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# Epidemiological Studies of Highly Pathogenic Avian Influenza in Vietnam

A dissertation presented  
in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy  
at Massey University

Phan Quang Minh  
2010

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Institute of Veterinary, Animal and Biomedical Sciences  
Massey University  
Palmerston North, New Zealand

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(Submitted 20 August 2010)

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# Abstract

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This thesis utilises data on highly pathogenic avian influenza (HPAI) subtype H5N1 from the Vietnamese national surveillance system and purpose-designed field studies to enhance the understanding of the epidemiological features of HPAI H5N1 in Vietnam.

The findings obtained from the first study show that the presence of a HPAI H5N1 human case was associated with an increase in the likelihood of disease being detected in poultry one and four weeks later, indicating that the occurrence of clinical disease in poultry is not a useful predictor of subsequent human cases in the same locality. The analyses from the second study demonstrate that the epidemiology of HPAI H5N1 in poultry in Vietnam has changed over time and the infection transmission occurs by a combination of local and long-distance spread. The findings from a cross-sectional survey of management practices of itinerant grazing ducks suggest that surveillance strategies for this type of duck management should focus on both layer and larger flocks as they are more likely to be moved outside of their home district, facilitating long-distance disease spread. The results from a matched case-control study in poultry identify factors associated with the presence of HPAI H5N1 and provide evidence that disease control strategies should emphasise the reduction of household-level, rather than village-level, risks for disease. In the last study, spatio-temporal interaction of disease risk in poultry was observed within a distance of 10 kilometres and 12 days following the detected onset of clinical signs. Household-to-household infection rate within a commune was approximately 50 times greater than the household-to-household infection rate between communes. These findings show that the predominant mechanism of HPAI H5N1 infection transfer was local spread.

The lessons learnt from the series of studies presented here should assist Vietnamese animal health authorities to implement the necessary systems and infrastructure that will allow novel and emerging disease syndromes to be investigated promptly and efficiently.



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## Nomenclature

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AHW	Animal Health Worker
AI	Avian influenza
AIC	Akaike Information Criterion
CI	Confidence interval
DAH	Department of Animal Health, Vietnam
DVS	District Veterinary Station
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FRD	Field running duck
GIS	Geographic Information System
GLEWS	Global Early Warning and Response System
HA	Haemagglutinin
HPAI	Highly pathogenic avian influenza
HPD	Highest posterior density region
IQR	Interquartile rage
LabNet	National laboratory network
LPAI	Low pathogenic avian influenza

MARD	Ministry of Agriculture and Rural Development, Vietnam
MCMC	Markov chain Monte Carlo
MRD	Mekong River Delta
NA	Neuraminidase
NIVR	National Institute of Veterinary Research, Vietnam
NVDC	National Veterinary Diagnostic Centre, Vietnam
OIE	World Organization for Animal Health
OR	Odds ratio
PDSR	Participatory disease surveillance and response
RAHO	Regional Animal Health Office
ROC	Receiver Operating Characteristic
RRD	Red River Delta
RRT-PCR	Real time Reverse Transcriptase Polymerase Chain Reaction
SDAH	Sub-Department of Animal Health
SE	Standard error
SEIR	Susceptible-Exposed-Infected-Recovered
SIR	Susceptible-Infected-Recovered
SIVR	Sub-Institute of Veterinary Research
TADinfo	Transboundary Animal Disease Information System
WHO	World Health Organization

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## List of Publications

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Minh, P. Q., Schauer, B., Stevenson, M. A., Jones, G., Morris, R., Noble, A. (2009). Association between human cases and poultry outbreaks of highly pathogenic avian influenza in Vietnam from 2003 to 2007: A nationwide study. *Transboundary and Emerging Diseases*, **56**, pp. 311 – 320.

Minh, P. Q., Morris, R. S., Schauer, B., Stevenson, M. A., Benschop, J., Nam, H.V., Jackson, R. (2009). Spatio-temporal epidemiology of highly pathogenic avian influenza outbreaks in the two deltas of Vietnam during 2003 – 2007. *Preventive Veterinary Medicine*, **89**, pp. 16 – 24.

Minh, P. Q., Stevenson, M. A., Morris, R. S., Schauer, B., Quy, T.D. (2010). A description of the management of itinerant grazing ducks in the Mekong River Delta of Vietnam. *Preventive Veterinary Medicine*, **94**, pp. 101 – 107.

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## Introduction

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Poultry production plays an important role in providing a valuable source of protein and income to millions of households in Vietnam. There were approximately 255 million domestic poultry distributed throughout the country in 2003 (General Statistics Office of Vietnam 2010). The scale and mode of poultry production are diverse throughout Vietnam. Sixty *percent* of the national poultry population is raised in small, privately owned backyard and free-range systems. The Red River Delta in the north and the Mekong River Delta in the south have the highest poultry densities. Compared with western countries, the movement of poultry in Vietnam is complex involving large numbers of small holders moving stock to and from traders, neighbours and local markets. An additional feature, unique to the Mekong River Delta — the major rice growing area — is that ducks are often moved from one location to another to allow insects, snails and leftover grains from harvested rice paddies to be grazed. These ducks form a key part of an efficient traditional integrated farming system. However, in addition, they potentially increase the risk of spreading infectious diseases such as avian influenza to new locations.

Outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 were first identified in Vietnam in late 2003 (DAH 2005*b*). The country has experienced a major epidemic of HPAI H5N1 in poultry during the winter of 2003/2004 with over 2,000 commune-level outbreaks recorded throughout the country. Seventeen *percent* (44 of 255 million) of the national poultry population was destroyed as a consequence of a pre-emptive culling strategy applied between January and March 2004. Although a number of strategies have been applied to control the disease (including pre-emptive culling and vaccination) the country has experienced recurrent HPAI H5N1 outbreaks among poultry, as well as fatal human cases. It is believed that several characteristics of the Vietnamese poultry industry — par-

ticularly the predominance of backyard and free-range systems, in addition to the large duck population — may play a key role in the persistence of H5N1 infection throughout the country.

Since September 2005, chickens and ducks in high-risk areas have been vaccinated for HPAI H5N1 twice yearly and culling has been limited to affected flocks only (FAO 2007*b*). The Vietnamese government also enforced a national ban on waterfowl hatching from February 2005 to February 2007 to reduce the influence of waterfowl as a reservoir of disease. The incidence of poultry outbreaks and human cases has decreased over time, suggesting that these control measures have been effective in reducing the incidence of clinical disease in both poultry and humans. The re-emergence of clinical sporadic HPAI H5N1 outbreaks in recent years is evidence of a present and continuing threat of disease in the country. The question is whether current control measures are sufficient to prevent further spread of the virus during future outbreaks. If not, additional disease control measures need to be applied. An understanding of the epidemiology of the disease and the effectiveness of control measures implemented during previous outbreaks is essential to fine-tune ‘standard’ control activities (i.e. culling and vaccination) and, to develop strategies designed specifically to block transmission pathways that are unique to the Vietnamese situation.

In this thesis I present a series of papers, either published or prepared for publication. Each chapter shows the stage of preparation each paper has reached at the date of thesis submission. Chapter 2 provides a concise overview of avian influenza, the global and Vietnamese HPAI H5N1 situation and strategies for disease control and prevention. The review is not intended to be exhaustive, rather to provide a context and background for the research chapters that follow. The following chapters investigate the complexity of HPAI H5N1 epidemiology in Vietnam from the national level down to the local level.

Chapters 3 and 4 describe spatial and temporal characteristics of HPAI H5N1 outbreaks in Vietnam using routine surveillance data. The data for these analyses were derived from the Department of Animal Health and the Ministry of Health of Vietnam. The specific objective of Chapter 3 was to assess the spatio-temporal relationship between poultry outbreaks and human cases of HPAI H5N1 in Vietnam at the national level. Human cases and poultry outbreaks of HPAI H5N1 have been reported in many countries (OIE 2009*a*, WHO 2010). However, at the time of writing the association between poultry outbreaks

and human cases were generally not documented or unavailable. Chapter 4 describes the spatio-temporal pattern of HPAI outbreaks in poultry and the efficacy of control measures applied in the two deltas of Vietnam. Findings from this work can be used to direct future research into HPAI epidemiology and serve as starting point for devising more effective programmes to control the disease. If the epidemiology of HPAI changes over time, disease control strategies should be adapted accordingly.

Chapters 5 and 6 present results of field studies conducted in the Mekong River Delta in 2008 and 2009. In Chapter 5, a cross-sectional survey was conducted to describe itinerant grazing (field running) duck flocks and their management. The motivation for this work was to provide a better understanding the importance of ducks and their potential role in the spread of HPAI H5N1 in the country. Information about management systems and practices might be used for future studies identifying risk factors for the disease transmission associated with farming practices. Chapter 6 presents the findings of a case-control study conducted to identify household- and village-level risk factors for HPAI H5N1 in the Mekong. The intention of this work was to identify characteristics for targeting interventions.

In contrast to earlier epidemiological studies of the spatial features of HPAI H5N1 in Vietnam which have used data aggregated to the commune level, the analyses presented in Chapter 7 are based on the point location of affected household flocks. Two analytical techniques were applied to these data. Firstly, the space-time K-function was used to quantify the level of spatio-temporal interaction in infection risk in households affected over a ten week period in early 2009 in the Mekong River Delta. Secondly, a fully Bayesian statistical stochastic epidemic model was used to estimate the disease transmission rates of HPAI H5N1 within and between communes. The results of this work provide insight into the characteristics of disease transmission in the Mekong River Delta, indicating the relative importance of local spread<sup>1</sup> in disease transmission.

