Epidemiology of the highly pathogenic avian influenza H5N1 in Northern Vietnam: applications for surveillance and control

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2012
I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

DESVAUX Stéphanie
Abstract

The epidemiology and the sanitary situation of avian influenza changed dramatically with the emergence of the highly pathogenic H5N1 virus (HPAI) in 1996. As a consequence, knowledge previously accumulated on the epidemiology and the ecology of the avian influenza viruses was questioned and was required to be updated to understand the current pandemic caused by the virus (Webster, 2007; Sturm-Ramirez, 2005).

This PhD combined a number of different epidemiological studies aimed at understanding the epidemiology of the H5N1 virus in the natural and human context of the Red River Delta area in Northern Vietnam.

Firstly, retrospectives studies were conducted to identify the determinants of occurrence of HPAI outbreaks at 2 different scales: provincial and regional. Those 2 approaches allowed us to study the influence of the poultry production systems (provincial scale) and the influence of environmental determinants (regional scale).

In addition, substantial field work was undertaken to monitor the serological and virological prevalence of HPAI in domestic poultry in our study area. After evaluation of the serological diagnostic tools being used, the data analysis contributed to a better understanding of the epidemiology of the H5N1 virus within a mass vaccination context. Furthermore, an evaluation of the vaccination strategy and implementation was also possible. In addition, to support our findings, a specific protocol to monitor the antibody kinetics of vaccinated poultry under field conditions was also conducted.

Finally, a study was undertaken, in collaboration with a sociologist, to better capture the way sanitary information was circulating within our community of poultry farmers and through the formal surveillance system.

Together with the results of our epidemiological work, this sociological study enabled us to propose measures to improve the surveillance and control of HPAI at the community level, to assist the people whose livelihoods were most affected.
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Acknowledgments

I sincerely thank my supervisors' team for their support and guidance during this thesis:

☞ To Stan Fenwick for being a very efficient and dedicated guide during all this process.
☞ To Trevor Ellis, for his meticulousness and efficiency in reviewing the papers.
☞ To John Edward, a role-model and a friend for several years now, who warmly welcomed me at Murdoch University.
☞ To François Roger from Cirad, a decisive encounter in my professional life, who introduced me to epidemiology and who is at the origin of this story. My sincere gratitude for the trust he puts on me since I am in his team.

I acknowledge the support from the French Ministry of Agriculture allowing me, as a civil servant, to conduct this PhD and the French Ministry of Foreign and European Affairs for project funding (Gripavi).

I thank the external examiners for their review and very useful comments.

I thank all the persons who contributed directly to this thesis and in particular:

☞ Pham Thi Thanh Hoa who was very precious for the data collection during all the time spent in Vietnam.
☞ Marisa Peyre, for her support on the evaluation of the vaccination.
☞ Annelise Tran from Cirad and Sebastian Tollis, master student in 2009 who processed the satellite images.
☞ Nguyen Cong Oanh who collected the field data related to the study on the risk of introduction from China (not an easy task!) and to Cecilia Henriquez, a master student who performed the initial descriptive analysis of the data.
☞ Vladimir Grobois for his skill in explaining statistics and his incredible patience to answer all the (stupid) questions I had during the last 2 years and Lucas Léger, for his perseverance in “R” and his help on spatial statistics.
☞ Muriel Figuié for the very rewarding collaboration we had that helped me to look at my work differently.

A special dedication to:

☞ the AGIRs team : c’est une chance de pouvoir mélanger le travail à l’amitié. Je vais quitter les collègues, mais je compte bien garder les amis.
☞ Carlène : pour le soutien mutuel et les échanges fructueux sur les subtilités des méthodes statistiques !
A ma petite famille qui équilibre ma vie,

A ma grand-mère qui va être rassurée que je termine ENFIN mes études...
Vietnam reported its first H5N1 Highly Pathogenic Avian Influenza (HPAI) outbreaks by the late 2003. By that time, the disease was already widespread (FAO, 2011). From this initial report and to the end of 2010, outbreaks were both clustered in time and space, with several waves being centred on the 2 river deltas, the Red River delta and the Mekong delta as illustrated by Figures 1.1 and 1.2 (Pfeiffer.D.U et al., 2007). It is estimated that 44 millions of poultry were culled during the first wave (Department for Agricultural Forestry and Fisheries Statistics and General Statistics Office, 2004). In 2009, this is around 124 thousands of poultry that either died of the infection or were culled (PAHI, 2010). Vietnam was also one of the countries that reported the highest number of human cases, together with Indonesia and Egypt (FAO-ECTAD, 2011). By the end of November 2011, 59 human fatal cases out of 119 reported cases were recorded (WHO, 2011). To limit the number of outbreaks and the risk of transmission to humans, the Government of Vietnam adapted a mass vaccination strategy by the end of 2005. Despite a period of about a year in 2006 without an outbreak on poultry or reported human cases, outbreaks reoccurred by the late 2006 in the South and by mid-2007 in the North. Since then, outbreaks on poultry and human cases continue to occur sporadically despite the vaccination being implemented on domestic poultry.
Figure 1.1. Spatial distribution of HPAI in Vietnam (2003-2010) (FAO-Vietnam, 2011)

Figure 1.2. Monthly distribution of reported outbreaks of H5N1 in Vietnam (2003-2010) (FAO, 2011)
1.2. THE VIETNAMESE POULTRY PRODUCTION SYSTEM

With more than 200 millions domestic poultry in the country, Vietnam presents areas of very high poultry density centred on its 2 deltas, which are also the area of highest human densities (Figure 1.3). The Vietnamese poultry production system is very diverse and involves many actors (Figure 1.4).

Within the framework of this PhD, a detailed review of the Vietnamese poultry production system was produced and published at the Hanoi Agricultural Publishing House, a Vietnamese publishing company specialized in agriculture (publication 1: a general review of the poultry production system in Vietnam) (Desvaux and Dinh, 2008). It gives a detailed description of the traditional and the semi-commercial farming systems (see Annex 1).

1.2.1. THE TRADITIONAL FARMING SYSTEM

This sector is defined as traditional or ‘backyard’ farmers. The number of birds per cycle is limited (typically less than 50). The vast majority of poultry farms in Vietnam fall into this category. According to the 2001 census, these farms produce about 65 percent of Vietnam’s chicken stock and 60 percent of its duck. Most chicken – 92 percent – are broilers, with the remainder kept for eggs production (GSO, 2004).

These farmers keep local breeds of poultry that generally wander freely. The animals are fed with household leftovers or locally procured inputs (paddy, bran, corn), perhaps supplemented with some industrial feed (GSO, 2004). This sector is characterized by low levels of investment and technical performance, self-producing of breeding chicks, the absence of sanitary or technical monitoring, and long farming cycles. Newcastle disease, the coccidiosis, the pasteurellosis and the fowl plague are common diseases on those domestic poultry.
Figure 1.3. Poultry density of Vietnam in 2006, extracted from (Desvaux and Dinh, 2008)

Figure 1.4. Diversity of the Vietnamese poultry production system
Most households in this sector are poor and their income is from rice and livestock production. Not all the birds are consumed by the household and the percentage of production sold, ranging from the vast majority to less than 50%, depends mainly on the location of the farm and its access to markets (Tung, 2005). The production is either sold to local markets (mainly from the same districts), at farm-gate to assemblers or at farm-gate to neighbours depending also on the location of the farm (Tung, 2005).

1.2.2. THE SEMI-COMMERCIAL SECTOR

The semi-industrial sector is defined here as a market-oriented production with improved technical inputs compared to traditional farming systems but still with a minimum to medium biosecurity level. This sector shows increased market integration compared to traditional farming systems and a wider marketing network (Agrifood, 2006). We consider a lower limit of around 50 birds per cycle but no upper limit. The differences between the farms lie in the size, the technical input and the market linkages. It is difficult to give a general limit; this depends on the type of production involved. This sector presents a great diversity based on the species (chickens, ducks and Muscovy ducks) and the type of production involved (breeders, layers or broilers). Breeders and layers are usually kept for at least one year; they will be called “long cycle”. Broilers are usually ready for sell within 2 to 6 months time, depending of the breed; they will be called “short cycle”.

A part of the duck production is highly seasonal and in relation with rice production (with 2 and sometimes 3 production periods a year according to the number of rice production cycles in the area). The ducklings are brought to the rice fields just after rice transplantation to control pest. When getting older, the ducks are driven out of the rice fields to canals, ditches, rivers and brought back to the rice fields during the days just after harvest for scavenging for weeds, crop residues, snails and fresh-water crustaceans.
(Desvaux and Dinh, 2008). The main periods for this production lie between March to July and September to December and vary according to the rice production seasonal calendar. In Northern Vietnam, the ducks are usually herded in the rice fields in one region (several communes) during the day but brought back in the same pen at night. Movement the duck herds between regions is not permitted. In Southern Vietnam, a similar system exists with ducks herded on rice fields but able to move from one province to another.
1.3. THEORETHICAL FRAMEWORK OF THE RESEARCH

1.3.1. RESEARCH QUESTIONS AND HYPOTHESIS

The main question was “what are the determinants and patterns of introduction, dissemination and persistence of HPAI H5N1 disease in the domestic poultry of the Red River Delta in Northern Vietnam?”. The general objective was to propose adapted surveillance and control options. Based on the initial existing knowledge related to the epidemiology of avian influenza in general, and the H5N1 subtype in particular, we had several hypotheses to guide our researches.

We hypothesized that:

- both persistence and introduction mechanisms were existing,
- low viral circulation in some domestic poultry populations with a sub-optimal induced immunity has an influence on the epidemiology of the disease,
- the natural environment is playing a role in the persistence and transmission of the virus,
- wild birds may play a limited role in the epidemiological cycle of the disease in the Vietnamese context.

1.3.2. CONCEPTUAL MODEL

In order to introduce the different protocols conducted to tackle our general question, we developed a conceptual model of the system to be studied (Figure 1.5). The system is defined as the compartments in which and between which the H5N1 HPAI infection may be transmitted or may persist in space or in time from the end of 2005 to 2010.
The system consists of:

- The poultry production system characterized by both the poultry production cycle (long cycle for breeders and layers and short production cycle for broilers) and the production management (semi-commercial flocks versus backyard production). Hatcheries, producing Day-Old-Chicks (DOC) and ducklings (DOD) are also part of the poultry production system.

- The environment, characterized by 3 compartments: the aquatic environment (including rice-field), the solid environment (crops other than rice, forest, roads, residential area) and the wild birds' population.

- The poultry trading actors (small and big traders) and main locations (live birds markets).

The model also includes actors that influence the system:

- The veterinary services that influence the system by their interventions: vaccination, culling, disinfection...

- China, from where the virus may be introduced.

- The consumers, as a dead end for the system.

Relationships between the compartments are complex and multiple. We modelled them simply, based on knowledge i) from existing reports (Agrifood, 2006) or ii) built from our own data collection, a study of the poultry production chain in 2008 within the framework of the Gripavi research project (project on the epidemiology and ecology of the avian influenza and Newcastle viruses in Vietnam and Africa). This study also initiated a basis for collaboration on: the characterization of the poultry production chain (Le Bas et al., 2008) (Annex 2), on the modelling of the flux of poultry production within the broiler chicken production system (Payne et al., 2009) (see Annex 3) and also on social network analysis of
the poultry and poultry products traders (see Annex 4 for a poster presented at the Epideomics conference).

The objective of this thesis was to understand how those compartments interact in space and time and to explain the pattern of the disease between the end of 2005 and 2010. To do so, different approaches were chosen to study particular parts of the system.

The system being too complex for an exhaustive approach, only some parts were studied in detail and a restricted geographical area was selected for its representativeness of the high risk areas in the Red River Delta region.

The combination of all those studies aimed to highlight the main mechanisms involved in the epidemiology of HPAI H5N1 influenza disease in Northern Vietnam and the influence of prevention programs (mainly vaccination) on this epidemiology.

Figure 1.5. Conceptual model of the system under study
1.3.3. STRUCTURE OF THE THESIS

The literature review (chapter 2) is limited to a few aspects of the epidemiology of the H5N1 HPAI disease that are not covered in the introduction of the other chapters. This chapter also gives a brief presentation of the phylogenetic analysis of the H5N1 virus in Vietnam.

General aim 1: to understand the mechanisms involved in HPAI H5N1 occurrence in Northern Vietnam

Chapter 3 presents and discusses the risk of introduction of HPAI H5N1 related to the introduction of illegal poultry from China. This chapter aimed to provide a qualitative description of illegal poultry imports from China in order to identify the season(s) or the poultry production sector(s) at risk of infection from poultry illegally imported from China.

Chapter 4 and chapter 5 investigate the local risk factors for disease spreading during the 2007 outbreaks wave (paper 1 published in Transboundary Animal Diseases) and the environmental factors possibly related to the maintenance of the virus in Northern Vietnam at a regional scale, respectively. Chapter 3 is based on a field study conducted in 2010 with the support of a Vietnamese researcher for data collection. Chapters 4 and 5 used two different data sets:

- One built from our own data collection for a case control study (in 2008) and looking at local risk factors of H5N1 outbreaks occurrence at a provincial level.
- One using reported data of outbreaks and using variables extracted from the interpretation of satellite images at a regional level.
- The Figure 1.6 illustrates those 2 scales of study: the provincial level (Bac Giang province) and the regional level (called “Great Delta” region).

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1 The numbering of illustrations in the chapters based on published papers is separated from the numbering of the rest of the document
Figure 1.6. The 2 scales of study for the determinants of HPAI H5N1 occurrence in Northern Vietnam

General aim 2: to evaluate some surveillance and control tools for HPAI

In chapter 6, we first evaluated the serological tools available in Vietnam for surveillance and evaluation of the vaccination status. We mainly focused our analysis on the evaluation of the HI test. We also evaluated an Elisa H5 test in order to discuss comparative advantages of those two types of diagnostic tests (paper 2 accepted in Veterinary Microbiology).

In chapter 7, we then evaluated the immunogenicity of the H5N1 vaccine used in Vietnam on a cohort of domestic poultry under field conditions. This study supports the results of the longitudinal study presented in the following chapter.

Chapter 8 presents the substantial part of our field work, made by repeated cross-sectional studies conducted between 2008 and 2010 on farmed domestic poultry to i) measure the virological and serological prevalence, ii) to evaluate the effectiveness of vaccination and iii) to evaluate the impact of the vaccination on the epidemiology of the disease (paper 3).
submitted to Epidemiology and Infection). The study presented in this chapter was also for the subject of a poster presentation at the SVEPM conference in 2010, in Nantes, France and an oral communication at the 7th conference on ‘Options for the Control of Influenza’, in 2010 in Hong Kong SAR, China.

In addition to the cross sectional studies conducted on the farms, a study in markets selling live birds was conducted to understand their role in the persistence of the virus.

The PhD student also collaborates on a study about the potential role of some wild bird species into the epidemiology of the disease in our study area, but those results, resulting from a collaborative work are not discussed in this PhD. The Annex 9 presents the main species studied, as well as raw laboratory results.

Finally, chapter 9 presents a study aiming at understanding the way that sanitary information related to H5N1 disease is handled by farmers was conducted in collaboration with a sociologist. This study was aimed at gaining a better understanding of the constraints related to the formal surveillance system for H5N1 in the socio-cultural context of Northern Vietnam, in order to propose adapted options (Chapter 9 is mainly based on paper 4 published in the proceedings of the International Conference on Animal Health Surveillance, ICAHS following an oral communication).

General aim 3: to analyse and combine the knowledge to propose an adapted control and surveillance protocol

In chapter 10 we discuss the different results in the perspective to propose adapted surveillance and control options for Northern Vietnam. Extrapolation of those results to other contexts will also be discussed.

All protocols were approved by CIRAD and Vietnamese’s veterinary services or research institutions before implementation.
CHAPITRE 2 - LITERATURE REVIEW

2.1. A BRIEF REVIEW OF INFLUENZA VIRUSES, IN PARTICULAR H5N1

2.1.1. GENERAL INTRODUCTION TO INFLUENZA VIRUSES

Influenza A viruses, members of the Orthomixoviridae family, infect a variety of animals, including wild and domestic birds, but also humans, pigs, horses and sea mammals. They are differentiated from type B and C influenza viruses on the basis of the major protein antigen, the nucleoprotein (NP) and the matrix (M1) proteins (Webster, Bean et al. 1992).

Avian influenza (AI) viruses can be categorized into subtypes and pathotypes. The subtype's distinction is based on serological typing of the two glycoproteins: the haemagglutinin (HA) and the neuraminidase (N). Sixteen antigenically different haemagglutinins (H1 to H16) and nine antigenically different neuraminidases (N1 to N9) are now recognized and all of them have been isolated in wild birds (Alexander, 2007) (Swane and Pantin-Jackwood, 2008). The pathotypes are based on the ability to produce disease and death in chickens Gallus domesticus. Some of the H5 and H7 subtypes, carrying multiple basic amino acids adjacent to the haemagglutinin cleavage sites, are responsible for severe and acute disease with high mortality in poultry, namely highly pathogenic influenza avian influenza (HPAI). Low pathogenic avian influenza (LPAI) viruses usually produce respiratory disease and decreased egg production in all types of poultry species.

Wild aquatic birds especially Anseriformes (ducks, geese and swans) and Charadriiformes (shorebirds, gulls, terns and auks) are natural reservoirs of LPAI viruses (Webster et al., 1992). They were so far not susceptible to the influenza viruses and they are suspected to be at the origin of the highly pathogenic viruses found in domestic poultry (Webster et al., 1992). However, since the appearance of HPAI H5N1 viruses in 1996 in Asia, illness and deaths have been observed in ducks and geese and a variety of captive and wild birds (Desvaux et al.,
viruses cause severe systemic disease and very high mortality.

2.1.2. ORIGIN AND EVOLUTION OF THE H5N1 HPAI VIRUS

The Asian epidemic of HPAI H5N1 disease in poultry started in 2003 and expanded its geographical range to affect poultry in East and Southeast Asia, becoming endemic in this region. The virus also spread to Central Asia, Europe, and Africa.

Antigenic drift in avian influenza viruses in their original aquatic bird reservoir is limited but, after the virus has spread into domestic poultry, it can become more frequent (Webster and Hulse, 2004). Thus, continued evolution of H5N1 viruses has resulted in the appearance of several distinct genotypes obliging the WHO/OIE/FAO H5N1 Evolution Working Group to adopt a nomenclature system of the HA lineage protein gene based on clade definition (WHO/OIE/FAO, 2008). A clade of viruses is based primarily on the phylogenetic characterization and sequence homology of the HA gene. The Asian outbreak was traced back to the (Gs/Gd)-like lineage, resulting from the evolution of the Goose/Guangdong/1/96 (Gs/Gd/96) virus, first H5N1 virus isolated in 1996 in the province of Guangdong, China (K.S.Li et al., 2004). Following the WHO/OIE/FAO nomenclature, the Goose/Guangdong/1/96 virus is referred as the HA clade 0.

A recent paper analyzed the dispersion and evolution of HA clades throughout the world (Pfeiffer et al., 2011) and emphasized that genetic variability of HPAI H5N1 viruses was much lower outside East and Southeast Asia than inside (Figure 1.7). This suggests that higher transmission frequencies occur in these regions, which are probably due to high population densities of terrestrial and aquatic poultry combined with special, specialised production and trading practices.
2.1.3. HISTORY OF H5N1 HPAI VIRUSES IN VIETNAM AND CONSEQUENCES FOR THE EPIDEMIOLOGY OF THE DISEASE

Vietnam officially declared its first outbreaks of HPAI H5N1 in 2003. The HPAI H5N1 viruses isolated in Vietnam from those initial declared outbreaks belonged to the HA clade 1 (genotype z) (Nguyen et al., 2008; Wan et al., 2008) and derived their HA genes from the Gs/GD/1/96-like lineage (Smith et al., 2006). Other H5N1 viruses were also detected in Vietnam before 2003, but did not seem to have evolved for a long time in the country (Wan et al., 2008). In 2008, a study identified that 6 different HA clades had circulated in the country (Wan et al., 2008) with clade 1 and clade 2.3.4 being the predominant ones (Nguyen et al., 2008). More recently, a novel HA clade was isolated from chickens seized at the border with China (Davis et al., 2010).
From phylogenetic and phylodynamic analysis of the H5N1 viruses identified in Vietnam, hypotheses can be made about the different epidemiological mechanisms explaining the global picture of the disease in this natural and human environment.

**There is persistence of the viruses between outbreaks**

After its first introduction in 2003, HA clade 1 viruses were still detected several years later and up to 2010 (Long et al., 2011; Nguyen et al., 2008). Based on routine surveillance sampling, this clade appeared to be predominant in the southern region indicating that this region probably acted as a reservoir for those clade 1 viruses, as did the other Mekong countries (Long et al., 2011; Nguyen et al., 2008; Pfeiffer et al., 2011). A period of silent spread or low level of incidence probably occurs between 2 epidemic waves to explain this maintenance of the virus in the region.

**There are regular introductions of viruses from China**

Based on genetic proximity with Chinese viruses, Chen et al demonstrated in 2006 that H5N1 virus had been introduced into Vietnam from Southern China on multiple occasions in 2001, 2003 and 2005 (Chen et al., 2006). More recently a study also described the genetic proximity of the 6 HA clades identified in Vietnam with precursor viruses isolated previously in mainland China and Hong Kong SAR, confirming regular introductions of viruses into Vietnam from those regions (Wan et al., 2008). The isolation of clade 7 viruses from chickens seized at the border with China is more proof of the regular introduction of new viruses from China (Davis et al., 2010).

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### 2.1.4. H5N1 PATHOBIOLGY AND CONSEQUENCES FOR THE SURVEILLANCE

Perkins et al (Perkins and Swayne, 2003) initially reported 4 distinct patho-biological groups for H5N1 HPAI viruses, based on virus replication, pathology, morbidity and mortality ranging from the most severe clinical expression (group 1) to the absence of infection and pathological consequences (group 4).
In chickens, the virulence of Asian lineage H5N1 HPAI viruses isolated after 1996 was 100% lethal, but the patho-biology, based on the Mean Death Times (MDT), varied slightly from 1.5 to 5.5 days by virus strains (Swane and Pantin-Jackwood, 2008).

In Ducks, the Asian lineage H5N1 HPAI viruses have evolved into multiple different strains with different patho-biological consequences. The initial strains isolated in Asia were in patho-biological group 4 while in 2001, some strains caused severe respiratory infection and occasional dissemination (group 3), in 2003 group 3 strains were isolated and, from 2004, group 1 strains (Swane and Pantin-Jackwood, 2008).

When extracting information about Vietnamese strains from laboratory trials (Table I), we can observe that the patho-biology of H5N1 strains in domestic ducks in Vietnam is very diverse and poses problems for recognition at the field level. Indeed, to find a uniform clinical case definition to be applied to ducks for the surveillance of HPAI H5N1 is very tricky.

Virus shedding in faeces and respiratory secretion is known to start from to 2 days post-infection (Spickler et al., 2008), making the transmission of the disease very easy within a flock, during transport of live birds or at a live bird market place.
Table 2.1. Mortality, MDTs and viral replication titres from oropharyngeal and cloacal swabs of ducks infected with Vietnamese-origin H5N1 influenza viruses (adapted from (Swane and Pantin-Jackwood, 2008), (Kim et al., 2008), (Hulse-Post et al., 2005) and (Sturm-Ramirez et al., 2005))

<table>
<thead>
<tr>
<th>Virus</th>
<th>HA clade</th>
<th>Pathobiology Group</th>
<th>Mortality</th>
<th>MTD (days)</th>
<th>Oral mean titres 3 dpi&lt;sup&gt;1&lt;/sup&gt; (no. shedding/no. sampled)</th>
<th>Cloacal mean titres 3 dpi&lt;sup&gt;1&lt;/sup&gt; (no. shedding/no. sampled)</th>
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<tbody>
<tr>
<td>A/duck/Vietnam/218/2005&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.3.4</td>
<td>1</td>
<td>8/8</td>
<td>2.7</td>
<td>6.5 (8/8)</td>
<td>3.3 (8/8)</td>
</tr>
<tr>
<td>A/duck/Vietnam/203/2005&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.3.2</td>
<td>1</td>
<td>8/8</td>
<td>3.4</td>
<td>4.8 (8/8)</td>
<td>1.5 (8/8)</td>
</tr>
<tr>
<td>A/Vietnam/1203/2004&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>7/8</td>
<td>4.2</td>
<td>4.9 (7/8)</td>
<td>2.0 (7/8)</td>
</tr>
<tr>
<td>A/Chicken/Vietnam/C58/04&lt;sup&gt;3&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
<td>4/6</td>
<td>na</td>
<td>Na (4/6) (8/6)</td>
<td>Na (4/6) (8/6)</td>
</tr>
<tr>
<td>A/Vietnam/3046/04&lt;sup&gt;3&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
<td>0/2</td>
<td>-</td>
<td>Na (2/2) (8/8)</td>
<td>Na (2/2) (8/8)</td>
</tr>
<tr>
<td>A/goose/Vietnam/113/2001&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3</td>
<td>3</td>
<td>0/8</td>
<td>-</td>
<td>1.8 (8/8)</td>
<td>&lt;1.6 (8/8)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Virus titres are expressed as the log<sub>10</sub> EID<sub>50</sub>/ml

<sup>2</sup> 2-week-old ducks inoculated

<sup>3</sup> 4- to 6-week-old ducks inoculated
2.2. SURVEILLANCE AND CONTROL OPTIONS FOR HSN1 HPAI IN BIRDS

2.2.1. GENERAL GUIDELINES RELATED TO THE SURVEILLANCE OF AVIAN INFLUENZA

HPAI of any subtype is a notifiable disease at the World Organisation for Animal Health (OIE). LPAI of the H5 and H7 subtypes is also notifiable. Together, they are named Notifiable Avian Influenza (NAI) in the Terrestrial Animal Health Code (OIE, 2010). Surveillance of LPAI H5 and H7 LPAI is justified by the fact that without detection, LPAI will spread and have a chance of mutation into HPAI viruses as it was probably the case in Italy and Netherlands (Capua and Marangon, 2000).

According to the OIE, a surveillance system should enable the detection and investigation of outbreaks of disease or NAI infection. Since the intensity and the nature of clinical signs resulting from an infection with a NAI virus depend on the subtypes, the strains and the infected species involved; detection of outbreaks of disease alone is not sufficient to determine the NAI status of a country, a zone or a compartment. Thus, virological surveillance of clinically suspect cases, at risk populations, positive serological results, vaccinated flocks or flocks epidemiologically linked with an outbreak, is a necessary tool in any surveillance programme for NAI.

Serological surveillance may also be used in a surveillance programme. If the serological surveillance is performed on non-vaccinated poultry, positive serological samples should be followed by virological and epidemiological investigations to confirm the presence of a NAI infection. Serological surveillance directed to the detection of antibodies against the neuraminidase can also be used on poultry vaccinated with a whole vaccine containing an influenza virus of the same HA sub-type but with a different neuraminidase from the field virus. This strategy, named DIVA for Differentiation of Infected from Vaccinated Animals, has been found efficient to support eradication programmes against LPAI viruses (Capua.I...
and Marangon.S, 2006) but is only possible if techniques related to the detection of antibodies directed against the neuraminidase are available in the country. Surveillance of vaccinated population may also be achieved via a clinical, serological and/or virological monitoring of sentinel birds, that is to say, of birds that are kept unvaccinated within vaccinated flocks. This system may be difficult to implement in the field and is considered rather impracticable, especially for the identification of sentinel birds in premises that contain floor-raised birds (Capua.I and Marangon.S, 2006).

2.2.2. BRIEF HISTORY OF CONTROL AND SURVEILLANCE STRATEGIES APPLIED TO AVIAN INFLUENZA OUTBREAKS: THE EUROPEAN EXPERIENCE

In 1992, the Council Directive 92/40/ECC introduced measures to be applied in the event of an outbreak of avian influenza in poultry in the European Community (Council of the European Communities, 1992). These measures included compulsory stamping out policy for the only HPAI viruses. Thus, when LPAI H7N1 outbreaks occurred in Italy in 1999, there were no legislative tools to prevent the spread of the virus and stamping out could not be applied on a voluntary basis for the high number of flocks involved in this epidemic. The dissemination of this LPAI H7N1 virus resulted in severe losses to turkey farmers where the highest number of outbreaks occurred and in the emergence of a HPAI H7N1 strain within few months. This latter outbreak had even heavier economical consequences (Capua et al., 2003).

Based on that experience, a decision was adopted by the European Commission to introduce compulsory surveillance programs in member countries, in order to early detect the outbreak of the H5 and H7 subtypes regardless of their virulence (Commission of the European Communities, 2002). For its first year of implementation, the aim of this surveillance programme was i) to perform an initial screening to detect infections with avian influenza virus subtypes H5 and H7 in different species of poultry and ii) to contribute
to a cost-benefit study in relation to eradication of all H5 and H7 subtypes from poultry envisaged by the change in definition of avian influenza in the regulation (Commission of the European Communities, 2002). This surveillance programme has been refined regularly in the light of acquired knowledge on the at risk species or production systems or in the light of new epidemiological events. This was the case for instance in 2005 with the evolution of the HPAI H5N1 avian influenza situation in Asia that resulted in the adoption of a Commission Decision. This decision planned to intensify the surveillance programme already planned for 2005/2006 by increasing sampling on migratory waterfowl along the flyways that could pose a risk for disease introduction (Commission of the European Communities, 2005). The surveillance programme is still in place nowadays.

Furthermore, following the changes in the Terrestrial Animal Health Code of the OIE in 2005 (in particular the introduction of the notification of LPAI of the H5 and H7 subtypes) and, following opinions delivered by the Scientific Committee on Animal Health and Animal Welfare and by the European Food Safety Authority, the Directive 92/40/ECC has been fundamentally reviewed in a new council directive (Council of the European Union, 2005) that introduced minimum measures for the prevention and control of avian influenza including the LPAI H5 and H7 infection. Among the control measures, vaccination is presented as an effective tool to supplement disease control measures and to avoid massive killing and destruction of poultry and other captive birds. Before the publication of this new regulation, special authorisations to practice vaccination were given to members states which requested for it to control epidemics of LPAI or HPAI. For instance, a vaccination programme was implemented by Italy to fight the re-emergence of a LPAI H7N1 virus in 2000 (Capua et al., 2003). These measures included emergency and prophylactic vaccination (Busani et al., 2007).
In Vietnam, only HPAI H5N1 is targeted for surveillance and control. Infection by LPAI viruses are not specifically monitored nor controlled.

Overview of the surveillance system in Vietnam
In 2006, a review of the existing surveillance systems for animal disease and a rapid evaluation of the surveillance for the HPAI H5N1 disease was produced within the framework of a FAO project in 4 pilot provinces (Desvaux, 2006). Table II summarises some of the findings of the review. The table lists all the actors involved in the surveillance of the animal diseases in Vietnam and the different constraints related to the rapid detection and the smooth report of disease suspect cases. Those actors are, from the grassroot level to the national level: the paraveterinarians (persons who usually received a training in animal heath and is able to provide basic care to husbandry animals); the commune veterinarians who are very often paravets receiving some responsibility from the commune administrative level and are a relay for the district veterinary services; the district veterinary services, the first link in the public veterinary services organisation; the provincial veterinary services and the national veterinary services organised within the Department of Animal Health (DAH).

The evaluation of the surveillance system in place for HPAI revealed that, in 2006, there were only few or no HPAI suspect cases for a long period in the pilot provinces. This was a clear indication that the surveillance system was not efficient since poultry mortalities due to acute diseases generally occur every year. Either the suspect cases were not detected which demonstrated a lack of sensitivity of the surveillance system or they were not reported which may indicate a problem in the reporting methodology and data management or, a politicization of the information related to AI in some areas where stakeholders may face political constraints to report to their technical hierarchy.
Different initiatives were undertaken to improve the surveillance of HPAI H5N1, especially in a context of vaccination where the case-definition had to be adapted compared to before the vaccination was implemented. Among those initiatives, a community-based surveillance pilot programme has been tested in 4 pilot provinces (Desvaux et al., 2009a) before being extended to a larger area. The community-based surveillance component aimed at improving the detection of HPAI suspect cases on poultry and strengthening the relation between the official veterinary services and the key informants of selected communes. The Community active disease surveillance was targeting the backyard sector (with lower vaccination coverage) and was based on clinical surveillance. The poultry health status was assessed every month in selected communes through semi-structured interviews with key informants (drug and feed sellers, heads of villages or paraveterinarians and human health workers) and by direct observations (between 5 to 10 families per village) by a team of two persons (head of paraveterinarians with a district veterinarian). The number of communes per district was determined according to the feasibility (human resources constraints) and the geography of the district. Criteria were used for selection of communes (previous occurrence of outbreak, poultry density, presence of main roads, presence of big live birds markets, low vaccination coverage for backyard sector, presence of wetlands known to host wild birds, no other active surveillance activities implemented, smugglings activities known to happen) (Desvaux et al., 2009a).

**Control options in place in Vietnam**

Culling of known infected flocks remains a core element of control programmes (Ministry of Agriculture and Rural Development and Ministry of Health, 2010). Before the end of 2004, preventive culling within a certain radius (about 3 km) around the infected farm was also used at different extent according to the provinces, but it is not a recommended option any more. Following an outbreak, movement controls are also imposed for 21 days and trade at
live poultry markets within 5 km of the site of an outbreak is suspended (Ministry of Agriculture and Rural Development and Ministry of Health, 2010).

A compensation scheme has also been adopted quite soon after the incursion of the disease but its implementation suffered from logistical constraints during the first waves of disease. The level of compensation and its implementation has been adapted, but the level paid does not cover the full cost of destroyed poultry (FAO, 2011).

Since the end of 2005, vaccination was used as a control measure when it became evident that existing measures were not preventing human cases (FAO, 2011). Vaccination was organized following 2 main campaigns per year spaced 6 months apart. The first round of vaccination started in October-November 2005 and since that date, is organised every year at the same period. This round of vaccination is programmed in order to achieve the peak population immunity by December, before the cool season and the New Year celebration (Têt celebration). A second round of vaccination is organized every year in the spring (April-May) with the goal to boost the immunity of longer-lived poultry or to prime any new long-lived poultry entered in the production system since the last campaign (Sims and Do Huu, 2009). Each vaccination campaign is organised within a 1 to 3 months period depending on the provinces. It is estimated that it costs at least USD 10 million per round (Ministry of Agriculture and Rural Development and Ministry of Health, 2010). Few vaccines were initially authorised for use on domestic poultry by the veterinary authorities: Re-1 vaccine, produced by reverse genetic from Harbin Vet Institute in China (H5N1), Nobilis Influenza inactivated vaccine from Intervet (H5N2), Gallimune flu inactivated vaccine from Merial (H5N9) (Peyre et al., 2009). A live fowl pox vector vaccine was also introduced to hatcheries for broiler chickens vaccination in late 2005 but its use was suspended because of some uncertainty about the extent of protection afforded by this vaccine in Vietnam (Sims and Do Huu, 2009). The H5N9 vaccine was dedicated to Muscovy ducks during one of vaccination campaign in 2008, but its use was also suspended. The H5N2 vaccine was used by some
large commercial farmers who preferred to use this vaccine because they received technical support from the European manufacturer (Sims and Do Huu, 2009). Currently, only chickens and ducks are vaccinated with Re-1 vaccine from Harbin Institute (Qiao et al., 2006).

The annual cost dedicated to the control and prevention of HPAI H5N1 in Vietnam and afforded by the government alone still runs into the tens of millions of dollars (FAO, 2011). It was reported that the resources devoted to this disease have at times been provided at the expense of the control and prevention of other important animal diseases such as foot-and-mouth-disease, rabies and porcine reproductive and respiratory syndrome. This has demonstrated the chronic under-resourcing of veterinary services in Viet Nam (FAO, 2011).

Vaccination was suspended after the second campaign in 2010 because the Re-1 vaccine was found not protective enough against the new predominant circulating strains (clade 2.3.2).

