prevalence and high sample size necessary for AIV isolation and the financial cost for using additional efficient molecular techniques. Taking into account the community of hosts will be important if the reservoir of LPAI or HPAI is not a single species but a complex interaction of bird populations (wild and/or domestic) in a specific spatio-temporal range. The concept of a multi-host reservoir (Haydon et al. 2002) could explain the maintenance of HPAI H5N1 in some ecosystems.

From this perspective, specific practices could be targeted to implement a compartment/community study. Domestic ducks in South-East Asia seem to act as a reservoir for HPAI H5N1. This is an interesting compartment since this domestic species is free-ranging during the day, in contact with the waterfowl compartment, and in farm buildings at high density at night. The potential for virus acquisition and selection is therefore high, and this interface compartment seems at risk. Mallard ducks raised for hunting in Europe may also be a potential "Trojan Horse" (Webster et al. 2007), since this wild species is kept under a domestic poultry management scheme for future release in the wild, with obvious differential selective pressures, and it plays an important role in LPAI epidemiology. The extensive farming of ostriches in Southern Africa is also of concern with recent HPAI H5N2 outbreaks: this wild but recently domesticated species, free-ranging and potentially in contact with wild populations has been highlighted as a potential mixing vessel for AIV (Abolnik et al. 2007). These practices promote contacts between domestic and wild bird populations and the created

interfaces associate the gene pool and the gene selection process which should lead to selection of HPAI strains.

Conclusion: describing the pathogen risk

HPAI H5N1 introduction pathways are a nightmare for epidemiologists (see recent introduction in UK or France as examples). The variability in individual bird migration coupled with intra-specific variability of susceptibility to HPAI H5N1 has been demonstrated by recent studies. More variability lies in the AIV interactions with their environment: other AIV strains, other pathogens, and the host immune system. Future researches have no options other than being local and specific.

We advocate a community ecology approach using evolutionary theory and phylogenetic analysis to build and test between-compartment interaction hypotheses. This ecological and epidemiological data can then be integrated into a risk analysis model (Goutard et al. 2007). Once compartment interactions are understood, the consequences of a virtual viral introduction in one of the compartments spreading to other compartments can be modelled and its risk estimated. Seasonally, pathways for virus spread in the system could be identified by simulations. The linkage of multiple local analyses could lead to a global understanding of the eco-epidemiology of the virus. Real and Childs (2006) have presented a similar approach for rabies studies at a global scale.

The community/compartment approach could be extended to human communities with the help of social sciences. Fasina et al. (2007) explains how peri-urban human populations in Africa are at higher risk of HPAI H5N1 infection than others. Such a human compartment has

specific interactions with the backyard poultry compartment and the wild bird compartment. This type of model could help describing the pandemic risk.

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