The analyses presented here use a range of techniques suitable for describing and explaining the spatial, temporal and spatio-temporal aspects of HPAI H5N1 outbreaks. These techniques can also be applied to other infectious diseases of livestock. The lessons learnt from the series of studies presented here should assist Vietnamese animal health

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<sup>1</sup>Local spread is the term coined to cover short distance (generally 10 km or less) spread between livestock units when there is no clear linkage other than geographical proximity (Sanson 1994).

authorities to implement the necessary systems and infrastructure that will allow novel and emerging disease syndromes to be investigated promptly and efficiently in future.

In Vietnam, data for HPAI H5N1 outbreaks in poultry are mainly derived from a system of passive surveillance, which relies on the reporting of suspected cases of HPAI H5N1 in domestic poultry. At the beginning of this programme of doctoral research, many important questions relating to the epidemiology of HPAI H5N1 had not been fully addressed, such as whether there is any association between human cases and poultry outbreaks of HPAI H5N1, whether the epidemiology of HPAI H5N1 has remained constant over time, what risk factors are associated with the infection and spread of HPAI H5N1, the role of domestic duck flocks in facilitating the spread of HPAI H5N1 infection, and the importance of surveillance during outbreak responses. The objectives of this thesis were to address these knowledge gaps.



## Literature review

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### 2.1 Introduction

Since its emergence in late 2003, HPAI H5N1 has attracted substantial public attention because the H5N1 virus has shown to cause disease in both animals and humans (OIE 2009*a*, WHO 2010). Although there is a substantial body of literature relating to the epidemiology of influenza viruses in both humans and animals, it is important to review the disease situation, surveillance, and control strategies from time to time. Updated information can be used to direct future research into HPAI epidemiology and devise more effective programmes to control the disease. It is also important to understand the current HPAI surveillance system and control strategies applied, and how these influence the development and course of HPAI H5N1 in Vietnam.

This chapter starts by presenting an overview of the aetiology and epidemiology of avian influenza and factors that may lead to the introduction, spread and persistence of HPAI viruses. The following sections review the emergence of HPAI, control and prevention activities, and surveillance strategies. Spatio-temporal patterns of HPAI H5N1 outbreaks between 2003 and 2007 are described utilising routine surveillance data that has been recorded by the Vietnamese Department of Animal Health (DAH). Illustrations of current disease surveillance and control strategies applied in Vietnam are emphasised. Some constraints facing the Vietnamese surveillance system will be discussed. In developing countries like Vietnam where resources for disease surveillance are limited, an extensive surveillance system may not always be feasible or effective. Therefore, it is important to identify the critical components of a surveillance system. Recommendations are proposed to show how to improve effectiveness of the existing surveillance system in the country,

and how routine outbreak data can be analysed to orient future research into HPAI. This literature review is not intended to be exhaustive, rather to provide a context and background for the research chapters that follow. Comprehensive literature reviews related to HPAI and disease surveillance have been provided by Capua & Marangon (2004), FAO (2004b), and Swayne (2008).

## 2.2 Avian influenza

### 2.2.1 Aetiology

Avian influenza (AI) is caused by type A strains of influenza virus. All AI viruses are members of the *Orthomyxoviridae* family. Type A influenza viruses are classified into different subtypes according to the antigenicity of their surface proteins, haemagglutinin (HA) and neuraminidase (NA) (Capua & Marangon 2004, Swayne 2008). There are 16 HA (H1 – H16) and 9 NA (N1 – N9) surface protein types that potentially form 144 HA × NA combinations, but only 103 of these combinations have been described. Various combinations have been detected in avian species (Webster & Hulse 2004, Alexander 2007).

AI viruses in poultry are classified as being either highly pathogenic (HPAI) or low pathogenic (LPAI) (OIE 2006). HPAI viruses are defined as those that kill 75% or more of 4- to 8-week-old chickens within ten days of inoculation (Alexander 2000). Only H5 and H7 subtype viruses can cause HPAI, although not all viruses of these subtypes are virulent (Alexander 2007). Outbreaks of HPAI H7N7 caused up to 100% mortality in chickens and ducks within a few days in The Netherlands during 2003 (Elbers et al. 2004). LPAI viruses (defined as those that kill less than 75% of 4- to 8-week-old chickens within ten days of inoculation) can include any of the 16 HA and 9 NA subtypes. LPAI viruses often go undetected and cause no clinical signs of infection. In some circumstances, low pathogenic strains can result in losses for poultry producers. For instance, during 2001 – 2002, a low pathogenic H6N2 strain caused disease and production losses in infected chickens and turkeys in California, USA (Cardona 2005). Of concern is that H5 and H7 LPAI viruses may mutate to be highly pathogenic and infect domestic poultry easily. Between 2002 and 2005, there were three outbreaks of HPAI in chickens in the

Americas, each of these outbreaks was caused by an H5 (USA 2004) or H7 (Chile 2002, and Canada 2004) virus that mutated from an LPAI virus after circulating in poultry for a period of two weeks to several years (Pasick et al. 2006, Senne 2006).

### 2.2.2 Epidemiology

#### Host range

All possible combinations of 16 HA and 9 NA can infect avian species but many infections do not show clinical signs, and some species are more resistant than others (Webster et al. 2006). One may find AI viruses circulating in wild birds through routine surveys and they generally are thought not to cause harm. Wild waterfowl are a natural reservoir of avian influenza A viruses, and these viruses are usually non-pathogenic in these species (Swayne 2008). Quails may be an important reservoir because they are susceptible to different subtypes of AI viruses (Makarova et al. 2003, Xu et al. 2007). Cross-species transmission of AI viruses can potentially cause infection in mammals including humans, hamsters, mice, pigs, ferrets, stone martens, dogs, domestic cats, tigers, leopards, civets, and macaques (Choi et al. 2005, Thiry et al. 2007, Lipatov et al. 2008). The turkey influenza virus isolates in the USA from 1980 to 1989 contained genes of swine origin and there was evidence that re-assortment of viruses from turkeys and swine had occurred (Wright et al. 1992). A wide range of host species presents difficulties in disease control and prevention if different species maintained are in close proximity. If there is a spillover of AI virus between species, it requires a wide range of disease control measures available for different management practices. It is essential that the development of disease control strategies depends on the species of birds at risk and those affected (chickens, ducks, or other species).

#### Virus excretion

AI viruses can be transmitted directly or indirectly by contact with infectious aerosols and other virus-contaminated materials. Frequent pathways for the transmission are respiratory or airborne and gastrointestinal routes (Kelsey et al. 1996). In poultry the greatest amount of virus is shed 2 – 3 days after infection (Suarez 2005). In pigs, AI viruses are

excreted only from the respiratory tract 1 – 5 days after inoculation (Lipatov et al. 2008). Most AI viruses have previously been found to preferentially replicate in the gastrointestinal tract of birds and transmitted primarily via the oral-faecal route (Webster et al. 1978). However, HPAI H5N1 viruses identified in the 2003 – 2004 epidemic in several countries in Asia have shown a reverse trend, with these viruses replicating at high levels in the trachea (particularly in ducks). Thus, the main path of transmission may have shifted from an oral-faecal route to more oral-oral route or even airborne route or both (Sturm-Ramirez et al. 2005). This change may increase transmissibility of the virus. It may also influence the epidemiology of HPAI H5N1 and disease surveillance strategies.

### **Source of infection and contributing factors for virus transmission**

It has been difficult to determine the precise origin of most HPAI outbreaks. Wild waterfowl and live-bird markets have been proposed as the most likely sources of infection in many HPAI outbreaks (Table 2.1). However, precise information on sources of infection has not been recorded sufficiently in numerous others. In outbreak investigations conducted in Japan (Nishiguchi et al. 2005), the Republic of Korea (Wee et al. 2006), and Israel (Balicer et al. 2007), the route of entry or source of virus was not conclusively proven. Insufficient data and late detection of index cases may have prevented analysis of many outbreaks to determine their source with certainty (Sims 2007).