The post-vaccination surveillance programme
Post-vaccination surveillance focused on the monitoring of antibody response in selected vaccinated flocks and on the detection of virus in unvaccinated poultry, smuggled poultry and poultry in markets (Sims and Do Huu, 2009). Detailed sampling protocol is issued annually by the Department of Animal Health (DAH) (Ministry of Agriculture and Rural Development and Ministry of Health, 2010). Sampling for virus detection in unvaccinated farms was mainly implemented on ducks and Muscovy ducks flocks in 19 provinces identified as high risk provinces, plus some districts of other provinces (Taylor and Dung, 2007).
<table>
<thead>
<tr>
<th>Administrative level</th>
<th>Technical person who received the sanitary information and who can/ has to transmit it</th>
<th>Identified weaknesses / constraints for information transmission</th>
<th>Solutions already tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village</td>
<td>Head of villages</td>
<td>Not aware i) to whom they have to report ii) in which cases they have to report</td>
<td>● Creation of professional Association</td>
</tr>
<tr>
<td></td>
<td>Paravets</td>
<td>● The poultry sector does not represent a big part of their activities, so they do not collect too much information on this sector Reasons = - not competent on poultry diseases - the farmers does not spend money for poultry treatment - the farmers are able to treat themselves ⚫ No link with the Commune Veterinary Board or with the head of paravets ● Not aware i) to whom they have to report ii) in which cases they have to report and not to treat (pb of case-definition, not aware of their duties regarding the regulated animal diseases) ● Has to financially afford the report by phone ● no incentive to report in comparison with the commercial risk to report ⚫ No link with official services ⚫ Not aware of their duties ⚫ Reporting is against their commercial interest</td>
<td>● Nomination of a head of paravet Awareness campaign ● Hotline (Ha Tay, Nam Dinh) with free access number ● Rewarding policy for the first paravet who will report (Nam Dinh)</td>
</tr>
<tr>
<td></td>
<td>Private vets and drug sellers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commune</td>
<td>People Committee / Agricultural cooperation office</td>
<td>● No motivation to collect information from paravets because they do not receive allowances</td>
<td>● Monthly allowances provided (Phu Tho = 120 000 vnd, South: from 120 000 to 300 000 vnd))</td>
</tr>
<tr>
<td>Level</td>
<td>Role</td>
<td>Issues</td>
<td>Actions</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Commune</td>
<td>Chief of Commune Veterinary Board</td>
<td>● No link with all the paravets of the commune</td>
<td>● Planned visits to communal head of paravets (Nam Dinh)</td>
</tr>
</tbody>
</table>
|        | Local authorities     | ● No clear understanding of the need to report to DVS and to control the outbreak  
                                ● No willing to take responsibility for economical losses in the commune | ● Budget for field travel expenses identified in the annual DVS budget  
                                ● Extra allowances provided according to the activities  
                                ● Contact the head of paravets or the chief by phone |
| District | District Veterinary staff | ● Do not go on the communes or villages for collecting data from head of paravets  
                                ● Budget constraints for DVS staff to organise data collection at commune level  
                                ● The link between DVS staff and CVB is not always strong or formalised | |

Table 2.2. Review of existing surveillance models in Vietnam (Desvaux, 2006)
China is part of our conceptual model and is suspected to be involved into the epidemiology of the HPAI H5N1 disease in Vietnam by its role as a source of new viruses.

To better understand this compartment and the interaction it has with the whole system, a descriptive study has been conducted to study the illegal poultry trade from China.

This chapter has not been submitted yet for publication to a peer review journal.
Figure 3.1. Extract from a Vietnamese Newspaper from 26th-28th January 2007 entitled “The difficult fight against illegal trading”, showing pedestrian transporters of poultry illegally imported from China at Lang Son border.
3.1. INTRODUCTION

All the 6 HA clades of the HPAI H5N1 viruses identified in Vietnam have precursor viruses isolated previously in mainland China and Hong Kong SAR which confirms regular introductions of viruses into Vietnam from those regions (Wan et al., 2008). The detection of clade 7 viruses from chickens seized at the border with China (Davis et al., 2010) is a further evidence supporting the fact that illegal trade of poultry from China is a major cause of regular introductions in Vietnam of new viruses emerging in China.

There have been regular communications about these illegal imports of live poultry from China, especially from the border at Lang Son province (see Figure 3.3 for localization) either in reports from international agencies (FAO, 2011) or in newspapers (see Figure 3.1, a newspaper’s page describing the difficult fight against illegal trade at Lang Son border giving among others, the example of illegal import of live chickens). Studies about the cross-border trading in Vietnam and other countries of the region were supported by the Food and Agriculture Organisation (FAO), but the report made for Vietnam is not public. Nevertheless, some information from this report was made available during a conference presentation where emphasis was put on the economic aspects of this trade, showing how much the trading of spent hens was lucrative for traders at the border (de Haan et al., 2011).

Thus, the risk of introduction of H5N1 viruses via the illegal poultry trade from China does exist but information related to the extent of this trade, its organization in space and time as well as the poultry types involved, are lacking. Without that information, it is difficult to assess the role of this trade into the epidemiology of the HPAI H5N1 disease in Vietnam. The present study aims at answering to the following questions:

- Is the risk of introduction of the HPAI H5N1 virus from China over one year period is negligible, low or high?
What are the more critical period(s) and product(s) related to this introduction from virus from China?

The specific objectives of this qualitative and short study were:

- to identify the type of poultry imported and the actors involved in this trade,
- to identify a possible seasonal pattern for these imports,
- to understand the drivers underlying this trade.

3.2. MATERIALS AND METHODS

3.2.1. QUESTIONNAIRE AND STUDY SITE

Two questionnaires were developed and administered (see Annex 5). One was dedicated to traders and the other one to farmers. The questions were related to the total amount of poultry bought by each informant, the monthly repartition of the Chinese poultry trading, the trading connections of the informants as well as general information about the trading practices (number of journey to China per year, duration of the transport), reasons for buying Chinese poultry and possible at-risk behaviour (disinfection of the cages, storage of the birds before selling).

The area of interest was the border between Vietnam and China at the Lang Son province (see Figure 3.3).
3.2.2. IDENTIFICATION OF KEY INFORMANTS AND SELECTION OF TRADERS AND FARMERS

Before selecting the persons to interview, the Vietnamese researcher, responsible for the field data collection, had to collect secondary information about known trade activities. He then had to identify key informants that would be aware of this trade. In each province, one or two persons were contacted thanks to personal relationships. Most of those initial key-informants were persons in charge of animal movements at the local or provincial veterinary services of the provinces located in the Red River Delta and North West administrative regions. Those persons were generally aware of the illegal trade in their area, but had very often limited power to control it. In some of the targeted provinces, those contact-persons were able to provide a list of traders' contacts. The researcher contacted those traders to collect general information and to identify key informants to be interviewed. One of the objectives in this selection was to get good representation in our sample of the different types of traders involved in this trade. We had the objective to personally interview 10 traders.

From the results of the traders' interviews, farmers raising poultry from China were identified and 10 were selected for face-to-face interview.

3.2.3. STUDY POPULATION

Following the selection procedure described above, a list of around 50 traders was built. Some traders were clearly identified and could be contacted by phone for a first general and informal interview. Information about traders importing poultry from China was obtained from Bac Giang, Bac Ninh, Hai Phong and Ha Tay provinces. No information could be gathered from Lang Son province. Then a selection, including both direct importers and provincial traders, was done according to the initial information collected by phone. Three or four initially selected traders refused to be interviewed and had to be replaced.
Face-to-face interviews were then organized in the province of origin of the 10 selected traders (6 in Bac Giang, 2 in Bac Ninh, 1 in Ha Tay and 1 in Hai Phong provinces). Following interviews with traders, 10 farmers were identified and interviewed in 4 different provinces (3 in Bac Giang, 1 in Ha Tay, 5 in Hung Yen and 1 in Thai Nguyen).

### 3.2.4. RISK ASSESSMENT FRAMEWORK

The risk assessment is the component of the analysis which estimates the risks associated with a hazard. Risk assessment should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases (OIE, 2010). Once the hazard has been described, the commonly accepted framework for risk assessment related to the introduction of disease is derived from the OIE Terrestrial Animal Health Code and involves four steps:

- the release assessment,
- the exposure assessment,
- the consequences estimation,
- the risk estimation.

The hazard identification consists in identifying the pathogenic agents which could potentially produce adverse consequences associated with the importation of a commodity. Risk assessment may be qualitative or quantitative. If qualitative, the risk estimation will end with a qualitative estimation of the risk being studied usually into four categories: negligible, low, medium and high.

This framework will be applied to our study. Due to the difficulty to collect data about illegal trade, only a preliminary qualitative assessment was targeted.
3.3. RESULTS AND DISCUSSION

3.3.1. IDENTIFICATION OF THE ACTORS INVOLVED IN THIS ILLEGAL TRADE

We identified three main types of traders involved in this illegal trade of Chinese poultry:

- Importing traders: traders importing directly poultry from China (4 were interviewed);

- Big provincial traders: traders buying poultry from the importing traders and selling mainly to other traders in different provinces (1 interviewed);

- Small traders: traders buying mainly from big provincial traders (5 interviewed).

Only two of the traders were trading only poultry from China, the others were also trading Vietnamese poultry.

From the informal interviews with the contact-persons from the local veterinary services and with the traders, we learnt that:

- Only a few importing traders were in each province. We estimated around 10 in each of the 4 provinces studied.

- The importing traders are persons with strong local political support.

- After the importing traders agreed in China with an exporting trader, they rely on transporters on foot or on motorbike to cross the borders (see Figure 3.1). All birds are then gathered on a place before being sent by truck or motorbike to their final destination (mainly to other traders who will then distribute them to farmers, markets or restaurants). At this stage, transporters can be stopped by the police and the commodities can be seized (see Figure 3.1 for illustration) but most of the birds finally enter in Vietnam without any official control.
- Birds can still be confiscated on the way to their final destination, but very often, drivers hired by the importing traders succeed in avoiding problems by paying bribes to the officials at the check points or at the market places.
- Importing traders pay for the birds only after checking their sanitary conditions by visual inspection once in Vietnam.
- The number of small provincial traders involved in this illegal trade is decreasing because of control strengthening by local veterinary services. This control is directed more against these small traders than against the big ones.

### 3.3.2. POULTRY IMPORTED

#### Poultry category and estimated volume imported

According to our interviews, the main poultry categories imported, in volume are:

- the spent hens,
- the Day-Old-Chicks (DOC),
- the ducklings.

In order to present an indication of the quantity of birds being imported from China, the annual quantity imported by the 4 importing traders interviewed are presented in the Table II. It is difficult to have a global estimation of the quantity imported by all illegal traders – and this was clearly not an objective of our study - and only non referenced sources can be quoted. From one of those sources, we learnt that at the border of Lang Son province, it is estimated that around 10 to 15 tons of spent hens are imported per day, 30 to 40 tons of DOCs and 30 to 40 tons of ducklings. At Quang Ninh province, the estimations are around 50 tons of spent hens per day and around 10 tons of ducklings per day. According to the data we have directly collected, those figures seems plausible or even under estimated (1.6 kg per bird multiplied 23 million birds imported by one of the trader interviewed, divided by 365 days = around 100 tons per day for the biggest of the importing trader interviewed).
Table 3.1. Repartition of the poultry imported by species and production type for the 4 interviewed importing traders

<table>
<thead>
<tr>
<th>Bird category</th>
<th>Spent hens</th>
<th>DOC</th>
<th>Duckling</th>
<th>Day Old goose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no birds imported</td>
<td>23,016,000</td>
<td>1,744,000</td>
<td>1,024,000</td>
<td>42,000</td>
<td>25,832,000</td>
</tr>
<tr>
<td>(number of importing traders</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
<td>(1)</td>
<td>(4)</td>
</tr>
<tr>
<td>involved)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of transports</td>
<td>25 (60)</td>
<td>17 (60)</td>
<td>22 (60)</td>
<td>8 (10)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>per year and per trader(max)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identified driving forces for illegal poultry trade

Different reasons for importing poultry from China have been identified. For the spent hens, the financial motivation is probably very strong for the importing traders. Indeed, de Haan et al (2011) reported that spent hens are bought in China at around 15,000 – 17,000 Vietnamese dong (VND) per kg. Those birds are then sold as meat chickens and sometimes even sold as local Vietnamese chickens at high prices as reported by traders interviewed (up to 80-90,000 VND per kg). Indeed, local Vietnamese chickens are preferred by consumers to industrial chicken broilers and the consumers can hardly make the difference between the spent hens and the local chickens. Despite expenses along the commodity chain (transport, bribes, storage) also described by de Haan et al (2011), the benefit must remain significant. The import of spent hens for meat consumption is also motivated by the high demand at certain periods of the years (see following section on seasonality).

The import of DOCs and ducklings from China is probably more related to a specific demand of Vietnamese poultry farmers for good genetic. Indeed, Chinese products have the reputation to present higher technical performances than Vietnamese DOCs and ducklings, probably because the private Vietnamese hatcheries do not provide good sanitary and genetic guaranties. It was also reported by Phan et al (2010) that Vietnamese supply was not enough for the local demand. Thus, some of the farmers interviewed
reported that laying hens from China produce more and bigger eggs and the resulting spent hens are heavier.

3.3.3. SEASONALITY OF THE CHINESE POULTRY TRADING

The import of Chinese poultry is organized all year long but there is some variation of the quantity imported according to the seasons. According to the traders interviewed, this seasonal variation is constant across the years. The mean seasonal repartition (in percentage) of the three main categories of Chinese imported poultry is presented in Figure 3.2. The seasonal variations can easily be explained by the demand of the Vietnamese poultry farmers and consumers.

The import of DOCs is more important during the period between August and November. Thus, the birds imported at this period will be ready for sell around the Tết celebration (Vietnamese New Year) in order to satisfy the high demand of the Vietnamese consumers at that period during which chicken is a popular meal.

The peak in the import of spent hens is also connected to the Tết celebration. Nevertheless, this percentage has been calculated for only 2 traders, and this peak was only significant for one of them. The other one, importing more than 20 millions of birds per year, presented a constant repartition of his import all over the year with only a slack period during the hot season from May to August. The seasonal tendency thus needs to be validated even if the intensification of illegal import of chickens at the end of the year was also described by other sources (as in the newspaper article reproduced in Figure 3.1).

The import of ducklings is more important around March and afterwards until July (Figure 3.2). From February to May-June, this is the period of the first annual rice crop in Northern Vietnam and from June to September-October, the second rice crop period. It is well known that duck production is connected to the rice production with the ducks being sent to the rice fields just after rice transplantation for pest control or just after the harvest for
scavenging the weeds, crop residues, snails and fresh water crustaceans (Desvaux and Dinh, 2008). Thus, the import of duckling is connected to the rice production calendar. In Northern Vietnam, there are not more than 2 rice crops per year and the first rice crop is the most important one since not all places can afford a second rice crop (see chapter 5 of this document).

![Figure 3.2. Annual repartition of the Chinese poultry purchase for the interviewed traders](image)

### 3.3.4. SPATIAL SPREADING AND RISK MANAGEMENT BY TRADERS AND FARMERS

From the interviews, it appears that traders were illegally importing poultry from China not only at the border at Lang Son province, but also at the province of Quang Ninh (Figure 3.3). Those two provinces are sharing their borders with Guangxi and Guangdong provinces in China. From the questions related to the places where the importing traders were selling the Chinese poultry, a map showing the spatial dispersion of those birds could be drawn (Figure 3.3). It shows that from only two main ports of entry, the Chinese

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poultry are then sent in most of the provinces of Northern Vietnam. Thus, the poultry, imported without any sanitary control and guarantee, can travel for quite long distances.

Birds are either transported by motorbike (from 500 to 700 adults or 2000 DOCs or ducklings per motorbike) or by truck (from 4000 to 6000 adults). According to the survey, birds are transported in average during 4 to 5 hours (min: 1 to 2 hours, max: 7 to 8 hours) by the traders.

Only 3 traders out of 10 declared proceeding to the disinfection of the material used for the poultry transports (cages and vehicle), the other ones were only using water for cleaning.

Seven out 10 traders declared that in normal situation, they needed to store the birds some hours or some days before selling (mean: 7 hours, min: 1 hour, max: 2 days). The birds are stored either in their house or farm or at the selling place (market) where they can be in contact with other poultry (in 6 cases out of 7). Six also declared they sometimes have unsold animals they bring back to their house for 1 to 3 days the time to sell them at a cheaper price to the local markets or to farmers in their village.

Farmers’ behaviour in case of mortality in their farm varies according to the situation and the type of poultry involved: farmers either organize a proper disposal of the dead birds (by burying or burning), use the dead birds, especially the young ones for feeding other animals (dogs, pigs, or fish, after cooking) or attempt to sell rapidly the remaining healthy birds if they are older. When disease is reported in the neighbourhood, farmers also reported some actions to protect their birds like disinfection of the farms, limitation of the visits into the house and farm or preventive treatment on the birds by antibiotics and “tonics”.

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Figure 3.3. Spatial distribution of the live poultry imported from China
3.3.6. QUALITATIVE RISK ASSESSMENT

The hazard under consideration in this study is the HPAI H5N1 virus circulating in China.

Release assessment

Considering that:

- China only declared around 100 outbreaks of HPAI H5N1 disease between 2003 and 2009 but, it is recognised that, not all cases are detected or reported and the disease is considered to be endemic in the country despite the mass vaccination program implemented (FAO, 2011; Martin et al., 2011).

- Illegal import of live poultry from China is a significant trade, with the quantity of poultry imported without any sanitary control being estimated to be of several thousand per day.

- Veterinary controls at the borders have limited impact on this trade.

- Importing traders are checking the sanitary conditions of the birds before paying to the exporting trader but:
  - some birds may be infected without clinical signs.
    - Considering that the protection induced by the vaccination with an inactivated vaccine does not last for more than 3 to 4 months under field conditions (see chapters 7 and 8), the spent hens imported may have received a vaccination against H5N1 in China during their production cycle, and thus they can still be protected against clinical expression of the disease but not against infection.
    - Young birds imported for breeding should normally be more susceptible to the disease and should express clinical signs if infected, but ducklings, as a reservoir species for avian influenza virus, may present some resistance to certain HPAI H5N1 strains (see chapter 2).
the birds have already entered into Vietnam once this visual inspection is being done and we have no clear details about the management of dead or sick birds by the exporting traders (proper destruction, disposal in the rivers, canals etc... or selling for consumption).

We conclude that the risk of release of HPAI H5N1 virus from China via the illegal trade of poultry is medium.

Exposure assessment

Considering that:

- The birds imported illegally from China are sent all over Northern Vietnam.
- Several middlemen are involved before the birds reach their final destination.
- Travel with each trader may last several hours.
- Spent hens are normally intended for consumption and thus are an epidemiological dead end, but:
  - the birds can be stored from 1 to several days at a trader’s house,
  - during the storage at a trader’s house, during transport or at the market places, the spent hens can be in contact with live poultry intended for breeding, from China or from Vietnam.
  - There is no proper disinfection of the cages or of the vehicles used for birds’ transport, and provincial traders visit several farms or markets.
  - Most of the traders involved in the trade of illegal poultry from China also trade poultry from Vietnam.
    - DOCs and ducklings are intended for breeding and they seem to be imported separately from the spent hens but ducklings may be a reservoir of virus able to contaminate the environment or the in-contact susceptible poultry.
Fomites or water contaminated by infectious faeces can be a source of indirect transmission of the virus for a quite extended period of time, depending on the environmental conditions (Brown et al., 2007; Stallknecht and Brown, 2009).

We conclude that the risk of direct or indirect exposure of the Vietnamese poultry population to the HPAI H5N1 virus released by one or several infected poultry illegally imported from China is high.

Consequence assessment
Considering that:

- Vietnamese poultry population is partially immunized because of the vaccination program in place.
- Farmers do implement preventive measures in their farms in case of mortalities reported in the neighbourhood, but they may also try to sell their flocks if they experience mortalities and thus can pose a risk of disease spreading along the commodity chain.
- Traders and farmers may not be fully aware of the risk they take by handling birds of unknown origin.

We conclude that the consequence for the Vietnamese poultry population is moderate but high for the human population.
3.4. CONCLUSIONS

The illegal trade of poultry from China unquestionably contributes to the epidemiology of the H5N1 HPAI disease in Vietnam by regular introductions of new avian influenza strains into the domestic Vietnamese poultry population.

The study conducted here enabled a better understanding of this trade and provided some clues to limit the risk of virus introduction.

First of all, it has to be acknowledged that, apart from financial motivation for the importing traders, there is also i) a farmers’ demand motivated by technical aspects and ii) consumers’ demand, especially at certain periods of the year such as religious holidays, that may not be satisfied by the national supply. Thus, efforts to stop this trade without tackling those issues will be in vain. Solutions have to be looked for in the improvement of DOC and duckling supplies and in licensing the import of spent hens from China for consumption, if birds are controlled and sent to the slaughter directly.

Secondly, traders being an obvious source of virus dissemination, efforts should be made to better control and supervise their working conditions. As an example, within a very short period of time the car taxi service in the big cities in Vietnam succeeded in organizing itself and in providing good service to consumers within a quite harmonized way. In the same way the poultry traders’ profession needs to be recognized and better organized. It would not be enough to only impose licensing on them, with corresponding sanitary requirements, as this alone might even contribute to a deterioration of their working conditions. Indeed, there is a risk that licenses will be distributed for money without guarantee that sanitary requirements are satisfied. Thus, a comprehensive solution should be sought in collaboration with the private sector and with the support of international donors contributing to the global effort to control and prevent the animal epidemics. Solutions such as installing cleaning and disinfection points in all main live
poultry markets, with standard approved cages to be used by all traders, might be a reasonable goal to achieve in the medium term.
This chapter aimed at investigating the local factors of occurrence of H5N1 HPAI outbreaks.

The case-control study conducted in one province in Northern Vietnam was published in Transboundary Emerging Diseases journal under the title "Risk factors of Highly Pathogenic Avian Influenza H5N1 occurrence at the village and farm levels in the Red River Delta region in Vietnam" (paper 1) (Desvaux et al., 2011).
Risk factors of Highly Pathogenic Avian Influenza H5N1 occurrence at the village and farm levels in the Red River Delta region in Vietnam

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Worked carried out in Vietnam
Abstract
A case-control study at both village and farm levels was designed to investigate risk factors for Highly Pathogenic Avian Influenza H5N1 during the 2007 outbreaks in one province of Northern Vietnam. Data related to human and natural environments, and poultry production systems was collected for 19 case and 38 unmatched control villages and 19 pairs of matched farms. Our results confirmed the role of poultry movements and trading activities. In particular, our models found that higher number of broiler flocks in the village increased the risk (OR = 1.49, 95% CI: 1.12-1.96), as well as the village having at least one poultry trader (OR =11.53, 95% CI: 1.34-98.86). To a lesser extent, in one of our 2 models, we also identified that increased density of ponds and streams, commonly used for waterfowl production, and greater number of duck flocks in the village also increased the risk. The higher percentage of households keeping poultry, as an indicator of households keeping backyard poultry in our study population, was a protective factor (OR= 0.95, 95% CI: 0.91-0.98). At the farm level, 3 risk factors at the 5% level of type I error were identified by univariate analysis: a greater total number of birds (P=0.006), and increase in the number of flocks having access to water (p=0.027, and a greater number of broiler flocks in the farm (P=0.049). Effect of vaccination implementation (date and doses) was difficult to investigate due to a poor recording system. Some protective or risk factors with limited effect may not have been identified due to our limited sample size. Nevertheless, our results provide a better understanding of local transmission mechanisms of HPAI H5N1 in one province of the Red River Delta region in Vietnam and highlight the need to reduce at-risk trading and production practices.

Key words: HPAI; H5N1; Vietnam; Risk factors
4.1. INTRODUCTION

Vietnam, with a poultry population over 200 million (Desvaux and Dinh, 2008), faced its first outbreaks of Highly Pathogenic Avian Influenza (HPAI) H5N1 at the end of 2003 (OIE, 2008). By the end of 2009, 5 epidemic waves had occurred in domestic poultry; with the latest waves being limited to the North or the South regions whereas the first waves had a national distribution (Minh et al., 2009). To limit the number of outbreaks and the risk of transmission to humans, the Government of Vietnam decided to use a mass vaccination strategy at the end of 2005. After a period of about a year without an outbreak, Northern Vietnam faced a significant epidemic in 2007 with 88 communes (administrative level made of several villages) affected in the Red River Delta administrative region (Minh et al., 2009). So far, most of the studies investigating the role of potential risk factors on the occurrence of HPAI outbreaks in Vietnam have been implemented at the commune level using aggregated data from general databases for risk factor quantification (Gilbert et al., 2008; Henning et al., 2009a; Pfeiffer.D.U et al., 2007). In Pfeiffer’s study of the 3 first waves (Pfeiffer.D.U et al., 2007) increased risk was associated with decreased distance from higher density human populated areas, increased land area used for rice, increased density of domestic water birds and increased density of chickens. In the same study, significant interaction terms related to the periods and the regions were also associated with the risk of HPAI emphasizing the importance of spatio-temporal variation in the disease pattern. Gilbert demonstrated that the relative importance of duck and rice crop intensity, compared to human density, on the risk of HPAI was variable according to the waves (Gilbert et al, 2008). Human-related transmission (as illustrated by human density being the predominant risk factor) played an important role in the first wave, whereas rice cropping intensity was the predominant risk factor in the second wave. For the third wave, duck and rice cropping intensity became less strong predictors probably due to control measures targeting duck populations during that period. Those studies provided a general understanding of the main mechanisms involved in the epidemiology of HPAI in this
region and their possible evolution over the different waves: in particular the role of human activities in the transmission process and the role of environment (mainly rice-related areas) as an indicator of the presence of duck populations or as a component of the transmission and maintenance processes. Previously, only one published case-control study has been carried out in Vietnam, at the farm level, following outbreaks in the South in 2006 (Henning et al., 2009b). There have been no studies investigating village-level indicators for HPAI infection. In order to define more detailed risk factors at a smaller scale (village and farm), this case-control study was carried out in one province in Northern Vietnam, Bac Giang, located 50 Km northeast of the capital Hanoi (Fig 1). Bac Giang had a poultry population estimated around 10 millions in 2007 (GSO, 2010) of which around 1 million were ducks. The province presents 3 distinct agro-ecological areas with one of them consisting of lowland, typical of the rest of the Red River Delta area in terms of agricultural practices and poultry density (Xiao, 2006; Desvaux and Dinh 2008). We focused our study in this lowland area since it is in this type of agro-ecological area that outbreaks in northern Vietnam were mainly concentrated (Pfeiffer.D.U et al., 2007; Minh et al, 2009). The objective of the study was to evaluate the risk factors related to the human and natural environments and the poultry production systems on the introduction; transmission or maintenance of the HPAI virus during the 2007 epidemic wave in Northern Vietnam, at both village and farm levels.
4.2. MATERIALS AND METHODS

4.2.1. STUDY DESIGN OVERVIEW

Two epidemiological units of interest were considered in this study: the village and the farm. Risk factors were investigated using a non matched case-control study for the villages and a matched case-control study, based on farm production type and location, for farms. Questionnaires were designed and administered between April and May 2008 and were related to outbreaks occurring in 2007. The epidemic wave period was defined as a window between February 2007 and August 2007 (DAH, 2008).

4.2.2. DATA SOURCE AND CASE AND CONTROL SELECTION

The initial data source used was provided by the Sub Department of Animal Health of Bac Giang province where the study was based. The data included information on 2005 and 2007 H5N1 outbreaks aggregated at the village level and included both villages with disease outbreaks and villages where only preventive culling had been performed. There was no precise indication of the number of farms infected or culled in the villages. In addition, some outbreaks were based on reported mortalities only whereas others also had laboratory confirmation of H5N1 infection. Laboratory confirmation was performed either by the Veterinary Regional Laboratory or the National Centre for Veterinary Diagnosis. Given these parameters, a village case was therefore initially defined as a village having reported H5N1 mortality and/or a village with laboratory confirmation reported.

Case and control selection at village level

In order to further refine the list of village cases, the list of infected village obtained was checked by field visits and discussion with local veterinary authorities (district and commune veterinarians) before the study commenced. When local veterinary authorities agreed on the HPAI status of a particular village, it was confirmed as a case. Where a discrepancy was found between our list and their reports, details were requested on the
mortality event in the village farms involved. A case-definition was then applied on the description of symptoms provided by the local veterinarians and the village was defined as a case if the following criteria were met in at least one farm in the village:

- per acute or acute disease (time from observed symptoms to mortality less than 2 days)
- mortality over 10% within 1 day
- neurological signs in ducks if ducks were involved in the outbreak (head tilt, uncoordinated movements)
- a positive result for a rapid diagnostic H5N1 test on sick birds if such a test had been applied (usually not reported on our initial list).

At the end of the field interviews and before analysis, a final check of the case villages included was carried out based on the answers to the village questionnaires. This enabled case villages where mortalities had occurred outside the epidemic wave period to be removed from the study.

The villages from communes with outbreaks in 2005 or 2007 were also excluded to take into account pre-emptive culling sometimes organized at a large scale. Control villages were randomly selected from the remaining villages in the study area. Two controls were selected for each case. The selection of control was stratified at the district level for administrative reason and to balance the number of case and control per district. A last check on the selection of controls was performed based on the answers to the questionnaire. Control villages reporting unusual poultry mortality in 2007 (anytime in 2007) were excluded from the analysis.

Case and control selection at farm level
The case farms were the first farms that had an outbreak in each of the case village. This was designed to investigate risk factors of introduction. If this farm was not available, the nearest farm (geographically) to be infected in 2007 was selected.
The matched control farms were selected among farms that never experienced an HPAI outbreak in the same village as the case farm (matched by location) and were also matched by species and by production type (broiler, layer or breeder).

Figure 1. Bac Giang province land cover map derived from composite SPOT image supervised classification
4.2.3. DATA COLLECTION

Questionnaires

Two questionnaires were developed, for the village and the farm levels. The village questionnaire, targeted at the head of the village, included general information about the village (number of households, presence of a live bird market within or near the village, presence of wild birds), the list of poultry farms in the village in 2007, the origin of day-old-chicks (DOC) in 2007, the vaccination practices, the description of mortality events that had occurred in previous years and a description of the HPAI outbreak for the village case (timeline, reporting, control measures). Where mortality events had occurred in previous years, we asked for estimates of the percentage of households involved and the date of this mortality event. The latter information was used to confirm the case or control status of the villages by eliminating cases with mortalities outside the defined epidemic period and controls with reported poultry mortality in 2007 (any report of poultry mortality by the head of the village was considered as an unusual event since only significant mortality event are generally noticed by local authority).

At the farm level, the questionnaire was targeted at the farmer or his/her family. The questions included information on the composition of the farm poultry population in 2007, trading practices (to whom they were selling and buying their birds), vaccination practices, and housing systems and for the cases, a description of the HPAI outbreak event. General opinions of the farmers were also collected regarding thoughts on why the farm had or did not have an HPAI outbreak.

Environmental and infrastructure data

As no Geographic Information System (GIS) map layers were available for the village administrative level, the density of variables possibly related to the transmission of virus (transport network, running water) or the persistence of virus (presence of rice fields and non running water) was calculated for a 500 m radius buffer zone from each village centre.
using GIS software (ESRI ArcGIS™, Spatial Analyst, Zonal statistics as table function). GIS layers including transport networks, hydrographic networks, lakes and ponds were bought from the National Cartography House in Hanoi. The density of transport feature (national roads and all roads) and animal production-related water features (canals, ponds and streams) were calculated within each buffer zone by dividing the number of pixels occupied by a specific feature by the total number of pixels in the buffer. The size of a pixel was defined as 20 x 20 meters. A land cover map derived from a composite SPOT (Satellite Pour l’Observation de la Terre) image supervised classification (Fig 1) was produced, validated by field visits and used to characterize the landscape of our study area (Tollis, 2009). The density of 5 different land cover types (water, rice, forest and fruit-tree, upland culture and residential areas) was calculated within each buffer.

4.2.4. DATA ANALYSIS

Univariate analyses

Statistical analyses were conducted using Stata 10 (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP) and R 2.11.1 softwares. The association between the outcomes (being a case or a control) and each explanatory variable was assessed using exact logistic regression (Hosmer and Lemeshow, 2000) (with the exlogistic command in Stata). A matched procedure was undertaken for the matched case-control study at the farm level. P-values for each variable were estimated using the Wald test (Hosmer and Lemeshow, 2000). Variables having a p-value ≤ 0.1 were candidates for inclusion in the multivariable model. All continuous variables were tested for linearity assumption by comparing two models with the Likelihood Ratio test: a model using a categorical transformation and a model with the same transformation but the variable treated as an ordinal variable. Different categories were tested: either a transformation based on quintile (or quartile depending on the distribution) or using equal range of values of the variable.
Multivariate analyses

For the unmatched case-control study at the village level only, an investigation of multivariate models was undertaken. The first step was to build a model including all the explanatory variables selected during the univariate step. We also included into this model one environmental variable with a p-value of less than 0.2. We then checked for collinearity among the variables in this model using -collin command in Stata, checking that tolerance was of more than 0.1 ([UCLA]2010). In order to take into account our small sample size we used a backward stepwise selection method based on the second-order bias correction Akaike Information Criteria comparison (AICc) (Burnham, 2004). Variables were removed sequentially. At each step, the variable which removal resulted in the largest AICc decrease was excluded. Goodness-of-fit of the final multivariate models was assessed using Pearson’s chi square test.
4.3. RESULTS

4.3.1. STUDY POPULATION

After initial field visits for infected village selection and confirmation, we ended up with a total number of 22 villages which had experienced an HPAI outbreak in Bac Giang in 2007. Among those 22 villages, 20 were targeted for interview (the 2 remaining ones belonged to 2 districts from more remote areas not targeted in our study as not representative of the Red River Delta region) and 40 control villages were selected. One village could not be interviewed and after reviewing the mortality criteria, a final total of 18 villages were included in our analysis as cases. The same procedure was followed to check control villages and 6 were omitted because they did not meet the definition for a control (unusual poultry mortalities was reported in 2007). In total, 18 case villages and 32 control villages were included in the final analysis.

Using the established criteria, a total of 18 pairs of matched farms remained for the analysis.

4.3.2. CHARACTERISTICS OF THE STUDY POPULATION

The village study population (18 cases and 32 controls) were located within 6 districts and 32 different communes. On average, the number of households per village was 218 (range 21-600).

The farm study population consisted of 18 pairs of case and control farms totalling 74 flocks, with farms having on average 2.1 flocks (range 1-4, median 2) of mixed poultry types. Duck flocks (N=34) had numbers of birds ranging from 10 to 1050 (mean 351; median 200) with the main breeds being Tau Khoang (N=11) and Super Egg (N=9). Chicken flocks (N=28) ranged from 10 to 2500 birds (mean 363; median 230) with the
main breeds being local (N=26). Muscovy duck flocks (N=12) ranged from 20 to 400 birds (mean 160; median 200) with all flocks derived from the French breed.

**Description of the case farms**

Outbreaks had occurred in the farms between 7th April 2007 and 23rd June 2007. Among the 18 case farms, clinical signs and mortality were reported from 63% of the flocks (24/38). At the farm level between 25 and 100% of the flocks were showing clinical signs and mortality. On average, 45% of the birds in the infected flocks died before the remaining ones were culled (n=24, range 5-100). The description of infected flocks by species, production type and age is given in Table I. The average age of infected birds was 66 days (range 20-120 days, median 60). Fourteen case farms out of 18 were reported to have been vaccinated against HPAI. The disease occurred on average 48 days after vaccination (range 7-92, n=7).

**Table I. Description of the infected flocks in the case farms**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. flocks</th>
<th>No. flocks with clinical signs or mortality</th>
<th>No. broiler flocks with clinical signs or mortality</th>
<th>No. breeder or layer flocks with clinical signs or mortality</th>
<th>Mean age of the affected flock in days (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>15</td>
<td>10</td>
<td>10/13</td>
<td>0/2</td>
<td>78 (30-120)</td>
</tr>
<tr>
<td>Duck¹</td>
<td>16</td>
<td>10</td>
<td>7/9</td>
<td>1/5</td>
<td>53 (20-90)</td>
</tr>
<tr>
<td>Muscovy Duck</td>
<td>7</td>
<td>4</td>
<td>4/7</td>
<td>0/0</td>
<td>71 (45-90)</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>24</td>
<td>21/29</td>
<td>1/7</td>
<td></td>
</tr>
</tbody>
</table>

¹The production type of 2 duck flocks with clinical signs was not recorded because the farmer answered globally for all his duck flocks
Description of the report and culling delay
On average the farmers declared the disease to official veterinarians 2.8 days (range 1-8, n=18) after the onset of the disease. There were on average 8.9 days between the onset of the disease at the farm and the culling of the flock (range 1-31, n=16).

Farmers’ behaviour and thoughts regarding HPAI source
Of 14 farmers who answered the question, 12 tried to cure their birds, 6 buried the dead birds, 4 threw the dead birds into a river, channel or fish pond, 1 ate the dead birds and 1 tried to sell the sick birds. The following possible causes of HPAI in the farm were quoted by the farmers:

- introduction from neighbouring infected farms (3 answers)
- contact with wild birds (2 answers)
- scavenging in rice fields (2 answers)
- contamination of the channel water due to animal burying nearby (1 answer)
- poisonous feed in rice field (1 answer)

Five farmers out of 18 did not believe their farm had HPAI even following veterinary authorities’ confirmation of the diagnosis.

4.3.3. VACCINATION PRACTICES IN THE VILLAGE STUDY POPULATION

Twelve percent (6/50) of the heads of village declared that vaccination was not compulsory, whereas it is; but only one head of village declared that no AI vaccination had been used in the village. In the majority of the villages (94% = 45/48), the small size farms had to take their birds to a vaccination centre. Those farms usually had less than 50 birds (56%=27/48 of the villages) or between 50-100 birds (35%=17/48). One village declared that farms up to 200 birds had to bring birds to the vaccination centre. The vaccination centre was located within each village. In most of the villages (90%) the head of the village declared that there was only one injection of HPAI vaccine per bird per campaign. Heads of
villages also reported that the vaccination coverage was not 100% due to difficulty in catching some birds in the farms and also because certain farmers with small number of birds did not want to vaccinate them.

4.3.4. ANALYSES AT THE VILLAGE-LEVEL

Twenty eight potential risk factors were individually tested using simple exact logistic regression method. Table II presents odds ratio (OR) estimation and their confidence intervals (CI). Then, eight variables with p≤0.1 and the only environmental variable with a p-value less than 0.2 were included in the initial multiple logistic regression model. Hatchery in the village (p-value of less than 0.1) was not included in the model because of the limited number of units in one category, which caused a problem with parameter estimation (Table II). The variable related to the number of flocks of more than 100 birds was of concern regarding collinearity (Tolerance=0.12). We tested the selection without this variable in the full model and came to the same result. Table III provides a summary of the 2 models obtained from the backyards selection based on the AICc. Those 2 models have an AICc that did not differ by more than 2 points and can thus be considered as describing the data with equivalent quality (Burnham, 2004). The lowest AICc model included three main predictors: percentage of households keeping poultry, presence of at least one poultry trader in the village and number of broiler flocks. The second lowest AICc model allowed the identification of risk factors of moderate effect. Indeed, model 2 identified two additional risk factors at the limit of significance: number of duck flocks and the percentage of village area occupied by ponds and small streams. These two final models fitted the data adequately (model 1: Pearson’s chi square = 37.33, df= 34, p value=0.3185; model 2: Pearson’s chi square = 25.66, df=37, p value=0.9198).
Table II. Results of univariate analysis using exact logistic regression for variables potentially associated with HPAI outbreaks at the village level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Case (mean)</th>
<th>Control (mean)</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General information on the village</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. households in the village in 2007 (N=49)</td>
<td></td>
<td>18 (260)</td>
<td>31 (195)</td>
<td>1</td>
<td>1-1.01</td>
<td>0.094</td>
</tr>
<tr>
<td>Percentage household keeping poultry (N=44)</td>
<td></td>
<td>16 (65%)</td>
<td>28 (83%)</td>
<td>0.98</td>
<td>0.96-1.00</td>
<td>0.053</td>
</tr>
<tr>
<td>Wild birds present in rice fields around the village (N=50)</td>
<td>A few</td>
<td>9</td>
<td>23</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A lot</td>
<td>9</td>
<td>9</td>
<td>2.51</td>
<td>0.65-10.03</td>
<td>0.216</td>
</tr>
<tr>
<td>Wild birds present in the village (N=50)</td>
<td>A few</td>
<td>13</td>
<td>23</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A lot</td>
<td>5</td>
<td>9</td>
<td>0.98</td>
<td>0.21-4.16</td>
<td>1</td>
</tr>
<tr>
<td>Live bird market present in the village in 2007 (N=50)</td>
<td>Yes</td>
<td>5/18</td>
<td>3/32</td>
<td>33.6</td>
<td>0.60-26.84</td>
<td>0.197</td>
</tr>
<tr>
<td>Presence of at least one poultry trader in the village in 2007 (N=50)</td>
<td>Yes</td>
<td>10/18</td>
<td>5/32</td>
<td>6.45</td>
<td>1.40-32.08</td>
<td>0.009</td>
</tr>
<tr>
<td>Presence of at least one bird hunter in the village in 2007 (N=49)</td>
<td>Yes</td>
<td>8/17</td>
<td>8/32</td>
<td>2.61</td>
<td>0.64-11.00</td>
<td>0.214</td>
</tr>
<tr>
<td>Presence of at least one hatchery (N=50)</td>
<td>Yes</td>
<td>3/18</td>
<td>0/32</td>
<td>7.55</td>
<td>0.77-inf</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Poultry production in the village in 2007</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. flock (from farms) of more than 100 birds (N=50)</td>
<td></td>
<td>18 (6.6)</td>
<td>32 (4.4)</td>
<td>1.31</td>
<td>1.11-1.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Percentage of farms vaccinated against HPAI (N=43)</td>
<td></td>
<td>14 (74%)</td>
<td>29 (79%)</td>
<td>0.98</td>
<td>0.95-1.02</td>
<td>0.341</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No chicken flocks (from the farms) (N=50)</td>
<td></td>
<td>18 (4)</td>
<td>32 (2.7)</td>
<td>1.18</td>
<td>0.95-1.48</td>
<td>0.141</td>
</tr>
<tr>
<td>No. duck flocks (from the farms) (N=50)</td>
<td></td>
<td>18 (4.3)</td>
<td>32 (2.3)</td>
<td>1.25</td>
<td>1.02-1.58</td>
<td>0.029</td>
</tr>
<tr>
<td>Presence of Muscovy duck flock(s) in the village (N=50)</td>
<td></td>
<td>13/18</td>
<td>8/32</td>
<td>7.43</td>
<td>1.81-35.98</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Production type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. broiler flocks (N=50)</td>
<td></td>
<td>18 (7.1)</td>
<td>32 (3.2)</td>
<td>1.38</td>
<td>1.14-1.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. breeder flocks (N=50)</td>
<td></td>
<td>18 (0.5)</td>
<td>32 (0.3)</td>
<td>1.30</td>
<td>0.56-3.00</td>
<td>0.606</td>
</tr>
<tr>
<td>No. layer flocks (N=50)</td>
<td></td>
<td>18 (2.2)</td>
<td>32 (1.8)</td>
<td>1.06</td>
<td>0.83-1.35</td>
<td>0.662</td>
</tr>
<tr>
<td><strong>Housing system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No enclosed flocks (N=50)</td>
<td></td>
<td>18 (2.2)</td>
<td>32 (3.3)</td>
<td>0.85</td>
<td>0.65-1.07</td>
<td>0.207</td>
</tr>
<tr>
<td>No. fenced flocks (outdoor access) (N=50)</td>
<td></td>
<td>18 (5.8)</td>
<td>32 (1.8)</td>
<td>1.49</td>
<td>1.18-1.98</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Spatial *a*

<table>
<thead>
<tr>
<th>Presence of scavenging flock(s) (N=50)</th>
<th>6/18</th>
<th>4/32</th>
<th>3.4</th>
<th>0.67-19.64</th>
<th>0.165</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of pixels with canals (N=50)</td>
<td>18 (0.8%)</td>
<td>32 (0.6%)</td>
<td>1.16</td>
<td>0.72-1.80</td>
<td>0.559</td>
</tr>
<tr>
<td>Percentage of pixels with ponds and streams (N=50)</td>
<td>18 (1.8%)</td>
<td>32 (1.1%)</td>
<td>1.25</td>
<td>0.91-1.75</td>
<td>0.170</td>
</tr>
<tr>
<td>Percentage of pixels with national roads (N=50)</td>
<td>18 (1.2%)</td>
<td>32 (1.1%)</td>
<td>1.04</td>
<td>0.77-1.38</td>
<td>0.773</td>
</tr>
<tr>
<td>Percentage of pixels with all kind of roads (N=50)</td>
<td>18 (2.4%)</td>
<td>32 (1.9%)</td>
<td>1.07</td>
<td>0.85-1.33</td>
<td>0.571</td>
</tr>
<tr>
<td>Percentage of pixels with water using SPOT (N=50)</td>
<td>18 (6.2%)</td>
<td>32 (5.5%)</td>
<td>1.01</td>
<td>0.95-1.06</td>
<td>0.790</td>
</tr>
<tr>
<td>Percentage of pixels with rice using SPOT (N=50)</td>
<td>18 (54.6%)</td>
<td>32 (59.1%)</td>
<td>0.99</td>
<td>0.96-1.02</td>
<td>0.452</td>
</tr>
<tr>
<td>Percentage of pixels with residential area using SPOT (N=50)</td>
<td>18 (23.6%)</td>
<td>32 (25.5%)</td>
<td>0.99</td>
<td>0.95-1.03</td>
<td>0.671</td>
</tr>
<tr>
<td>Percentage of pixels with forest and fruit trees using SPOT (N=50)</td>
<td>18 (11.5%)</td>
<td>32 (5.7%)</td>
<td>1.02</td>
<td>0.99-1.06</td>
<td>0.228</td>
</tr>
<tr>
<td>Percentage of pixels with upland culture production using SPOT (standardized) (N=50)</td>
<td>18 (4%)</td>
<td>32 (4.2%)</td>
<td>1</td>
<td>0.92-1.07</td>
<td>0.982</td>
</tr>
</tbody>
</table>

*a* variables are expressed for a 500m radius buffer around village centroids
Table III. Result of the final logistic regression models at village level using two selection methods

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Model 1 (AICc =40.14)</th>
<th>Model 2 (AICc =40.61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Percentage household keeping poultry</td>
<td>yes</td>
<td>0.95 (0.91-0.98)</td>
<td>0.006</td>
</tr>
<tr>
<td>Presence of at least one poultry trader in the village</td>
<td></td>
<td>11.53 (1.34-98.86)</td>
<td>0.026</td>
</tr>
<tr>
<td>No. duck flocks (from the farms)</td>
<td></td>
<td>1.39 (0.96-2.01)</td>
<td>0.079</td>
</tr>
<tr>
<td>No. broiler flocks</td>
<td></td>
<td>1.60 (1.14-2.24)</td>
<td>0.007</td>
</tr>
<tr>
<td>Percentage of pixels with ponds and streams</td>
<td></td>
<td>2.35 (0.79-6.98)</td>
<td>0.125</td>
</tr>
</tbody>
</table>
4.3.5. ANALYSIS AT THE FARM-LEVEL

Three factors were significantly influential at the 5% level: the total number of birds in 2007 (p=0.005), number of flocks having access to water (p=0.027), and the number of broiler flocks in the farm in 2007 (p=0.049). Two factors could be considered as significantly influential at the 10% level: the presence of more than one species in the farm (p=0.065) and the total number of flocks in 2007 (p=0.089) (Table IV). No multivariate model was built due to limited sample size.

Table IV. Results of univariate analysis using exact logistic regression for variables potentially associated with HPAI outbreaks at the farm level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Case (mean)</th>
<th>Control (mean)</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General information on the farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of more than one species in the farm</td>
<td>yes</td>
<td>14/18</td>
<td>7/18</td>
<td>4.5</td>
<td>0.93-42.80</td>
<td>0.065</td>
</tr>
<tr>
<td>The different species are separated</td>
<td>yes</td>
<td>2/14</td>
<td>0/8</td>
<td>1</td>
<td>0.03-inf</td>
<td>1</td>
</tr>
<tr>
<td>The farmer vaccinates against Newcastle disease</td>
<td>yes</td>
<td>9/17</td>
<td>9/18</td>
<td>1.33</td>
<td>0.22-9.10</td>
<td>1</td>
</tr>
<tr>
<td>The farmer vaccinates against the main poultry diseases</td>
<td>yes</td>
<td>16/18</td>
<td>16/17</td>
<td>2</td>
<td>0.10-117.99</td>
<td>1</td>
</tr>
<tr>
<td>The farm used H5N1 vaccination</td>
<td>yes</td>
<td>14/18</td>
<td>17/18</td>
<td>0.26*</td>
<td>0-0.41</td>
<td>0.25</td>
</tr>
<tr>
<td>Person in charge of the H5N1 vaccination</td>
<td>farmer</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>veterinarian or paravet.</td>
<td>12</td>
<td>15</td>
<td>0.5</td>
<td>0.01-9.61</td>
<td>1</td>
</tr>
<tr>
<td><strong>Trading activity of the farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The farm is trading with a trader</td>
<td>yes</td>
<td>10/14</td>
<td>17/18</td>
<td>0.25</td>
<td>0.01-2.53</td>
<td>0.375</td>
</tr>
<tr>
<td>The farm is trading with a market</td>
<td>yes</td>
<td>2/16</td>
<td>2/18</td>
<td>1</td>
<td>0.07-13.80</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of poultry product sold to a collector</td>
<td></td>
<td>14 (59%)</td>
<td>18 (76%)</td>
<td>0.99</td>
<td>0.96-1.01</td>
<td>0.313</td>
</tr>
<tr>
<td>Percentage of poultry product sold to another farmer</td>
<td></td>
<td>14 (29%)</td>
<td>18 (17%)</td>
<td>1.01</td>
<td>0.99-1.05</td>
<td>0.311</td>
</tr>
<tr>
<td>Percentage of poultry product sold to a market</td>
<td>14 (4%)</td>
<td>18 (7%)</td>
<td>0.99</td>
<td>0.93-1.03</td>
<td>0.625</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
<td>-----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>The farmer has a trading activity</td>
<td>yes</td>
<td>0/18</td>
<td>1/18</td>
<td>1*</td>
<td>0-39</td>
<td>1</td>
</tr>
<tr>
<td>No. of laying and breeding flocks in the farm in 2007</td>
<td>18 (0.5)</td>
<td>18 (0.5)</td>
<td>1</td>
<td>0.29-3.38</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No. of broiler flocks in the farm in 2007</td>
<td>18 (1.9)</td>
<td>17 (1.7)</td>
<td>3.27</td>
<td>1-24.87</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Total no. of flocks in the farm in 2007</td>
<td>18 (2.4)</td>
<td>18 (1.7)</td>
<td>1.98</td>
<td>0.92-5.51</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td>No. of chicken flocks in the farm in 2007</td>
<td>18 (0.9)</td>
<td>18 (0.7)</td>
<td>2.49</td>
<td>0.52-23.06</td>
<td>0.359</td>
<td></td>
</tr>
<tr>
<td>No. of duck flocks in the farm in 2007</td>
<td>18 (1.1)</td>
<td>18 (0.8)</td>
<td>3.36</td>
<td>0.74-31.09</td>
<td>0.148</td>
<td></td>
</tr>
<tr>
<td>No. of Muscovy duck flocks in the farm in 2007</td>
<td>18 (0.4)</td>
<td>18 (0.3)</td>
<td>2</td>
<td>0.29-22.11</td>
<td>0.688</td>
<td></td>
</tr>
<tr>
<td>Total no. of birds in 2007</td>
<td>18 (954)</td>
<td>18 (406)</td>
<td>1</td>
<td>1-1.01</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Total no. of production cycles in 2007</td>
<td>18 (2.8)</td>
<td>18 (2.2)</td>
<td>1.32</td>
<td>0.80-2.43</td>
<td>0.324</td>
<td></td>
</tr>
</tbody>
</table>

**Housing and feeding system and water source**

<table>
<thead>
<tr>
<th>No. of flocks having housing without access to water</th>
<th>18 (0.6)</th>
<th>18 (0.7)</th>
<th>0.86</th>
<th>0.22-3.07</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks having housing with access to water</td>
<td>18 (1.7)</td>
<td>18 (1.1)</td>
<td>5.81</td>
<td>1.11-236.82</td>
<td>0.027</td>
</tr>
<tr>
<td>Source of drinking water</td>
<td>well</td>
<td>11</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pond or river</td>
<td>7</td>
<td>3</td>
<td>5.28*</td>
<td>0.66-inf</td>
</tr>
</tbody>
</table>

* Median unbiased estimates (MUE) reported instead of the conditional maximum likelihood estimates (CMLEs)
4.4. DISCUSSION

Our results confirm the role played by poultry movements and trading activities, detailed by different indicators both at village and farm levels. Our results also suggest the role played by certain water bodies in virus transmission or as a temporary reservoir. The precise influence of vaccination was difficult to investigate due to limited data available.

4.4.1. METHODOLOGY

Both studies suffered from low statistical power that probably led to conclude that some potential risk factors did not have effect whereas they had one (type II error).

We especially faced some limitations in the analysis of the matched case-control study at farm level. Indeed, the effective sample size is reduced by the matching procedure with only discordant pairs included into the analysis ((Dohoo et al., 2003)). The number of farm cases could not be increased since we had initially targeted all cases in our study area, but we should have tried to increase the number of matched controls per case in order to increase the effective sample size. We also recognize that for some questions recall bias may have occurred. This is particularly obvious for the questions related to the detailed implementation of the vaccination (date and number of injections). However, for most of the questions related to the structure of the village or the farm, no bias was suspected in the answers. The selection biases were limited by our checking of the status at different steps of the study: field verification after initial selection and elimination criteria based on mortality events after interviews and before inclusion into the analysis.
4.4.2. INTENSITY OF POULTRY MOVEMENTS AND TRADING ACTIVITY AT THE VILLAGE AND FARM LEVEL

A higher number of broiler flocks was found to be a significant risk factor for HPAI outbreaks at both the village and farm levels. Broiler production is characterized by a high turnover of birds because of the short production cycle and by a high number of trading connections and poultry movements, with several DOC supplies per year and visits by multiple traders when a flock is being sold. Furthermore, H5N1 vaccination in Vietnam is normally carried out during 2 main campaigns per year, in March-April and October-November (FAO, 2010). In some areas vaccination is also organized between those campaigns to better suit the production cycles but Bac Giang province was following the bi-annual vaccination strategy in 2007. Thus, some broiler flocks could have been produced between the main vaccination campaigns and thus not protected against the infection as demonstrated by serological study of the vaccination coverage (Desvaux et al, 2010). Therefore, we can hypothesize that in Vietnam the number of broiler flocks is a risk factor of H5N1 introduction because of the high poultry trading movements related to this production type and because of the low vaccination coverage. Broiler flocks may also better reveal virus circulation than layer flocks that are better vaccinated as illustrated by the distribution of flocks affected in the case farms (Table I). Indeed, infected not vaccinated flocks show a more typical HPAI clinical picture. Paul et al (2010) found that density of broiler and layer ducks and, to a lesser extent, density of boiler and layer chickens was associated with the risk of HPAI in Thailand where vaccination against HPAI is not applied. In our study we found that only the number of broiler flocks is associated with this risk.

The presence of at least one poultry trader in the village was found to be significantly associated with the risk of HPAI at the village level. This variable is an indicator of the
poultry movements within the village that may contribute to disease introduction and transmission. Traders are usually carrying poultry on their motorbikes or on small trucks without significant biosecurity measures (Agrifood Consulting International, 2007). They also often bring birds at home for few days in order to gather enough animals for selling. Those practices probably contribute to the introduction of virus within the village which can then be easily transmitted to village farms by animal and human movements. The presence of a trader was not tested as a potential risk factor in previous studies.

We also found that a higher percentage of households keeping poultry was a protective factor at the village level. In our sample of villages there was no correlation between the number of poultry farms and this percentage meaning that it is more an indicator of the percentage of backyard poultry in the village. Backyard production is defined as a poultry production of small size with low level of investment and technical performance ((Desvaux and Dinh, 2008)). Thus, villages with high percentage of households keeping backyard poultry are probably more rural and with a smaller human density than others (human density figures were not available for our villages but we found a tendency for negative correlation between household density and this percentage in our sample). The protective effect of low human density on the risk of HPAI has been reported in previous studies ((Minh et al., 2009; Paul et al., 2010; Pfeiffer.D.U et al., 2007)). Another observation that can be made from this result is that even if the percentage of households keeping backyard poultry increases in a village, the risk of HPAI does not increase. This could be explained by the backyard production system having less trading activities and connections than semi-commercial farms. This result is also in accordance with Paul et al’s (2010) results. It is also possible that people keeping backyard poultry pay less attention to their birds than larger farmers. Thus, we cannot exclude the possibility that detection of HPAI suspect cases is less efficient in this sector.
Finally, all the variables found positively associated with the risk of HPAI outbreaks in our study explain how the disease can be spread from one village or farm to another, thus they are indicators of the distribution mechanism.

4.4.3. FARM-LEVEL FACTORS

Apart from a higher number of broiler flocks, an increased number of birds and a greater number of all poultry flocks were both also identified as potential risk factors by the univariate analysis at the farm level. Size of the farm has already been described as a risk factor for HPAI infection ((Thompson et al., 2008)). This may be explained by an increased frequency of potentially infectious contacts (e.g. by traders, feed or DOC suppliers). Furthermore, viral transmission was also found to be dependent on an increased number of birds ((Tsukamoto et al., 2007)). Thus a big farm may have more chance to develop a typical H5N1 case with most of the birds being infected and showing symptoms and subsequently being detected as a HPAI case.

The presence of more than one species in the farm was also positively associated with the risk of HPAI. This variable may simply be an indicator of a farm having several flocks or an indicator of the role of waterfowl in the increased risk of HPAI as discussed later.

Most of the farmers declared that their flocks were vaccinated against H5N1, but we can suspect a bias in this answer since, as the vaccination was compulsory; the tendency might be to declare that the flocks were vaccinated. Furthermore, there were too many missing data related to the date of vaccination or the number of injections received to categorize the farms according to those criteria or to observe this having an influence on the protection of the birds. The poor recording system, both at farm or veterinary services levels, did not allow us to fully investigate the influence of vaccination except indirectly by showing that broiler flocks, known to be less vaccinated, are also related to an increased risk of infection.
4.4.4. ENVIRONMENTAL AND INFRASTRUCTURE VARIABLES AT VILLAGE AND FARM LEVEL

At the village level, a higher percentage of the village surface occupied by ponds and small streams (defined as a 500 meters radius buffer zone around the village centroids) was found to increase the risk of H5N1 outbreak in one of our models. At the farm level, a higher number of flocks having a housing system with access to outdoor water was found to be a risk factor by the univariate analysis. The farm level result corroborates the result at the village level since the water bodies involved in the poultry farming of ducks and Muscovy ducks in Vietnam are usually ponds, canals or small streams, with the birds being kept in a restricted area (around a pond or within part of a canal or small river) or with the ducks ranging in the rice fields, canals and rivers during the day ((Desvaux and Dinh, 2008)). It was also known, and reported by one of our interviewed farmers, that dead birds may be thrown into canals or rivers by farmers, contributing to contamination of this possible reservoir of virus. In our study, the density of canals within the 500 m buffer zone was not identified as a significant risk factor probably because canals are more frequent outside the village than inside contrary to the ponds. Direct and indirect contact with wild birds through the aquatic environment can also be hypothesized even if in Vietnam infection from wild birds to domestic poultry has not been proven. Our results support the previous work that faecal-oral transmission by contaminated water is a mechanism of avian influenza transmission ((Brown et al., 2007)), and our results suggest that contaminated water can play a part in the transmission of the virus within a flock and also between flocks sharing the same environment at the same time or at different periods ((Brown et al., 2009; Brown et al., 2007; Tran. et al., 2010)).

Our study area was limited to few districts in one province and thus the heterogeneity of spatial variables was limited. This may explain why we did not find any significant
relationship between our outcome and variables related to transport networks as shown in previous studies ((Fang et al., 2008; Paul et al., 2010).

Density of waterfowl was recognized previously as a risk factor for disease occurrence, possibly due to their potential role as a reservoir of infection ((Biswa et al., 2009; Fang et al., 2008; Gilbert et al., 2006; Paul et al., 2010; Pfeiffer.D.U et al., 2007)). Nevertheless, in our study, the number of duck flocks was at the limit of significance at the village and farm levels, indicating that this species was not a predominant risk factor for disease occurrence in 2007 in our study area. This might be explained in the Vietnamese context by the prevention measures applied to that species (vaccination) and also to the H5N1 strains circulating in North Vietnam. Indeed, as ducks were recognized as a silent carrier in a study conducted in 2005 ((Diagnosis, 2005)) the veterinary services took the decision to vaccinate this species. Thus, in 2007 ducks in Vietnam were better protected against infection than in the earlier waves of infection. Another significant change relates to the predominant strains circulating in North Vietnam in 2007 (clade 2.3.4) ((Nguyen. et al., 2008)) which are more pathogenic for ducks than the original clade 1 strain ((Swane. and Pantin-Jackwood., 2008)) and may limit the role of silent carrier played by non-vaccinated ducks.
4.5. CONCLUSIONS

Our results provide a better understanding of the local transmission mechanisms of the HPAI H5N1 virus in one province of the Red River Delta region by confirming and detailing the role played by poultry movements and trading activities as well as water bodies in the introduction and transmission of the H5N1 virus at the village and farm levels. Despite limited statistical power and possible unrecognized risk factors of more limited effect, we were able to characterize the villages that may be more at risk of H5N1 outbreaks based on the structure of their poultry production (a higher number of broiler flocks), the presence of a poultry trader and a higher surface area of ponds or small streams. It was interesting to note that broiler flocks are also those known to be less well vaccinated against H5N1 due to their short production cycle. Thus, despite intensive mass communication and awareness campaigns organized in Vietnam by different programs since HPAI first occurred, there are still considerable at-risk behaviours and local disease transmission is still difficult to avoid. Nevertheless, it should also be noted that detection of an H5N1 case may also be more challenging for farmers and local veterinarians since clinical expression is probably altered in partially immunized populations. We also recognize the limitation of classical epidemiological studies for investigating the effect of vaccination in the absence of good recording systems. Use of modelling approaches to test effect of different vaccination strategies on populations or capture-recapture methods using different information sources may be more suitable techniques in that context. Finally, it is vital that the scientific knowledge acquired is transformed into appropriate actions in terms of prevention and surveillance. In this respect, better use of sociological approaches could also help to change high risk practices.
Acknowledgments

We thank the French Ministry of Foreign and European Affairs for funding the Gripavi project in the frame of which this work was done. We are grateful to the provincial veterinary services of Bac Giang province that supported us for data collection and to Mrs Pham Thi Thu Huyen for the data entry.
References


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The aim of this chapter was to study the possible role of the environmental compartment in the epidemiology of the HPAI H5N1 disease in Northern Vietnam.

We previously formulated a hypothesis related to the role of the environment as a source of indirect contamination which may contribute to the maintenance of the HPAI H5N1 viruses within the domestic poultry population (see page 24). Thus, we studied the influence of environment by describing statistical associations between some environmental features and reported outbreaks of HPAI H5N1. Some of the environmental variables tested were extracted from the interpretation of satellite images.

Another approach, to complete the statistical approach, was also adopted in close collaboration with a modeller, Edouard Amouroux from the Institute of Research for the Development (IRD). This modelling approach was aimed at developing a “virtual laboratory” to test the influence of the environment on the persistence of the HPAI H5N1 virus over a one-year period within the domestic poultry population of a selected commune in Northern Vietnam. This approach is not presented in this thesis since it needs further development. Nevertheless, the modelling process was very useful to formulate the hypothesis and questions related to the role of the environment in the epidemiology of the HPAI H5N1 disease in Northern Vietnam. A poster prepared for an international conference and presenting the conceptual model is given in Annex 6 (Use of Individual-Based Modelling for a better understanding of HPAI epidemiology in North Vietnam: approach proposed and description of GAMA platform).
5.1. INTRODUCTION

There has been some evidence for the role of the environment on the epidemiology of HPAI H5N1 disease in Asia. The two main studies which used field data of disease outbreaks in Vietnam found significant associations between the outbreaks occurrence and the relative importance of rice fields at commune levels (Gilbert et al., 2008; Pfeiffer.D.U et al., 2007). Rice fields were considered by Gilbert et al (2008) either as an indicator of the presence of ducks population or as a component of the transmission and maintenance processes. It is interesting to note as well that the relative importance of duck and rice crop intensity on the risk of HPAI, compared to human density, was variable according to the outbreak waves (Gilbert et al, 2008): human-related transmission (as illustrated by human density being the predominant risk factor) played an important role in the first wave of outbreaks, whereas rice cropping intensity was the predominant risk factor in the second wave. In the same study, duck and rice cropping intensity became less strong predictors for the third wave, probably due to control measures targeting duck populations during that period.

It is difficult to clearly support the hypothesis of indirect transmission via the environment or to evaluate the importance of this indirect transmission only by studying the statistical association between outbreaks and environmental variables. Thus, a modelling approach was used in some studies to explore more precisely the role of environment on the epidemiological cycle of the avian influenza disease. From different mathematical models (Breban et al., 2009; Roche et al., 2009; Roche and Rohani, 2010), the persistence of viral particles in the environment and the resulting indirect transmission were found to be necessary to explain avian influenza dynamics in wild bird populations.

Since domestic ducks may act as a reservoir for some HPAI H5N1 strains (D.J.Hulse-Post et al, 2005; Pantin-Jackwood and Swayne, 2007), they can contribute to the contamination of
the environment without any visible clinical signs for the farmers or the veterinarians. Chickens and ducks vaccinated with the Re-1 vaccine, currently used in Vietnam can also be infected and excrete virus without clinical expression (Pfeiffer et al., 2010). Those birds increase the range of the domestic poultry population able to contaminate the environment and, as a consequence, they contribute both to the indirect transmission process of the disease and to direct transmission through contact with susceptible populations.

Thus, a good characterisation of the environment using detailed spatial variables and an exploration of the relationship between those variables and the risk of occurrence of HPAI H5N1 outbreaks, were necessary steps in our understanding of the epidemiology of the disease in Northern Vietnam. Compared to other studies that investigated spatial variables, we used a set of spatial environmental variables specially produced for Northern Vietnam and we also applied our analysis to the last major epidemic wave which occurred in that region in 2007. Previous investigation of spatial variables were only conducted on 2005 outbreaks (Gilbert et al., 2008; Pfeiffer.D.U et al., 2007) or did not include a study of spatial determinants (Minh et al., 2009).
5.2. MATERIALS AND METHODS

5.2.1. STUDY AREA AND STUDY PERIOD

The study area was defined as the “Great Delta” made of 15 provinces of the Red River Delta and North East administrative regions. This area comprises 3075 communes. The period of interest was from 1st October 2005 to 30th June 2009 and the epidemiological unit was the commune.

Figure 5.1. Study area for descriptive and analytical analysis
5.2.2. OUTBREAK DATA

For the year 2005, outbreak data was collected from the OIE website (OIE, 2008b). From December 2005, data were regularly collected from the Department of Animal Health’s (DAH) website (DAH, 2010). For the whole period, the presence of HPAI H5N1 was confirmed by the Veterinary Regional Laboratories and the National Centre for Veterinary Diagnosis (Minh et al., 2009).

From the end of 2003, several epidemic waves occurred in Vietnam. For our study period, they are defined as follows:

- wave 3 (W3)- from 1\textsuperscript{st} October 2005 to 31\textsuperscript{st} December 2005,
- wave 4 (W4) - from 1\textsuperscript{st} December 2006 to 30 January 2007 (only occurred in the South);
- wave 5 (W5) - from 1 February 2007 to 30\textsuperscript{th} September 2007,
- wave 6 (W6)- sporadic outbreaks, from 1\textsuperscript{st} October 2007 to 30\textsuperscript{th} June 2009.

The definition of the waves was based on a previous description (Pfeiffer, 2007) and observed temporal patterns (FAO, 2011) (see introduction section).

5.2.3. ENVIRONMENTAL VARIABLES

Environmental features, possibly related to the transmission of virus (roads and running water), its persistence (lakes and ponds) or the presence of duck population (rice fields) were extracted from GIS layers and the land cover maps produced from the interpretation of satellite images.

GIS layers including transport networks, hydrographic networks, lakes and ponds were obtained from the National Cartography House in Hanoi. All polyline-type layers were first transformed into raster-type layers and classified into 2 classes, presence or absence. The size of a pixel was defined as 20 x 20 meters.
The density - defined as the percentage of pixels occupied by each variable - of transport features (national roads and all roads) and animal production-related water features (canals, ponds and streams) were calculated for each commune using the mean value obtained from 'Zonal statistics as table' function from Spatial analyst of Arctoolbox in ArcGIS software v.9.3 (ESRI Inc). For the layers including several categories, the function "Tabulate area" was used instead and percentage of area occupied by each category was then calculated.

The detailed methodology related to the interpretation of the satellite images processing is provided in Annex 7. In summary, two temporal series (respectively for 2005 and 2007) of Terra-MODIS (Moderate Resolution Imaging Spectroradiometer) eight days composite images (product 'MOD09A1 Surface Reflectance 8-Day L3 Global 500m', Land Processes Distributed Active Archive Center, http://lpdaac.usgs.gov), were processed to map respectively for 2005 and 2007 i) the flooded areas and the annual duration of flood, ii) the paddy rice agriculture areas and the annual intensity of cropping, following the method developed by Xiao et al. (2006) and iii) the forest area. This processing was validated by field visits, and, from those maps, the density of five different land cover types was calculated within each commune: water, paddy field with one crop per year, paddy field with two crops per year, forest and permanent water. The mean number of weeks of flood for each commune was also calculated for the two years.

5.2.4. POULTRY POPULATION'S DATA

Poultry population data at the commune level were obtained from the DAH, and resulted from a survey conducted in order to get a better estimate of the poultry population in the main provinces of Northern Vietnam. This study was conducted in 2009-2010, and the estimations were related to poultry populations for that period. In order to limit the imprecision of the estimation for our period of interest, we used a transformation of this variable into categories. Because not all provinces of the Great Delta region were covered
by this census, we built 2 different datasets, with and without poultry population variables, and proceeded to a separate random selection of the control communes based on the number of case communes included in each dataset. Results of the models were compared.

5.2.5. TEMPORAL AND SPATIAL PATTERNS

The number of outbreaks was plotted for the whole period of interest.

Communes’ centroids were first computed using the ‘Calculate geometry’ function in ArgGIS that provides the X and Y coordinates of a polygon’s centroid. The mean centres of the communes’ centroids with at least one outbreak for the W3 and W5 epidemic waves were computed and mapped using ‘Mean center’ function from Spatial statistics of Arctoolbox in ArcGIS. A mean centre is a point constructed from the average x and y values for the input feature centroids. It is a useful measure for tracking change in the distribution of a geo-referenced event (Mitchell, 2005). The standard deviational ellipses were also computed for W3 and W5 case communes’ centroids using the ‘Directional distribution’ function of Arctoolbox in ArcGIS. It shows whether a distribution of features exhibits a directional trend. Mean centres and directional distribution of the two main waves of outbreaks were compared in order to identify similarities or dissimilarities between those two epidemics.

5.2.6. STATISTICAL ANALYSIS

The associations between HPAI H5N1 occurrence at commune level and the potential environmental risk factors were explored using multiple logistic regression modelling. In case of low prevalence values for the response variable (<10%), the logistic regression model performance metrics may be biased (McPherson et al, 2004, quoted in (Gilbert et al., 2008)). We thus decided to use a case control study design, with four controls randomly selected for each case among the remaining non-case communes. The models
were built separately for the W3 and the W5 waves. No model was built for W6 due to the limited number of reported cases for that wave.

Because spatial autocorrelation contradicts the assumption of independence between observations, the initial fitted logistic regression model has to be checked for evidence of spatial autocorrelation in the residuals (Pfeiffer et al., 2008). Testing spatial autocorrelation on residuals from a logistic regression model with a Moran test is not appropriate and no statistical tools are available to properly test this autocorrelation (only residuals from a linear regression can be tested with an appropriate Moran Test) (R. Bivand, personal communication). Observation of the semivariogram, showing dependence as a plot of semivariance versus distance, is usually used instead (Pfeiffer et al., 2008). Nevertheless, observation of the semivariogram only gives a rough indication of the spatial structure of the model residuals. Thus, we decided to include a random effect in the model to take into account spatial dependency of the response variable, and we then checked if the intra-group coefficient of correlation, Rho, for this clustering variable was significantly different from zero. We finally observed the semivariogram of the residuals of this generalized linear mixed model. District was the variable selected for the random effect because cases might be clustered in space due to environmental variables and local transmission, but also because of the reporting policy and the detection capacity of the veterinary services. The first level of veterinary services in Vietnam being the district, this variable was found to be pertinent. Goodness-of-fit of the final multivariate models was assessed by calculating the Area Under the Curve (AUC).

All continuous variables were transformed into categorical variables. Categories were defined similarly for all datasets (W3 and W5) for ease of interpretation and comparison. Categories were defined to better suit the variable distribution into the different datasets. The effect of each variable on the HPAI occurrence was tested by using a univariate logistic model including the random effect term. Variables having a p-value $\leq 0.25$ were
candidates for inclusion in the multivariable model. Then, non significant predictors were removed using a backward stepwise selection method based on the Akaike Information Criteria comparison (AIC).