Although several factors have been identified to play important roles in the spread of HPAI viruses such as movement of birds and trade of domestic poultry, the mechanisms for virus transmission of HPAI are not clearly defined. Wild birds are carriers of LPAI viruses that may not be pathogenic for poultry and humans. However, if these birds can also survive infection with HPAI viruses, they may spread the pathogenic virus over long distances during migration (Truszczyński & Samorek-Salamonowicz 2008). Climate change and consequent variations in wild bird migratory routes may also influence the incidence of AI introductions in domestic poultry (Capua & Marangon 2004). Wild birds have been claimed to play an essential role for new introduction of AI virus into domestic poultry. Once AI viruses penetrate domestic poultry flocks, wild birds may no longer play a crucial role for spread of infections within and between flocks. Infections are mainly spread by movement of poultry and poultry products (Webster et al. 2006). AI viruses can maintain in live bird markets, water sources or anywhere poultry have been present if good

**Table 2.1:** Summary of possible sources and contributing factors for selected HPAI outbreaks in domestic poultry.

Outbreak	Subtype	Source	Contributing factors
Cambodia 2004	H5N1	Unknown	Large-scale poultry flocks (Otte et al. 2008)
Canada 2004	H7N3	Unknown	Mutation of LPAI H7N3 viruses; high density of poultry; movement of birds (Pasick et al. 2006).
Chile 2002	H7N3	Unknown	Mutation of LPAI H7N3 viruses; densely populated poultry (Max et al. 2006).
China 2004 – 2006	H5N1	Unknown	Environmental factors: closeness of lakes and wetlands to highways; annual average precipitation (Fang et al. 2008).
Israel 2005	H5N1	Unknown	Shared vehicles, personnel, migratory birds (Balicer et al. 2007).
Italy 1999 – 2000	H7N1	Unknown	High density of susceptible animals; structure of poultry industry; circulation of LPAI viruses (Capua & Marangon 2000).
Japan 2004	H5N1	Wild birds	Sharing sheds and slaughterhouses (Nishiguchi et al. 2005).
Nigeria 2005	H5N1	Waterfowl	Farming practices; mixing of different poultry species (Meseko et al. 2007).
Republic of Korea	H5N1	Wild birds	Vehicles and farming equipment (Wee et al. 2006).
Romania 2005 – 2006	H5N1	Wild birds	Transport of infected poultry and movement of contaminated poultry products (Ward et al. 2008).
Thailand 2004 – 2005	H5N1	Unknown	Free-ranging ducks (Gilbert et al. 2007). Large-scale poultry flocks (Otte et al. 2008).
The Netherlands 2003	H7N7	Wild birds	High density and close proximity of poultry farms (Capua & Marangon 2004, Thomas et al. 2005).
USA 2004	H5N2	Live-bird markets	Mutation of LPAI H5N2 viruses (Senne 2006). Live-bird markets (Pelzel et al. 2006).

biosecurity measures are not put into practice. In some circumstances, infected domestic poultry can transmit HPAI viruses to wild birds which are in close contact (Philippa et al. 2005). This occurrence may introduce a potential threat of virus transmission to other domestic poultry populations.

During the 2004 – 2005 epidemic there was a very strong association between HPAI H5N1 outbreaks and the spatial distribution of ducks, human population, and rice cropping intensity in Thailand and Vietnam (Gilbert et al. 2008). Free-ranging ducks increase the risk of introduction and spread of AI viruses in Southeast Asian countries where ducks scavenge in rice fields under rice-duck production systems. The geographical focus of the scavenging system is not only around the villages where duck owners are living, but also distant villages. Ducks are often raised in rice fields to scavenge for leftover rice grains, insects and snails. Ducks can also be in close contact with village poultry during field movements. Hence, scavenging ducks may play an important role in transmission of HPAI viruses.

Although the precise route of introduction and spread of HPAI viruses have not been

clearly defined in many affected countries, wild birds are considered to play a key role in the spread of HPAI viruses in the wild and contribute to the introduction of viruses into domestic poultry. Human activities, especially those associated with poultry management and trade practices, are considered a more likely mode for transmission of the virus between domestic poultry populations. Trade in poultry and poultry products may facilitate the spread of disease across international boundaries. Detailed epidemiological studies are needed to provide reliable knowledge on the routes of introduction and spread of HPAI viruses to poultry populations.

### **Silent spread of H5N1 virus**

The term 'silent spread' refers to the transmission of H5N1 infection without detectable disease and death (Brown et al. 2008). Several factors have been associated with the silent spread of H5N1 virus. First, wild and domestic waterfowl have been recognised as potential carriers of at least some strains of H5N1 virus as they may excrete virus without showing obvious clinical signs of infection (Chen et al. 2004, 2006). Sturm-Ramirez et al. (2005) exposed ducks to H5N1 virus samples isolated from the outbreaks that occurred in Hong Kong, Vietnam, and Thailand in 2003 – 2004 to determine whether waterfowl are resistant to disease and can act as asymptomatic carriers. Results from this experiment showed that HPAI H5N1 viruses causing minimal signs of disease in ducks and that they have the potential to spread disease silently. In Southeast Asian countries, ducks are mainly raised freely and they can be in close proximity to other species, including humans. The probability of infection occurring in ducks without clinical sign may explain why several human cases of HPAI H5N1 in Vietnam could not be traced to contact with diseased poultry.

Secondly, live bird markets often harbour multiple AI subtypes, which may influence expression of clinical H5N1 infection (Imai et al. 2007). Seo & Webster (2001) showed that the cross-reactive cellular immunity induced by H9N2 influenza viruses protected chickens in live bird markets from lethal infection, but permitted shedding of H5N1 virus in the faeces. Furthermore, H5N1 infection of poultry may be undetected in live bird markets if circulation levels are low (Nguyen et al. 2005, Chen et al. 2006) since this may not induce a detectable increase in poultry mortality. Additionally, H5N1 infection may not be detected if infected birds are sold during the incubation period.

Vaccination may act as a factor facilitating silent transmission of H5N1 viruses, and contribute to a failure to detect disease in affected poultry. Savill et al. (2006) showed that if vaccination coverage is only sufficient to induce partial flock immunity, mortality of infected flocks may not rise above typical levels, whilst virus shedding and transmission still occurs. However, even if vaccination coverage is insufficient, vaccination may reduce the amount of virus shedding.

### **Virus evolution**

Although H5 and H7 viruses are not all highly pathogenic, they can mutate from low pathogenic to highly pathogenic forms and can infect a number of avian species (Philippa et al. 2005). For instance, outbreaks of H5N2 influenza viruses occurred in Pennsylvania, USA. For the period of April to September 1983, the disease spread throughout the chicken population with low mortality since the virus was of low pathogenicity. However, clinical infections with high mortality were reported in October of the same year and the virus was confirmed as highly virulent. H5N2 outbreaks then reoccurred in some north-east states of the USA through live bird markets (Alexander 2000). The occurrence of LPAI viruses mutating to highly pathogenic forms was also recognised in Mexico in 1994 and Italy in 1999 (Capua & Marangon 2004). Therefore, control efforts should not only target HPAI viruses but also LPAI viruses in domestic poultry.

Different AI virus subtypes may exchange their gene segments to generate reassortments that may produce potentially pandemic strains. There is evidence of antigenic drift of H5N1 virus samples isolated in 2003 and 2004 from Hong Kong, Thailand, and Vietnam (Sturm-Ramirez et al. 2005). Xu et al. (2007) demonstrate that reassortment between H9N2 and H5N1 subtype viruses has generated reassortments of both subtypes that have circulated in Southern China. Quails may have played an important role in facilitating the reassortment of the H5N1 virus in the 1997 Hong Kong epidemic (Guan et al. 1999). Lipatov et al. (2008) proposed that HPAI H5N1 viruses in poultry can reassort in pigs with human influenza viruses and adapt to efficient transmission in humans. In conclusion, mixing of different species is thought to increase the chance of reassortment and interspecies transmission of AI viruses. It remains difficult to predict further virus evolution, so strategies are required to prevent the evolution of AI viruses and the emergence of

pandemics. Proposed measures include the separation of species, increased biosecurity, better understanding of the virus, and improved vaccination strategies.

### **Human infection**

All subtypes of the 16 HA and 9 NA combinations have been isolated from birds, but only a few subtypes have been isolated from mammals (Peiris et al. 2001, Hien et al. 2004, Choi et al. 2005). It has been recorded that different AI virus subtypes can infect humans (Stegeman & Bouma 2004, Pasick et al. 2006, Swayne 2008). Although there is no direct evidence to indicate that HPAI H5N1 virus can develop the capacity to transmit between humans, there is evidence to indicate that H5N1 virus can be transmitted from domestic poultry to humans (Claas et al. 1998, Swayne 2008).

The literature has indicated that most human cases of H5N1 infection are directly introduced from a poultry source. Consumption of raw or contaminated products can potentially transmit AI viruses to humans. Pigs may play an important role in transmission of AI viruses to humans, because pigs can be infected by both avian and human influenza viruses (Vahlenkamp 2008). Humans infected with AI viruses may also propagate the virus over long distances and this could lead to further infections of poultry or other susceptible species. Because the H5N1 virus is endemic in poultry in some countries, it is vital to improve surveillance strategies for early detection of poultry infection and prevention of a pandemic influenza. Data on human cases and poultry outbreaks of HPAI H5N1 that have come to the attention of animal health and human health authorities should be analysed using appropriate methods to define any association, if present.