5.2.7. STUDY POPULATION FOR RISK FACTOR ANALYSIS

Only 2939 of the 3075 communes in our defined area were covered by the MODIS images (including 226 out of 237 case communes) (Figure 5.1). Of those communes, some had inconsistent ID codes and were removed. A total of 2914 communes were used in the analysis (including 223 case communes). We also decided to remove all the communes from provinces which had no cases declared because it might be considered as an indication of under-reporting by the province. Thus, the provinces of Lang Son and Ha Tay were removed. Lang Son had no cases declared whereas outbreaks occurred at its border and Ha Tay only declared one case (detected at a market place) whereas it is the most intensive poultry production province in Northern Vietnam and outbreaks of HPAI are known to occur without declaration (see chapter 9). Ha Noi was also removed despite one case was reported because this province is the capital and it is not representative of the Delta region. Finally, we used 1893 non case communes, 151 case communes for W3 and 57 case communes for W5 in the analysis. Only 6 communes declared outbreaks for more than one wave.
5.3. RESULTS

5.3.1. LAND COVER MAP

The method for rice cropping extraction initially developed by Xiao et al (2006) was adapted to our context (see annex 6 for details) and validated by field visits. An illustration of the map produced for the 2005 MODIS images processing in our study area is provided in Figure 5.2. A similar map was also produced for the 2007 temporal series.

![Land cover map from 2005 MODIS images processing](image)

**Figure 5.2.** Result from 2005 8-days composite MODIS images temporal series processing
Figure 5.3. Spatial patterns of the W3 and W5 epidemic waves
5.3.2. TEMPORAL AND SPATIAL PATTERNS

The risk of outbreaks at commune level decreased from W3 to W6 (Table VIII) and if W3 and W6 occurred during the cool season around the Têt celebration, W5 presented a different seasonality by being centred on the month of June (Table IX). Nevertheless, the spatial patterns of W3 and W5, characterized by their mean centre and deviational ellipse, exhibit similarities, with mean centres of the 2 waves very close to each other and the directional distributions also almost merged with a similar rotation of the long axis (Figure 5.3). Figure 5.3 also shows communes with outbreaks declared during the first 10 days of each wave, as well as the 6 communes which reported outbreaks in both waves. Most of those communes are along one national road coming from Quang Ninh province.

Table 5.1. Summary statistics of reported HPAI H5N1 outbreaks in the Great Delta Region
(3075 communes)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>No. outbreaks</td>
<td>299</td>
<td>0</td>
<td>72</td>
<td>24</td>
<td>395</td>
</tr>
<tr>
<td>No. communes affected by at least one outbreak</td>
<td>163</td>
<td>0</td>
<td>60</td>
<td>24</td>
<td>237</td>
</tr>
<tr>
<td>Proportion of commune with declared outbreaks</td>
<td>5.30%</td>
<td>1.95%</td>
<td>0.78%</td>
<td>7.70%</td>
<td></td>
</tr>
<tr>
<td>Average number of outbreak / commune</td>
<td>1.8</td>
<td>1.2</td>
<td>1.0</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>
5.3.3. RISK FACTOR ANALYSIS

The results of the final generalized linear mixed models including the district as a random effect are presented in Table IX. For each wave, the 2 final models resulting from 2 different datasets are presented. It is interesting to note that with or without the poultry population as a potential determinant of the occurrence of HPAI H5N1 reported cases in the variable selection process, the results of the models were very similar. The only difference was related to Wave 3, where the final model resulting from the use of the dataset with the poultry population included the duck population density as an explanatory variable instead of the percentage of pixels with paddy fields with 2 cultures per year (Table IX). This indicates that percentage of pixels with paddy fields with 2 cultures per year can be a proxy of the duck population density. Finally for W3, apart from the duck population density or its proxy, identified as strong risk factors for the occurrence of HPAI H5N1 cases, 2 other risk factors were identified: the percentage of
pixels of the commune with ponds and lakes and the percentage of pixels of the commune occupied by national roads. For models using data from W5, the final explanatory variables were different from the ones selected in the W3 models. Two variables were selected by both final models for W5: the average number of weeks of flood in the commune and the percentage of pixels with transport routes. A higher number of weeks of flood in the commune significantly increased the risk of occurrence of W5 outbreaks at the commune level, whereas a medium percentage of transport networks in the commune decreased the risk of occurrence compared to a low percentage of this variable. For all the 4 models built, the intra-class correlation coefficients, Rho, measuring the correlation of the response variable at the district level, were significantly different from 0, confirming the choice of this variable as a clustering variable. The semivariograms of the 4 models did not present signs of autocorrelation of the residuals (Figure 5.5).
Figure 5.5. Case and control mapping
Semivariogram computed using the residuals derived from the final generalized linear mixed models presented in Table IX.
The dashed lines show the pointwise 95% confidence limits constructed for 1000 simulations where the residuals were randomly allocated to commune locations and the semivariogram computed for each simulation.

Model without poultry population  Model with poultry population

Figure 5.6. Semivariogram of the four models presented in Table IX.
Table 5.2. Final generalized linear mixed model computed by adaptive Gauss-Hermite quadrature, presented with all variables tested

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</thead>
<tbody>
<tr>
<td></td>
<td>Model without poultry population, n= 755</td>
<td>Model with poultry population, n=524</td>
<td>Model without poultry population, n=284</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value*</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Variables extracted from 2 temporal series (respectively for 2005 and 2007) of Terra-MODIS eight days composite images</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of pixels with no identified land cover</td>
<td>Cont.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of pixels with paddy fields with one crop per year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 %; 0–20 %; 21-40%; 41-60%;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of pixels with paddy fields with two crops per year</td>
<td>0 %; 0–20 %; 21-40%; 41-60%; &gt;60%</td>
<td>2.55 (0.96-6.75) 0.060</td>
<td>2.86 (0.10-8.18) 0.050</td>
</tr>
<tr>
<td>Percentage of pixels with forest</td>
<td>0.5 %; ≥ 6 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of weeks of flood</td>
<td>0-10 11-20 ≥21</td>
<td>Ref 6.10 (1.66-22.47) 0.007</td>
<td>3.68 (0.99-13.68) 0.052</td>
</tr>
<tr>
<td>Variables extracted from GIS layers</td>
<td>0-3 %; 4-5 % 6-10 %; 11% -max</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Percentage of pixels with ponds and lakes

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>0-2%</td>
<td>1.75 (0.95-3.25)</td>
<td>0.076</td>
</tr>
<tr>
<td>&gt;2%</td>
<td>2.61 (1.15-5.96)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

### Percentage of pixels with all types of transport (railways, highways, national roads, provincial roads; streets; other roads)

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1%</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>1-2%</td>
<td>1.92 (0.93-3.97)</td>
<td>0.017</td>
</tr>
<tr>
<td>&gt;2%</td>
<td>3.97 (1.28-12.30)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

### Percentage of pixels with national roads

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>0-800; 801-1000; 1001-1500; &gt;1500</td>
<td>1.46 (0.83-2.54)</td>
<td>0.185</td>
</tr>
<tr>
<td>&gt;0%</td>
<td>3.31 (1.66-6.59)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Human density (persons/ km²)

<table>
<thead>
<tr>
<th>Human density</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-800; 801-1000; 1001-1500; &gt;1500</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>0.017</td>
<td>0.017</td>
<td></td>
</tr>
</tbody>
</table>

### Variables related to the poultry population

#### Chicken population density (birds / km²)

<table>
<thead>
<tr>
<th>Chicken density</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1,000; 1,001-2,000; 2,001-4,000; &gt;4,000</td>
<td>0.074</td>
<td></td>
</tr>
</tbody>
</table>

#### Duck population density (birds / km²)

<table>
<thead>
<tr>
<th>Duck density</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-100</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>101-400</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>401-1000</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>&gt;1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Muscovy duck population density (birds / km²)

<table>
<thead>
<tr>
<th>Muscovy duck density</th>
<th>Ref (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50; 51-100; &gt;100</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Rho (95% CI)</th>
<th>AUC (std deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56** (0.42-0.70)</td>
<td>0.932 (0.0088)</td>
</tr>
<tr>
<td>0.59** (0.42-0.75)</td>
<td>0.936 (0.0111)</td>
</tr>
<tr>
<td>0.40** (0.19-0.66)</td>
<td>0.920 (0.0189)</td>
</tr>
<tr>
<td>0.38** (0.16-0.66)</td>
<td>0.947 (0.0199)</td>
</tr>
</tbody>
</table>

*Wald's test  **p-value of the likelihood ratio test of rho=0 ≤ 0.001
5.4. DISCUSSION

Land cover map as a tool for risk mapping

This study enabled the production of detailed land cover maps by the interpretation of temporal series of MODIS satellite images and the aggregation of existing GIS layers to present the spatial distribution of different potential risk factors for the occurrence of the occurrence of HPAI H5N1 outbreaks at a regional level. The resulting maps are ready to be used by the animal health services of Vietnam for disease control purposes, e.g. for identification of at-risk areas. Furthermore, the methodology developed initially by Xiao et al (2006), and adapted in our study for the agricultural conditions of Northern Vietnam, could be used for annual monitoring of the land surface changes and for identification of the zones where duck populations are probably concentrated, using the rice cropping intensity as a proxy for the estimation of that population.

Poultry trade as a factor of initial disease spreading

The analysis of temporal and spatial patterns highlighted the similar spatial distribution of HPAI H5N1 declared outbreaks during the two last major epidemic waves in Northern Vietnam, despite their occurrence at different periods of the year. This finding supports the idea of recurrent determinants explaining the spatial pattern of the disease at a regional scale. It is interesting to note a cluster of outbreaks along the national road from Quang Ninh province. Many early outbreaks of both waves were reported in that area and this is also one zone were recurrence of outbreaks at the commune level is more frequent than in other zones (Figure 5.3). Minh et al (2009) described this area as the most likely cluster for W3 in the Red River Delta administrative region, but not for W5. Indeed, for the 2007 epidemic wave, the clustering is less obvious but communes with outbreaks in this area were among the first to be declared infected.

This pattern may be explained by the illegal trade of poultry from China, known to occur from Quang Ninh province. This hypothesis is supported, for the 2007 outbreak wave, by
the fact that one of the first communes which declared an outbreak during that wave (Mong Cai commune in Quang Ninh province) is one of the 5 communes where illegal trade from China was listed by traders interviewed during our study on the risk of introduction of HPAI H5N1 from China (Chapter 3). The spatial dispersion of the outbreaks within a window of 10 days also favours dissemination by poultry movements.

In the case of the 2007 epidemic wave, if the virus had been first introduced into Vietnam by a batch of infected poultry (possibly ducklings) illegally imported from China to the Mong Cai commune, the resulting dispersion can then easily be explained by trade movements. Indeed, poultry illegally imported from China are then sent to most of the provinces of Northern Vietnam as explained in Chapter 3.

**Water bodies as main drivers of the occurrence of the outbreaks**

The analysis of the spatial determinants of the 2005 and 2007 epidemic waves ended with different predominant predictors. Nevertheless, for both wave’s, outbreak occurrence was associated with the presence of water.

In 2005, in addition to the rice fields with 2 crops per year, we found that increased surface area occupied by ponds and lakes increased the risk of occurrence of the disease. This result was also described in our case control study (chapter 4) but was not as significant as in the present study. The water bodies involved in the poultry farming of ducks and Muscovy ducks in Vietnam are usually ponds, canals or small streams, with the birds being kept in a restricted area (around a pond or within part of a canal or small river) or with the ducks ranging in the rice fields, canals and rivers during the day (Desvaux and Dinh, 2008). It is also known, and reported by some farmers we interviewed (see chapter 4), that dead birds may be thrown into canals or rivers by farmers, contributing to contamination of this possible reservoir of the virus. Thus, our result supports the hypothesis that contaminated water can play a part in the transmission of the virus within a flock and also between flocks sharing the same environment at the same time or at different periods (Brown et al., 2009; Brown et al., 2007; Tran. et al., 2010).
the 2007 epidemic, we found that a higher average number of weeks of flood increased the risk of occurrence of the disease.

We also found that presence of national roads in the commune increased the risk of occurrence of the disease in 2005. This result is in agreement with previous findings and suggests the role of this kind of transport network in the disease transmission (Paul et al., 2010; Ward et al., 2008). This is also in accordance with our hypothesis related to the spatial distribution of the outbreaks, partly explained by poultry trade. On the other hand, in 2007 we found that communes with an intermediate level of transport networks (including all types of transport networks, not only the national roads) were less at risk than communes with a lower percentage of surface area occupied by these networks. This is in accordance with previous findings (Paul et al., 2010) for medium level of transport network density. In our dataset, this variable is highly correlated with the percentage of built-up land. Thus those communes may have less land available for agriculture and may be involved in different activities than other more rural areas.

5.5. CONCLUSIONS

From the analysis at a regional scale, we confirmed the role played by the environment on the occurrence of HPAI H5N1 outbreaks. Using the data from reported outbreaks, it seems that the environment is involved in the transmission of the virus during an epidemic wave. After an epidemic started, it appeared that places where water bodies were more widespread were more likely to declare an outbreak. Water probably acts as a reservoir of virus facilitating viral transmission from one flock to another. From this type of data alone however, it is not possible to clarify the role of the environment as a major source of virus for the emergence of an epidemic. On the other hand, the description of the spatial and
temporal patterns of the 2007 epidemic gave evidence supporting the initial introduction of the virus from China, before wider dissemination in the Northern provinces.

While a source of considerable information, a study of reported outbreaks alone does not enable to identify the occurrence of silent virus circulation among vaccinated poultry or among reservoir species. Thus, a further longitudinal study was developed and conducted using serological and virological testing (Chapter 8).

Before we present the results of this longitudinal study, we will present 2 additional studies that were considered necessary i) to have a non biased estimation of the seroprevalence on our domestic poultry population (chapter 6 on the evaluation of the serological test used on our samples) and ii) to support our findings on the vaccinated population (chapter 7 on the immunogenicity of the vaccine under field conditions).
The main object of this chapter was to evaluate the performances in terms of sensitivity and specificity of the serological test used in Vietnam to measure the H5N1 seroprevalence on the domestic poultry population. More generally, the evaluation of several serological tests was an opportunity to discuss the tools available for surveillance in the context of Vietnam.

This chapter is based on a paper published in Veterinary Microbiology journal in November 2011 (paper 2).
Evaluation of serological tests for H5N1 avian influenza on field samples from domestic poultry populations in Vietnam: consequences for surveillance.

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Abstract

In Vietnam, serological post H5N1 vaccination surveillance using the HI test is applied to assess the efficiency of the vaccination in addition to virological monitoring. In this paper we report on the evaluations of the performances of the haemagglutination inhibition (HI) test and of a H5-ELISA, using chicken and duck field samples. The evaluations were conducted by comparison with a pseudotyped-based virus neutralization test (H5pp VNT) performed in a reference laboratory and considered as a “gold standard” and also by using methods developed for imperfect reference test. Their global accuracy and best cut-offs were also estimated. Results from the HI test for several haemagglutinin subtypes and from a commercial type A influenza competition ELISA were also compared.

The results showed that performance of the HI test was very good in comparison with the H5pp VNT. Data also clearly supported the cut-off of ≥4log₂ used for the HI test for chickens but, a 3log₂ positivity cut-off would be more appropriate for ducks. When compared with the VNT, the H5-ELISA showed poor specificity when using the positivity cut-off specified by the manufacturer but could be used as a screening test if confirmed by the HI test or the H5ppVNT which presents some interests for large scale testing (no need for biosafety level 3 conditions and high performance).

A general and highly sensitive pre-screening can also be achieved using the detection of NP-specific antibodies with a competition ELISA. This appears of little interest in a context of high subtypes diversity where only a subtype is targeted for surveillance and control.

Key words: avian influenza, H5N1, vaccination, Vietnam, serology, evaluation tests, influenza pseudotyped lentiviral particles.
6.1. INTRODUCTION

H5N1 Avian Influenza (AI) virus is a type A influenza virus from the Orthomyxovirus family. The H5N1 strains circulating intensively in domestic poultry in Asia since 2003 are highly pathogenic AI viruses (HPAI) (Peiris, 2009). Observation of poultry immune responses against the AI virus are commonly used either as a way to detect evidence of infection or to evaluate the vaccination efficiency. In order to correctly interpret results of serological tests, it is important (1) to understand the immunology of the population under surveillance or monitoring and (2) to know the performances of the tests being used. The performance of the test is defined here by its sensibility and its specificity.

Influenza viruses type A genome encodes for 10 viral proteins that can be divided into 3 main categories: the surface proteins (haemagglutinin HA, neuraminidase NA and matrix 2 (M2) the internal proteins (3 polymerase proteins PA, PB1, and PB2; the nucleoprotein (NP), the matrix 1 (M1) and the nonstructural proteins 2 (NS2)); and finally, the nonstructural protein 1 (NS1) that is not packaged into the virus particle (Suarez and Schultz-Cherry, 2000). While the surface proteins (HA and NA) are the only antigens capable of inducing neutralizing antibodies and therefore a protective immune response, M2, NP and M1 proteins can also induce antibody response (Aymard et al., 1998; Suarez and Schultz-Cherry, 2000). The NP and M1 antigens have high sequence conservation that allows the detection of antibody from birds infected with any type A influenza viruses (Suarez and Schultz-Cherry, 2000). Several experimental infections conducted in chickens using low pathogenic strains showed that antibodies against HA, NA and NP protein have the same kinetic profile whereas the anti-M2 response showed a different profile by being of shorter duration and disappearing more rapidly (Marche et al., 2010).

The most commonly used serological tests target the NP protein when the objective is to have a non sub-type specific test (e.g.: agar gel immunodiffusion (AGID), commercial or in-house enzyme-linked immunosorbent assay (ELISA)), or the HA protein when a sub-type
specific test is required (e.g.: hemagglutination inhibition (HI), virus neutralization test (VNT) or HA-specific ELISA) (WHO, 2002). Detection of antibodies against subtype-specific NA protein is also used but not routinely. Similarly, detection of antibodies against NS1 and M2 proteins are used to differentiate infected from vaccinated animals (DIVA strategy), but no routine tests are available (Siting et al., 2005).

Neutralizing antibodies are participating to protection; those directed towards HA are the more potent (Garcia et al., 2010; Suarez and Schultz-Cherry, 2000). In contrast, irrespective of their neutralizing activity, antibodies against HA, NA and NP are marker of infection. Some authors even indicate that detection of antibodies against NP protein provides a more sensitive test than detection of antibodies against HA protein (Marche et al., 2010).

Vietnam experienced severe epizootics of HPAI H5N1 from 2003 to 2005 before adopting a mass vaccination strategy to control the number of outbreaks in domestic poultry and to limit the number of human cases. With implementation of the vaccination, serological post-vaccination surveillance became an important tool to assess the efficiency of vaccination. Serological surveillance currently applied in Vietnam uses the HI test and aims at evaluating the immunity induced by the H5N1 vaccine on vaccinated birds and in some circumstances at detecting the circulation of H5N1 virus on non vaccinated ones. In addition, virological monitoring in market places and in non vaccinated population is also being applied. The use of sentinel birds in vaccinated flocks to detect virus circulation was not adopted in the country.

In this study, antibodies against HA were used as a marker for both infection and vaccination since we collected samples from partially vaccinated domestic poultry in Vietnam. Because the vaccine used in Vietnam is generated from a genetically modified reassortant H5N1 low pathogenic virus (referred to as Re-1) (Qiao et al., 2006), distinction between infected and vaccinated birds is not possible when serological response against
HA antigen is measured. In this paper we report on the evaluations of the performances of several diagnostic techniques under field conditions considering the two main species present in the country: chicken and duck. In particular, we have evaluated the performance of the HI test as well as of an H5-ELISA for its rapidity and easiness of implementation compared to the HI test. Results from the HI test for several haemagglutinin subtypes and from a commercial type A competition ELISA (detecting the NP antibodies) were also used for our evaluation. The evaluation of these tests were conducted by comparison with an influenza H5 pseudotyped based VNT performed in a reference laboratory as a reference assay given true serological status and also by comparing results of the different tests using methods developed for imperfect reference test. The neutralization assays are considered to be a sensitive and specific test for both animals and humans (WHO, 2002). The VNT applied in our study uses a H5-pseudotyped lentiviral particle for the neutralization-based assay (H5pp VNT assay) (Garcia et al., 2010) and was used instead of the conventional neutralization assay because it is recognized this method is at least as sensitive as the conventional method (Garcia and Lai, 2011; Tsai et al., 2009), does not need biosafety 3 level conditions, and is less labor intensive. Evaluation of the sensitivity and specificity of serological tests using field samples will be valuable for routine AI surveillance and post-vaccination evaluation in Vietnam.

6.2. MATERIALS AND METHODS

6.2.1. FIELD DATA

Four repeated cross sectional surveys were conducted over one year (2008-2009), in order to study the H5N1 HPAI seroprevalence in the domestic poultry population of the Red River Delta (Northern Vietnam). Around 1000 birds were sampled during each campaign with the farms (for farm poultry) or villages (for backyard poultry) being randomly selected in the study area. Fifteen birds were sampled from each selected
epidemiological unit providing a total of 4356 sera. The population was known to be partially immunized against H5N1 virus with the Re-1 vaccine produced by Weike Biological Company of the Harbin Veterinary Research Institute (Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China). This vaccine derives its HA and NA genes from GS/GD/96 virus (belonging to H5N1 clade 0) (Qiao et al., 2006).

Influenza H5 seroprevalence was estimated on the 4356 sera by the HI test specific for the H5 subtype performed at the National Institute of Veterinary Research (NIVR) in Hanoi, Vietnam (results not presented nor discussed in this paper). Our sera were classified by species and production types (broiler and breeder) and other serological tests were also applied on different subsets of those sera.

One subset of sera was used for the evaluation of HI and ELISA tests as follows:

- 406 sera randomly selected from the chicken and duck breeder and broiler populations were tested using the H5pp VNT performed at HKU-Pasteur Research Centre.
- From those 406 sera, a subsample of 230 from the chicken and duck breeder populations (96 and 134 respectively) was also tested using an H5-ELISA kit performed at the NIVR.

Another subset of sera was used to explore the possible cross reactivity of the H5-ELISA between HA subtypes. Initially 1103 sera randomly selected were tested by an influenza type A ELISA test kit, and from the positive samples, a subset of 260 sera were further tested by the H5-ELISA and by the HI test for H5 and other available subtypes (H3, H4, H6 and H9).

### 6.2.2. SEROLOGICAL TESTS

The HI test was used to estimate the H5N1 seroprevalence on all sera samples collected considering that the main H5 subtype in Vietnam is the H5N1 HPAI and that the only
vaccine being used is generated from a H5N1 virus. The analyses were performed at NIVR in Hanoi, Vietnam. The test used a HA clade 1 antigen (A/Dk/Vietnam/6/03 H5N1) following the protocol described in the OIE manual. All sera were first heated-inactivated at 56°C for 30 min. This method uses the ability of influenza virus to agglutinate red blood cells and measures inhibition of this process by anti-HA antibodies specific to the viral strain. Serum titers were expressed as log2 values of the highest reciprocal dilution that showed complete inhibition of haemagglutination. All sera with a titer ≥ 4log2 were initially defined as positive following the most commonly used cut-off (OIE, 2008a). The HI test was also used for 4 other AI subtypes commonly infecting the domestic poultry in the region: H9, H3, H6 and H4 (A/Dk/HK/Y280/97 H9N2; A/Dk/Vietnam/12/03 H3N2; A/Teal/HK/W312/97 H6N1; A/Dk/Siberia/378/01 H4N6).

A subtype specific ELISA (ID-Screen® Influenza H5 Antibody Competition) was also applied on a selection of sera in order to evaluate the performances of this test. This test detects anti-H5 antibodies. Under the manufacturer's instructions, a sample is considered to be positive if it gives a result less than or equal to 50% competition and negative if it gives a result more than or equal to 60% competition. The competition percentage was determined by the following formula: (OD of the sample divided by the OD of the mean value of the negative control) x 100, but results were presented using the inhibition percentage (100 - competition percentage).

A competition ELISA kit based on a blocking procedure and detecting antibodies against the internal nucleocapsid (NP) of influenza A virus (ID-Screen® Influenza A Antibody Competition) was used to estimate the Influenza A seroprevalence. Under the manufacturer's instructions, a result is considered positive if it displays a result lower or equal to 45% of competition and negative if it gives a result more than or equal to 50% competition. The competition and inhibition percentages were calculated as described above.
Finally, 406 randomly selected sera (out of 4357) were also tested using as reference test, an influenza A (H5) pseudotyped lentiviral particle-based (H5pp) VNT performed at HKU-Pasteur Research Centre (Du et al., 2010; Garcia et al., 2010; Nefkens et al., 2007). The H5pp VNT was performed as described by Garcia et al. (2010). Briefly, two-fold serial dilutions of sera were incubated for 1 hour with luciferase encoding H5 pseudotyped lentiviral particles before transfer to a monolayer of Madin-Darby canine kidney (MDCK) cells and incubated at 37 °C in 5% CO₂. After 48h infection, Steady-Glo substrate (Promega) was added and luminescence read on a Microbeta luminometer (Perkin-Elmer). H5 antigen was derived from the HA clade 1 A/Cambodia/408008/2005 virus. The neutralization titer was determined as the dilution of serum that results in the inhibition of 50% of signal [as compared to negative (absence of virus) and positive (absence of sera) controls considered as 100% & 0% neutralization, respectively].

6.2.3. DATA ANALYSIS

General methodology for evaluating the Se and Sp

Sensitivity (Se) is the proportion of diseased animals correctly identified by the test. Specificity (Sp) is the proportion of healthy animals correctly identified by the test. Se and Sp were evaluated separately for chicken and ducks in order to take into account possible differences in the tests’ performance. Those differences are expected because of species specific natural inhibitory substances in the samples (a known source of trouble in the HI assays) or because the diversity of virus that could infect duck (and other aquatic birds) is theoretically much higher than for chicken and therefore may affect the match between the antigen used in the assays and the antigens that triggered the antibodies in the case of infection.

We calculated the Se and Sp of HI test using 3 methods: (1) Se and Sp and their 95% exact binomial Confidence Intervals (CI) were calculated using results from the H5pp VNT at a positivity cut-off of titer ≥80 as the true status; (2) adjustment on the Se and Sp were made
using Staquet equations (Enoe et al., 2000; Staquet et al., 1981) assuming that the reference test is imperfect but with known Se and Sp and that the test to be evaluated and the reference test are conditionally independent given the true disease status (we fixed the Se and Sp of H5pp VNT using the cut-off titer of 80 at 0.90 and 0.99 respectively following the estimations made by Garcia (Garcia et al., 2010); and (3) we estimate the Se and Sp with their 95% probability interval by a Bayesian analysis for 2 dependent tests and 2 populations using code developed by Branscum et al (Branscum, 2003; Branscum et al., 2005). The 2 populations were either chicken broilers and chicken breeders; or duck broilers and duck breeder.

The Se and Sp of the H5-ELISA test were calculated using frequentist methods only (non Bayesian methods). Doubtful results from the ELISA test were not included into the Se and Sp calculation.

**Bayesian inference**

Bayesian analyses were performed on OpenBUGS (Spiegelhalter et al., 2007). Beta prior distributions were defined using informative prior information for the Se and Sp of the H5pp VNT test (based on Garcia and al, 2010) and the prevalence of the 2 populations (unpublished data from author Desvaux) (see Table 1 for details). Non informative priors were used for the Se and Sp of HI test and the correlation between tests (beta distributions (1,1) equivalent to uniform distributions (0,1)). A large sample of the posterior distributions was generated by a Markov Chain Monte Carlo (MCMC) algorithm, and the median of this sample is presented as a Bayesian estimate of our parameters. We presented the median together with the 2.5 and 97.5 percentile points that define the 95% probability interval of our parameters.

**Receiver Operating Characteristic (ROC) analysis**

ROC analysis was used to globally assess the accuracy of the tests to be evaluated and to define their optimal cut-off points. ROC analyses were performed using `roctab` command
in Stata (non-parametric ROC analyses). ROC curves were plotted using empirical data and the Area Under the Curve (AUC) was calculated. The AUC is a global (i.e. based on all possible cut-off values) summary statistic of diagnostic accuracy that is independent of the prevalence. A ROC curve is obtained by calculating the sensitivity of the test at every possible cut-off point, and plotting sensitivity against 1-specificity (Akobeng, 2007); the greater the AUC, the better the test. An AUC of 0.5 or less means the test is not able to differentiate cases and non cases (Akobeng, 2007). The best cut-off was then calculated using the "closest-to-(0,1)" criterion which is the cut-off that gives minimal value for \((1-Se)^2 + (1-Sp)^2\).

Table 1. Input information used to define beta prior distributions of the 2 Bayesian models

<table>
<thead>
<tr>
<th>Bayesian analysis for chicken population</th>
<th>Bayesian analysis for duck population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td><strong>95% sure the parameter is</strong></td>
<td><strong>95% sure the parameter is</strong></td>
</tr>
<tr>
<td><strong>Mode</strong></td>
<td><strong>Mode</strong></td>
</tr>
<tr>
<td>Prevalence of chicken breeders population</td>
<td>Prevalence of duck breeders population</td>
</tr>
<tr>
<td>&gt; 15% 25%</td>
<td>&gt; 25% 30%</td>
</tr>
<tr>
<td>Prevalence of chicken broilers population</td>
<td>Prevalence of duck broilers population</td>
</tr>
<tr>
<td>&lt; 30% 10%</td>
<td>&lt; 30% 10%</td>
</tr>
<tr>
<td>Se H5pp VNT &gt; 85% 90%</td>
<td>Se H5pp VNT &gt; 85% 90%</td>
</tr>
<tr>
<td>Sp H5pp VNT &gt; 95% 99%</td>
<td>Sp H5pp VNT &gt; 95% 99%</td>
</tr>
</tbody>
</table>
6.3. RESULTS

6.3.1. EVALUATION OF THE HI TEST

Evaluation of HI performances using defined cut-off

3.2.1 Evaluation of HI performances using defined cut-off

Using H5pp VNT at a cut-off of ≥80 as a reference test, we evaluated the HI performance for detecting H5 neutralizing antibodies at a cut-off of ≥4 Log₂. We estimated that the Se of the HI test performed in Vietnam for chickens and ducks varies between 83% and 88% when both species are considered. However, when evaluating chicken and duck samples separately, we found that Se for H5 antibody detection in chickens was higher, whatever the calculation method used (between 91% to 100 % for chickens and between 74% to 81% for ducks) (Table 2).

The AUC of the ROC curves (Table 2) were greater than 0.9 indicating high accuracy of the HI test when compared to the H5pp-based assay performed in the reference laboratory.
Table 2. HI test performances using H5pp VNT at a cut-off of ≥80 as a reference test

<table>
<thead>
<tr>
<th></th>
<th>All species (n=406)</th>
<th>Chickens (n=200)</th>
<th>Ducks (n=206)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value (95% CI)</td>
<td>Value (95% CI)</td>
<td>Value (95% CI)</td>
</tr>
<tr>
<td>Se (1)</td>
<td>83% (72.1% - 91.4%)*</td>
<td>100%* (79% - 100%)*</td>
<td>78%* (64% - 89%)*</td>
</tr>
<tr>
<td>Sp (1)</td>
<td>94% (90% - 96%)*</td>
<td>89%* (83% - 93%)*</td>
<td>99%* (97% - 100%)*</td>
</tr>
<tr>
<td>PPV</td>
<td>71% (60% - 81%)</td>
<td>43% (27% - 61%)</td>
<td>98%</td>
</tr>
<tr>
<td>NPV</td>
<td>97% (94% - 98%)</td>
<td>100%</td>
<td>93%</td>
</tr>
<tr>
<td>Kappa</td>
<td>0.72 (0.62-0.82)</td>
<td>0.55 (0.43-0.68)</td>
<td>0.83</td>
</tr>
<tr>
<td>AUC</td>
<td>0.94 (0.90-0.96)*</td>
<td>0.98 (0.94-0.99)*</td>
<td>0.93</td>
</tr>
<tr>
<td>Se adjusted (2)</td>
<td>88%</td>
<td>100%</td>
<td>81%</td>
</tr>
<tr>
<td>Sp adjusted (2)</td>
<td>80%</td>
<td>82%</td>
<td>79%</td>
</tr>
<tr>
<td>Se adjusted (3)</td>
<td>na</td>
<td>91% (83%-93%)**</td>
<td>74% (60%-87%)**</td>
</tr>
<tr>
<td>Sp adjusted (3)</td>
<td>na</td>
<td>88% (83%-93%)**</td>
<td>98% (95%-100%)**</td>
</tr>
</tbody>
</table>

*  Exact Binomial CI
** Probability interval
(1) Estimation of Se and Sp using H5pp VNT as a reference test given true serological status
(2) Adjustment using equations for Se and Sp proposed by Staquet et al
(3) Adjustment using Bayesian analysis assuming conditional dependence
a, b Different lower-case superscript letters indicate a significant (p<0.05) difference between groups (per row) with the use of a Student—t-test with unequal variance
Best cut-off estimation

When applying the “closest-to-(0,1)” criterion in the ROC analysis, we confirmed that the cut-off \( \geq 4\log_2 \) is well suited for chickens in our population, but a cut-off of \( \geq 3\log_2 \) for ducks would be more appropriate (Figure 1). For this \( \geq 3\log_2 \) cut-off, the HI Se increases from 78% to 88% and the HI Sp decreases from 99% to 94.23%.

<table>
<thead>
<tr>
<th>Chicken</th>
<th>Duck</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chicken ROC curve" /></td>
<td><img src="image2.png" alt="Duck ROC curve" /></td>
</tr>
</tbody>
</table>

**Figure 1. Determination of the optimal cut-off for HI test using “closest-to-(0,1)” criterion**
6.3.2. EVALUATION OF THE H5 ELISA

Evaluation of H5 ELISA performance using defined cut-off

Using H5pp VNT at a cut-off of ≥80 as a reference test, we evaluated the H5-ELISA performance for detecting H5 antibodies at the cut-off defined by the manufacturer (Table 3 and 4). We estimated that the Se of the H5-ELISA was 100% but the Sp varied from 58% to 70% according to the species and calculation methods used. The Sp value for ducks was lower than for chickens (between 55% to 58% and 69% to 70 respectively) (Table 4).

Despite, low agreement (Kappa < 0.5) between both tests using the manufacturer’s cut-off for H5-ELISA, the AUC of the ROC curves were superior to 0.9 indicating a global high accuracy of this ELISA test when compared to the H5pp-based assay performed in the reference laboratory. This indicates that different cut-offs may give better agreement for this ELISA as described below.

Table 3. Contingency table for the comparison between H5-ELISA and H5pp VNT assays including both chicken and ducks species

<table>
<thead>
<tr>
<th></th>
<th>H5pp VNT positive</th>
<th>H5pp VNT negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5-ELISA positive</td>
<td>48</td>
<td>66</td>
<td>114</td>
</tr>
<tr>
<td>H5-ELISA negative</td>
<td>0</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td>H5-ELISA doubtful</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>182</td>
<td>230</td>
</tr>
</tbody>
</table>
Table 4. H5-ELISA test performances using H5pp VNT at a cut-off of ≥80 as a reference test given true status.

<table>
<thead>
<tr>
<th></th>
<th>All species (n=221)</th>
<th>Chickens (n=92)</th>
<th>Ducks (n=129)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value (95% CI)</td>
<td>Value (95% CI)</td>
<td>Value (95% CI)</td>
</tr>
<tr>
<td>Se (1)</td>
<td>100% (93%-100%)*</td>
<td>100% (72%-100%)*</td>
<td>100% (91%-100%)*</td>
</tr>
<tr>
<td>Sp (1)</td>
<td>62% (54%-69%)*</td>
<td>69% (58%-79%)*</td>
<td>55% (45%-66%)*</td>
</tr>
<tr>
<td>PPV</td>
<td>42% (33%-52%)</td>
<td>31% (16%-48%)</td>
<td>47% (36%-59%)</td>
</tr>
<tr>
<td>NPV</td>
<td>100% (97%-100%)</td>
<td>100% (94%-100%)</td>
<td>100% (93%-100%)</td>
</tr>
<tr>
<td>Kappa</td>
<td>0.41 (0.31-0.52)</td>
<td>0.35 (0.19-0.50)</td>
<td>0.42 (0.28-0.56)</td>
</tr>
<tr>
<td>AUC</td>
<td>0.92* (0.88-0.95)</td>
<td>0.94* (0.87-0.98)</td>
<td>0.91* (0.85-0.95)</td>
</tr>
<tr>
<td>Se adjusted (2)</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Sp adjusted (2)</td>
<td>64%</td>
<td>70%</td>
<td>58%</td>
</tr>
</tbody>
</table>

*95% exact Binomial CI
(1) Estimation of Se and Sp using H5pp VNT as a reference test given true serological status
(2) Adjustment using equations for Se and Sp proposed by Staquet et al
\[ n = 230, 2\ n = 96, 3\ n = 134 \]
\[ a, b \] Different lower-case superscript letters indicate a significant (p<0.05) difference between groups (per row) with the use of a Student—t-test with unequal variance

Best cut-off estimation “closest-to-(0,1)” criterion

When applying the “closest-to-(0,1)” criterion in the ROC analysis, we found that a different cut-off than the one proposed by the manufacturer should be selected. When both species are considered together, a positivity cut-off of ≤18% which gives a Se of 90% and a Sp of 82%, should be applied. A slightly different cut-off could be applied for chickens and ducks (21% and 16% respectively) to get a Se of 100% for chickens and 84% for ducks and a specificity of 86% for chickens and 89% for ducks. This cut-off, defined in comparison with H5pp VNT on field samples, is very different from the one proposed by the manufacturer (50%).
Supporting data from influenza type A Elisa

Of the 1103 samples randomly selected from our total number of sera, the overall type A seroprevalence on all species was estimated at 43% (95% CI: 40%-45%).

Among those 1103 samples, 12% (23/185) of the sera positive by HI test for H5 were negative for the ELISA A, giving indication of a possible higher sensitivity of the HI test. Those 23 discordant sera presented an average mean H5 HI titer of 5.5 log₂.

From the subset selection of 230 samples also tested by the H5-ELISA, less than 1% of the H5-ELISA positive sera were negative for the ELISA A, giving indication of good concordance between the 2 ELISA tests for the positive results (Table 5).

The comparison between HI test for different subtypes and H5-ELISA on 260 samples of ELISA A positive sera is detailed in Table 6. In this sample, 56% of the ELISA A positive sera were not identified by the HI test using H5, H6, H9, H3 or H4 antigens. Furthermore, from those 260 samples of ELISA A positive sera, around 10% of the H5-ELISA positive sera were positive by the HI test for HA subtypes other than H5.

Table 5. Concordance between the ELISA-A and the H5-ELISA positive results

<table>
<thead>
<tr>
<th></th>
<th>H5-ELISA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>ELISA A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>57</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>46</td>
<td>111</td>
</tr>
<tr>
<td>Doubtful</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>107</td>
<td>114</td>
</tr>
</tbody>
</table>
Table 6. Results of the HI tests applied on 260 ELISA type A positive samples and comparison with H5-ELISA results

<table>
<thead>
<tr>
<th>H5-ELISA</th>
<th>HI results</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>doubtful</td>
</tr>
<tr>
<td>H3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H3 H4</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>H4</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>H4 H9</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H5</td>
<td>0</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>H5 H4</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>H5 H4 H6</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H5 H4 H9</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>H5 H6</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>H5 H9</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>H6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H6 H9</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H9</td>
<td>19</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Not identified</td>
<td>68</td>
<td>68</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>152</td>
<td>15</td>
</tr>
</tbody>
</table>
6.4. DISCUSSION

The aim of this study was to evaluate the performance of two H5 antibody detection methods based on field samples collected from a partially immunized population in Vietnam in comparison with a more sensitive and specific neutralization test used as reference.

We found that performance of the HI test performed at NIVR was very good in comparison with an H5pp-based assay at the influenza reference laboratory in Hong Kong. The globally lower Se for ducks might be explained by the use of an inappropriate positivity cut-off for that species. Data clearly supported the cut-off of ≥4log$_2$ used for the HI test for chickens but, a 3log$_2$ positivity cut-off would be more appropriate in the domestic duck population in comparison with the reference test used. By changing the cut-off of the HI test for ducks we increase the Se of this test on that population but as a consequence, we slightly reduce its specificity.

The Bayesian analysis, evaluating the HI test with some uncertainty related to the H5pp VNT’s performance, also confirmed the global tendency of a higher Se for chickens compared to ducks.

When compared with the H5pp VNT, the H5-specific ELISA showed a major specificity problem at the manufacturer’s positivity cut-off. Several hypotheses can be put forward to explain this difference. One of them could be that the H5-specific ELISA has a better cross-reactivity than the H5ppVNT to detect a variety of H5 strains. This hypothesis cannot be excluded but is also not fully supported by our data, since we can increase the agreement between the H5-specific ELISA and the reference test just by adapting the cut-off of the Elisa (Kappa increasing from 0.41 to 0.58 for the cut-off determined by the best-cut-off estimation, data not shown). We also increased the agreement between both tests by using a different cut-off (40 instead of 80) for the H5pp VNT (data not shown) indicating that
disagreement between the two techniques mainly occurs for the sera with low titer considered to be non-specific by the reference method. Furthermore, the observation that 10% of the H5-ELISA positive are actually positive to subtypes other than H5 by the HI test, supports the hypothesis that H5-specific ELISA cross-reacts, to a certain extent, with other HA subtypes. In conclusion, the best cut-off estimations for the H5-specific ELISA would be in the high positive range in comparison to the manufacturer's recommendations, so the test could only be considered to be accurate in identifying birds giving a high positive reaction. To date, no other studies are available on the assessment of H5-specific ELISA test either under experimental or field conditions.

The HI testing with selected subtypes on a subset of type A ELISA positives showed around 55% of the sera could not be subtyped by HI test when the most common HA subtypes for poultry in the region were used. Either the type A blocking ELISA is more sensitive for detecting birds exposed to influenza viruses than the HI test for specific subtypes or there are other HA subtypes circulating that were not tested for. This difference of results between samples tested with a competitive or blocking type A ELISA detecting NP antibodies and the HI test, suggesting an apparent higher sensitivity of the ELISA method, was described and discussed previously for studies using field samples from different bird species (Perez-Ramirez et al., 2010; Starick et al., 2006). Experimental studies also indicated that competitive type A ELISA tests were able to detect an antibody reaction earlier than the HI test (Song et al., 2009; Starick et al., 2006). Those findings are supported by the observation of the NP antibody kinetic profile after infection using the same type A ELISA kit (Marche et al., 2010). Therefore, to assess those observations it would have been necessary to sample the birds at a later date or to conduct HI tests using all the other AI subtypes as well as representatives of the main H5 clades. Nevertheless, it is difficult to justify that around 12% (23/185) of the H5 HI positive sera (out of the 1103 sera tested by the type A ELISA) were negative for the type A ELISA. This would either suggest a higher sensitivity of the HI test compared to the type A ELISA as described for a
blocking ELISA on an experimental trial on ducks (Spackman et al., 2009), or this would indicate a lower specificity for the HI test (perhaps some sera with a low HI titer were false positives).

### 6.5. CONCLUSIONS

The strategy currently applied in Vietnam that uses the H5 HI test on sera samples for estimating the proportion of birds responding to vaccination against H5N1 or exposed to the virus, proved to be good in comparison with a H5ppVNT using the same HA clade. Nevertheless the cut-off for ducks needs to be changed to obtain a non biased estimation of the proportion of seropositive birds. Differentiation between vaccinated and non vaccinated birds remains an issue but can be by-passed by appropriate record of vaccination status and regular virological monitoring.

From the study it can also be concluded that a H5 ELISA with a good sensitivity could be used as a screening test in a surveillance programme aiming at determining the proportion of birds having significant antibody titers to H5N1 viruses as a result of prior infection or H5N1 vaccination as long as positive sera are being re-tested by a more specific method. The H5 HI and/or the H5ppVNT could be suitable options for confirmation. The H5pseudotyped based VNT, even if more costly than the HI test, presents the advantages of having a less subjective reading as well as better performances, and does not need biosafety level 3 conditions. This test could be particularly interesting for large scale testing in the context of highly pathogenic strains surveillance where a specific subtype is targeted. Furthermore, there is a need to validate the manufacturer positivity cut-off for the H5-ELISA and possibly to adapt it to the study population. In complement to H5 HI or H5ppVNT, a N1-specific ELISA could be an interesting option to support the identification of the strains circulating on non vaccinated birds but needs to be validated on the poultry population of interest.
In addition, in a context where the diversity of subtypes is known to be low, a general and highly sensitive pre-screening can be achieved using the detection of NP-specific antibodies with a competition ELISA. It also presents the advantages of being less subject to reader interpretation and can be implemented in an ordinary laboratory (no need to work on a biosafety level 2 or 3 conditions). In the epidemiological context of Vietnam with a high seroprevalence of type A influenza virus resulting from the circulation of a diversity of avian influenza subtypes, this type of test appears of little interest because the surveillance needs to specifically targets the sub-types involved in the national disease surveillance and control programme.

Finally, to adequately fit the antigens being used for serological surveillance, regular virus detection and characterisation, as this is being done in Vietnam, is an essential component of the surveillance programme.

**Acknowledgments**

We thank the French Ministry of Foreign and European Affairs for funding the Gripavi project in the frame of which this work was done. We acknowledge funding from the Area of Excellence Scheme of the University Grants Committee (grant AoE/M-12/-06) of the Hong Kong Special Administrative Region Government which supported the laboratory work in Hong Kong. We also thank Mrs Pham Thi Thanh Hoa and Mrs Huong Ho Thu, for their contribution to the field data collection and laboratory testing, and Dr Marisa Peyre and Dr Emmanuel Albina for their contributions to the discussion.
References


http://mathstat.helsinki.fi/openbugs/Manuals/Manual.html


This chapter aimed to support our analysis of the seroprevalence of vaccinated birds presented in the following chapter. Because the estimated seroprevalence of our initial cross sectional studies presented levels much lower than expected in a vaccinated population, we decided to investigate the immunogenicity of the vaccine used in the domestic poultry population to support the interpretation of our results.

This chapter has not yet been submitted for publication to a peer-reviewed journal, but will be proposed for publication in the future, possibly together with experimental trials conducted in Vietnam on the same vaccine.
7.1. INTRODUCTION

Evaluation of a vaccine's immunogenicity under field conditions is an important step in the choice of an appropriate vaccine and vaccination strategy. Immunogenicity refers to the ability of a vaccine to induce an immune response (antibody and/or cell-mediated immunity) in a vaccinated animal (Hannoun et al., 2004). The immunogenicity of a vaccine can vary because of the vaccine being used (live versus inactivated vaccine), because of the vaccination administration protocol being used (single versus multiple doses, age at vaccination) or because of characteristics of the target population (maternal immunity, immunosuppression, sanitary status, genetic factors...) (Peyre et al., 2009). The immunogenicity of the AI vaccine is commonly assessed by the serological immune response produced and the serological response is commonly measured by the analysis of HI titres in vaccinated birds using an antigen of the same subtype as that used in the vaccine (Suarez and Schultz-Cherry, 2000).

The objective of our study was to evaluate the level and the kinetics of the serological response in domestic poultry vaccinated under field conditions, using the same protocol as that used by the local veterinary services.

7.2. MATERIALS AND METHODS

7.2.1. FARMS AND BIRD SELECTION

We targeted our study at the most common chicken and duck breeds in the semi-commercial production systems in our study area. For chickens, the Luong Phong breed and for ducks, the Super Egg breed were selected (Desvaux and Dinh, 2008).

Initially, 3 chicken breeder farms (A, B and C) and 3 duck breeder farms (F, G and H) were selected for this study. Those farms were selected from farms of 2 communes in Ha Tay province where the longitudinal study was organized (Figure 1, chapter 8). Farmers were
identified by the commune veterinarians of those 2 communes in order to get new flocks which had not yet received H5N1 vaccination. The protocols to be used were explained in detail to the farmers in order to receive their approval for repetitive samplings of their birds. Compensation for each birds sampled was proposed.

We did not intervene in the vaccination schedule of the selected farms and the birds were vaccinated as usual by the commune veterinarian of their area, with the inactivated vaccine produced by Weike Biological Company of the Harbin Veterinary Research Institute (Chinese Academy of Agricultural Sciences, Harbin, Peoples’s republic of China), which is used in the whole country. This vaccine is generated from a genetically modified re-assortant H5N1 low pathogenic virus, A/Harbin/Re-1/2003 (referred to as Re-1) (Qiao et al., 2006) that derives it’s HA and NA genes from GS/GD/96 virus referred to as HA clade 0.
7.2.2. SAMPLING PROTOCOL

In the selected flocks, 15 to 20 birds were sampled for serological analysis according to the following schedule: at \( t=0 \) weeks (before vaccination), \( t=3 \) weeks post vaccination (prior-booster), \( t=6 \) weeks post vaccination (after booster), \( t=3 \) months after vaccination, \( t=4 \) months after vaccination and \( t=6 \) months after vaccination. All samples were tested by the HI test to measure the quantity of antibodies against H5N1 (see details on the test procedure in the following section). Samples were collected from April 2009 to October 2009.

After the initial visits and first samplings, a Vietnamese veterinarian was contracted to perform the subsequent sampling. All birds were individually identified using initially a plaster on the leg (in addition to a colour print on their back for easy recognition within the flock). The plaster was then replaced by a metallic printed wing tag.

Whereas we succeeded in following the 3 chicken farms up to 6 months after the first vaccination, we had difficulty in following the duck flocks. Two of the selected farmers withdrew from the study after the second sampling. The third farm had a sanitary problem (not identified) and sold its flock after the first sampling. We then had to find alternative duck farms with non vaccinated flocks, and this proved difficult. Finally, only one duck flock was followed up for a shorter period than the chicken flocks.

For each sampling date, not all tagged birds were sampled since some could not be caught, had lost their tag or had died. Table XVI gives a summary of the bird samples used for the kinetic assessment.

7.2.3. DATA ANALYSIS

The HI titre kinetics were graphed based on the time elapsed since first vaccination and the mean HI titres of the vaccinated birds.
7.3. RESULTS

The antibody response kinetics (HI titres) for the 3 chicken farms under monitoring shows that at the last sampling date (around 6 months after initial vaccination) a low percentage of sampled birds had a positive HI titre (Figure 7.1).

The same response kinetic for ducks was only built up to around 3 months after initial vaccination. Nevertheless, we observed that despite a second vaccination, the mean HI titre of the sampled birds remained quite low. Furthermore, at 3 months after initial vaccination, this mean HI titre was already below the positive HI threshold defined for ducks (3 \( \log_2 \) instead of 4 \( \log_2 \) for chickens) (Figure 7.2).

### Table 7.1 Repartition of the birds sampled per date (in days) and farm

<table>
<thead>
<tr>
<th>Age at vaccination</th>
<th>Delay between 2 injections</th>
<th>T0</th>
<th>W3</th>
<th>W6</th>
<th>M3</th>
<th>M4</th>
<th>M6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td>91</td>
<td>28</td>
<td>20</td>
<td>19</td>
<td>17</td>
<td>a</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Farm B</td>
<td>45</td>
<td>41</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>a</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Farm C</td>
<td>90</td>
<td>31</td>
<td>20</td>
<td>19</td>
<td>17</td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Farms F and G</td>
<td>64</td>
<td>na</td>
<td>28</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm H</td>
<td>54</td>
<td>52</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>103</td>
<td>88</td>
<td>62</td>
<td>23</td>
<td>34</td>
<td>27</td>
<td>337</td>
</tr>
</tbody>
</table>

a the samples collected were removed from the analysis due to inconsistency between the date of sampling and date of laboratory reception. The person in charge of sampling did not correctly complete the sampling form and the exact sampling date could not be certified.
Figure 7.1 Variation of the mean HI titre for each sampling date by chicken farm

Figure 7.2. Variation of the mean HI titre for each sampling date by duck farm
7.4. DISCUSSION

This study provides useful information on the antibody response, under field conditions, of domestic poultry vaccinated with the inactivated vaccine produced by Weike Biological Company of the Harbin Veterinary Research Institute and extensively used in Vietnam and China (where other HA strains are used).

We observed that, under field conditions, the chicken and duck antibody responses to that vaccine fell under the positive HI threshold value at about 4 months after initial vaccination. For ducks, it appeared to be even faster, but as we only had the results from one farm, this was difficult to verify.

The mean HI antibody titres measured in chickens at 3 weeks after initial vaccination are in accordance with data produced by laboratory trials when measured by the HI test using a heterologous antigen (Tian et al., 2010). On the other hand, the antibody kinetic differs significantly from data published earlier by the same author (Tian et al., 2005), since the HI antibody titres were described in that paper to last up to 10 months after a single dose. In our field trial, we have seen that the HI titre decreased much faster. Even if one considers that levels of HI antibody titre to heterologous virus are about 8- to 32-fold lower than those to homologous virus (Tian et al., 2010), the titres are so low after 4 months post vaccination that antigen used for the HI test cannot be the only explanation.

The mean HI antibody titres measured in ducks at 3 weeks after initial vaccination confirmed previous findings that ducks present a lower level of HI titre compared to chickens (Pfeiffer et al., 2010) and that the antibody titre also decreases faster than in chickens.

Thus, it is suspected that chicken and duck breeder farms have H5 HI antibody titres below the optimum protective level during most of their laying period.
If correlation between clinical protection and immunity level is clear for chickens, this correlation needs to be further discussed for ducks.

Without challenge, we cannot make definite conclusions about the clinical protection and the reduction of virus shedding by those ducks following natural infection. Nevertheless, a study of recent laboratory trials indicated that clinical protection of ducks, despite absence of measured HI titres following vaccination with Re-1 vaccine, only occurred when clade 2.3.2 or 2.3.4 antigens were used for the HI test (Pfeiffer et al., 2010) and was more a problem of cross-virus detection than an absence of immunogenic response. Another trial described an absence of correlation between measured HI titre and clinical protection in ducks, but this trial did not use the Re-1 vaccine (Kim et al., 2008). It used a vaccine produced similarly but deriving its HA protein from a clade 2.3.4 virus, suspected to produce antibodies less detectable by the HI test due to a serine residue at position 223 in the virus (Kim et al., 2008).

Finally, since an immune response was measured in ducks in our study, no cross-reactivity issue is suspected and there is no reason that protection should not be correlated with this immunity level.

The vaccination protocol used by the different farms (i.e. age at first vaccination and length of delay between first and second vaccinations) are different. It seems that farmers and commune veterinarians do not strictly follow the vaccination protocol approved by the district veterinary services. Farmer B even reported that the commune veterinarian does not give him advice on the date of vaccination. Nevertheless, based on our questionnaire to commune veterinarians (see chapter 8), we know that the doses used are the same for all commune veterinarians and a fortiori, within a commune, and thus some aspects of the protocol are adhered to.
7.5. CONCLUSIONS

The results obtained from this small study highlight the limitations in terms of immunogenicity of the inactivated vaccine used in Vietnam for domestic poultry protection against the HPAI H5N1 virus.

The study also reveals the need to be able to better correlate the immune response with protection against clinical disease and infection.

Because cross-reactivity among H5N1 strains may not be total, protocols and guidelines to evaluate the vaccination should address this issue. Clear indication should be provided regarding the minimum immunity level that should be expected after vaccination when birds are tested with the heterologous antigen. Experimental trials to answer this question should be part of any vaccine agreement procedure with the manufacturer.

In the case of Vietnam, the HI test using a clade 1 antigen appears to give good results to evaluate the immune response induced by the Re-1 vaccine. The literature review also supports the hypothesis that measured HI titres in these conditions are good indicators of the birds' protection. Nevertheless, experimental trials to test this hypothesis and identify the protective threshold, at least for chickens, would be of use for a proper evaluation of the population protection after vaccination.
This chapter is based on longitudinal field data collection conducted in Vietnam from the end of 2008 to the middle of 2010.

All the data collection was organized and implemented by the PhD student in collaboration with the veterinary services of the study area and with the support of a Vietnamese researcher under her responsibility. Only the last sampling campaign in 2010 was coordinated by the Vietnamese researcher alone with the support of another colleague from Cirad and a master student.

The data collected consisted in:

- serological and virological samples from domestic poultry,
- individuals data related to the birds sampled,
- information on the farm management of the birds sampled,
- information on the villages where the birds were sampled.

We present in this chapter the paper submitted to Epidemiology and Infection the 30th August 2011 (paper 3) which describes and discuss the most significant results of the longitudinal study. This study was also a support for a poster presentation at the SVEPM conference in 2010, in Nantes, France (Annex 8) and an oral communication at the 7th conference Options for the Control of Influenza, in 2010 in Hong Kong SAR, China. In those two communications we presented the overall seroprevalence of type A influenza, estimated around 40 % (see Annex 9 for details).
Apart from the study of the farm and village domestic poultry, parallel protocols were also set up to monitor the virological prevalence of HPAI H5N1:

- in the local and big live birds markets of our study area,
- on targeted wild birds species also present in our study area.

The initial results of the monitoring of the live birds market are presented in Annex 8. Some laboratory results being still pending, this study will not be presented into details into this thesis.

The wild birds study being the result of a collaborative work, it will not be presented and discussed in this thesis (see Annex 10 for short presentation). From the initial results obtained, no clear conclusion can be drawn on the role of bridge species (species having contact with both domestic and wild bird populations) for the epidemiology of H5N1 HPAI in Vietnam.

Pictures illustrating the field work are given in annex 11.
Evaluation of the vaccination efficacy against H5N1 in domestic poultry in the Red River Delta in Viet Nam between 2008 and 2010 and effects on the epidemiology of the disease

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Running head: H5N1 vaccination and epidemiology in Vietnam
Abstract

The domestic poultry population in Vietnam has been vaccinated against Highly Pathogenic Avian Influenza (HPAI) H5N1 since 2005. Since then, outbreaks continue to occur without clear understanding of the mechanisms involved.

The general objective of this study was to understand the epidemiology of the disease in the context of vaccination and to draw some conclusions about vaccination efficacy in the domestic poultry population of the Red River Delta area. Five cross-sectional surveys to measure the serological and virological prevalence on vaccinated and non-vaccinated poultry were performed from the end 2008 to June 2010. The global seroprevalence was 24% (95% CI: 19.9%-28.2%). Determinants of the vaccine immunogenicity were identified separately in chickens and ducks as well as determinants of the seroconversion in non-vaccinated birds.

Our results highlight the difficulties in maintaining good flock immunity in poultry populations using inactivated vaccine in the field with 2 vaccination rounds per year, and in preventing circulation of virus in co-existing non vaccinated poultry.
Vietnam, with a poultry population over 200 million (Desvaux and Dinh, 2008), faced its first outbreaks of Highly Pathogenic Avian Influenza (HPAI) H5N1 at the end of 2003 (OIE, 2008). By the end of 2009, 5 epidemic waves and then sporadic outbreaks had occurred in domestic poultry (FAO, 2011; Minh et al., 2009). The H5N1 HPAI viruses isolated in Vietnam from the initial outbreaks belonged to the haemagglutinin (HA) clade 1 (genotype z) (Nguyen et al., 2008; Wan et al., 2008) according to the nomenclature system of the HA lineage protein gene (WHO/OIE/FAO, 2008) and those viruses derived their HA genes from the Gs/GD/1/96-like lineage (Smith et al., 2006).

To limit the number of outbreaks and the risk of transmission to humans, the Government of Vietnam decided to use vaccination from the end of 2005. Despite a period of about a year without an outbreak, Northern Vietnam faced a significant epidemic in 2007 (Minh et al., 2009) and since then, outbreaks continue to occur sporadically without clear understanding of the mechanisms involved: low level virus circulation among the vaccinated population; regular re-introduction from neighboring countries; or both?

The general objective of this study was to evaluate the level of virus circulation in the context of vaccination and to draw some conclusions about vaccination efficacy in the domestic poultry population of the Red River Delta area.

The specific objectives were 1) to assess, through a serological monitoring, the effect of the vaccination strategy (protocol and vaccine used) on the immunity of the population; 2) to identify the determinants of the vaccine immunogenicity under field conditions through an investigation of the variation in the H5N1 HI titers in vaccinated birds (Suarez and Schultz-Cherry, 2000) 3) to measure the level of virus circulation in vaccinated and co-existing non vaccinated populations and its determinants, by the means of virological
follow up of the whole population and serological monitoring of the non vaccinated population.

The domestic poultry population has been vaccinated since 2005, following a bi-annual vaccination campaign organized by the veterinary services. The vaccine is provided free of charge to farmers who only have to pay for the vaccination service. During our study period, chickens and ducks were vaccinated with a vaccine produced by WeiKe Biological Company of the Harbin Veterinary Research Institute (Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China). This vaccine is generated from a genetically modified reassortant H5N1 low pathogenic virus, A/Harbin/Re-1/2003 (referred to as Re-1) (Qiao et al., 2006) that derives its HA and NA genes from GS/GD/96 virus referred to as HA clade 0.
8.2. MATERIALS AND METHODS

8.2.1. STUDY DESIGN OVERVIEW

In 2008-2009, repeated population-based cross sectional surveys were conducted in order to study the patterns of H5N1 HPAI serological and virological prevalences over one year in the domestic poultry population of the Red River Delta region (Northern Vietnam). Initially, four sampling campaigns were performed: mid December 2008 (C1), end of January 2009 (C2), end of March 2009 (C3) and early June 2009 (C4). Around 1000 birds were sampled at each campaign, with the flocks (for farm poultry) or villages (for backyard poultry) randomly selected within our study area. Fifteen birds were sampled from each selected epidemiological unit, providing a total of 4356 sera. Then, in 2010, a cross-sectional study was performed on the same population in order to evaluate the serological prevalence in an outbreak recrudescence context (DAH, 2010).

8.2.2. STUDY SITES

The study site consisted of 9 communes located within 4 districts from 2 provinces (Figure 1). These communes were selected because they were considered to be at risk for HPAI infection due to previous virus circulation at the early stage of the epidemic waves.

Those communes also provided a good representation of the poultry production systems of the Red River Delta area, with Bac Giang province representative of the agricultural practices in the Delta region and Ha Tay province being the main poultry production area in Northern Vietnam, especially for breeders. Day-ld-Chickens (DOC) and ducklings from this province are sent to most of the Northern provinces.
Figure 1. Study area showing the selected provinces and communes within the delta region (rice production area)

8.2.3. SAMPLING STRATEGY

For each campaign we adopted a one-stage clustered stratified design. The population was stratified into 3 production systems (Desvaux and Dinh, 2008):

- backyard poultry system;
- semi-commercial long cycle (including breeding and laying flocks);
- semi-commercial short cycle (including broiler flocks).

A proportional stratified random sampling was applied, with the number of units sampled within each stratum being proportional to the total number of units in the stratum. The
sampling frame, consisting of all flocks and villages in our study area, was updated by the commune veterinarians before each sampling to take into account the known seasonal variation of the poultry population. Within each stratum, flocks or villages were first randomly selected using the surveytool box software. Then, birds were randomly sampled within each selected flocks or village. During the study visits, selected farms that had no birds in their selected flocks were replaced by a flock of the same category in the same village, if possible, or with one from another village of the same commune.

8.2.4. SAMPLE SIZE CALCULATION

Based on available experimental trials (Veits et al., 2008), we hypothesised that virological prevalence could be up to 0.15 of the birds in our partially vaccinated population. We set the expected seroprevalence at 0.5 (the prevalence for which the sample required to reach a given precision is the largest). Based on those hypotheses, we computed the sample size required to estimate a virological prevalence up to 15% with precision of 3% at the 95% confidence level, and a seroprevalence up to 50% with a precision of 5% and a the 95% confidence level. In order to determine the sample size needed to reach the target precision in the case of a cluster sampling and in absence of data related to the variance of HPAI prevalence within and between clusters, we applied a multiplying factor of 2 to the estimated sample size corresponding to the design effect (Killip et al., 2004). We obtained a minimum number of birds to be sampled at each campaign equal to 1090 birds. The birds sampled were then randomly selected within each cluster were selected. To be able to detect the presence of virus with 90% confidence if the within-cluster prevalence was over 15 %, 15 birds were sampled in each selected cluster (flock or village).

In 2010, only serological samples were collected and the total number of samples was increased to 1500 in order to improve the precision of the seroprevalence estimation. Furthermore, 10 birds were sampled per flock or village.
8.2.5. DATA COLLECTION

Four different questionnaires were designed. Two were administered respectively to the flock owners and to the heads of each village visited. One was filled by the commune veterinarians between two sampling campaigns with data related to the date of H5N1 vaccination in the commune and about poultry mortality events during the period elapsed. Questions to the farmers were related to: the vaccination status, the size and the characteristics of the flock (species, breed, age, origin of the birds), the housing system, the source of water supply, the sanitary conditions of the birds sampled, the way the farmer was normally selling the birds (at market or at farm gate), the delay since new birds were last entered into the farm, the delay since birds were last sold out of the farm, the existence of contacts between different species and the observation of mortality during the last 4 weeks. The questions to the head of the village were related to the number of households and poultry farms in the village, and the presence of a poultry trader, a live bird market or a hatchery. In addition, in 2010 a questionnaire was administered to the commune veterinarians including questions on the vaccination protocol for H5N1.

For each selected bird, a blood sample was collected from the wing vein, as well as 1 cloacal swab and 2 oropharyngeal swabs for campaigns 1 to 4. Oropharyngeal and cloacal swabs from 3 birds were pooled in 2 ml of virus transport medium. The remaining oropharyngeal swabs were pooled separately. During the field visits, samples were stored in cool boxes filled with ice. All samples were sent within 1 or 2 days to the National Institute of Veterinary Research, Hanoi, Vietnam (NIVR) where swabs were stored at –80°C until further processing. Sera were centrifuged the following day and were stored at -20°C until serological tests were performed.
District and commune veterinarians were trained in data collection and sample collection before the survey started. For each campaign, 2 persons from CIRAD and 1 person from the provincial veterinary services (except for Ha Tay province) accompanied the teams, which included one district and one commune veterinarian.

8.2.6. LABORATORY TESTS

Serological tests
The HI test was used to estimate the H5N1 seroprevalence on all sera samples collected. The analyses were performed at the NIVR. The test used a HA clade 1 antigen (A/Dk/Vietnam/6/03 H5N1) following the protocol described in the OIE manual (OIE, 2008a). All sera were first heat-inactivated at 56°C for 30 min. Serum titres were expressed as Log$_2$ values of the highest reciprocal dilution that showed complete inhibition of haemagglutination.

The sensitivity (Se) and specificity (Sp) of the HI test performed at NIVR on our population were evaluated by comparison with a reference test, and the best cut-off values for the positive threshold were found to be at 4Log$_2$ for chickens and at 3Log$_2$ for duck (unpublished observations). We used these positive cut-off values to define seropositivity as a result of prior infection or significant vaccination responses for chickens and ducks respectively.

Virological testing
Viral RNA extraction (using Qiagen® RNeasy Mini Kit) and reverse transcription-polymerase chain reaction (RT-PCR) were carried out at the NIVR on the pooled containing oropharyngeal and cloacal swabs. Every positive result for the RT-PCR for viral matrix protein (M) was subjected to RT-PCR for the HA gene of subtype H5. 2.7 Data analysis
8.2.7. DATA ANALYSIS

For prevalence estimation at the bird level a sampling weight was applied to each individual in order to obtain an unbiased estimation of the overall prevalence in the poultry population despite the stratified sampling strategy (Dohoo et al., 2003). The sampling weights were calculated as the inverse of the probability of being selected. The probability of selection was calculated as follows: (number of epidemiological units selected in the strata / number of epidemiological units in the strata) x (number of birds selected in the epidemiological unit / number of birds in the epidemiological unit).

All analyses used a robust calculation of the standard errors that accounted for potential intracluster (flock or village) correlation (Rogers, 1993). In the investigation of the determinants of prevalence in the non-vaccinated birds, potential intracluster correlation was accounted for by including a flock or village as a random effect in the statistical models.

Immunity level, vaccination coverage and vaccination implementation effectiveness

The estimation of the overall bird-level seroprevalence in the whole population, computed as described in the above section, was considered as the maximum immunity level (considering that seropositivity always resulted from vaccination). The theoretical vaccination coverage was assessed from farmers reports on the vaccination status of the sampled birds. Comparison of the odds of being seropositive between categories of birds was performed by means of univariate logistic regression. To evaluate the vaccination implementation effectiveness at the flock level, we defined a protected flock as having at least 70% of the sampled birds with positive titers and having a Geometric Mean Titer (GMT) ≥20 (Ellis et al., 2006; Peyre et al., 2009).

Vaccine immunogenicity
Immunogenicity refers to the ability of a vaccine to induce an immune response (antibody and/or cell-mediated immunity) in a vaccinated animal (Hannoun et al., 2004). Only birds vaccinated for at least 21 days were considered in the analysis to allow the HI titer to reach a maximum level and to be constant (Marche et al., 2010). The HI titer kinetics was then graphed based on the number of months elapsed since vaccination and the mean HI titers of 2360 out of 2945 vaccinated birds. We analyzed the determinants of the vaccine immunogenicity with a zero-inflated-Poisson regression model separately for vaccinated chickens and ducks. HI titer was considered as dependent variable. Zero-inflated-Poisson regression models allow addressing both the factors that distinguish the seroconverted birds from the non-seroconverted ones by fitting a logistic regression model and the factors that explain the different levels of antibody titers among the seroconverted birds by fitting a Poisson model (Dohoo et al., 2003). In order to limit bias due to the misclassification of the birds (farmer declaring the flock was vaccinated whereas it was not), only birds from flocks declared as vaccinated and presenting at least one seropositive sampled bird were included into this analysis. Birds showing discrepancy between their date of vaccination and their current age were removed from analysis. Different predictors were initially considered. They were related 1) to the breed 2) to the vaccination implementation (time elapsed since the last vaccination, age at the first vaccination and number of injections) or 3) to the farming management that may influence the quality of the vaccination administration and seroconversion of the birds (the number of birds in the flocks was used as an indicator of the specialization of the farmer and the housing system an indicator of exposure to diverse microbiological pressure that may limit the immune system reaction). The first step was to build a model including all explanatory variables in both components of the model. If no further adjustment significantly improved the model (variation of more than 2 points of Akaike Information Criteria comparison (AIC) when one variable was removed) then the full model was presented in order to get the adjusted coefficients. Once the model was fitted,
we performed Vuong test to assess the validity of using a zero-inflated Poisson model instead of a standard Poisson model (Dohoo et al., 2003).

**Determinants of the seroconversion in non-vaccinated birds**

A random-effect logistic model was built to study the determinants of the seroconversion of the non vaccinated birds. Flocks or villages were included as a random-effect in order to take into account intracluster correlation in the birds’ seroconversion. The variables tested were related 1) to the birds characteristics (species, production type and age); 2) to the flock characteristic (number of poultry within the flock); 3) to the village characteristic (number of layer-breeder duck flocks in the village at the sampling time, presence or not of duck broiler flocks in the village at the sampling time) and 4) to the estimated H5N1 immunity coverage of vaccinated poultry at commune level at the time of sampling.

We used our sampling frame, updated for each campaign, to estimate the number of duck flocks per village. The immunity coverage of vaccinated poultry in the commune was estimated by the seroprevalence at the bird level in the vaccinated birds in our study sample. The other variables were extracted from the farmer questionnaire.

8.3. RESULTS

8.3.1. STUDY POPULATION

In total, 5880 domestic birds were sampled from 447 epidemiological units (C1: 69, C2: 75, C3:74, C4:76, C5: 153). All of them were tested for HI antibody to H5N1 virus and only the birds sampled from C1 to C4 (n=4354) were tested by RT-PCR. The sample consisted of 2489 chickens, 2201 ducks, 1133 Muscovy ducks, 18 geese and 39 birds without clear species identification. The breakdown of the total number of flocks in our study area between December 2008 and June 2009, representing our sampling frame for flock selection, showed that the duck broiler population increased significantly during the first rice harvesting season in June (Figure 2), as already described (Desvaux and Dinh, 2008) .
Figure 2. Breakdown of flock numbers in our study area between December 2008 and June 2009.