## **2.3 The emergence of HPAI**

### **2.3.1 Worldwide situation**

HPAI, subtype H7, was first observed to cause highly lethal disease in Italian poultry in 1878 (Swayne 2008). In late 19<sup>th</sup> and early 20<sup>th</sup> centuries, HPAI viruses spread throughout Europe, Middle East, Northern Africa, Asia and North and South America. Recently, there has been an increase number of reported of HPAI outbreaks in domestic poultry. The



emergence and widespread of HPAI outbreaks have resulted in economic losses and great public concern. Outbreaks of different HPAI H7- and H5- subtypes have been reported in many countries in recent years. Outbreaks of HPAI have been reported in America (Mexico 1994 – 2003 H5N2, Guatemala 2000 H5N2, El Salvador 2001 H5N2, Chile 2002 H7N3, USA 2004 H5N2, and Canada 2004 H7N3), Europe (Italy 1997 – 1998 H5N2, Italy 1999 – 2000 H7N1, The Netherlands 2003 H7N7, Belgium 2003 H7N7, and Germany 2003 H7N7), Oceania (Australia 1997 H7N4), and Asia (Pakistan 1995 – 2003 H7N3) (Capua & Marangon 2004, Senne 2006). Although several subtypes of AI viruses had been identified in Hong Kong and Southern China in the 1970s and 1980s, the HPAI H5N1 virus was first isolated in poultry in November 1996 in southeast China. The first HPAI H5N1 outbreak was recognised in Hong Kong in April 1997 (Sims et al. 2003). Approximately 1.5 million poultry in all chicken farms and poultry markets were destroyed as the result of massive control measures applied in Hong Kong during that year. No H5 virus was isolated in samples taken in 1998. However, the virus was continuously isolated in Hong Kong from swab samples taken in poultry markets and poultry farms in 1999, 2000, 2001, and 2002 (Sims et al. 2003). The detection of virus indicates the persistence circulation of H5N1 virus for a prolonged period. In 2001, H5N1 viruses were isolated in duck meat imported into the Republic of Korea from China (Chen et al. 2004). Updated information about global HPAI situation in poultry can be found on the website of the World Organization for Animal Health (OIE).<sup>1</sup>

In late 2003, a HPAI H5N1 poultry epidemic spread throughout Asia and was considered to be the first epidemic wave in the region. In early 2004, HPAI outbreaks were simultaneously reported in nine Asian countries: South Korea, Vietnam, Japan, Thailand, Cambodia, Laos, Indonesia, China, and Malaysia (Li et al. 2004). HPAI outbreaks in birds have now been reported in more than 60 countries. Cases of HPAI in humans have been identified in 15 countries (WHO 2010). The disease has then been reported in both wild birds and domestic poultry. Figure 2.1 shows that at the beginning of the current HPAI H5N1 epidemic, reported outbreaks were mainly recorded in domestic poultry. This suggests one of two things. Firstly, the virus did not cause clinical disease in wild birds at that time. Secondly, surveillance programmes in place at the time had a greater sensitivity to detect the disease in domestic poultry than in wild birds. Although the emergence of

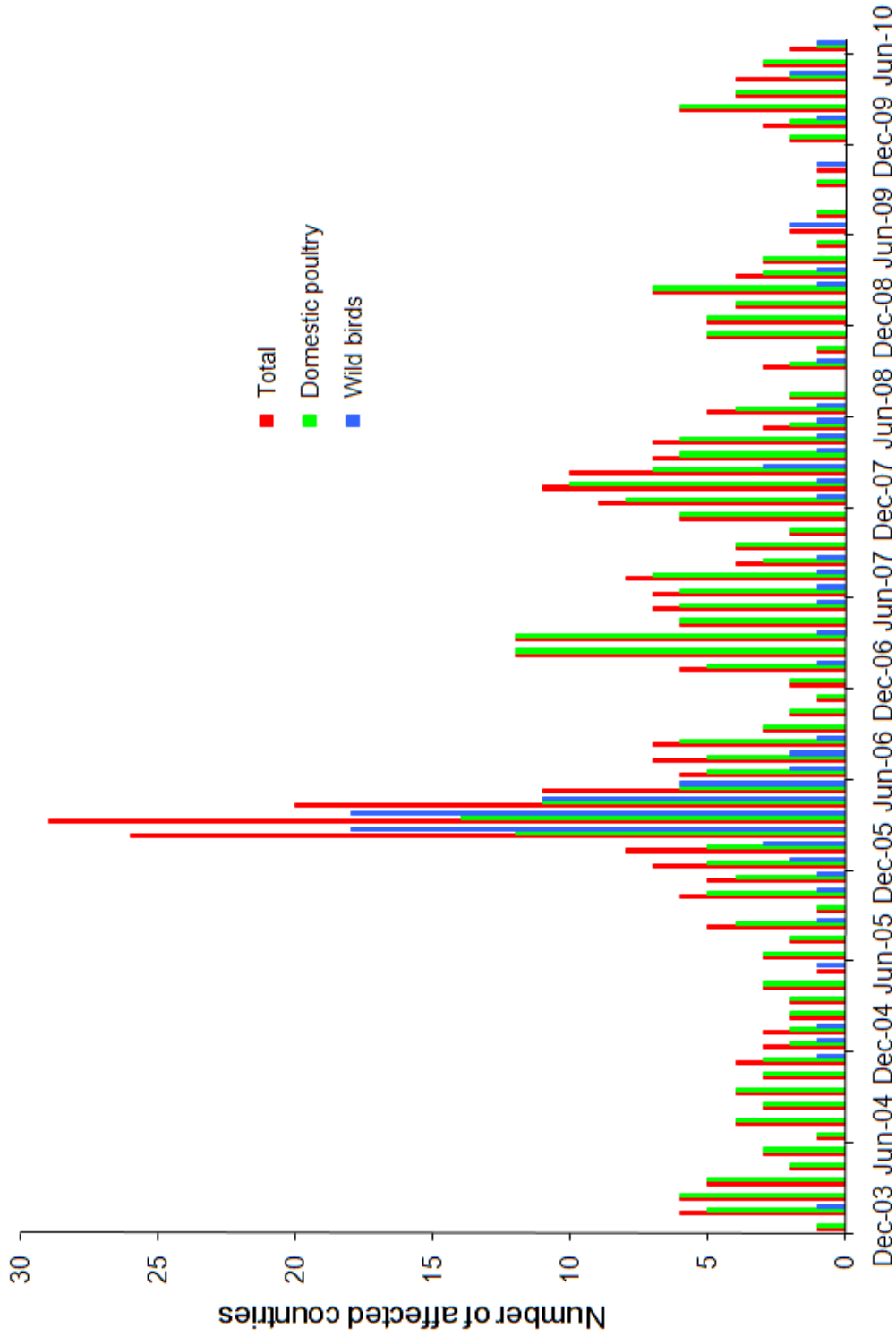
<sup>1</sup>[http://www.oie.int/download/AVIAN%20INFLUENZA/A\\_AI-Asia.htm](http://www.oie.int/download/AVIAN%20INFLUENZA/A_AI-Asia.htm)

HPAI H5N1 outbreaks in Asia has been presumed to relate to a virus that infected geese in Guangdong province in China in 1996 (Guan et al. 2002, Martin et al. 2006), most H5N1 viruses isolated in this period were antigenically different from H5N1 viruses circulating in the region before the end of 2003 (Aubin et al. 2005). Between 2003 and 2010, HPAI H5N1 outbreaks were reported in Asia, Africa, Europe, and the Middle East, affecting wild birds, domestic poultry, human and other mammals. In some countries, HPAI H5N1 outbreaks have been detected in wild birds but not in domestic poultry (OIE 2009b). This provides evidence to suggest that the virus has been introduced via migratory birds. If a disease surveillance system is capable of detecting virus in wild birds, and disease control measures are promptly applied, further outbreaks in domestic poultry can be effectively prevented.

In the twentieth century, three human pandemics of AI have been recorded: (1) H1N1 ‘Spanish flu’ in 1918 – 1919 causing deaths of about 50 million worldwide, (2) H2N2 ‘Asian flu’ in 1957 – 1958, and (3) H3N2 ‘Hong Kong flu’ in 1968 – 1969 (Ligon 2005). Sporadic human cases of various AI subtypes have been reported in recent years (H5N1, H7N2, H7N3, H7N7, and H9N2). Although there was no direct contact between human cases of HPAI H5N1 and affected chickens, the occurrence of HPAI H5N1 outbreaks in chickens and human cases in Hong Kong in 1997 has attracted international concern due to the possibility that this might provide sufficient conditions to initiate an influenza pandemic in humans. Between 2004 and 2005, H5N1 human infections were confirmed by laboratories in Vietnam, Thailand, Cambodia and Indonesia. The H5N1 subtype is great concern because, as 22 July 2010, it has caused 501 human cases in 15 countries (WHO 2010). Updated details of the number of cases of HPAI in humans is available on the website of the World Health Organization (WHO)<sup>2</sup>. To date, although no sustained human-to-human transmission of the H5N1 has been reported, the occurrence of epizootic outbreaks remains an important potential public health risk.