8.3.2. VIRAL CIRCULATION OVER A ONE-YEAR PERIOD

The overall pool prevalence of type A influenza viruses for C2, C3 and C4 was 0.08 (C2: 2/374; C3: 1/365; C4: 6/396). No type A influenza positive or suspect samples were detected for the 1036 individual oropharyngeal samples collected during the first campaign. The overall H5 influenza pool prevalence was 0.02 (2/1135) (see Table 1 for details).
Table 1. Detailed information related to the positive and suspect H5 RT-PCR results

<table>
<thead>
<tr>
<th>H5 PCR pool (ct value)</th>
<th>Farm (No)</th>
<th>Species and production type (breed)</th>
<th>Vaccination status (delay since last vaccination in days)</th>
<th>Campaign</th>
<th>No birds with HI titre / No birds sampled in the farm</th>
<th>Mean HI titre of the positive pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (33.31)</td>
<td>1</td>
<td>Duck broiler (Bau Canh Tran)</td>
<td>Said not vaccinated</td>
<td>C4</td>
<td>1/15</td>
<td>0.66</td>
</tr>
<tr>
<td>Positive (34.7)</td>
<td>1</td>
<td>Duck broiler (Bau Canh Tran)</td>
<td>Said not vaccinated</td>
<td>C4</td>
<td>1/15</td>
<td>3**</td>
</tr>
<tr>
<td>Suspect (38.09)</td>
<td>1</td>
<td>Duck broiler (Bau Canh Tran)</td>
<td>Said not vaccinated</td>
<td>C4</td>
<td>1/15</td>
<td>3**</td>
</tr>
<tr>
<td>Suspect (38.54)</td>
<td>2</td>
<td>Duck breeder (Super egg)</td>
<td>Said vaccinated (31)</td>
<td>C4</td>
<td>0/15</td>
<td>0/3</td>
</tr>
<tr>
<td>Suspect (37.27)</td>
<td>3</td>
<td>Duck layer-breeder (Super egg)</td>
<td>Said vaccinated (114)</td>
<td>C4</td>
<td>10/15</td>
<td>5.33</td>
</tr>
</tbody>
</table>

*based on farmers’ reports

**titre of the only seropositie bird

8.3.3 VARIATION IN VACCINATION PRACTICES

The commune veterinarians questionnaire's results revealed variations among the 9 communes in the way vaccination against H5N1 was implemented (Table 2). However, doses used were homogeneous in the 2 provinces (0.3 ml and 0.5 ml for chickens and ducks of less than 35 days olds respectively; 0.5 ml and 1 ml for chickens and ducks of more than 35 days old respectively).
Table 2. Vaccination practices in the 9 communes interviewed

<table>
<thead>
<tr>
<th>Birds category</th>
<th>do not vaccinate</th>
<th>Provide 1 injection per campaign</th>
<th>Provide 2 injections per campaign</th>
<th>Average age in days for first injection (min-max)</th>
<th>Average delay between 2 injections (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken layers and breeders</td>
<td>0/9</td>
<td>6/9</td>
<td>3/9</td>
<td>19 (14-35)</td>
<td>56 (20-120)</td>
</tr>
<tr>
<td>Duck layer and breeders</td>
<td>0/9</td>
<td>4/9</td>
<td>5/9*</td>
<td>20 (14-43)</td>
<td>24 (20-28)</td>
</tr>
<tr>
<td>Chicken broilers</td>
<td>0/9</td>
<td>8/9</td>
<td>1/9</td>
<td>19 (14-35)</td>
<td>20</td>
</tr>
<tr>
<td>Duck broilers</td>
<td>1/9</td>
<td>7/9</td>
<td>1/9</td>
<td>16 (14-43)</td>
<td>21</td>
</tr>
</tbody>
</table>

* One commune declared that some farmers were using 3 injections before the laying period

8.3.4. CHANGE IN SEROPREVALENCE OF THE STUDY POPULATION OVER THE STUDY PERIOD

The seroprevalence over the 5 cross-sectional surveys of the overall population, without consideration of the reported vaccination status of the birds and estimated by methods accounting for the survey design (sampling weight and clustering) was 24% (95% CI: 19.9%-28.2%). The change in seroprevalence over sampling dates is represented in the Figure 3.
The seroprevalence estimations by species or production categories are given in Table 3. The odds of being seropositive did not differ between the chickens and the ducks (p=0.226) but was lower for Muscovy ducks when compared to chickens (OR: 0.24, 95% CI: 0.10-0.56, p=0.001). The odds of being seropositive were not significantly different for backyard and layer-breeder production types (p=0.077) and for backyard and broiler flocks (p=0.243) but the odds of being seropositive was significantly lower for broilers when compared to layer-breeders (OR: 0.37, 95% CI: 0.18-0.74, p=0.005). The same difference between broilers and layer-breeders was noted on the vaccination coverage based on farmers’ reports, with 70.9% of the layer-breeders said vaccinated (95% CI: 64.2-77.6, n=3561) against 38.9% of the broilers (95% CI: 25.9-51.8, n=1576). The change in the level of immunity for layer-breeders and broilers for the different sampling dates is shown in the Figure 4. The odds of being seropositive did not differ between the 2 provinces (p=0.166).
Table 3. Stratum specific bird level seroprevalence corrected according to sampling design

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Seroprevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td>2489</td>
<td>24.2% (95% CI: 17.7-30.7)</td>
</tr>
<tr>
<td>Ducks</td>
<td>2201</td>
<td>29.9% (95% CI: 23.3-36.5%)</td>
</tr>
<tr>
<td>Muscovy ducks</td>
<td>1133</td>
<td>7.1% (95% CI: 2-12.3)</td>
</tr>
<tr>
<td>Backyard poultry</td>
<td>733</td>
<td>20.2% (95% CI: 12.2-28.2)</td>
</tr>
<tr>
<td>Layers and breeders</td>
<td>3561</td>
<td>29.7% (95% CI: 23.9-35.5)</td>
</tr>
<tr>
<td>Broilers</td>
<td>1576</td>
<td>13.5% (95% CI: 6.1-21)</td>
</tr>
<tr>
<td>Province 1</td>
<td>2994</td>
<td>26.8% (95% CI: 20.6-30)</td>
</tr>
<tr>
<td>Province 2</td>
<td>2886</td>
<td>21% (95% CI: 15.6-26.5)</td>
</tr>
<tr>
<td>Birds said vaccinated</td>
<td>2945</td>
<td>36.9% (95% CI: 30.4-43.5)</td>
</tr>
<tr>
<td>Birds said vaccinated for at least 21 days</td>
<td>2502</td>
<td>36.1% (95% CI: 29.1-43.1)</td>
</tr>
<tr>
<td>Birds said not vaccinated</td>
<td>2561</td>
<td>10.3% (95% CI: 6-14.5)</td>
</tr>
<tr>
<td>Vaccinated layers and breeders</td>
<td>2280</td>
<td>36.9 (95% CI: 29.8-44)</td>
</tr>
<tr>
<td>Vaccinated broilers</td>
<td>603</td>
<td>31.6 (95% CI: 16.5-46.7)</td>
</tr>
</tbody>
</table>

Figure 4. Variation of the H5N1 bird-level seroprevalence for layer-breeder and broiler birds
8.3.5. LIMITED VACCINATION IMPLEMENTATION EFFECTIVENESS

At the population level

The overall seroprevalence of the birds declared vaccinated was only 36.9\% (Table 3). Its variation by sampling date is presented in Figure 3. The odds of being seropositive was significantly higher for vaccinated when compared to non-vaccinated birds (OR: 5.1, 95\% CI: 3-8.7). The odds of being seropositive did not differ between the vaccinated chickens and the vaccinated ducks (p=0.642) or between the vaccinated broiler and layer-breeder poultry (p=0.294).

At the flock level

Considering the chicken and duck flocks which were said to have been vaccinated at least 21 days previously (n=182), the mean within flock proportion of seropositive birds was 29.2\% (95\% CI: 24.3-34.1\%). The mean within flock mean HI titer for the same sub-population was only 1.7 log\(_2\) (95\% CI: 1.4-2.1 log\(_2\)). Finally only 11.5\% of those flocks (21/182) could be defined as protected (70\% of the birds sampled seropositive and a GMT \(\geq\)20).

In order to limit bias due to wrong vaccination status reports we also had those parameters computed for the chicken and duck flocks said to have been vaccinated at least 21 days previously and with at least one seropositive bird (n=107). For that sub-population, the mean within flock proportion of seropositive birds increased to 49.7\% (95\% CI: 44.0-55.3\%). The mean within flock mean HI titer for the same sub-population increased to 2.8 log\(_2\) (95\% CI: 2.5-3.1 log\(_2\)) and 19.6\% of those flocks (21/107) could be defined as protected.
8.3.6. DURATION OF (PROTECTIVE) IMMUNITY AGAINST H5N1 UNDER FIELD CONDITIONS

The kinetics of antibody responses measured by HI titer in vaccinated flocks is presented in Figure 5 and Figure 6 for both chicken and duck layer-breeders and broilers respectively. Those data show that HI titers and their upper confidence intervals were not consistently over the seropositive titer defined in the Materials and Methods more than one month after vaccination. The jump in antibody titers observed in Figure 5 at 4 months after vaccination might be explained by the booster vaccination occurring between 1 and 2 months after primary inoculation. For duck broilers, although there was no available data after 3 months post-vaccination, the upper confidence interval of the HI antibody responses never reached the defined seropositive level by two months after vaccination.

Figure 5. Antibody response kinetics (HI titres) for all layer and breeder birds said vaccinated for at least 21 days and from flocks with at least one seropositive bird

Figure 6. Antibody response kinetics (HI titres) for all broiler birds said vaccinated for at least 21 days and from flocks with at least one seropositive bird
8.3.7. MODELING OF THE DETERMINANTS OF THE VACCINE IMMUNOGENICITY

Due to limited size of some categories, we finally decided to fit a model only for the birds at 2 and 3 months post-vaccination. Three potential determinants of the immune response were tested in a zero-inflated Poisson regression model for vaccinated chickens and ducks: the vaccination protocol (age at vaccination and number of injections); the number of poultry within the flock; and the housing system. The breed could not be tested due to limited variability of breeds within our selected samples.

The determinants of immunogenicity were only studied for the last campaign for which detailed information about the number of injections per vaccination course was recorded in addition to the vaccination status and date of vaccination.

Determinants of the immunogenicity in vaccinated chickens

The only factor in the final logistic component of the model, was the vaccination protocol (Table 4). Chickens vaccinated before 20 days old with 1 injection had a zero HI titer much more often than chickens vaccinated after 20 days with 2 injections (OR: 45.98, p=0.000). To a lesser extent, and at the limit of the significance level, being vaccinated after 20 days old with 1 injection also increased the chance of getting a zero HI titer compared to a vaccination after 20 days old with 2 injections (OR=2.62, p=0.061). Regarding the variables influencing the HI titer of the seroconverted birds, we found an effect of vaccination protocol with a mean HI titer higher on the birds vaccinated before 20 days with 1 injection than on those vaccinated after 20 days old with 2 injections (IRR=1.35, p=0.000). We detected the same trend, at the limit of the significance, for chickens vaccinated after 20 days old with only 1 injection (IRR=1.22, p=0.056). The housing system also influenced the level of the immune response of the seroconverted birds, with
scavenging birds having a lower HI mean titer than birds kept in a closed building all day long (IRR=0.78, p=0.007) (Table 4).

Table 4. Final zero-inflated Poisson model\(^1\) for the HI titres in chickens vaccinated 2 and 3 months previously (between 31 to 120 days post vaccination) (120 observations used)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Poisson regression(^2)</th>
<th>Inflated(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR (95% CI)</td>
<td>p value</td>
</tr>
<tr>
<td>Vaccination protocol</td>
<td>Chicken vaccinated after 20 days with 2 injections</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>Chicken vaccinated after 20 days with 1 injection</td>
<td>1.22 (0.99-1.50)</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>Chicken vaccinated before 20 days with 1 injection</td>
<td>1.35 (1.17-1.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Housing system</td>
<td>Birds in a closed building all day long</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Birds with an outdoor closed pen</td>
<td>0.95 (0.76-1.19)</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>Scavenging birds</td>
<td>0.78 (0.65-0.93)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

\(^1\) Vuong test of a zero-inflated Poisson versus a standard Poisson model: z = 5.77, Pr>z = 0.0000  
\(^2\) Modeling the ratio of the HI titre mean  
\(^3\) Modeling probability of zero titre

Determinants of the immunogenicity in vaccinated ducks

Only ducks of the Super Egg breed were represented in the population of ducks sampled at 2 or 3 months post-vaccination. The only factor differentiating the probability for a Super Egg duck of having a zero HI titer at 2 and 3 months post-vaccination was the size of the flocks (the two categories with the higher number of ducks had a lower probability of getting a zero titer than the category with the lowest number of ducks per flock) (Table 5). Regarding the variables influencing the HI titer of the seroconverted birds, we found an
effect of the vaccination protocol with a lower mean HI titer on the birds vaccinated after 20 days with 1 injection than on those vaccinated after 20 days old with 2 injections. We did not detect any significant difference in the mean HI titer between the ducks vaccinated with 2 injections before or after 20 days old. Because none of the sampled birds had been vaccinated with only one injection before 20 days of age, we could not assess the performance of this protocol. We also detected an influence of the size of the flock, with birds from large flocks having a mean HI titer lower than birds from small flocks (Table 5).

Table 5. Final zero-inflated Poisson model\textsuperscript{1} for the HI titres in Super Egg ducks vaccinated since 2 and 3 months (139 observations)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Poisson regression\textsuperscript{2}</th>
<th>Inflated\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Vaccination protocol</td>
<td>Duck vaccinated after 20 days with 2 injections</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td></td>
<td>Duck vaccinated after 20 days with 1 injection</td>
<td>0.76 (0.62-0.95)</td>
<td>2.01 (0.77-5.23)</td>
</tr>
<tr>
<td></td>
<td>Duck vaccinated before 20 days with 2 injections</td>
<td>0.96 (0.83-1.12)</td>
<td>1.32 (0.33-5.24)</td>
</tr>
<tr>
<td>Number of poultry in the flock</td>
<td>≤ 150 birds</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td></td>
<td>Between 150 and 250</td>
<td>0.88 (0.69-1.11)</td>
<td>0.03 (0.01-0.09)</td>
</tr>
<tr>
<td></td>
<td>More than 250</td>
<td>0.73 (0.59-0.90)</td>
<td>0.18 (0.08-0.40)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Vuong test of zip vs. standard Poisson: z = 9.18, Pr>z = 0.0000
\textsuperscript{2} Modeling the ratio of the HI titre mean
\textsuperscript{3} Modeling probability of zero titre
8.3.8. SEROLOGICAL EVIDENCE OF EXPOSURE TO THE H5N1 VIRUS IN NON-VACCINATED POULTRY

The overall seroprevalence for the non vaccinated poultry corrected by the cluster effect and the sampling weights was 10.3% (Table 3). Its variation over the study period is presented in Figure 3. The species-specific seroprevalence was 10.6% for non vaccinated chickens (95% CI: 6.6-15.2, n=986), 13.4% for ducks (95% CI: 0.4-26.7, n=608) and 6.5% for Muscovy ducks (95% CI: 0.7-12.3, n=946).

The prevalence at flock level (one flock being positive if at least one bird was seropositive at the defined cut-off value) was 20.6% (95% CI: 14.3-27, n=160). The species specific flock seroprevalence was 27.4% for chickens (95% CI: 14.8-40.1, n=51), 25.6% for ducks (95% CI: 12.3-40.1, n=42) and 12.1% for Muscovy ducks (95% CI: 4.1-20.2, n=66). Only of them had been declared having experienced mortality in the month before sampling.

8.3.9. DETERMINANTS OF THE SEROPOSITIVITY IN THE NON-VACCINATED BIRDS

The variables having a significant effect on the seroconversion of the non-vaccinated animals were 1) the age: the probability of seroconversion increased with age (OR=1.15 for a 30 days increase in age, p-value=0.000); 2) the poultry category: duck layer or breeder and backyard muscovy duck had a higher probability of seroconversion compared to chicken layer or breeder (respectively OR=14.67, p-value=0.026 and OR=28.12, p-value=0.081); 3) the number of layer-breeder duck flocks in the village at the time of sampling: higher probability of seroconversion was observed when the number of layer or breeder duck flocks in the village was medium than when this number was low (OR= 5.59, p-value=0.019); 4) the presence of at least one duck broiler flock in the village at the time of sampling (OR=5.38, p-value=0.010); 5) the immunity coverage of the poultry declared vaccinated in the commune during the same sampling campaign: having between 50% to
70% of the vaccinated poultry in the commune above the defined positive H5 HI antibody titers decreased the probability of seroconversion of non-vaccinated birds (OR=0.01, p-value=0.000) and 6) the time period: the probability of seroconversion was higher in June 2009 and June 2010 than in December-January 2009 just before the Têt celebration (OR=7.39, p-value=0.015 and OR=4.62, p-value=0.042 respectively). To a lesser extent, higher numbers of birds in the flock from which birds were sampled increased the probability of seroconversion (OR=1.005, p-value=0.063) (Table 6).
Table 6. Final random-effect logistic model for the seroconversion of the non-vaccinated birds (2124 observations)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry age</td>
<td>Continuous variable</td>
<td>1.005 (1.00-1.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Poultry category</td>
<td>Chicken layer-breeder ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken broiler</td>
<td>2.20 (0.18-26.44)</td>
<td>0.535</td>
</tr>
<tr>
<td></td>
<td>Chicken backyard</td>
<td>9.90 (0.56-174.50)</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>Duck layer-breeder</td>
<td>14.67 (1.38-155.28)</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Duck broiler</td>
<td>0.40 (0.02-7.40)</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td>Duck backyard</td>
<td>0.94 (0.04-21.68)</td>
<td>0.968</td>
</tr>
<tr>
<td></td>
<td>Muscovy duck layer-breeder</td>
<td>7.66 (0.62-95.14)</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>Muscovy duck broiler</td>
<td>1.32 (1.0-10^-9)</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Muscovy backyard</td>
<td>28.12 (0.66-1198.63)</td>
<td>0.081</td>
</tr>
<tr>
<td>Number of poultry within the flock</td>
<td>No duck layer-breeder flock ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between 1 and 5</td>
<td>5.29 (1.32-21.22)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>More than 5 flocks</td>
<td>0.31 (0.05-2.02)</td>
<td>0.225</td>
</tr>
<tr>
<td>Presence of at least one duck broiler flock in the village one month before sampling</td>
<td>Yes</td>
<td>5.38 (1.50-19.26)</td>
<td>0.010</td>
</tr>
<tr>
<td>H5N1 immunity level of the vaccinated birds at commune level at the sampling time</td>
<td>≤ 50% ref</td>
<td>0.01 (0.001-0.090)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>≥ 70%</td>
<td>0.84 (0.07-9.99)</td>
<td>0.893</td>
</tr>
<tr>
<td></td>
<td>Before 2009 Têt celebration (Dec 08 – Jan 09) ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 2009 Têt celebration (Mar 09)</td>
<td>1.03 (0.21-5.09)</td>
<td>0.972</td>
</tr>
<tr>
<td></td>
<td>During 2009 high duck broiler production season (June 09)</td>
<td>7.39 (1.47-37.03)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>During 2009 high duck broiler production season (June 10)</td>
<td>4.62 (1.06-20.21)</td>
<td>0.042</td>
</tr>
<tr>
<td>Intra-cluster (intra-flock) correlation</td>
<td></td>
<td>0.51* (0.36-0.66)</td>
<td></td>
</tr>
</tbody>
</table>

*Likelihood-ratio test of rho=0: p=0.000
8.4. DISCUSSION

Our results highlight the difficulties in maintaining good flock immunity all year long in poultry populations from Northern Vietnam using an inactivated H5N1 vaccine. The results also provide an evaluation of the vaccination implementation and Re-1 vaccine immunogenicity under field conditions. We were able to detect some determinants of immunogenicity of this vaccine for chickens and ducks respectively. Finally, the assessment of vaccine effectiveness in the field demonstrated that vaccination levels being achieved did not prevent circulation of virus in co-existing non vaccinated poultry.

The limitations of the study in terms of methodology and the limitations and issues associated with the existing vaccination program are discussed before conclusions are drawn.

Limitations of the virological testing
According to the virus titers shed in H5N1 infected non vaccinated chickens and ducks from various experimental studies (at least $10^2$ EID$_{50}$/0.1ml of virus) (Pfeiffer et al., 2010; Tian et al., 2005) it is likely that a swab from one infected non vaccinated bird in a pool of swabs from 2-3 birds would still give a positive A RT-PCR result. However, Pfeiffer et al. (Pfeiffer et al., 2010) showed that viral excretion in birds vaccinated with Re-1 vaccine may be as short as 3 days post challenge, making the window for detection of circulating H5N1 virus very short for the vaccinated population.

Bias related to the vaccination status
Bias related to the reported vaccination status of the birds was suspected and avoided by developing an adapted case definition at flock level, with a vaccinated flock being defined as a flock reported vaccinated with at least one seropositive bird.
Measure of the serological response using a clade 1 H5 HA antigen
Cross reactivity between the clade 1 antigen used in the HI test and the antibodies induced by a clade 0 vaccine antigen is expected to be good. Indeed, clade 1 was found to be a good antigen for detection of HI antibody responses (Kim et al., 2008). Previous studies at the Agriculture Fisheries and Conservation Department in Hong Kong also confirmed this good cross-reactivity (Trevor Ellis, personal communication). Nevertheless, we may suspect that an HI test in those conditions may result in slightly lower measured HI antibody titers (Tian et al., 2010).

Relationship between HI titer and protection
In our study we considered cut-off values for the HI titers to define seropositivity as a result of prior infection or significant vaccination responses, but the data did not allow us to define a HI titer that would provide protection for clinical disease or infection. Nevertheless, the literature gives us an indication of the correlation between HI titer and protection. Pfeiffer et al (Pfeiffer et al., 2010) found that immunity induced by Re-1 vaccine measured with homologous antigen was correlated to protection in chickens. In the same trial, the virus shedding duration in vaccinated ducks was found to be correlated to the HI titer. Another study suggests that a clade 2.3.4 virus vaccine may not induce substantial HI antibody responses in ducks, even measured with homologous antigen, although the vaccine provided complete protection against H5N1 challenge (Kim et al., 2008). However, the Re-1 vaccine has been shown to induce substantial HI antibody responses in ducks and chickens (Tian et al., 2010).

Limits of the vaccination strategy to confer a high herd immunity level
The seroprevalence measured by the presence of HI H5 antibodies over a defined positive cutoff on a random sample of our studied population was below 30% for all the sampling campaigns. This measure is actually an overestimate of the acquired immunity due to vaccination since among the seropositive birds some immune responses, especially in waterfowl, are likely to be due to natural infection. Nevertheless, this immunity level is far
below the expected herd immunity threshold following a bi-annual mass vaccination campaign and the previous estimations made in Vietnam (Taylor and Dung, 2007). Several factors may contribute and explain this low immunity level.

Firstly, because of the high population turnover in poultry production systems, a bi-annual vaccination campaign does not allow the vaccination of all the birds. Indeed, the farmer reports reveal lower vaccination coverage for the two sampling dates between the main vaccination campaigns and, especially in March, just before the second main vaccination campaign. The lower seroprevalence in broiler poultry at that time is also probably a consequence of the low frequency of vaccination sessions relatively to the turnover rate of the poultry population. Broilers are produced within a 3 to 4 month period, and some flocks are not vaccinated if started just after a vaccination campaign. If the bi-annual vaccination campaign was strictly followed by one of the two provinces under study, flocks were vaccinated between the campaigns in the second province. Nevertheless, we did not detect a significant difference between these two provinces in the odds of a bird being seropositive.

Secondly, this limited population immunity level can also be explained by the post-vaccination drop in H5 HI antibody titers below the positive cutoff value observed in the present study (Fig 5 and 6). The antibody kinetics shows that under field conditions, without sufficient booster doses, it is not possible to keep a good immunity level with inactivated vaccines for more than a few months. Even if the antibody kinetic curves don't take into account the differences in vaccination protocol that may influence the level of the seroconversion, they give a good indication of the mean population HI titers in the months following vaccination. The questionnaires to the commune veterinarians also revealed that poultry are not vaccinated during their laying period and that all inoculations are given before the point-of-lay. Thus, the layer and breeder poultry flocks probably have H5 HI antibody titers below the optimum protective level during most of their laying period.
Finally, our results also indicate that preventable failures in the vaccination implementation probably occur. Indeed, when estimating the seroconversion at flock level, we observed that only a limited percentage of the birds from a vaccinated flock showed a positive serological titer. Even in the vaccinated flocks with at least one seropositive bird the seroprevalence was still below the expected target prevalence or herd immunity threshold (60 to 80%) needed in a vaccinated flock to prevent an outbreak (Bouma et al., 2009; Rudolf et al., 2010). This indicates that the level of vaccine failure was substantially greater than the intrinsic, non-preventable, primary vaccine failure rate and that all possible causes of preventable vaccine failures have to be sought (Chen and Orenstein, 1996): for instance, problems with the cold chain that could have a direct consequence on the effect of the vaccine, or wrong injection technique or incorrect dosage that could lead to birds not receiving the appropriate amount of antigen.

**Effect of the vaccination protocol and farming system on the vaccine immunogenicity**

The level of antibody responses in vaccinated birds at approximately one month post-vaccination, calculated as mean HI titers, is in accordance with results obtained in clinical trials using a heterologous virus as HI antigen, 3 weeks post vaccination in chickens (Tian et al., 2010). The observation of lower mean HI titers in ducks as compared to chickens is also in accordance with previous reports (Pfeiffer et al., 2010). The investigation of the determinants of vaccine immunogenicity highlights an important effect of the age at first injection in chickens. Indeed, a protocol using only one injection before 20 days of age greatly impaired the chance of getting an immune response compared to a protocol using 2 injections after 20 days of age. We did not observe any differences in the odds of getting a HI zero titer between protocols with one or two injections for birds vaccinated only after 20 days of age. The inhibition of antibody induction in young chickens might be explained by the presence of maternal antibodies in those birds as recently described under experimental conditions (Maas et al., 2011). On the other hand, we did not detect an effect of the vaccination protocol on the probability of seroconversion for ducks, but we
observed lower mean HI titers for seroconverted ducks with 1 injection after 20 days when compared to 2 injections.

We also demonstrated that farming management may influence effective immunization by this vaccine. In chickens, we found that scavenging birds with an immune response had a lower mean HI titer than birds kept all day long in a closed building. In scavenging birds, possibly submitted to higher microbial pressure than birds in a closed building, the vaccine specific immune responses may suffer from competition as demonstrated previously (Ellis et al., 2006; Hao et al., 2008).

In ducks, flock size influenced both the chance of being a non-responder and the mean HI titer of the seropositive birds. Thus, bigger flocks were less at risk of having poor HI antibody responders than smaller flocks. This might be due to a higher technical capacity of farmers in the vaccination implementation that led to less frequent preventable vaccine failure. However, the relationship between antibody production and flock size was not linear with the highest mean HI titer observed in large flocks but not in the largest ones. More intensive management practices for the largest flocks may induce more stress for the birds and, as a consequence, a lower level of immunological response.

Evaluation of the vaccine effectiveness

Based on the H5 HI antibody seroprevalence and antibody titers under field conditions, we demonstrated deficiencies in the vaccination strategy in Northern Vietnam and we have tried to analyse reasons for this. However, because our study area and period were limited, our conclusions about the vaccine effectiveness or the efficacy of the vaccination strategy cannot be directly generalized to other contexts. Furthermore, understanding the link between the population immunity level and the vaccine effectiveness is a challenging task that requires assessing the direct (protection of the vaccinated population) and indirect (protection of the non-vaccinated population by the vaccinated population) effects of immunization (Chen and Orenstein, 1996; Committee for proprietary medicinal
products, 1997). Moreover, vaccine effectiveness is dependent on the vaccine efficacy but also on the conditions under which the vaccine is used and the characteristics of the target population.

The virological investigations and serological monitoring of non vaccinated birds gives some indications of the level of protection of the non vaccinated birds by the vaccinated population. In the study area suspect positive results for H5 by PCR testing were found on only two vaccinated farms. Those results do not exclude the possibility of H5N1 virus circulation in vaccinated birds, but indicate that this circulation is probably at a low level and might be at the limit of detection. Furthermore, the virological results may have been biased by technical issues as explained above. On the other hand, we have detected strong H5 PCR positive pools from healthy non vaccinated ducks, confirming again the potential role of H5N1 virus reservoirs in waterfowl (Pantin-Jackwood and Swayne, 2007; Perkins.L.E and Swayne.D.E, 2002). Finally, this low virological prevalence measured on random samples at the farm level is in accordance with another study conducted in the southern part of Vietnam a year before (Henning et al., 2010). Because virological detection of H5N1 infection in poultry flocks not showing disease has some technical and logistical limitations, serological markers of infection are more useful to understand the epidemiology of H5N1 HPAI in non vaccinated populations. Whereas the presence of antibodies against H5N1 without clinical signs is common in waterfowl, it is less frequent in chickens. Nevertheless, low pathogenic H5N1 and H5N2 have been detected in Vietnam (DAH, personal communication) and might explain these observations together with possible false positive reactions (cross-reaction with another HA subtype).

The study of the determinants linked to H5 seropositivity in non vaccinated birds indicates that there was indirect protection of non vaccinated birds by immunization of the vaccinated birds. Indeed, if the vaccinated population showed seroprevalence levels of between 50 and 70%, the risk for a non-vaccinated bird of the same commune to be seropositive, that is to say, to have been exposed to the virus, significantly decreased
compared to situations where the seroprevalence level of the vaccinated population was below 50%. However, in our final multivariate model, we did not detect a similar effect for a population seroprevalence level over 70%. One hypothesis might be that having more birds clinically protected because of vaccination makes the detection of H5N1 HPAI virus circulation more difficult for farmers. Indeed, recent studies proved that birds vaccinated with the Re-1 vaccine and clinically protected upon challenge may shed virus (Pfeiffer et al., 2010; Tian et al., 2010). Even though the above mentioned studies reported quite different levels of virus shedding, both indicated that virus shedding is not fully prevented by the vaccination, even under optimal laboratory conditions. The virus shedding even persisted for as long as 11 days in ducks vaccinated with the Re-1 vaccine and challenged with a clade 2.3.4 virus (Pfeiffer et al., 2010). Furthermore, a modeling approach also demonstrated that the time taken to report outbreaks had increased in the period where vaccination was used compared to previous periods (Walker et al., 2010) giving support to the hypothesis of “silent spread” of infection in vaccinated birds (Savill. N. J et al., 2006). We also identified other determinants of seroconversion of non-vaccinated birds. We showed that non-vaccinated birds have more chance of being seropositive in June than in December-January. June is the period of the year at which the duck broiler population reaches its maximum size. The last big epidemic wave in the North in 2007 occurred during that period (Minh et al., 2009). Confirming the influence of the duck broiler population in supporting viral circulation, we found that the presence of at least one duck broiler flock in the village around the time of sampling significantly increased the risk for a non-vaccinated bird being seropositive. The number of layer or breeder duck flocks in the village seemed also to influence the level of seroconversion. A bird in a village with 1 to 5 duck layer or breeder flocks was 5.3 times more likely to be seropositive than a bird in a village without any layer or breeder duck flock. Finally, long cycle non-vaccinated duck (layer or breeder) had a higher chance of seroconversion compared to long cycle non-vaccinated chicken. Thus, non-vaccinated long cycle ducks (and to a lesser extent long
cycle non vaccinated Muscovy ducks) are involved in the maintenance of the virus in the poultry population. Moreover, non-vaccinated duck broilers probably contribute to virus dissemination because of their farming management, as they are allowed to scavenge all day long in rice fields. This may explain why seroconversion of non-vaccinated birds is more frequent when duck broilers are produced.

8.5. CONCLUSIONS

The study highlights the difficulties in maintaining good herd immunity all year long in poultry populations using an inactivated H5N1 vaccine in Northern Vietnam. Improvements might still be obtained by limiting the preventable vaccination failures and by optimizing and harmonizing the protocols being used separately for chickens and ducks. Our study provided insights into the epidemiology of the HPAI H5N1 virus within a vaccination context by providing indirect evidence that vaccinated populations with less than optimal levels of immunity can contribute to persistence of the virus within the poultry population. More precisely, we hypothesized that virus is maintained in non-vaccinated long cycle ducks (and to lesser extent long cycle non vaccinated Muscovy ducks) and that non-vaccinated duck broilers probably contribute to virus dissemination because of their farming management.

Acknowledgments

We thank the French Ministry of Foreign and European Affairs for funding the Gripavi project in the frame of which this work was done. We are grateful to the veterinary services of the 9 communes under study for their support for data collection, to Thomas Beuscart for his contribution to the data collection in 2010, to Mrs Pham Thi Thu Huyen for the data entry. We would like also to thank Lucas Leger from CIRAD for the fruitful exchanges on statistical analysis.
REFERENCES


This chapter is based on a study conducted in collaboration with a sociologist from CIRAD, and presented orally during the first International Conference on Animal Health Surveillance (ICAHS) in Lyon, France, in May 2011.

We have built this last chapter around a paper published in the proceedings of that conference (paper 4) but we have added 2 sections: a general introduction and perspectives. The introduction presents the analytical grid developed to interpret the results and to build the perspectives. Supplementary information is also provided in annex 11.
9.1. INTRODUCTION TO THE STUDY

9.1.1. THE “HUMAN” COMPONENT OF THE SURVEILLANCE SYSTEMS

Surveillance has become an essential tool for national and international sanitary governance. At the national level, most countries have adopted surveillance and control programs for animal diseases with the most significant impact on animal health, economy or public health. Farmers are sometimes forced by law to follow those programs.

At the international level, countries which are members of OIE (World Organization for Animal Health) are, following articles 1.1.2 and 1.1.3 of the Terrestrial Animal Health Code (Terrestrial Code) (OIE, 2010), obliged to notify any occurrence of listed diseases (OIE recommended listed diseases are provided in volume 2 of the Terrestrial Code) and also of an emerging health event.

National sanitary authorities rely on the participation of different actors, especially for reporting a suspect case of one of the diseases under surveillance and control. Among those actors, the farmers are the first responders of the animal disease surveillance and veterinarians are involved soon after. Thus, OIE adopted a resolution during its 76th general session supporting the participation of small farmers in animal health programs.

It has to be recognized that a farmer’s decision process to report or not report a disease or to participate in a preventive program might be complex. As a consequence, a low level of farmers’ participation, either into preventive control programs (application of biosecurity measures) or surveillance programs, became the subject for study by sociologists, animal disease health specialists (Casal et al., 2007; Elbers et al., 2010; Heffernan et al., 2008; Palmer et al., 2009) and plant disease specialists (Prete, 2008).

The levers that the sanitary authorities can use to improve farmers’ participation in a surveillance program seem to be quite limited. Two main systems are usually used:
• The regulation: Some diseases are listed as notifiable diseases and reporting suspect cases of those diseases by farmers and private veterinarians becomes not only a professional but also a legal responsibility.

• The incentives: Financial compensations are planned for farmers reporting a disease and to support losses incurred due to animal deaths or culling.

Apart from these two methods to “encourage” disease reporting by farmers, it has to be recognized that other factors contribute to a farmer’s decision to report or not report a disease.

Socio-anthropology may contribute to capture those factors.

9.1.2. APPLICATION OF SOCIO-ANTROPOLOGY TO THE SURVEILLANCE OF HPAI IN VIETNAM

A low level of reporting of HPAI suspect cases by small farmers has been identified as an issue in Vietnam by local experts and by FAO (FAO, 2011). HPAI is a notifiable disease in Vietnam and a compensation scheme has been adopted by the Government for poultry farmers having their birds culled.

In this study we attempted to address the issue of reporting among two communities of poultry farmers in Vietnam. We tackled the subject by trying to assess the interest of farmers for epidemiological information, and by describing the effective way this information was then used by farmers, private veterinarians and official veterinarians; that is to say to describe any possible information system (informal surveillance system) in place, apart from the formal surveillance system aimed at collecting and gathering data to the national level. We then tried to explore the benefit to the farmers if they had to use one or the other system. We tried also to explore the farmers’ reactions when facing a sanitary problem and finally to describe the trigger that each actor in the system used to either take action or report information to another link in the chain.
From this perspective, we consider the surveillance system on animal disease as a network of stakeholders, more or less strongly connected and with possible convergent or divergent interests. Capturing the nature of the information, the channel it uses to circulate, and the actions resulting from this information becomes a way to understand the underlying social structure and social norms.

Before to develop the study, we present in the following section the analytical grid developed to support the interpretation of the results.

9.1.3. ANALYTICAL GRID USED FOR INTERPRETATION OF THE RESULTS

Previous sociological works already provide us with a hypothesis about what may contribute to the decision process of a farmer to report or not report a suspect case of notifiable disease. Based on previous studies, an analytical grid was developed to assist with the interpretation of the study.

To interpret the observation we can consider:

- The farmer's awareness

One of the first aspects to consider is the level of awareness and understanding related to the disease under surveillance that the farmer may have. Indeed, it may be tempting to think that if the farmers really understand the importance of the disease and its prompt reporting, they would undoubtedly participate into the collective effort to monitor and control it. This approach, called in a study of Australian sheep farmers as an educative approach (Palmer et al., 2009) denotes a science-centred or risk-centred stance which does not allow for an alternative view, leading to accusations of arrogance and public alienation.

In Vietnam, most of the projects dedicated to the control and prevention of HPAI H5N1 have a farmer's or animal health worker's Information Education and Communication
(IEC) component. In 2006, a survey conducted to assess the training needs for poultry farmers and other actors in poultry production indicated that most of the farmers interviewed had a good knowledge of avian influenza in birds and human (National agricultural extention center, 2006).

In this context, it is interesting to ponder if the suspected low level of reporting is still a question of awareness?

- The farmer’s risk framing

Framing is a tool used to describe the way an event (disease) is perceived, named and handled (Rosenberg, 1992).

More broadly, the same event might not be framed as a problem for all stakeholders. Framing an event as a problem or even as a risk, reflects what people consider to be desirable or not, fair or unfair and can vary among different levels of society. In this perspective, differences in risk perception might not be considered as different levels of awareness but as a reflection of different values, interests, logics etc…(Rosenberg, 1992)

Again, if there is an important gap between farmer’s representation of the risk of the disease under surveillance and the effort the national authorities are requesting from him, the chances are high that the farmers do not really feel the necessity to report. More and more, studies have been conducted to explore the farmer’s risk perception as part of an attempt to better understand farmer’s behaviour (Casal et al., 2007; Elbers et al., 2010; Palmer et al., 2009), or to assess the reaction of a population exposed to a new situation (Barennes. H, July 2007). For instance, Elbers et al (2010) showed that Dutch farmers do not recognize LPAI to be a problem: “it is something created by politicians just to bother the farmers” explained one of the farmers interviewed. In those circumstances, it is difficult to achieve a high level of participation in the surveillance of LPAI. Those studies are sometimes conducted in order to plan “corrective” measures that may change farmers’
perception of the risk. But often, those measures do not fully consider the drivers of the farmers’ risk framing and may use the wrong levers. In the latter example, we might suspect that farmers do not really understand the animal and public health issues at stake in the surveillance of LPAI (e.g. circulation of an LPAI strain capable of mutation to become a HPAI strain). However, we may also consider this issue is too far from their daily routines and preoccupations and that some alternatives have to be found.

In the Vietnamese conditions, we can also expect that the public health issue related to the surveillance for H5N1 HPAI (risk of a human pandemic) is far removed from the day to day preoccupations and experience of farmers. How is asking farmers to support this issue of international concern important to them when other immediate risks are perceived to be much more likely? More generally, what are the components of the H5N1 risk framing for Vietnamese farmers? Do the farmers only consider the epizootic component of the risk of an H5N1 outbreak, or do they also consider the risk for public health or even the pandemic risk?

- Social incentive

Social incentive to report or not to report should not be disregarded. If we consider the surveillance systems as a network of stakeholders, we may also consider that the norms which guide the behaviour of people in their daily lives are also present in the way the surveillance system functions: what about social norms such as transparency, solidarity, collective and individual responsibility, prevention or precaution? The question here is to define what the social norms are for a group of farmers in relation to the fact of reporting or not reporting a disease. Recent studies have supported the idea that the social consequences of a disease notification by a farmer do carry weight in their behaviour. In Europe, the norm seems to be constructed around the fact of having or not having a disease. Elbers (2010) reported that a Dutch farmer reporting a case of LPAI believes that his or her personal image might be destroyed, whereas Heffernan (2008) reported that UK
cattle and sheep farmers consider that “good farmers keep a watchful eye and therefore do not suffer from endemic disease problems”.

On the one hand, Vietnam recently left collectivism for individual ownership, and private business seem to be very energetic and free of political constraints. On the other hand, political messages from the party are still intensively displayed through propaganda (mass organization, loudspeakers etc) and individuals may be under pressure to follow the national directives. Thus, it is difficult to foresee if reporting a suspect case of HPAI will be regarded as dishonour, as betrayal of neighbouring farmers who may suffer from preventive culling or as civic behaviour.

- Trust in the official services

Mistrust is described as a possible barrier for good participation of farmers in a national surveillance system. Elbers (2010) indicated that some Dutch farmers perceived state veterinarians as people with limited knowledge on animal disease control and thus were not encouraged to report their next possible case of LPAI. Furthermore, those farmers also believed that disease prevention measures launched by government bodies were not consistent and hence not fair. Palmer (2009), in support to Wynne (Wynne, 1992, 2006), also insisted that while a high level of mistrust persists, increased knowledge and understanding will not necessarily achieve the outcome animal health bodies desire and that messengers may be of more importance than the (scientific) content of the message.

Thus, it is of interest to HPAI control to assess the trust that Vietnamese poultry farmers have in state veterinarians and the general HPAI control policies. The following paper describes the results of a study to identify aspects of farmers’ understanding or knowledge that might influence their behaviour.
9.2. FORMAL AND INFORMAL SURVEILLANCE SYSTEMS: HOW TO BUILD BRIDGES?

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**Keywords:**  
Surveillance, influenza, socio-anthropology, Vietnam

**Summary**

Within the framework of highly pathogenic avian influenza (HPAI) surveillance in Vietnam, a number of interviews were carried out with poultry breeders and local animal health operators in 2 communes of the Red River Delta (RRD) with a view to documenting the circulation of sanitary information concerning poultry (content of the information; method, scope and speed of circulation; actors involved; actions triggered as a result of the information received; and the economic and social incentives for disseminating or withholding information. The main results demonstrate that (1) active “informal” surveillance networks exist, (2) the alert levels vary and the measures applied by the breeders are myriad and often far-removed from the official recommendations and (3) the commune veterinarian represents an interface between the formal and informal systems.
9.2.1. INTRODUCTION

Against a backdrop of growing emergence or re-emergence of sanitary problems, surveillance has become an essential tool of international sanitary governance: “without well-functioning surveillance and reporting systems, we are stuck” declared Dr D. Nabarro, United Nations System Influenza Coordinator (1), in 2009. In the case of animal health, numerous problems are associated to the low level of breeders’ participation in the surveillance networks and their reluctance to implement recommended biosafety measures (2, 3). We thus occasionally call on the social sciences to explain this fact based on individual perceptions and local cultures. These disciplines are nevertheless somewhat unwilling to be made the tools of the normative procedures underlying these calls and are reluctant to participate in the associated education projects (modifying perceptions by means of “awareness”) of social groups deemed to be poor implementers of strategies defined by the actors of the public area (veterinary services, international community etc. in the present case).

The study presented here is the result of collaboration between the fields of socio-anthropology and epidemiology. Socio-anthropology, as reflected by the works of J.-P. Darré (4) is called upon initially to identify the operators’ practices and rules governing these practices and to understand the specific rationales underlying them. In the context of the present study, it is a question of analysing the dynamics at work to assess and confront the sanitary risks in a community of breeders. Particular attention is paid to the role of sanitary information produced and circulating locally. These results are then discussed from an epidemiological standpoint: comparing the reasoning of the breeders with the rationales of the parties responsible for implementing national or international surveillance networks.

In Vietnam, at present, the breeders have to declare cases of HPAI (as well as cases of porcine reproductive and respiratory syndrome – PRRS – and foot and mouth disease).
These declarations must be made to the commune veterinarian who then refers them to the local authorities, the communal People's Committee. From the committee, the information has to be sent to the district authorities, and then to the provincial authorities and finally to the Ministry of Agriculture. Theoretically, confirmation of the existence of one of these diseases leads to the zone being placed in quarantine and the animals may be culled. This action is accompanied by compensation measures, officially variable over time and place, and for which operational implementation is somewhat unclear.

9.2.2. MATERIALS AND METHODS

Our study examines two communities of breeders on the front line of the fight against the emergence of sanitary problems: the poultry breeders of two communes in the RRD in Vietnam facing HPAI outbreaks.

The choice of the communes studied was dictated both by the importance of poultry breeding in the local production systems and by familiarity acquired with farmers and local authorities during previous research works. These two communes will be referred to as A and B. Commune A, highly specialised in poultry breeding, is located in one of the provinces early and seriously affected by the H5N1 virus when it appeared in Vietnam in 2003 and 2004. However, since then no outbreak has officially been declared in this province. In the province where commune B is located, outbreaks have regularly been declared during the subsequent epidemic waves.

The breeders in these communes breed poultry (chickens, ducks and Muscovy ducks) by combining different production systems (meat, eggs and chicks). While certain breeders have relatively "large" farms in the local context (more than 500 heads), the vast majority of breeders work on a more limited scale (100-400 heads). We eliminated from our study families with only a small number of poultry primarily intended for home consumption.
In 2010, we interviewed 19 breeders as well as commune veterinarians (private veterinarians with a public mission) and veterinary drug sellers working in the areas concerned.

The interviews dealt with the circulation of sanitary information concerning poultry: content of the information; method, scope and speed of circulation; actors involved; actions triggered as a result of the information received; the economic and social incentives for disseminating or withholding information and for treating animals; the role of the veterinarians, etc. The interviews were recorded and a written interview sheet was produced for each interview.

### 9.2.3 RESULTS

**Active “informal” surveillance networks**

The first observation from our interviews is that an informal sanitary information network exists. The information circulating within this network concerns the symptoms observed on different farms (mortality, diarrhoea, etc.); it does not relate exclusively to poultry but also to pigs, common in this area. It also includes technical economic information (prices of animals and inputs, breeding techniques, etc.). It is shared between neighbours and parents, on markets and during encounters with other breeders in the veterinary drug store. According to the breeders, the volume of sanitary information circulating since the appearance of avian influenza has increased.

What we call here the breeders’ epidemiological territory (which we define as the radius within which the information is considered useful by the breeder and may trigger the implementation of measures on his own farm) is nevertheless limited (from 500 m to 3 km). The information relating to more remote farms, which nevertheless share the same stakeholders for feed or chicks supply, do not seem relevant by the breeders interviewed, showing that they consider the disease dissemination more by proximity than by the value chain.
The breeders claim to be satisfied by this informal network (nature, scope, speed, reliability). They judge the information issued from this network more useful than that disseminated by the veterinary services by the loudspeakers placed in residential areas and through the intermediary of the commune veterinarians because it is considered to arrive late and to be too general in nature.

It is interesting to note that the breeders clearly distinguish two types of information: (a) information relating to common diseases (for example Newcastle Disease, Ga Ru and Gumboro Disease, Gum), which the breeders feel they can control (even if they cause numerous deaths) and (b) information concerning new diseases or symptoms with regard to which the breeders feel powerless to act. PRRS falls into this second category. However, while HPAI belongs to this category in commune B, this is not the case in commune A. How can this be explained?

A variable alert level and differing measures, often far-removed from the official recommendations.

In commune A, breeders mention frequent cases of avian influenza among their entourage. These events would appear to be a part of the breeders’ routine; they believe that they are capable both of clearly identifying HPAI cases (in particular due to the speed at which mortalities occur) and of coping with them. However, the criteria used to identify the disease vary considerably from one person to the next. There is no fear of possible consequences for human health and the measures taken by the breeders are essentially aimed at protecting the health of their animals and limiting economic losses: the breeders can thus decide to anticipate the date of the booster vaccination against avian influenza (the poultry vaccination seems to be common practice except in backyard farms), to increase disinfection measures in the poultry pens and their immediate surroundings and to limit their own movements. The animals can also be given vitamins and various supplements. However, this information can also trigger destocking measures if the animals have a commercial value: to avoid potential losses, the farmers sell broilers close
to their sale weight or laying hens close to the end of their production life. Animals which are already infected or dead are often sold (to the usual collectors) even if the prices are very low. We thus see that numerous measures are taken by the breeders (and that, in their own way, they act as risk managers), but that the main measure officially recommended is not mentioned, i.e. report to the commune veterinarian. According to the breeders themselves, they feel confident that they can manage this situation, : “with experience; we have succeeded until now in controlling the extent of the epidemic with outbreaks here and there, so there is no need to inform the district or the province” explained one breeder. This is even more so the case as they consider the public sector veterinarians (including the commune veterinarian) to be incompetent. On the other hand, the breeders are more willing to consult veterinarians in the private sector who give them medicines and advice. Furthermore, there is nothing to indicate that the breeders concerned are trying to evade administrative authority or social control by hiding sanitary events. This is supported by two facts: first because, in their own words, it is important for breeders to provide each other with information in order to be protected and, in any case, it would be impossible to hide a massive number of animal deaths in the context of very close living conditions of Vietnamese villages. Second, because these cases only rarely result in the implementation of restrictive measures by the authorities.

In commune B, however, breeders indicate no cases of avian influenza other than the last cases officially declared in 2007. The breeders therefore have only a very limited experience which would explain why avian influenza is referred to as a new disease which is dangerous to people and with regard to which breeders feel powerless to act. The breeders state that in the event of new cases, they would immediately inform the commune veterinarian as they would not know what to do.
The commune veterinarian, an interface between the formal and informal systems

Despite apparently playing a limited role in the local information networks, the commune veterinarians nevertheless claim to be well informed of the sanitary situation of the farms, in particular via the drug sellers who are at the heart of the information circulating within the commune and have no problem about sharing the information. So why are there not more control measures or official declaration in this commune? In all probability, it is the result of economic considerations as the province is an important source of poultry and chicks for the capital Hanoi and the Northern provinces. The drug seller admits that it is important to give the breeders the chance to sell their animals before taking the matter to the next level. Similarly, the People’s Committee would also appear to exercise its own judgement concerning the speed at which the information is to be communicated in the official network. Furthermore, while the breeders claimed several times to be sure of their own diagnoses, the commune veterinarians pointed to the fear to launch a false alarm which would discredit them in the eyes of their superiors.

It can therefore be seen that the logic of the commune veterinarian, and probably of the local authorities as well, is primarily to temporise. This does not enter into conflict with the rationale of the breeders. In this way, the commune veterinarian has found a compromise between the position of the breeders and the demands of the official system, acting as an interface between the two.

9.2.4. DISCUSSION

From an epidemiological point of view, if we consider the objective of monitoring and controlling the disease, the situation described reveals numerous obstacles to a fully operational national HPAI surveillance system in a context where the disease has become endemic.
From the point of view of surveillance, the cases recognised as HPAI would appear to take varying forms depending on the actors and their experience. It would appear that the breeders keep a case definition close to the outbreaks experienced before the vaccination starts, involving massive and sudden mortalities, and cannot imagine that the disease can take a different form among a partially immunised population. The epidemiology of the disease therefore changes more quickly than the knowledge of local breeders. Similarly, in a national context which aims to identify and index every case, the logical strategy would be to adopt a sufficiently sensitive case definition. However, at local level, key actors – the commune veterinarians – only trigger an alert when they are absolutely sure of their clinical diagnosis, which can nevertheless prove to be problematic for this disease in certain contexts.

From the point of view of control, a local body of knowledge was quickly created within this breeders’ community focussing on the recognition and monitoring of outbreaks of what, rightly or wrongly, they associate to HPAI. This knowledge, which we could compare to that of the experts in order to assess its real efficiency, corresponds to a means of managing an endemic disease. This is out of step with the crisis management approach still applied by the government, in particular in response to pressure from the international community (5). This discrepancy between control policy, the current epidemiology of the disease in certain areas and the vision of the local actors hampers the constitution of expert knowledge, primarily because the sanitary information relating to this disease remains sensitive.

If the breeders do not necessarily see any interest in declaring cases as they feel confident in their management approach, do they nevertheless feel any obligation? The legal framework governing the incentive or obligation to report suspected cases of regulated diseases is a pivotal question in a surveillance system. In the case of a commune where the disease is no longer exceptional, the only incentive to declare a case would appear to be the social incentive to inform neighbours so that they can protect themselves. It is rarely a
question of a legal obligation. While it exists and is recognised (the breeders know that they are supposed to inform the commune veterinarian), the regulatory incentive framework is ineffective. However, in the case of commune B where the disease is still an exceptional occurrence and the breeders have yet to learn how to manage it themselves, the commune veterinarian would appear to be the favoured contact partner to whom they turn. Consequently, while the surveillance system is based on the declaration of specific diseases or syndromes, the breeders identify levels of “seriousness” and “loss of control” which justify recourse to the commune veterinarian and thus to the official system.

Finally, the local objectives do not always appear to correspond to the national objectives of the surveillance and control system. Locally, it would seem that a balance between the economic interests of the commune and the control of the disease is reached. The objective being to keep the disease to a level considered to be acceptable by the operators. Our study was unable to clearly identify this level, although it would appear to correspond to outbreaks capable of causing high mortality rates but the progression of which is contained or diminished. At the central level, an accurate estimation of the disease prevalence throughout the entire territory is a key element for the assessment of control policies. However, local management of cases using criteria defined locally gives a biased vision of the real epidemiological situation.

In conclusion, the commune veterinarians, who represent the interface of the two systems, must therefore reconcile the technical demands of the ministry which they represent with the political and economic requirements of the local authority (under whose direct control they fall) and with the individual rationales of the breeders. As repositories of valuable sanitary information, they should be given more responsibility in their role by their technical superiors while following a more comprehensive professional training with a view to increasing their legitimacy vis-à-vis the local operators.
With regard to the breeders it would appear necessary to accompany them in redefining the risk, in particular by providing them with more information concerning the sanitary risk linked to the value chains. This could thereby extend their epidemiological territory and the number of operators to whom, professionally speaking, they feel committed.

References


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9.3. PERSPECTIVE ARISING FROM THE STUDY: HOW TO BUILD BRIDGES?

9.3.1. REDUCE THE GAP BETWEEN EXPERIENCE-BASED VERSUS PANDEMIC-BASED POLICY BY WORKING AT ACHIEVING AN EPIDEMIOLOGICAL CONSENSUS

This study demonstrated the existence of a gap between the experience-based knowledge of farmers facing recurrent outbreaks of what they consider, rightly or wrongly, to be H5N1, and the scientific-based knowledge, which underlies the national surveillance and control policies. The experience-based knowledge of farmers from one of the communes studied was built after seven years of regular exposure to the disease. If, during the first waves, farmers were totally helpless, they gradually improved their capacity to protect their flocks against the disease and thus to limit the extent of an outbreak. Listed in the paper are the different actions that farmers adopt to limit the spread of disease. Disinfection of the poultry pens and their surroundings, anticipation of the date of the booster vaccination and limitation of the movements are probably among the most efficient measures to prevent a flock from infection, and farmers now consider H5N1 to be an endemic disease, in the same way as Newcastle Disease. Furthermore, since the number of human deaths was much lower than initially expected and announced, and since not all places with H5N1 poultry cases experienced human deaths, H5N1 has lost its ‘exceptionally dangerous’ nature and is being “domesticated”.

Nevertheless, the national policy was backed up by scientific-based knowledge dominated principally by the threat of a human pandemic. If Vietnam ranks second among the top ten donor-recipient countries, with 115 million dollars in total avian influenza-related aid commitments from foreign donors (Vu, 2009), it is because of the strong central commitment to fight the disease according to recognised international standards. The strategy for response to the disease was mainly produced by experts and researchers in animal health, epidemiology, human health, communication and agronomy, with a
predominance of animal health experts, as explained by Vu (2009) in his detailed description of the Vietnamese response to avian influenza. Thus, these national policies to combat H5N1 were influenced and supported by the international agencies and legitimized by the seriousness of the threat for human health. Little space was given to public debate and, if government offered many opportunities for discussion with international agencies and experts, farmers, businesses and consumers had no, or very limited, opportunities to express their views.

Therefore, it is not surprising that national policy is neither understood nor accepted by farmers, who have an intimate experience of the disease far removed from the international and public health dimensions of the problem given by the central Government. Thus there is a need to reach an epidemiological consensus around this disease. Probably both participants should move on to a new position as the next step forward. Government has already backpedalled on the culling policy by relaxing the obligation to cull birds in a 3 km-radius around an outbreak, instead opting for culling of only the infected flock. Nevertheless, the original policy was still being implemented in 2007 and farmers may not yet have realized that a change has been made. In addition, there should be also a change in the nature of the Government’s communication to farmers to take more consideration of their problems when facing disease in their flocks than for international public health issues which are only theoretical for poultry farmers in Vietnam, as shown in the study of surveillance for LPAI among Dutch poultry farmers (Elbers et al., 2010). On the other hand, farmers should also consider that their experience-based knowledge does not always give them a complete picture of the situation and they should consider the risk posed to public health and animal health when selling sick or dead birds.
9.3.2. ENHANCE TRUST INTO INSTITUTIONS

The interviews also showed that farmers do not trust the national compensation policy for HPAI.

Farmer 6 « it does not change anything to report to the commune vet or not. If we report, we do not have compensation, so farmers prefer to sell to get some money »

Farmers had a bad experience about the way this policy was implemented after the first disease waves (FAO, 2007). Even if the Government recognized those problems and tried to improve the situation, the farmers were left with memories of their first bad experience. Thus, it is a Government responsibility to better communicate about this new compensation policy, to test and promote its smooth implementation to farmers and to advocate for the global economical benefit to declare and be compensated compared to having losses and being obliged to sell remaining live poultry at low prices.

A higher transparency on the HPAI control policy may also contribute to limiting farmers’ uncertainties about what is going to be done and how, in the case of notification.

9.3.3. STRENGTHEN CREDIBILITY AND RESPONSIBILITY OF THE ACTORS AT THE INTERFACE

Stronger technical background for the commune veterinarian

Limited credibility of commune veterinarians in regards to poultry health issues was a recurrent element in the farmer’s discussions. Thus, commune veterinarians are not seen to be by farmers of any assistance when they are facing poultry diseases. Farmers do not see any advantage in contacting them and even they consider that commune veterinarians do not have the necessary qualification to help them. Thus, it was demonstrated that trust and credibility are crucial in the relationship between different groups for a message to really be effective (Wynne, 1992). Village and commune veterinarians should be given better training so that they become integral to the control of diseases spreading in their
areas. In order to support them in their relationship to the farmers, they could for instance be given the responsibility to propose a rapid diagnosis tool once a suspected case has been reported. Elbers (2010) reported the Dutch experience regarding the surveillance of LPAI. By separating the diagnosis of a suspect case from the notification and the isolation of the farm (that normally occurred after a suspicion was reported), they encouraged farmers to report more suspect cases. They also proposed that diagnostic testing was free of charge for farmers. In one of our communes, we knew from previous field work that rapid AI tests were sometimes used by the veterinary services in case where disease was suspected. We do not know if this practice is still being used and we do not have the details of its use. Nevertheless, a good use of rapid tests may help to better control the spread of the disease by ruling in or ruling out a suspicion of H5N1 in a farm at the very early stage. Providing such a service to farmers might encourage them to report and to report sooner. Indeed, if the suspicion is ruled out, farmers would know they may have a chance to treat the flock (if vaccination against other viral diseases has been performed) and thus, they do not need to sell the flock before the end of its production cycle. If ruled in, farmers should have the guarantee of rapid compensation after the birds have been culled.

To enhance their credibility, veterinarians should also be encouraged and supported to relay farmers’ dissatisfaction or constraints to higher technical and political levels.

**Better use of the available sanitary information by the commune veterinarian**

As already demonstrated, commune veterinarians are repositories of valuable sanitary information. Unfortunately, it appears that the use they make of this information is not optimal. Indeed, they react only when the situation is out of control for the farmers as illustrated by the following 2 declarations:
Farmer 6 « Generally, the only useful source of information is the one circulating between farmers; the veterinarians and the loudspeakers are always late. When the information is given in the loudspeakers, it means the epidemic is already severe”

Village veterinarian of commune A « If we lose 60% of the poultry population in the village within 3-4 months, we do not report to the district, this is not a serious epidemic»

Thus, the commune veterinarian could be more pro-active by applying rapid preventive measures (distribution of disinfectants, announcements via loudspeakers, encouragement to vaccinate, advice about the best way to dispose of dead birds, etc.) as soon as a suspect case is identified. To do so, the sanitary information should lose its political nature and get back its technical nature, and commune veterinarians should be given more responsibility to act without the risk of retribution from the political hierarchy.

9.3.4. ASSIST FARMERS TO RE-FRAME THE DISEASE

We have seen that in a context where the disease became endemic, farmers had a framing of the disease that only recognized the local risk of transmission. Thus, even if farmers have a good awareness about the disease and experience in the way to protect their flocks, this knowledge and experience presents some limits. One specific element which may be missed in the way they handle the disease is the long distance transmission mode. Either when they buy a new flock, or when they sell sick birds, they do not seem to consider the risk of long distance transmission of the disease. Thus, farmers do not seem to be professionally committed, or linked to communities outside of their close neighbourhood. Working on an extension of their professional responsibility may make a difference compared to the general and basic messages usually spread by awareness campaigns.
9.3.5. COMMON CONSTRUCTION OF THE OBJECT UNDER SURVEILLANCE: HARMONIZATION AND ACCEPTATION OF THE H5N1 CASE DEFINITION

Another important issue is that nobody involved in the surveillance systems (both formal and informal) is looking at the same object. Again, farmers built their case-definition based on their early experience of the disease in 2004-2005. Commune veterinarians adopted a very cautious attitude by reporting to the formal system only when they are absolutely sure the outbreak is due to H5N1.

_Village veterinarian of commune B «When I am sure of the diagnosis, I inform, because we should not talk nonsense »_

Technically this situation is a weak link with regard to the national strategy for early detection of cases of H5N1. Furthermore, neither the farmers nor the commune veterinarians seem to acknowledge the possibility that an H5N1 case could only cause limited mortality (contrary to the clinical picture of the first waves) in a partially vaccinated population. Thus, there is a need for a common construction of what is being under surveillance: i.e. what should be the threshold level to report to the surveillance system?

This case definition should be in accordance with the control capabilities of the veterinary authorities. There is no need to set up a very sensitive case definition at the farm or village level if the official veterinary authorities do not have the human or financial resources to respond to every notification of suspected cases.

It is also important that commune, district or provincial authorities reporting a case are not blamed in the case of a misdiagnosis. Commune veterinarians seemed to dread more the consequences of a false alarm than the possibility that some cases were not identified. This behaviour is probably a consequence of the reaction of the central government that regularly blamed provincial services for not doing a good job. As an example, Tu, the
Deputy Minister in 2008, pointed out in 2010 that the provincial Government was the problem: “the epidemic has returned because many provinces are not focused and determined in spreading propaganda, preventing and fighting the disease”. Government should accept that controlling H5N1 outbreaks is very demanding and blaming provinces for reporting of cases that are subsequently proven to be misfounded might be the worst thing to do.

Thus, in this study, it has been shown that in areas where HPAI seems endemic, farmers and local actors are disseminating and using valuable sanitary information. National government would get advantages to be connected to this informal surveillance system.
10.1. MAIN DETERMINANTS OF THE OCCURRENCE OF H5N1 HPAI IN VIETNAM

10.1.1. CHINA, A RECURRENT SOURCE OF INTRODUCTION OF VIRUSES

The study we conducted on the spatial determinants of the 2007 epidemic indicated that HPAI viruses were very probably introduced from China before being spread all over Northern Vietnam. At that period, the spring season, the conditions were very favourable for disease spreading among domestic poultry populations, particularly via the ducks grazed in the rice fields for scavenging.

We found that the illegal trade from China is very significant, is seasonal, and has many drivers. Without tackling the drivers for the Vietnamese demand for poultry from China, it would be of little use to try to limit imports in order to manage the risk of future virus introduction. Two key drivers that we identified were the high demand for chickens around the Vietnamese Tết Celebration and the demand for good quality DOCs and DODs.

10.1.2. WATER BODIES, RESERVOIRS OF VIRUSES DURING EPIDEMICS

We have found a significant association between the occurrence of HPAI H5N1 and the presence of water in two of our studies. Depending on the epidemic studied and the scale of study, both a higher surface area occupied by ponds and lakes, and communes with longer flood periods, were identified as risk factors. It is difficult to clarify the role of water bodies as a long term reservoir of virus (possibly maintaining viruses alive for a few weeks or months between epidemics), but it is obvious from our results and from previous findings (Brown et al., 2009; Brown et al., 2007; Rohani et al., 2009) that water acts as a short term reservoir of virus during an epidemic, facilitating the transmission of infection between flocks.
10.1.3. TRADERS, MAIN SPREADERS OF VIRUSES

Our case control study that identified the presence of poultry traders in a village as a risk factor for occurrence of disease, and the spatial analysis of the last 2 major epidemics in Northern Vietnam, gave support to the role of traders and trading activity (including trading from China) on HPAI H5N1 introduction into a village and on the spread of the disease at a regional level. The role of growers and dealers in disseminating and perpetuating the infection in the rural poultry production chain was also highlighted during the LPAI epidemics in Italy (Cecchinato et al., 2011), despite probable stricter biosecurity measures imposed on those activities compared to what is practiced in Vietnam. This emphasises the difficulty in imposing good biosecurity practices for all activities related to the poultry trade and in preventing all possible risky behaviours of actors involved in this trade.

10.1.4. BROILERS FLOCKS, SENTINELS OF THE VILLAGES OR DANGER FOR THE VILLAGE?

Villages with broiler flocks were more at risk for occurrence of HPAI H5N1 in 2007, but we found in the longitudinal study that unvaccinated layers and breeder ducks were more at risk of infection, as detected by sero-conversion. Nevertheless broiler flocks may still pose a risk at the village level because of the trading activities they involve. However, we also think that broiler flocks, generally not as well vaccinated as layer and breeder flocks, are acting as effective sentinels in a village by showing clinical signs after infection, whereas layer and breeder flocks with sub-optimal vaccine protection may continue to excrete virus without symptoms. Of course, this is not always true for ducks because they may be resistant to clinical expression after infection with some HPAI H5N1 strains.
10.1.5. DUCK PRODUCTION, A SIGNIFICANT DETERMINANT FOR THE OCCURRENCE OF OUTBREAKS AND FOR VIRUS CIRCULATION

Several of the results related to studies of the determinants of occurrence in declared outbreaks, or of the seroconversion of unvaccinated poultry, are related to the presence of ducks. Thus, duck population density was found to be a significant predictor for the probability of occurrence of declared outbreaks in 2005; unvaccinated layer and breeder ducks were more at risk of sero-conversion in 2009/2010 than layer and breeder chickens; and the sero-conversion of unvaccinated poultry increased during the spring season, which is the high season for the production of broiler ducks. All those findings support the hypothesis that ducks play a significant role in the epidemiology of H5N1 HPAI in Vietnam as suggested previously (Gilbert et al., 2008). The role, in the epidemiology of avian influenza in China and Hong Kong, of domestic ducks and faecal-water-oral transmission between flocks was already suggested in the 80’s in a study highlighting the great diversity of influenza A viruses isolated from domestic ducks over a 4-year period in that region (Shortridge, 1982). Our work contributed to clarifying this role by providing evidence that layer and breeder ducks are contributing to the silent spread of the virus, whereas broiler ducks probably contribute to the dissemination of the viruses via rice fields, canals and ponds.
10.2. VACCINATION AS A CONTROL TOOL IN AN ENDEMIC COUNTRY: THE DRAWBACKS

We discuss here the difficulties and problems related to the H5N1 vaccination we have identified during our work. We were not able to address the question related to selection of new viruses due to vaccination pressure. In several countries where vaccination has been used, antigenic variant virus have been detected, although it is not yet clear whether the vaccine was responsible (FAO, 2011). To address this problem, countries using vaccination should be able to i) detect rapidly any antigenic drift in circulating viruses and ii) to introduce new vaccine antigens to answer to this antigenic drift.

Vietnam is regularly monitoring the viruses isolated from domestic poultry and decided to change the vaccine antigen in 2010/2011 to better fit new identified circulating strains (Re5 strain was selected instead of the Re1 strain used since the vaccination started).

10.2.1. MINIMUM HERD IMMUNITY THRESHOLD DIFFICULT TO REACH DESPITE HUGE HUMAN AND FINANCIAL INVESTMENTS

Mass vaccination was organized in Vietnam at high financial (the cost of a campaign was estimated around USD 10 million) and human costs (all veterinary services were dedicated to the implementation of the bi-annual vaccination campaigns). The direct evaluation of this vaccination programme that we conducted by measuring the serological response of the targeted population, suggests that the level of protection, at a population level, is far from an expected herd immunity threshold to limit virus circulation. This low level of immunity at a population level can easily be explained by i) the difficulty in maintaining a protective immune response in birds with a vaccine that needs regular boosters and ii) logistical issues which contribute to limit the level of sero-conversion of the vaccinated population. In addition, a programme based on bi-annual vaccination campaigns is not suitable given the high turnover of the poultry population.
10.2.2. INACTIVATED VACCINES NEED TO BE IMPROVED TO INDUCE LONGER IMMUNITY IN DOMESTIC POULTRY

The results of the study into the antibody kinetics of birds vaccinated with the Re-1 vaccine clearly indicates that, under field conditions and following the vaccination protocol currently being used by the Vietnamese veterinary services, the serological protective response does not last for more than 3 or 4 months (and even less for ducks). Thus, there is a need to test new vaccination protocols in order to obtain better results for antibody duration under field conditions. Increasing the dose might be an option, but the cost of such a measure should be carefully measured. Live vaccine delivered to DOCs and DODs would be the best solution to enhance the serological protective responses and to limit most of the logistical constraints related to the vaccination of poultry already in production.

10.2.3. NEED FOR HARMONIZED PROTOCOLS TO EVALUATE THE IMMUNOGENICITY UNDER FIELD CONDITIONS

In the evaluation of the serological diagnostic tests and in the observation of the antibody kinetics following vaccination, we raised the issue related to the appropriate test(s) and antigen(s) to be used for measuring serological response to avian influenza viruses. Because cross-reactivity among different H5N1 HA clades is not total, guidelines should be issued for each commercial vaccine available, specifying the strains to be used for serological testing and the minimum serological titer needed for protection after vaccination. Furthermore, to estimate antibody titers against a specific HA clade when measured with a different clade, a correlation coefficient should be estimated. Antigens presenting good cross-reactivity against a variety of different HA clades should be identified and preferably used for experimental trials. In ducks, for which antibody responses seemed to vary significantly according to the antigen used for serological testing, special care should be taken before any conclusions are drawn.
10.3. PERSPECTIVES: CONTROL AND SURVEILLANCE OPTIONS FOR A H5N1 HPAI ENDEMIC COUNTRY WITH LIMITED RESOURCES

10.3.1. VIETNAMESE ENDEMIC AREAS, AN EXAMPLE OF LIVING WITH DISEASE?

During our investigation on the way farmers managed the sanitary information related to suspect cases of HPAI, and the actions they triggered once such information was received, we provided an example of how a farming community was living with this disease. As described previously for ostrich farmers in South Africa (Mather and Marshall, 2011), influenza infection was approached as a normal risk associated with rearing poultry in a semi-commercial way. After several years of experience, Vietnamese farmers from the community studied developed some risk reduction measures when the epidemiological conditions met some criteria they defined by themselves. From a purely technical point of view, those measures seem adequate in preventing the disease from entering into the farms, but the conditions of their implementation and the triggers the farmers use may need to be validated and refined.

This example reflects the idea of living with disease, as described by Mather et al (2011) and previously by Hinchliffe (Hinchliffe, 2007). The authors discuss an alternative to the single globalised approach to animal health leading countries to adopt international best practices to control serious contagious (animal) diseases. Indeed, the potential for a human pandemic, and even the current zoonotic dimensions of the H5N1 HPAI disease, were not present in the risk framing of the farmers living with the disease, whereas those aspects are the foundations for the international responses to the H5N1 HPAI panzootic. As a consequence, the national policies influenced and supported by international agencies, and legitimized by the seriousness of the threat for human health are neither fully understood nor accepted by those living with the disease.
There is certainly room to reduce the gap between the way some farmers, other actors in the poultry commodity chain and central government are framing HPAI. Reaching an epidemiological consensus should be a goal of the long term control policy for this disease. Furthermore, national policies should be built more on the existing knowledge and practices of farmers and other local actors.

10.3.2. ZONING APPROACH

Except when most of the domestic poultry population is being culled as it was the case in Hong Kong in 1997 (FAO, 2011), stamping out can hardly limit the spread of virus within an area once the disease has been detected. This was obvious during the first waves of epidemics in Vietnam during which the number of outbreaks was very high. With vaccination, the number of reported clinical cases during the last major epidemics in 2005 and 2007 was more limited. Nevertheless, disease spreads rapidly over a large geographical area and some outbreaks may not have been declared. As experienced previously by The Netherlands and Italy, preventing the spread of virus to unaffected areas may be more achievable than preventing the spread of virus within an area, especially for areas with a high flock density (Stegeman et al., 2004; Zanella et al., 2001). The problem with a country like Vietnam is that areas with high flock densities are very large and, due to trading connections, all provinces are connected with each other. If vaccination is limiting the virus dissemination within highly populated areas, it can also mask the first signs of infection in non-affected areas. This is where zoning, defined by OIE as subpopulations of distinct health status defined on a geographical basis (using natural, artificial or legal boundaries), would be useful (OIE, 2010). It would be possible to define a zone made of several districts or provinces where vaccination is necessary to limit virus spread once it has entered into the domestic population. Ducks should be the priority for vaccination in a zoning policy since they can easily transmit the disease because of their management system, and also because, even without vaccination, they can act as a
reservoir of viruses. In other areas, vaccination should not be used, in order to facilitate the early detection of HPAI cases.

If we consider the spatial distribution of the last 2 major epidemics in Northern Vietnam, we can identify the zone where vaccination may be necessary. This zone follows the national road from China, where outbreaks were clustered and where recurrence was more frequent. Then, 2 pertinent options for the border with China can be proposed:

- For the sake of early detection of any new introduction of viruses from China, the border zone could be left unvaccinated. This option only makes sense if surveillance in this area becomes reliable enough that any suspect clinical cases of HPAI in domestic poultry are reported without delay. This is where sentinel chicken flocks could be employed. Those sentinel flocks would be localised in communes known to be the port of entry of the imported poultry from China, such as Mong Cai commune in Quanh Ninh province (Figure 10.1), and would be kept under strict and regular monitoring by the local veterinary services.