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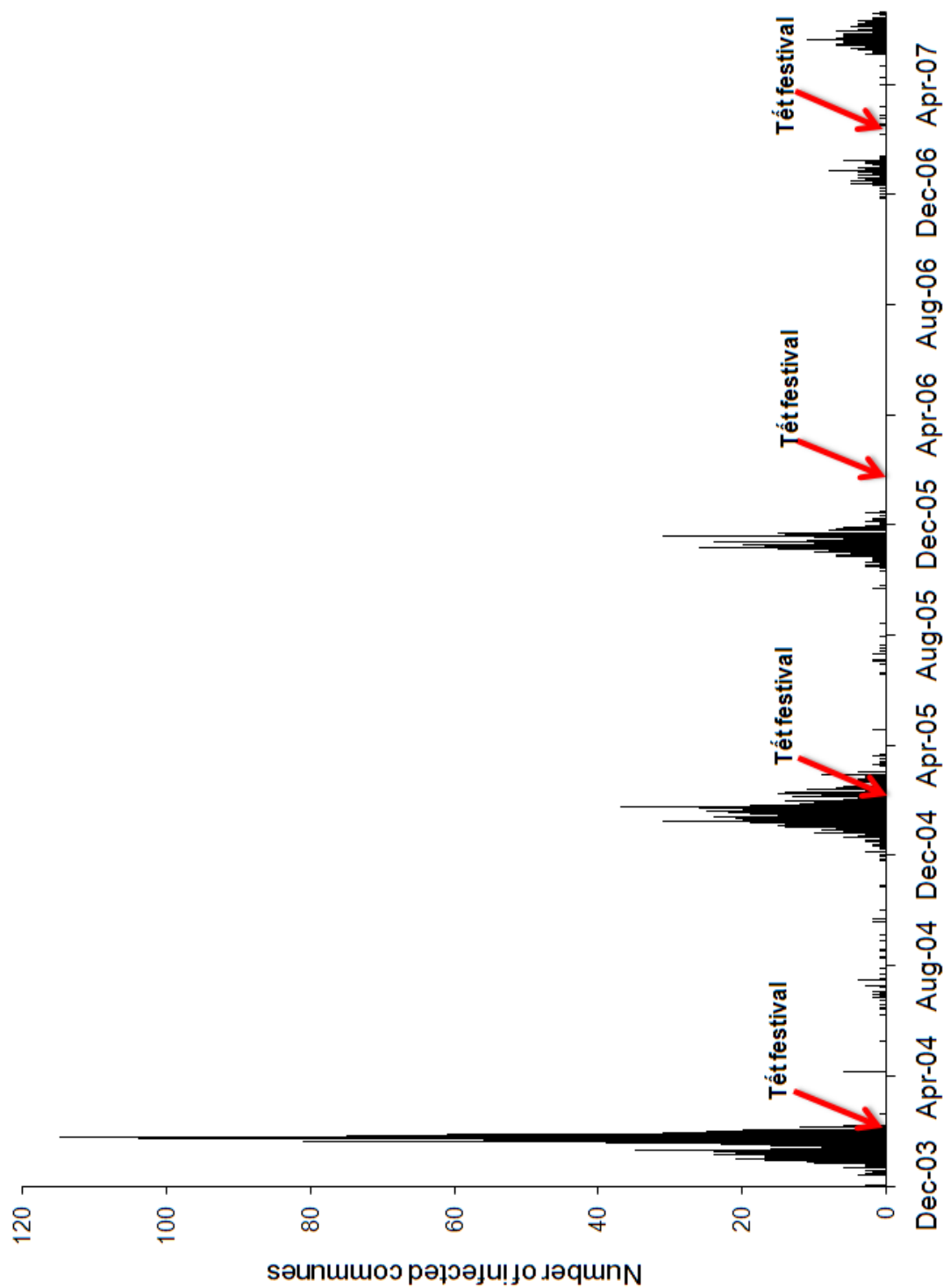
<sup>2</sup>[http://www.who.int/csr/disease/avian\\_influenza/en/index.html](http://www.who.int/csr/disease/avian_influenza/en/index.html)



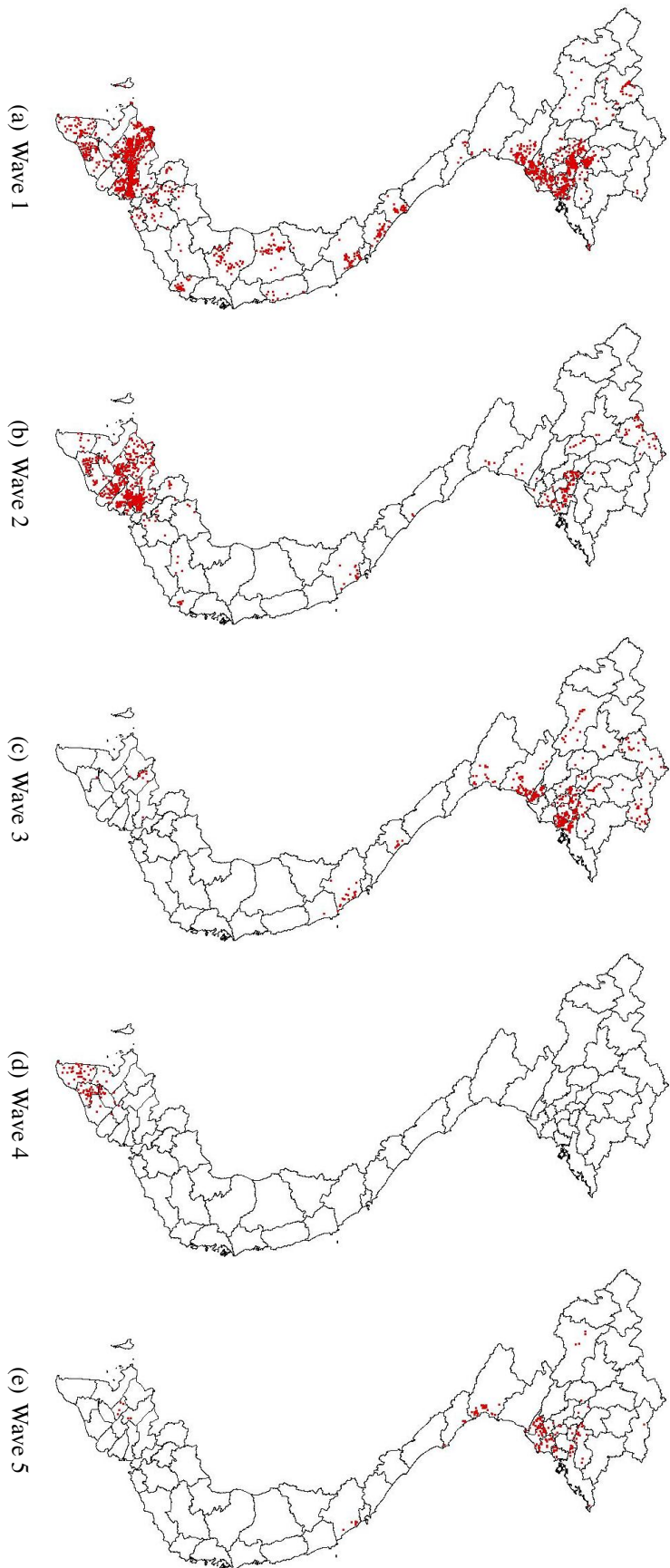
**Figure 2.1:** Graph showing the monthly number of HPAI H5N1 affected countries between December 2003 and June 2010. Outbreaks may affect both domestic poultry and wildlife in a country.

### 2.3.2 Outbreaks of HPAI H5N1 in Vietnam

AI viruses are known to have been present in Vietnam before the 2003 – 2004 HPAI H5N1 epidemic since virological investigation detected several AI subtypes circulating in healthy ducks and geese in live bird markets in 2001 (Nguyen et al. 2005). The first outbreaks of HPAI H5N1 were reported in Vietnam in late 2003. A sequence of epidemic waves has occurred since. The spatial and temporal pattern of HPAI H5N1 outbreaks reported between 2003 and 2007 in Vietnam is shown in Figures 2.2 and 2.3. Five distinct epidemic waves can be identified, with the first two occurring around the Têt (Vietnamese New Year) festival, the next two finishing about one month before Têt, and the last occurring in summer 2007 (May – June). The duration of epidemic waves varied between one and four months. This duration decreased over time, which may be indicative of the effectiveness of control measures applied in the country to bring the epidemic under control. Apart from the fourth epidemic wave which was characterised by comparatively limited spatial spread in several southern provinces, the spatial and temporal pattern of HPAI H5N1 outbreaks shows that outbreaks simultaneously occurred in different provinces of Vietnam, concentrating in the Red River Delta (RRD) in the north and the Mekong River Delta (MRD) in the south. The occurrence of sporadic HPAI H5N1 outbreaks between 2007 and 2010 indicates that there is a persistent circulation of H5N1 virus in the country. Concurrent with poultry outbreaks, the HPAI H5N1 virus has caused a total of 119 human cases of disease, with 59 fatalities in Vietnam since December 2003 (WHO 2010). Close contact with infected poultry is the main source of transmission of H5N1 to humans (Dinh et al. 2006). In Vietnam, poultry are mainly sold at local markets or to farm gate traders. Farm gate traders may move poultry over large distances, exposing people in areas well away from the area where the birds are raised. Most human cases of HPAI H5N1 in Vietnam occur in rural areas, and are thought to be related to local marketing and poultry management practices. Though no sustained human-to-human transmission has been reported, the greater and longer the period of exposure of humans to HPAI H5N1 virus, the greater likelihood that the virus can mutate and cause a more virulent form of the disease in humans.



**Figure 2.2:** Epidemic curves showing the daily number of HPAI H5N1-infected communes reported in Vietnam between 2003 and 2007. The Tét festival was on 9 – 11 February 2004, 11 – 13 February 2005, 29 – 31 January 2006, and 16 – 18 February 2007.



**Figure 2.3:** Point maps showing the locations of of HPAI H5N1-infected communes in Vietnam stratified by epidemic waves: (1) 12 December 2003 – 15 April 2004, (2) 04 December 2004 – 01 April 2005, (3) 01 October 2005 – 25 December 2005, (4) 16 December 2006 – 22 January 2007, and (5) 14 May 2007 – 29 June 2007. Provincial boundaries ( $n = 64$ ) are shown as black lines.