- If surveillance cannot be organised in such a way that early reporting of any suspect case of HPAI is guaranteed, it may be preferable to create a buffer zone where vaccination is organised to protect domestic poultry from infection. The current vaccination protocol with a bi-annual vaccination campaign should be adapted in order to guarantee a high annual immunity level for the population.
Figure 10.1. Zoning for targeted vaccination
10.3.3. IMPROVE BIOSECURITY AND SURVEILLANCE AT THE LOCAL LEVEL, A COMMONPLACE BUT NECESSARY STEP

All actions related to the surveillance and control of H5N1 HPAI should be regarded as part of the global effort to limit the burden and circulation of virus with zoonotic potential within the poultry population and between poultry and human populations. Today, H5N1 is regarded as a major threat for poultry production and public health; tomorrow this will be another virus. In our work, we have demonstrated that the overall seroprevalence of avian influenza viruses was very high (around 40%) and that H9 subtype was widespread on ducks. Cases of H9 infection in humans have been regularly detected in Hong Kong and this virus may be also of concern in Vietnam. Thus, improvement of the conditions of poultry production and trading is a necessary step to sustain this sector and to protect, in a long term perspective, the public health.

Farmers, already on the right track

Vietnamese farmers changed their practices since the first outbreaks of H5N1 HPAI occurred in 2003/2004. We have demonstrated that in places where HPAI is regularly suspected, farmers have adopted a risk reduction approach to deal with the threat of this disease. Furthermore, we consider that their behaviour contributed to the reduction of the extent of the last epidemics and that their contributions should be fully acknowledged.

Surveillance at local level, need for improved official reaction and harmonised case definition

We also observed that farmers very often implement preventive measures before official channels have spread information related to the presence of HPAI in the neighbourhood. Thus, local management of information and of the risk should be enhanced by giving commune veterinarians stronger technical training and responsibility. We have demonstrated that commune veterinarians are at the interface of the informal and formal systems of disease control and thus, they could contribute more effectively to support more comprehensive and efficient responses to any suspect case of HPAI.
We also insisted on the need for an adapted and harmonised case definition of HPAI suspect cases. It is necessary that key actors, especially the commune veterinarians and the drug sellers, receive updated information on what a suspect case of HPAI may be in a context of vaccination. Alert thresholds of key actors should adapt to variable epidemiological situations.

**Professionalization of the trading activities: a tricky but achievable task**

Traders actively contribute to the introduction and dissemination of HPAI virus in Vietnam. Apart from being sometimes obliged to pay fees to receive certificates (which have little technical value), the poultry trader’s activities are not governed by any technical regulations obliging them to follow a minimum standard of good practices. It has been demonstrated in Hong Kong that improving hygiene in market places by introducing a compulsory retail market rest day on which all birds in all retail outlets were sold or slaughtered so that the stalls could be thoroughly cleaned and disinfected, contributed to the reduction of the virus circulation (Kung et al., 2003; Sims et al., 2003). Having good practices adopted for the poultry trading profession in the context of Vietnam is not an inconceivable objective. The private sector in Vietnam is very dynamic and Government could rely on this attribute to encourage the emergence of a new more professional and more responsible form of poultry trading activity. It is clear however that any regulations related to the poultry trading activity should not be issued by the Government alone. Consultation with the private sector should be organised with haste to identify and adopt measures that are realistic, understandable and acceptable by those who may have to implement them.

**Controlled supply of DOCs and DODs**

We have demonstrated that illegal import of DOCs and DODs from China is motivated by the demand for good quality stock, and probably by the insufficient local supply. Others have also described how much of the local supply is not produced under good sanitary conditions (Phan Dang T. et al., 2010). Despite all the programmes financed by
international agencies dedicated to the control and prevention of HPAI, it seems that this part of the value chain has not been improved significantly. Initiatives such as the STOP AI project (STOP AI project, 2011) funded by the United States Agency for International Development (USAID) and that developed good practices and regulations for poultry farming and processing, could be extended to the production of DOCs and DODs. Of course, such initiatives that rely on the creation of labels and on the accreditation of producers need an independent and reliable quality control system. This is probably the most challenging part of the project for the current Vietnamese system.
GENERAL CONCLUSION

By combining diverse approaches and tools, we have gathered in this thesis different pieces of information leading to a better understanding of the epidemiology of H5N1 HPAI disease in Northern Vietnam, and we were able to draw perspectives in terms of surveillance and control in the context of an endemic country.

The epidemiology of H5N1 HPAI is complex as is the poultry production system, and controlling the disease requires sustained efforts and innovative approaches. Thus, eradication of the virus in a context such as Vietnam is a long-term objective as admitted by FAO (FAO, 2011), and efforts should concentrate on the limitation of the consequences of the disease and the global improvement of the production and trading conditions. The avian influenza crisis was an opportunity for veterinary services and animal production departments of many countries to receive more attention and financial support. This is a good start to pave the way for a global improvement of public health, in relation with the cultural and socio-economical constraints of each country. Strengthening partnerships between research and development should be a priority of international agencies or national veterinary services in order to lead to adapted solutions for prevention and control.

Finally, this thesis was a privileged occasion to acquire a diversity of concepts and techniques that make up the foundations of modern epidemiology. It was also an opportunity to collaborate with other disciplines such as modeling, socio-anthropology, virology etc... This is all the interest of epidemiology, to be at the crossroad of different views.
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Annex 1 - Poultry production system review

Annex 2 - Poultry production chain

Extracted from Le Bas et al (2008).

High Pathogenic Avian Influenza in Poultry Production Systems in Vietnam:
TENTATIVE APPROACH FOR AN HACCP-LIKE RISK SCORING METHODOLOGY

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ABSTRACT

The circulation of the HPAI virus can be studied at farm level, but relevant data gathered at other points in the poultry production chain, such as markets or collection points, help create a clearer picture of the virus' circulation, since these points are often more accessible than the farms. There is also potential for the virus to become concentrated at these points. Observation of critical points along the chain should draw a good picture of the virus' circulation. Either this could be used as a comparison with farm survey data, or it could provide more efficient and rapid detection of HPAI in a given context and production sector.

The objectives of the present study are to analyze and describe flows and practices from the top to the bottom of the poultry marketing chain in two Provinces of North Vietnam to evaluate a HACCP-like risk scoring methodology and to identify critical points for the detection of pathogen circulation along these marketing chains.

The qualitative description of poultry production sectors was based on questionnaires compiled between May and August 2008 on 240 poultry semi-commercial production farms and 60 collective interviews of backyard producers and 60 traders in Ha Tay and Bac Giang Provinces. Possible observation points were identified. The HACCP-like approach with a risk scoring methodology, tested on two points from the layer production sector, was able to discriminate between these two points. Our result confirms that this method could be used to identify a few critical observation points with a higher risk of virus circulation in the chain, where the early detection of HPAI virus would be most effective.

Keywords: High-Pathogenic Avian Influenza; Risk scoring; HACCP; Poultry production; Poultry traders; Chicken; Duck; Vietnam

INTRODUCTION

Poultry marketing chains converge to a single geographical point. Birds come in from many village farms in a given geographic zone to singular pool of markets and collection points. At these locations, birds are usually accessible than farms, they may be suitable points for observing the circulation of pathogens, such as high-pathogenic avian influenza virus, throughout the supply chain. The propensity of small farmers in developing countries to sell-off sick animals as a precautionary measure - even when faced with only a vague suspicion of risk, likely leads to a concentration of pathogenic prevalence that can be easily detected in poultry available at central markets compared to the likelihood of detecting such prevalence in poultry still being raised on farms. Therefore, inspecting certain key points along the marketing chain can create a...
Figure 1: Schematic representation of the stakeholders (COPs) in the chicken and duck LAYER production chains in Bac Giang and Ha Tay Provinces: INFLOWS to semi-commercial broiler farms and backyard producers.

Figure 2: Schematic representation of the stakeholders (COPs) in the chicken and duck LAYER production chains in Bac Giang and Ha Tay Provinces: OUTFLOWS from semi-commercial layer farms and backyard producers.
Annex 3 - Modeling the chicken broiler flux

Paper published in the journal "Epidemiologie et Santé Animale", resulting from a master student training course under the co-supervision of D.Bicout and S.Desvaux (in French).

Epidémio. et santé anim., 2009, 55, 137-152 Flux de volailles et propagation de l'influenza aviaire dans la filière avicole au Vietnam

FLUX DE VOLAILLES ET PROPAGATION DE L'INFLUENZA AVIAIRE DANS LA FILIERE AVICOLE AU VIETNAM**

Ariane Payne 1, Stéphanie Desvaux 2, Karine Chalvet-Monfray 1,
Jean-François Renard 2 et Dominique J. Bicout 1

RESUME
Depuis 2003, le Vietnam connaît l'incidence d'influenza aviaire hautement pathogène la plus élevée d'Asie. La transmission du virus influenza entre volailles se produit à l'occasion de nombreux contacts qui ont lieu au sein des réseaux de distribution. Notre objectif est de modéliser la dynamique de ces flux de volailles à l'échelle d'un réseau communal d'une province du Nord du Vietnam à partir de données recueillies sur place, et de développer une modélisation de la propagation du virus influenza forcée par la dynamique des flux. Les résultats de simulation montrent comment évoluent les quantités de volailles au cours du temps sur une période de 2 ans. Nous visualisons la plasticité du système (population de volailles au sein d'un réseau) face aux événements perturbant les flux d'échanges.

Mots clés : Influenza aviaire, filière avicole, modélisation, Vietnam.

SUMMARY
Since 2003, Vietnam has experienced the highest incidence of highly pathogenic avian influenza in Asia. The influenza virus spread through numerous contacts occurring between birds in those networks. This study was designed first to model the local poultry flux dynamics using field data from a province in Northern Vietnam. Then, we developed a disease transmission model within a poultry network, driven by flux dynamics. Simulation results illustrated the changes in poultry livestock over a two - year period. Various aspects of changes in the poultry livestock occurring in response to events perturbing the network could be studied thanks to the flux dynamics model.

Keywords: Avian influenza, Poultry, Modelling, Vietnam.

* Texte de la communication orale présentée au cours des Journées scientifiques AEEMA-AESA, 4-5 juin 2009
** Nous dédions ce travail à notre collègue ami, J. F. Renard, qui nous a quittés cet été.

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Annex 4 - Modeling the social network of the poultry trader

Poster presented at the Epidemics conference in December 2009 in Athens, Greece. (poster session 2, poster number 9) (www.epidemics.elsevier.com).

**Simulation of avian influenza propagation in the poultry production network of the North Vietnam**

Raphael Dubas, Bruno Bonté, Stéphanie Desvaux, Vladimir Grosbois

Contact: raphael.dubas@cirad.fr

Highly Pathogenic Avian Influenza (HPAI), subtype H5N1, is now endemic in the Red River Delta, North Vietnam. Presence and control of the virus circulation in poultry remain a challenge. One shared hypothesis is that poultry trading network drives the spread of the virus at the scale of the delta.

Networks were constructed in a sub-area of the Red River Delta, Vietnam, in 2009 in order to perform a Social Network Analysis (SNA). A representative sample of semi-commercial farms and backyard poultry producers was interviewed in 5 communes (Figure 1) and their trading connections with poultry markets and traders were recorded. The majority of the exchanges are performed by traders. As illustrated by Figure 2, the poultry trading network is highly interconnected if we don’t consider the traders’ activity. Unfortunately, traders identified by the first survey were very difficult to trace. Therefore, a survey was performed on a representative sample of the population of traders and provided informations at the commune level. We know the communes in which a trader sells and/or buys to markets, backyards or farms. Nevertheless, we can not identify those markets, backyards or farms.

**The model**

We model the spread of a disease in a graph. Nodes are either farms, backyards or markets. Links are oriented and represent poultry flows between nodes. The modeled network is a graph of linear finite state automata.

Nodes: Each node is specified with the following set of states:

\[ N = \{ S, E, I, R \} \]

with the following set of transitions:

\[ T = \{ S \rightarrow E, E \rightarrow I, I \rightarrow R \} \]

and the following associated life spans:

\[ S = \text{infectious period; } E = \text{infectious period; } R = \infty \]

Poultry flow dynamics: Every time step, each node sends an event to the nodes it is connected with. With a probability \( P \), this probability is defined as the inverse of the frequency of interactions with the same type of interaction. The transition between \( S \) and \( E \) occurs when an event comes from one infected connected node.

**Experimental Design**

We test the influence of 3 factors (Table 1):

- sells frequency
- network density (traders’ connectivity)
- intra-communal structure

Time step is one day and simulation period is 1 month. We perform 10 replicates for each factor combination.

**Simulation Results**

We build a simulation model of the avian influenza propagation in a representative poultry production network.

We then simulate the epidemic (Table 2) and test the influence of those networks on the final size of the epidemics.

**Analysis**

Table 2 provides the main results of an analysis of variance. The objective is to quantify the impact of the three factors modulated on the final size of the epidemic. As shown by table 2, the trader connectivity density and frequency of sells are highly significant regarding the final size of the simulated epidemic. The interaction between these two factors is less important, even if it is significant. The different graph topologies generated are not significant.

**Conclusion**

This preliminary study indicates that the identification of the traders clients and suppliers at the individual level is not necessary to accurately reproduce an epidemic in the studied network. The knowledge of the type and the number of clients and suppliers in each commune, as well as the frequencies of interactions is sufficient.
Annex 5 - Questionnaires of the study on illegal poultry trade from China

QUESTIONNAIRE TRADERS

Nb de fiche: ...........................................
Date: ..............................................

1. INFORMATIONS GÉNÉRALES

Nom du trader enquêté: ..............................................................
Province: .................................................................
District: ............................................................
Commune: ...........................................................
Village: ............................................................

Remarque pour l’enquêteur:
- Il faut comprendre si les traders vendent des animaux de Chine aux éleveurs moins chers que des animaux du Vietnam
- Il faut savoir s’ils informent les éleveurs que les animaux viennent de Chine ou bien s’ils cachent cela aux éleveurs

2. ACHAT

Indiquez les types de produits achetés, la quantité moyenne par mois et sa répartition par types de lieux d’achat

<table>
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<th>Produit</th>
<th>Pays</th>
<th>Quantité moyenne achetée</th>
<th>Poissons (%)</th>
<th>Charcuterie (%)</th>
<th>Pies de bétail (%)</th>
<th>Pies de volailles (%)</th>
<th>Activité (%)</th>
<th>Total</th>
<th>Origine de volailles (vue par province)</th>
<th>Vaccinations H5N1 en Chine (oui, non ou ne possible)</th>
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ADRESSES ACHETS: où achetez-vous essentiellement vos produits (Nom, province, district, commune, village)
3. VENTE : indiquez les types de produits vendus, la quantité moyenne par an et les proportions (%) vendues en fonction des types de lieux de vente.

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<tr>
<th>Produits</th>
<th>Pays</th>
<th>Quantité moyenne vendue</th>
<th>Grand marché (marché de consommation)</th>
<th>Petit marché (marché de consommation)</th>
<th>Point d'échange</th>
<th>Grand marché (marché de commerce)</th>
<th>Petit marché (marché de commerce)</th>
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<th>Restaurant</th>
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<th>Autre</th>
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ADRESSES VENTES où vendez-vous habituellement vos produits (Nom, province, district, commune, village)

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<th>Petit marché (marché de consommation)</th>
<th>Point d'échange</th>
<th>Grand marché (marché de commerce)</th>
<th>Petit marché (marché de commerce)</th>
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4. FLUX

La répartition des achats de volaille de Chine (% au mois) et ses raisons durant l’année 2009

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</tbody>
</table>

Est-ce que les variations saisonnières sont les mêmes tous les ans? Oui ☐ Non ☐
Si oui, précisez les raisons: _________________________________________________________________
Si non, précisez les raisons: _________________________________________________________________

232
Quantité transportée par an

<table>
<thead>
<tr>
<th>Produit</th>
<th>Pays</th>
<th>Nb MAX de produits transportés par an</th>
<th>Nb MAX de transport jusqu'à destination finale</th>
<th>Nb MOYEN de produits transportés par an</th>
<th>Nb MOYEN de transport jusqu'à destination finale</th>
<th>Nb MIN de produits transportés par an</th>
<th>Nb MIN de transport jusqu'à destination finale</th>
</tr>
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<td>Chine</td>
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</tbody>
</table>

Etes-vous de volaille?  Oui ☐  Non ☐
Si oui: complétez l'information dans le tableau ci-dessous

<table>
<thead>
<tr>
<th>Produit</th>
<th>Nombre par an</th>
<th>Nombre de bandes</th>
<th>Nombre le jour d'angélique</th>
<th>L'origine</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Comportement du trader/Pratique à risque

- En ce qui concerne vos animaux malades?  Oui ☐  Non ☐
Si oui:
  Quels types d'animal(s)?
  De quelle manière en général sont-ils atteints?
  Quantité moyenne d'animaux malades achetée/an?
  Ont-ils été nouvellement achetés et vendus?
  Utilisez-vous alors les mêmes animaux d'élevage ou d'autres?
  Précisez:
  Et vous vendez les mêmes prix (estimation de 1% du prix normal)

- Comment effectuez-vous l'organisation des arrivées et des ventes?
  Achat:
  Vente:
  Présentez-vous le matériel que vous utilisez pour le transport des volailles (cage, panier, ...)
  Désinfection du matériel de transport?  Oui ☐  Non ☐
Si oui: Quel produit et Quand?

Avez-vous un stock de volailles de Chine?  Oui ☐  Non ☐
Si oui: Combien de temps vous gardez les animaux de chine?
Minimum: ____________________ (heures)
Moyenne: ____________________ (heures)
Maximum: ____________________ (heures)
En où est-il conservé ?

☐ Sur le lieu de vente
☐ À la maison/exploitation
☐ Autre ; précisez : ........................................

- Pendant le stockage les volatile(s) ont-ils en common avec d'autres volatile(s) ? ☐ oui ☐ non

Si oui, détail (combien, durée, contact direct ...) : ........................................................................................................

- Desinfection du lieu de stockage ? ☐ oui ☐ non

Si oui, Quel produit et Quand ? Précisez : ........................................................................................................

- Combien de temps vous gardez les animaux vivants de Chine ?
  Minimum ..................(jour)
  Moyenne ............... (jour)
  Maximum .................. (jour)

+ Et où sont-ils conservés ?

☐ Retournés à la maison ou à l'exploitation
☐ Confisés à un tiers
☐ Broyés en fin de marché
☐ Autres ; précisez : ........................................

- En cas de mortalité importante de volatile(s) que vous vendez, que faites-vous ? (plusieurs possibilités)

☐ Brise les carcasses
☐ Les enterrer
☐ Les jette (bord de champ, rivière, canal, ...)
☐ Les consomme (avec ou sans partage avec parents et voisins)
☐ Cherche à les vendre même à prix réduits
☐ Cherche à vendre rapidement les survivants
☐ S'adresse au vétérinaire ☐ Autre ? ..............
Annex 6 - Individual-based modelling applied to the epidemiology of HPAI

Use of Individual-Based Modelling for a better understanding of HPAI epidemiology in North Vietnam: approach proposed and description of GAMA platform

The understanding of the Highly Pathogenic Avian Influenza (HPAI) epidemic in South East Asia is still incomplete. In particular, the persistence mechanisms of the virus between two outbreaks issues in country like Vietnam are of particular interest. We propose in our research project to combine classical epidemiological studies in North Vietnam together with Individual-Based Modelling (IBM) to better understand these mechanisms. IBM models are based on entities that represent individuals and their interactions in the system modeled. They provide an interesting way for exploring, analyzing, understanding a process from the interactions of local components. The IBM will be used to create a "virtual laboratory" where disease evolution could be observed in a manner of time in different agro-systems integrating domestic poultry population and their natural and human environments. It could be used as tool to test different prevention and control measures if the transmission of the disease is successfully modeled.

Research questions in computer science

How to develop?
- A generic IBM-based simulation framework = validate model = definition of the modelling concepts, their properties and their relations.
- A specialized MAS simulation platform
- GIS support
- Agro-systems in a (possibly complex) environment (spatial & social)
- A tool easy of use for non computer scientist = Modeling language

GAMA: an IBM simulation platform
http://gama-platform.sourceforge.net

GAMA, is a spatially explicit multi-agent System, MAS, a subcategory of IBM. This platform fulfils different criteria needed to address the research questions in a multi-disciplinary approach involving computer scientists, epidemiologist and field actors.

Modeling process

1. Study frame description
2. System without disease: iterative modeling / proposal / epidemiologists & actors validation
3. System with disease:
   a) study the persistence mechanisms
   b) study the transmission pattern
4. Comparative validation / Validation with existing dataset
5. Virtual lab: Understanding persistence mechanism / epidemic (prediction) / Targeted monitoring network / targeted prevention and control measures
6. Validation of the tool to non computer scientists

Fig 1. Diversity of the poultry production system

Research questions in epidemiology

1. Persistence mechanisms?
2. Environmental reservoir?
3. Contact (or wild) reservoir? (of complex production cycles)
4. Or a combination of both?
5. Which agro-system could maintain the virus over the months?

Modeling the transmission
- To identify the most critical parameters on which control/prevention measures could be applied?
- To predict (validation from data / other mathematical models)
- To identify the most critical parameters on which control/prevention measures could be applied?

Space scale for persistence mechanisms: commune (6 villages / 8 km ?)
Space unit: 10 meters
Time scale: 1 year
Time step: 1 hour

Poster presented at the Bangkok International Conference on Avian Influenza 2008: Integration from knowledge to control January 23-25, 2008 Bangkok, Thailand
 Annex 7 - Processing of MODIS images

A.Tran, Cirad, 2011

Pre-processing: cloud masking and temporal interpolation of MODIS time series

We used the quality reflectance files provided with 8-days composite MODIS images and an additional restriction on blue and green reflectance values \( \left( \frac{\rho_{\text{blue}} - \rho_{\text{green}}}{\rho_{\text{blue}} + \rho_{\text{green}}} \right) \geq -0.2 \) to mask cloudy and cloud shadows pixels in each MODIS scene. Then, a temporal interpolation was performed for each of the seven reflectance bands to simulate the missing data.

Detection of water bodies

A spectral index suited for water bodies’s extraction (Tran. et al., 2010) was computed for each MODIS image: the Modified Normalized Difference Water Index (MNDWI) calculated from the bands 4 (green) and 6 (middle infrared). Free water areas were delineated by thresholding MNDWI images (MNDWI threshold = -0.4). The yearly flood duration was estimated for each pixel by computing the number of images for which MNDWI value exceeded this threshold.

Detection of paddy rice fields

Following Xiao et al. (2006), we computed for each MODIS image three spectral indices suited for the characterization of the temporal dynamics of paddy rice fields: the Normalized Difference Vegetation Index (NDVI), the Land Surface Water Index (LSWI), and the Enhanced Vegetation Index (EVI) (for a description of these indices see (Xiao et al., 2006). First, we used the value of annual flood duration to identify pixels with a flood duration incompatible with rice agriculture (annual flood duration > 30 composite periods instead
of 10 in initial algorithm). Classified as ‘persistent water bodies’, those pixels were not included in the procedure of identification of paddy rice fields.

Then, a mask of ‘evergreen vegetation’, including tropical and mangrove forests, was generated using both NDVI and LSWI time series data: pixels having NDVI values greater than 0.7 over at least twenty 8-day composites during the year or having no LSWI values less than 0.10 during the year were classified as evergreen vegetation.

Finally, pixels were identified as ‘rice pixels’ if the temporal dynamics of the three indices showed a temporary inversion of the vegetation indices (NDVI and EVI) with the LSWI, which may be a signal for flooding in paddy rice fields, and if this inversion was followed by a rapid growth of vegetation (Xiao et al., 2006; Xiao et al., 2005). Pixels with LSWI+0.05 ≥ EVI or LSWI+0.05 ≥ NDVI were considered as ‘flooding and transplanting’ pixels. From those pixels, pixels for which EVI value has reached the half of the maximum EVI value (observed about two months after transplantation) within 40 days following the date of flooding and transplanting, were identified as ‘rice pixels’. This procedure allows also determining for each ‘rice pixel’ the annual number of crops.


Annex 8 - Poster presented at the SVEPM conference, March, 2010

Avian influenza seroprevalence in North Vietnam

Stéphanie Desvaucels1, Marisa Peyre1, Pham Thi Thanh Haut1, Nguyen Tien Dung2, François Roger1
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3 National Institute of Veterinary Research, Hanoi, Vietnam

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Highly Pathogenic Avian Influenza (HPAI), subtype H5N1, is now endemic in Vietnam. Surveillance and control of the virus circulation in poultry remain a challenge. In 2005, the Government of Vietnam decided to launch a massive vaccination campaign to control the number of outbreaks and the transmission to Humans. The domestic poultry are currently vaccinated against H5N1 virus using a H5N1 inactivated vaccine and following a national program with 2 main vaccination periods during the year (October-November and April-May). The objective of the national services being to reduce the size of the susceptible population with at least 50% of the flocks to be vaccinated.

SAMPLING METHOD

In order to study the pattern of seroprevalence over one year time, repeated population based cross sectional surveys were conducted on domestic poultry in the Red River Delta in North Vietnam.

Four sampling periods were defined: mid December 2008 (C1), and January 2009 (C2), end March of 2009 (C3) and finally, June- July of 2009 (C4). Around 1000 birds were sampled for each campaign with the farms for farm poultry or villages (for backyard poultry) being randomly selected in our study area (see Figure 1). 15 birds were sampled for each selected epidemiological unit.

SERODOLOGICAL DIAGNOSIS METHOD

Influenza A seroprevalence was estimated using a competition Elisa kit that detects antibodies against the internal nucleoprotein of influenza A virus (ID-Screen® Influenza A Antibody Competitive). A subtype specific Elisa was also used to compare the results with H5S (ID-Screen® Influenza H5 Antibody Competitive).

Influenza H5 seroprevalence was estimated using Hemagglutination inhibition test (HI test). All sera with a titer 48IgG were defined as positive.

H5 SUBTYPE SEROREPREVALENCE

INFLUENZA TYPE A SEROREPREVALENCE

CONCLUSIONS

Seroreprevalescence of type A on domestic poultry is not only due to H5 subtype in Vietnam and demonstrates a high circulation of influenza virus on ducks (especially H5 – data not shown).

The global individual vaccination coverage of domestic poultry in the Red River Delta is around 20% of the birds whatever the period of the year (close or not from the mass vaccination campaign). This protection level is much lower than coverage expected from a massive vaccination but may be explained by:
- population turn over
- duration of the immunity
- practical implementation of the vaccination.

As a consequence of the population turn over, the protection of the breeder population is lower than the breeder-layer population (same for ducks, data not shown).

Difference between vaccination and infection may be suggested for some animals said not have been vaccinated but showing serocoversion.

PERSPECTIVES

The serological diagnostic tests need to be better evaluated for the different species. Seroneutralisation test is going to be used on two populations and Se and Sp will be evaluated using Bayesian or frequentist methods.

Correlation between serocoversion and protection needs to be better investigated for ducks.
The general initial objective of this study was to detect the circulation of influenza virus H5N1 within the domestic poultry population sold at the local market places and to compare the virus detected in those places with the virus detected on farm and village domestic poultry, sampled at the same period.

The initial results indicated that virological prevalence on those local small markets was low and we decided to develop a new protocol for studying prevalence of infected flocks sold on the biggest live bird market in our study area.

**Study population**

The study population was made of live domestic poultry sold:

- at local small and medium size markets of the longitudinal study area, from the 5\textsuperscript{th} December 2008 to the 5\textsuperscript{th} June 2009,

- at Ha Vi market in Ha Tay province, the biggest live poultry market in Northern Vietnam, from the 26\textsuperscript{th} March 2010 to 24\textsuperscript{th} November 2011.

In total, 1300 birds were collected for the first part of the study (17 visits in small and medium local markets) and 3810 for the second part (3 visits at Ha Vi market).

**Samples and data collection**

Cloacal and tracheal samples were collected for each bird.

In addition to the samples collected, we have collected data related to the birds’ description (species, breed and production type and possibly one estimation of the age).

In the study at Ha Vi market, we also tried to collect the origin of the birds as precisely as possible (type of production: backyard: farm less than 1000 birds or more than 1000 birds, province, district, commune, village).

**Initial results from the local small market study**

Out of the 240 pools tested so far, 3 pools were positive for Newcastle disease (local chickens) and 1 pool was positive for H4 avian influenza (ducks).
Hypothesis to be tested

Some peri-domestic wild bird species that have contact with both wild birds and domestic birds, may have a role in the transmission of AI virus and especially HPAI virus H5N1 between wild and domestic birds.

Research questions

- Can the peri-domestic wild bird species be infected with HPAI H5N1 in a context of regular virus circulation?
- What is the sero-prevalence of antibodies against HPAI H5N1 Avian influenza virus in peri-domestic wild bird species?
- What is the prevalence of influenza A virus and HPAI H5N1 Avian influenza infection in peri-domestic wild bird species?
- Do the AI viruses circulating within wild waterbird, peridomestic wild bird and domestic birds have phylogenetic relations?

Protocol

Identification wild bird species

Small terrestrial birds are potentially important hosts in AIV (H5N1) ecology because many of these birds may be in contact with wild birds, domestic waterfowl and poultry. However, reports of their susceptibility to AIV infection in particular to H5N1 and their potential to transmit these viruses are limited.

A group of common resident terrestrial birds is:

**Seedeater passerines** (in particular the *tree sparrows, Passer montanus*) are potentially in contact with poultry in villages (chicken, domestic ducks, Muscovy duck) because they feed both in villages and in paddy fields, hence they share the same habitats. This bird species is commonly seen in villages and paddy fields where the AI out breaks are endemic in domestic birds. Therefore, Tree sparrow is one bird species that we are interest in this study.

The second species getting much concern in this study are **White-rumped Munia** (*Lonchura striata*) and **Scaly-breasted Munia** (*Lonchura punctulata*). These birds are common resident birds fed in paddy fields.

Other species that share the wetlands of domestic duck population are waterbirds (*Egrets, Herons*). The most common waterbirds present in agro-ecosystems in Vietnam and can be observed at close distance in paddy fields, small ponds or lakes are **little Egret** (*Egretta garzetta*) and **Chinese Pond Heron** (*Ardeola bacchus*). These birds can be considered in this study.

Main criteria for selecting these species:

- Feeding behaviour:
- Abundance: some of the most abundant terrestrial (sparrows and munias) and aquatic (herons) wild bird species in agroecosystem of the Red River Delta region.

- Epidemiology: natural infection with H5N1 HPAI has occurred in wild herons and sparrows; in experimental infection trial, sparrows have been found susceptible to H5N1 HPAI infection.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reproduction period</th>
<th>Resident / migratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese pond heron</td>
<td>Mars to May</td>
<td>Resident and wintering birds</td>
</tr>
<tr>
<td>Little egret</td>
<td>June to August</td>
<td>Mainly resident birds</td>
</tr>
<tr>
<td>Munia</td>
<td>Theoretically all year long, but reproduction may stop during coldest months in Northern Vietnam</td>
<td>Resident birds</td>
</tr>
<tr>
<td>Sparrow</td>
<td>Theoretically all year long, but reproduction may stop during coldest months in Northern Vietnam</td>
<td>Resident birds</td>
</tr>
</tbody>
</table>

**Selection of study area**

Samples of wild birds are taken in the areas where HPAI H5N1 is studied on domestic birds:

- Phu Xuyen district, Ha Tay province
- Yen Dung, Viet Yen and Hiep Hoa districts, Bac Giang province

**Study period**

This study will be implemented for one year period starting from August 2008.

**Catching and sampling methods**

For Heron and Egret, it is proposed to collect faeces during the 2 main sampling seasons at different places where those species are gathering at night for sleeping. Number of faeces samples collected: between 100 and 150 fresh faeces samples (pooled by 5).

Due to high pressure of hunting in Vietnam, sparrow and munia are very difficult to catch by misnet. However, these birds are captured easily by local hunters with drop nets. Therefore, wild birds catching for the study will be mainly based on local hunting.

**Sampling period**

Time for collecting samples of studied bird species depends on the season the birds are available.

It is proposed to collect samples in relation with the identified at-risk periods that is to say when virus is usually circulating on domestic poultry and when migratory wild birds are moving from China to the South.

In the past 2 years, the peaks of outbreaks in the North were either during the pre-Têt period (February) or in May-June.
It could be also interesting to have a “control” period, when circulation on domestic birds is normally low or absent to check the presence of virus on the wild birds population and before migratory period.

According to the availability of the different target species, we planned the following sampling agenda:

- one period after migration starts but before high peak of outbreaks on domestic birds (Nov/Dec),
- one period after the second period of outbreak (June),
- 1 sampling during low risk area (as a reference),
- an annual collection for heron and Egret to be sure to reach a minimum amount of samples.

Laboratory protocol

All samples will be analysed with the IDEXX antibody detection kit (IDEXX FlockChek™).

Positive results will be kept for further advanced serological testing (western blot or micro-neutralisation).

Swabs will be stored until serological results will be known. Species population with positive results on serology will be tested for virology (RT-PCR, isolation and genetic sequencing)

Results

<table>
<thead>
<tr>
<th>Birds sampled</th>
<th>Blood</th>
<th>Individual tracheal sample</th>
<th>Individual cloacal sample</th>
<th>5 pooled Cloacal samples</th>
<th>5 pooled tracheal samples</th>
<th>3 pooled tracheal and cloacal samples</th>
<th>Faeces from roosting site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Munia</td>
<td>306</td>
<td>42</td>
<td>159</td>
<td>35</td>
<td>35</td>
<td>115</td>
<td>99</td>
</tr>
<tr>
<td>Sparrow</td>
<td>316</td>
<td>0</td>
<td>130</td>
<td>40</td>
<td>40</td>
<td>15</td>
<td>99</td>
</tr>
<tr>
<td>Heron</td>
<td>142</td>
<td>28</td>
<td>28</td>
<td>40</td>
<td>40</td>
<td>115</td>
<td>99</td>
</tr>
<tr>
<td>Egret</td>
<td>22</td>
<td>7</td>
<td>7</td>
<td>15</td>
<td>15</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>786</td>
<td>77</td>
<td>324</td>
<td>75</td>
<td>75</td>
<td>130</td>
<td>99</td>
</tr>
</tbody>
</table>

Laboratory results

All sera tested with the Elisa kit were negative.

All faeces samples collected were inoculated and all were negative.
<table>
<thead>
<tr>
<th>Annex 11 - Pictures from field work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Farmers helping for catching the birds</strong></td>
</tr>
<tr>
<td><strong>Sampling at market (Voi market, Bac Giang province)</strong></td>
</tr>
<tr>
<td><strong>Presentation of the study protocol to the district veterinarians by Mrs P.T.T.Hoa, research assistant on the project</strong></td>
</tr>
</tbody>
</table>
Notes about the farmers’ risk framing

The farmers’ risk framing depends on the epidemiological context. In commune B where H5N1 cases are rare, the disease has kept its exceptional character whereas in commune A, where farmers reported frequent cases of what was, rightly or wrongly, associated with H5N1 outbreaks, the risk has been ‘domesticated’. We mean that outbreaks are no more an unusual event asking for new skills, they have been gotten into the routine.

See table 1 for detailed description of the representation of the disease by farmers.

Representation of the H5N1 disease by farmers of the 2 communes studied.

<table>
<thead>
<tr>
<th>Commune B</th>
<th>Commune A</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 is a sporadic event, a threat</td>
<td>H5N1 is now part of the routine</td>
</tr>
<tr>
<td>Zoonotic and epizootic risk</td>
<td>Epizootic risk only</td>
</tr>
<tr>
<td>Farmers feel helpless, look for assistance</td>
<td>Farmers have a high level of perceived self-</td>
</tr>
<tr>
<td>through the formal system</td>
<td>efficacy</td>
</tr>
<tr>
<td>Fear the direct consequence of the disease</td>
<td>Fear the indirect consequences of the</td>
</tr>
<tr>
<td></td>
<td>disease (ban of transport)</td>
</tr>
</tbody>
</table>

Notes about case-definition used by farmers

Farmers had very variable levels of alert as illustrated by some quotations:

Farmer 4 « if 30% of the Muscovy ducks are closing their eyes, have diarrhoea and stop eating, I sell the remaining ones”
Farmer 6 « with 60% of mortality and typical symptoms, farmers have concluded it was avian influenza »

Farmer 5 « If I only have 20% mortality, I do not inform, because this is not a big loss »

Farmer 9 « I can make the difference between avian influenza and Newcastle disease. With AI, the birds have a sudden fever and they shake the head. For Newcastle, there is a massive mortality and we can pull out the feather as easily as they had been put in boiling water »

Notes about the limited central power over local governments

Our study also illustrates the way local governments are implementing the central policies. In the commune B, we understood that the central policy was recognized and applied. At every level, actors recognize the need to inform the formal surveillance system without delay. On the other hand, in the commune A, a compromise has been found between local economical interest, control of the disease and national policy. This is an example of the Vietnamese political system paradox with a central government’s power much more limited than one would expect in a one-party communist state as explained by Vu (2009).

“This is not uncommon that local governments interpret central policies any way they like”

(Vu, T., in Avian influenza: science, policy and politics, 2009)