## 2.4 HPAI control and prevention

In countries where HPAI H5N1 outbreaks have caused high mortalities in affected poultry flocks and losses due to culling, the aims of disease control efforts are to prevent further spread of the disease, confine the disease to infected areas, and gradually achieve disease eradication (FAO 2007*b*). The threat of a human influenza pandemic has drawn global attention to control HPAI H5N1 outbreaks in poultry in order to limit opportunities for exposure of humans to the virus and minimise the possibility of virus mutation to more virulent forms.

Early detection of infection and rapid response to that infection are essential components of the effective control of an HPAI outbreak. Early detection can be achieved by pre-planned surveillance programmes. Rapid response aims to implement control procedures following identification of infected flocks. Once outbreaks of HPAI have been identified, control measures should be applied including destruction of infected poultry and pre-emptive culling of poultry at risk, movement management, cleaning and disinfection, tightening of farm bio-security activities, and (in some situations) vaccination. In countries where the veterinary infrastructure is weak or unable to enforce laws related to disease control, a modified approach should be applied to control rather than to eradicate HPAI H5N1 virus (Sims 2007).

It is not possible to control any HPAI outbreak if comprehensive measures are not applied. Stamping out of poultry flocks is intended to reduce the numbers of infected and susceptible poultry in outbreaks and surrounding areas, but it is only applicable where there are sufficient compensation policies and it must take into account issues relating to animal welfare. Movement control measures are also applied to minimise disease transmission over long distances. Stamping out of infected poultry and pre-emptive culling may result in the elimination of important sources of household income and disposal of large number of carcasses may damage the environment. Therefore, this policy should only be applied when the number of affected flocks is low. When the disease is endemic, vaccination strategies can be applied simultaneously with other control measures to reduce HPAI incidence and minimise the risk of human exposure to infection (FAO 2007*b*). Importantly, vaccination must be used in accordance with guidelines to ensure that vaccines are of assured quality. In addition, appropriate monitoring of immune response and infection

status of vaccinated flocks must be considered. It is not practical that one control strategy for HPAI can be applied for every country and all poultry species. Effectiveness of HPAI control programmes is influenced by many factors such as status of HPAI, the composition of affected species within a geographical area, financial resources, and veterinary infrastructure. All of these factors vary between (and often within) countries.

### **2.4.1 Control and prevention strategies in other countries**

To effectively prevent and control HPAI, a transboundary response is required to contain and eliminate the disease at its source. It is essential that countries progress towards harmonising their control and prevention strategies. Based on experiences and lessons learned from involvement in the global control of HPAI outbreaks, the Food and Agricultural Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE), in collaboration with the World Health Organization (WHO) and other relevant agencies, have proposed a global strategy for prevention and control of HPAI that identifies priorities and strategic approaches for national, regional and global level control and eradication (FAO 2007*b*). The strategy proposes three priorities related to country HPAI status: (1) reduction of the incidence of HPAI in endemic countries, (2) eradication of HPAI in countries where the disease is sporadic, and (3) improvement of HPAI preparedness and capacity for early detection and response. Depending on its own situation, a country may apply the most appropriate approach for disease control and prevention. It may require a long-term strategy to achieve successful control and eradication of HPAI H5N1.

Different approaches have been applied to control HPAI outbreaks including stamping out, vaccination, movement control, closure of live bird markets, and enhancement of biosecurity. Massive control strategies have remarkably reduced the incidence of disease and outbreaks have been limited to certain areas in some countries. A stamping out policy has proven to be effective in preventing and eliminating establishment of HPAI H5N1 virus in developed countries where poultry are raised under intensive conditions and veterinary capacity is able to detect infection early (Sims 2007). However, stamping out may not be the main tool used in countries where the disease is endemic. In these situations alternative strategies must be applied.



It has been demonstrated that vaccination against HPAI can protect poultry by providing resistance to infection, reducing the number of susceptible poultry and minimising virus excretion. Vaccination was a key strategy for controlling the HPAI H5N2 outbreaks in Mexico in 1994 – 1995 and Central America in 2001 – 2002. Outbreaks have not been recorded in those countries during recent years (Senne 2006, OIE 2009*b*). China, Indonesia, and Vietnam have used vaccination as a key strategy for controlling recent HPAI H5N1 outbreaks (Capua & Marangon 2004, FAO 2007*b*, Sims 2007). In combination with other measures, these countries have achieved substantial success in disease control. Recently, vaccination has been applied in other countries including Russia and Egypt (Sims 2007). A combination of stamping out, vaccination and movement controls have proven to be successful in controlling the HPAI H5N1 outbreaks that occurred in Nigeria and Egypt during 2006 (FAO 2007*b*).

Approaches for disease control and prevention can be modified according to the disease situation of individual countries. For instance, during the first phase of HPAI H5N1 epidemic during January 2004, Thailand applied a massive stamping out policy where poultry were slaughtered on infected premises and surrounding areas within a radius of 5 kilometres. This distance was reduced to 1 kilometre for later outbreaks. When the incidence of disease was sporadic, only poultry on infected premises were slaughtered (DLD 2006). In Hong Kong, culling of the entire poultry population was applied in the 1997 HPAI H5N1 epidemic, while a combination of measures were applied in the 2002 – 2003 epidemic including culling of infected flocks, vaccination and movement controls. Both control strategies eliminated the virus (Sims 2007). Destruction of infected and at-risk poultry, in combination with movement controls and other measures, can lead to success in prevention of secondary spread of the disease. However, it is costly and requires a large amount of resources. In a country where the disease is endemic, it may not be feasible to proceed with massive culling. In this situation, only infected flocks should be slaughtered, and vaccination considered a suitable option. With application of massive control strategies involving a combination of stamping out, movement control, screening, and disinfection of infected premises France, Sweden, Germany and Denmark were able to eradicate the emergence of HPAI H5N1 during 2006. However, the disease was re-introduced in Germany during 2007 – 2008 (OIE 2009*a*). Although the use of combined control measures including stamping out, enhanced biosecurity, and movement

control had been applied, it was difficult to eliminate HPAI viruses in Russia, Kazakhstan, Albania, Azerbaijan, Serbia, and Turkey (FAO 2007*b*).

In summary, effective control and prevention strategies for HPAI require a combination of disease control methods. Early detection of incursions of virus can prevent establishment of disease in countries with proper surveillance systems. When the disease is emerging, massive culling of infected and at risk flocks is the appropriate response in countries with strong veterinary services and financial resources. In countries where detection of disease is delayed, disease may be widely spread at the time of detection. Control measures should take this issue into account. Most of the countries experiencing HPAI outbreaks have been able to eliminate the disease in poultry and prevent human infection. However, the re-introduction of disease has occurred in some countries recently. The disease may be difficult to detect and control in countries with deficient veterinary services and resources.

#### **2.4.2 Control and prevention strategies in Vietnam**

Since the first HPAI H5N1 epidemic in 2003 – 2004, the Vietnamese government has implemented aggressive control policies to control the disease. The total numbers of birds destroyed during the first epidemic wave were 43.9 million, accounting for 16.8% of the total poultry population, of which 30.4 million were chickens and 13.5 million were waterfowl (DAH 2005*a*, To et al. 2007). A massive culling approach reduced the impact of disease at the time, however this did not eliminate the virus and disease reoccurred soon after. It has been shown that culling is useful in eliminating and preventing establishment of the virus if incursions into domestic poultry are detected early as was seen in Japan and the Republic of Korea (Nishiguchi et al. 2005, Wee et al. 2006). However, if the disease is widespread and farmers are not able (or willing) to cooperate, massive culling may not be an appropriate tool for disease control and prevention. Lessons learned from the HPAI H5N1 epidemics in Vietnam have shown that immediate stamping out is most likely to be successful in controlling the disease when it is combined with movement controls and disinfection of affected premises. In addition, vaccination has been used as a tool to control the disease and to reduce the viral load in the environment.

After the reoccurrence of HPAI H5N1 outbreaks in 2004 – 2005, the Vietnamese government has focused on controlling rather than eliminating the disease. For that purpose,

chickens and ducks in high-risk areas have been vaccinated for HPAI H5N1 twice yearly since September 2005, whilst culling was limited to affected flocks only (FAO 2007a). Due to increasing evidence that domestic waterfowl may silently spread HPAI infection (Chen et al. 2004, Sturm-Ramirez et al. 2005), the Vietnamese government enforced a national ban on waterfowl hatching between February 2005 and February 2007 and has implemented movement controls for itinerant grazing (field running) duck flocks since March 2007. The use of restricted culling combined with other control measures has shown to be more effective than mass culling. The incidence of human cases and poultry outbreaks has decreased over time, indicating that these control measures have been effective in reducing clinical disease in both poultry and humans. The general public has been involved in the disease control process through a nationwide education campaigns on how HPAI H5N1 virus is introduced and spread and how the disease can be prevented. The control of risky behaviours and actions such as close physical contact between humans and poultry, live bird markets, and marketing practices has greatly reduced the spread of HPAI H5N1 virus in Vietnam since 2006 (Chen, Jin, Yu, Dan, Zhang, Song & Chen 2007). The re-emergence of clinical sporadic HPAI H5N1 outbreaks in 2009 provides evidence of a present and continuing threat of disease in the country. In order to control and eliminate the disease, the country should adopt an integrated control programme using the combination of measures best suited to its existing situation. This includes animal culling of affected flocks, movement control, and vaccination. It is unknown to what extent H5N1 virus is still circulating in the country as the true incidence of H5N1 can only be determined by means of rigorous active surveillance.

Vaccination has been found to be effective in reducing disease incidence in poultry and the risk of transmission to humans in Vietnam. Vaccination campaigns have targeted high-risk areas (areas of high poultry density and areas that had experienced outbreaks in the past). In Vietnam, the poultry production system is characterised by smallholder and village poultry sectors with a low level of biosecurity. Therefore, HPAI viruses from an infected flock can easily propagate to neighbouring villages, wild birds, or domestic ducks. In this situation, vaccination is applied to minimise disease dissemination and to prevent a risk to human health. Vaccination approach requires post-vaccination monitoring to assess vaccine efficacy and flock protection levels. Surveillance programmes are also crucial to detect the presence of field virus in vaccinated flocks and to ensure that

existing vaccine strains provide protection against field viruses. Middleton et al. (2007) conducted an experimental study to test effectiveness of a monovalent H5N3 oil emulsion vaccine and a bivalent H5N9 + H7N1 oil emulsion vaccine in ducks. Results show that both vaccines protect against morbidity, and the monovalent vaccine provides effective protection to H5N1 challenge virus (A/Muscovy duck/Vietnam/435/2004). Despite the cost and logistic challenges of delivering a comprehensive vaccination campaign, vaccination policy is recommended for Southeast Asian countries where the policy of stamping out of infected poultry may result in the removal of a major source of food for humans (FAO 2004a). This said, while HPAI viruses may still infect and replicate in vaccinated poultry (Van der Goot et al. 2005), proper vaccination may protect clinical disease in vaccinated poultry and reduce the levels of virus load in the environment. Although the precise impact of vaccination cannot be assessed, the use of vaccination in combination with other control measures has resulted in a reduction in the number of HPAI H5N1 poultry outbreaks and human cases in Vietnam. One of the spin-offs from vaccination is that the quality of animal health data (e.g. records of household location and flock size) are regularly updated because veterinarians regularly visit households.

In summary, the goals for HPAI control and prevention strategies may be different depending on the country and its disease status. Single measures cannot lead to successful control and elimination of HPAI H5N1 virus. In Vietnam, outbreaks of HPAI H5N1 have been controlled by a combination of measures. The strengths and weaknesses of all control measures should be considered and the most applicable methods applied to specific conditions. Vaccination has not eliminated H5N1 virus from the country, but it appears to have reduced the severity of outbreaks. Culling of poultry also reduces the level of infection and its spread in infected areas. If the surveillance system can ensure early detection of HPAI H5N1 infection, the use of restricted culling involving only infected flocks combined with targeted vaccination, movement controls and biosecurity measures is likely to be effective. In addition to the disease control and prevention measures listed above, it is important to establish routine monitoring and surveillance activities to reduce risk and facilitate the early detection of HPAI infections or evidence that virus may be circulating in some localities or poultry species.

## 2.5 HPAI surveillance

Disease control programmes cannot be effective without appropriate surveillance strategies, and effective control strategies require good surveillance data. Epidemiological surveillance has been defined as the continuing and systematic collection, analysis, interpretation, dissemination of health data and the application of these data to disease prevention and control (Centers for Disease Control and Prevention 1986, Thacker & Berkelman 1988). Early and successful control of HPAI requires effective surveillance programmes. The reason some countries have difficulty in applying effective controls is due to weak veterinary capacities and insufficient capability to identify major risk factors for HPAI infection and transmission. The objectives of HPAI surveillance, as proposed by FAO (2004*b*), are to detect early incursions and/or clinical disease and infection, understand the epidemiology and ecology of AI, assess the temporal and spatial patterns of infections, understand the evolution of HPAI strains, define and control risks to public health, monitor for antigenic drift in AI viruses, demonstrate freedom from clinical disease and absence of infection in a country or compartment and assess the efficacy of vaccination. The speed of disease control depends on how quickly the initial cases of HPAI are detected and how promptly control measures implemented (Swayne 2008). Surveillance programmes contribute to early detection of HPAI infection and risk factors, monitoring of vaccination programmes, understanding of disease patterns, allowing authorities to adjust disease prevention and control measures. Surveillance has been described as either 'passive' or 'active'. Recently, the terms 'scanning' and 'targeted' surveillance have been used in veterinary surveillance (Scudamore 2002). While scanning surveillance refers to a continuous watch to estimate the extent of disease in animal populations, targeted surveillance refers to structured surveys to gather particular information about a disease in groups or subpopulations of animals.

Surveillance data on HPAI can be obtained either passively or actively. Passive data collection system refers to data gathered from reporting of clinical suspicion outbreaks (Lilienfeld & Stolley 1994). Thus, the quality of data may be influenced by the willingness of reporters (farmers, traders, animal health workers) to report the presence of disease. The awareness and knowledge level of disease also influences the ability to report. People tend to report a disease with high mortality and frequency or significant clinical

signs rather than the one with lower levels and they may be more aware of diseases that are more severe. In this situation, the proper application and interpretation of diagnostic tests is critical for confirmation of cases and management of surveillance activities (Capua & Alexander 2006). During the 2001 epidemic of foot-and-mouth disease (FMD) in the United Kingdom, suspected cases were mostly picked up by passive surveillance where farmers reported to authorities the presence of suspicious clinical signs in their stock (McLaws et al. 2006). Results obtained from passive surveillance may enhance disease control activities. For instance, results from a routine surveillance programme showed that H5N1 viruses had circulated among apparently healthy ducks in mainland China from 1999 to 2002. This finding might have induced authorities to take immediate actions to prevent the transmission of HPAI viruses from healthy ducks into chicken flocks (Chen et al. 2004). Obviously, one cannot rely on passive surveillance or outbreak reports to estimate the prevalence of H5N1 viruses in duck populations (for example), as it may be underestimated. Nevertheless, passive surveillance may contribute to findings of new HPAI cases occurring in a population, and then more solid active surveillance will be implemented.

Active surveillance is based on specific targeted investigations. While a passive surveillance system is not based on any formal process of sampling, an active surveillance system depends on a defined sampling scheme (Christensen 2001). As consequence, the quality of data will be greatly influenced by the sampling design actually used. These essentially depend on the event of interest and its expected disease prevalence and incidence as well as the availability of diagnostic tests. Therefore, active surveillance can be very costly if HPAI infection is rare and the prevalence of disease is extremely low. In this situation one has to use a large sample size and apply diagnostic tests of sufficient sensitivity and specificity. Active surveillance is also essential to detect LPAI infections of H5 and H7 subtypes that could change to HPAI (Elbers et al. 2004). Active surveillance can be used to confirm disease events detected through passive surveillance, improving the accuracy and effectiveness of passive reporting. In some circumstances, a standard surveillance strategy may be harmonised for some countries. Otherwise individual countries can design their own surveillance programme with particular priorities depending on epidemiological considerations and available resources.

Surveillance for HPAI in wild birds provides an important early warning of an impend-

ing HPAI incursion into a domestic poultry population. Wildlife surveillance campaigns aim to determine the role of wild birds in maintenance and spread of HPAI viruses, guide strategies to prevent infections in humans and domestic poultry, and provide better understanding of the mechanism of virus spread internationally. These help to identify regions where the likelihood of HPAI incursion and establishment is high. Risk-based and targeted resources should be mobilised to prevent domestic poultry and/or humans from HPAI infection in those areas. Wildlife surveillance for HPAI can be a comprised of a combination of passive and active surveillance activities. Samples can be collected opportunistically from different sources, particularly from sick and dead birds. This sampling strategy may not require large financial resources. Dead bird surveillance plays an important role in the early detection of West Nile virus in the United States of America (Eidson et al. 2001). However, collected samples from passive surveillance may not present for bird populations and species. In contrast, targeted surveillance can ensure a predetermined level of accuracy (Guberti & Newman 2006). In active surveillance programmes, samples can be collected from birds that are captured by trapping and/or hunting activities. A sampling frame is defined for a targeted species or group of species. In general, surveillance for HPAI in wild birds should focus on appropriate wildlife habitats. Munster et al. (2005) proposed some factors to consider when developing wildlife surveillance programmes: the frequency of detection of various HPAI viruses in a given bird species, species habitat, species characteristics, poultry contact, links between HPAI infected areas and surveillance areas, movement patterns, migratory pathways, and the population size of survey species in a given area. In conclusion, HPAI surveillance for wild birds can play a key role in the early detection and preparation for disease threats. Wildlife surveillance is hard and complex. It may require a combination of active and passive surveillance methods to obtain valuable information.

Similar to surveillance in wildlife, HPAI surveillance in domestic poultry can be comprised of both passive and active approaches. Passive surveillance generally gathers details from reporting of clinical suspicion outbreaks of HPAI in domestic poultry. Active surveillance is based on specific targeted investigation of at-risk populations for evidence of HPAI infection. For the purpose of early warning of HPAI incursion and detailed information on HPAI status, surveillance programmes should target high-risk areas such as borders and international entry points, domestic waterfowl, and live bird markets. For

countries, where HPAI vaccination is applied, it is important to conduct surveillance programmes to monitor vaccination strategies and ensure that vaccinated poultry achieve protective levels of immunity.

Appropriate surveillance programmes require adequate information. Apart from information on HPAI infection, it is crucial to obtain available information on uninfected individuals within the population. Data on population at risk is important for epidemiological analysis, but not broadly available in many Asian countries where village poultry are often free-grazing (Morris & Jackson 2005). Census information for numbers of households with poultry is difficult to obtain because of difficulty in identifying all poultry owners. In some circumstances, a practical approach for getting around this problem would be to simulate a poultry population based on human census data. Animal census data is often aggregated at large administrative areas such as the provincial level in Vietnam (General Statistics Office of Vietnam 2010). In addition, census data is only obtained on yearly basis. Therefore, it may not accurately reflect the current situation. In this situation, outbreak investigations are desired for collecting data on the population at risk within affected areas. Data obtained from appropriately structured investigations is adequate for the purpose of epidemiological analysis. It is also crucial to obtain information on poultry movements. Because movement patterns of domestic birds may differ between areas and poultry populations, they may influence the maintenance and transmission of HPAI. Furthermore, various surveillance strategies may be applied for specific purposes such as risk-based surveillance for early detection of HPAI infections, and virology surveillance to track evolution of HPAI viruses. Adequate surveillance data allow one to quantify transmission patterns and risk factors for HPAI.

### **2.5.1 Surveillance programmes in other countries**

Depending on epidemiological considerations and available resources, countries may choose to apply surveillance approaches with particular priorities or intensive surveillance programmes. For instance, HPAI free countries may focus on surveillance at international borders and on wildlife to detect incursions of HPAI. For HPAI affected countries, surveillance priorities may focus on the understanding of epidemiology, ecology and evolution of HPAI strains. Different countries have different regulations concerning disease surveil-



lance strategies. As a result, surveillance quality and the capacity of veterinary services also vary greatly between countries and regions.

Surveillance programmes are not only restricted to an individual country's border but also to regional or inter-continental networks, especially for transboundary infectious diseases like HPAI. In 2006, a large-scale surveillance study of wild birds was launched in several countries of Eastern Europe, the Middle East, and Africa to evaluate the perpetuation of HPAI H5N1 virus in wild birds (Gaidet et al. 2006). Field surveillance activities were conducted in the collaboration of various institutions and participants including international organisations, national and local agencies in participating countries, ornithologists, and veterinarians. Initial results obtained from this extensive surveillance campaign indicate the need to improve the understanding of the host ecology of AI viruses in sub- and tropical environments. European Union Member States have implemented annual surveillance programmes for AI in domestic poultry and wild birds since 2002 (Pittman et al. 2006). According to guidelines of the European Union (EU), the occurrence of HPAI in poultry must be reported to competent authorities of the member countries, and outbreaks of HPAI infection must be reported to the European Commission via the Animal Disease Notification System. Under threat of HPAI H5N1 subtypes from Southeast Asia, intensified wild bird investigations have been conducted focusing on high-risk species and examinations of dead birds. To ensure early detection of the virus and enhance collaboration, veterinary authorities, national reference laboratories, and the EU Reference Laboratory liaise closely. As a consequence, these surveillance strategies have contributed to efforts to prevent major HPAI outbreaks in domestic poultry in EU member countries (OIE 2009*b*). At the time of writing this review no human cases of HPAI H5N1 have occurred in the EU (WHO 2010).

Various surveillance strategies have been conducted in the Americas. During the H7N3 epidemic in 2003, Chile conducted nationwide surveys to evaluate the potential spread of the virus in poultry populations (Max et al. 2006). Sentinel birds were placed in previously infected farms to monitor silent spread of the virus. Results from these surveys demonstrated that the influenza infections of poultry in Chile in 2002 were the first isolation of AI viruses in poultry in South America. Chilean viruses were distinct from existing H7 AI viruses, and a novel mechanism-recombination between genes of the LPAI virus resulted in a virulent shift to cause clinical disease in chickens (Suarez et al. 2004).

Intensive active and passive surveillance programmes in the USA detected evidence of 13 H (H1 – H13) and 9 N subtypes of AI viruses in live-bird markets and poultry farms during 2002 – 2005 (Senne 2006). The Texas Poultry Federation conducted a serologic surveillance programme in participating commercial flocks during 1995 – 2004. Samples were taken from all areas of the state. However, the index flock of the 2004 HPAI H5N2 outbreak in Texas was not a participant in the surveillance programme (Pelzel et al. 2006). In general, the sensitivity of HPAI surveillance programmes in individual countries is greatly influenced by the level of testing and reporting capacity.

Biosecurity authorities in New Zealand have conducted many AI surveillance programmes in wild birds and domestic poultry (Rawdon et al. 2010). Though the country has never recorded a case of HPAI in wild and domestic birds, LPAI H5 and H7 subtype viruses have been detected in healthy wild waterfowl (Stanislawek et al. 2002, Frazer et al. 2009, Langstaff et al. 2009). In order to plan future surveillance activities, Rawdon et al. (2010) conducted a serological survey to determine LPAI infection status of chicken and turkey farms. No evidence of H5 or H7 viruses was detected in the surveyed farms at the time of sampling. However, H5 subtype positive samples were identified in three free-range chicken farms, suggesting a historical local exposure of these chickens to virus from wild birds. The authors suggest that free-range layer farms are a risk sector for exposure to AI, and screening surveillance programme should be applied for this sector where targeted surveillance is used for other commercial poultry sectors in New Zealand.

In Asia, community-based and participatory surveillance programmes have been applied for HPAI detection. In 2004, Thailand launched an intensive active clinical surveillance programme (the so-called ‘X-ray’ strategy) to gather information of HPAI situation in the country (Gilbert et al. 2006). Thousands of trained inspectors participated in this survey. They searched for evidence of HPAI in village poultry during October and early November 2004. All sick and dead poultry in surveyed villages were reported to local authorities. As a consequence, the number of reported outbreaks increased remarkably soon after the announcement of the X-ray survey. This suggests that HPAI viruses were still circulating in the country, and the increase in number of HPAI outbreaks were likely influenced by the intensity of surveillance activities. In Indonesia, a participatory approach for HPAI surveillance has been applied to track where and when HPAI H5N1 outbreaks have occurred (Normile 2007). Trained teams formally diagnose AI in the field by using

rapid tests (Desvaux et al. 2008). Trained teams of veterinarians visit door to door asking villagers about outbreaks among their backyard chickens, and then persuade their cooperation in disease control efforts. Information obtained from villagers provides details of the epidemiological features of HPAI and contribute to control strategies. Although community-based and participatory surveillance approaches appear to be successful in Thailand and Indonesia, these surveillance programmes required great investment and efforts for implementation at national level.

In summary, HPAI surveillance capacity has been enhanced to allow early diagnosis of HPAI cases in many countries. Appropriate surveillance strategies provide better understanding of risk factors and the epidemiology of HPAI, poultry production and marketing systems, and the ability to assess the effectiveness of vaccination programmes. To ensure successful control and eradication of HPAI, a country should consider applicable surveillance approaches according to its own situation and available resources. Results of national surveillance should be shared to provide neighbouring countries knowledge of regional disease and infection status. Some of the data sharing may go on through international organisations like the WHO, FAO, and OIE. The three organisations have jointly developed the Global Early Warning and Response System (GLEWS), a linking network to improve global surveillance system for zoonotic disease.<sup>3</sup>

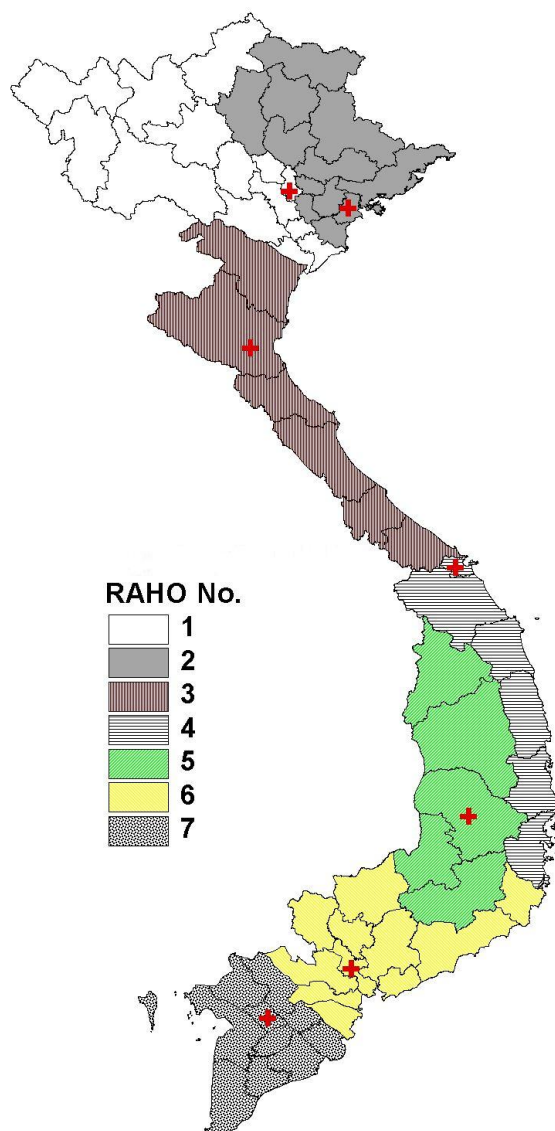
## 2.5.2 Surveillance for HPAI in Vietnam

### Information system

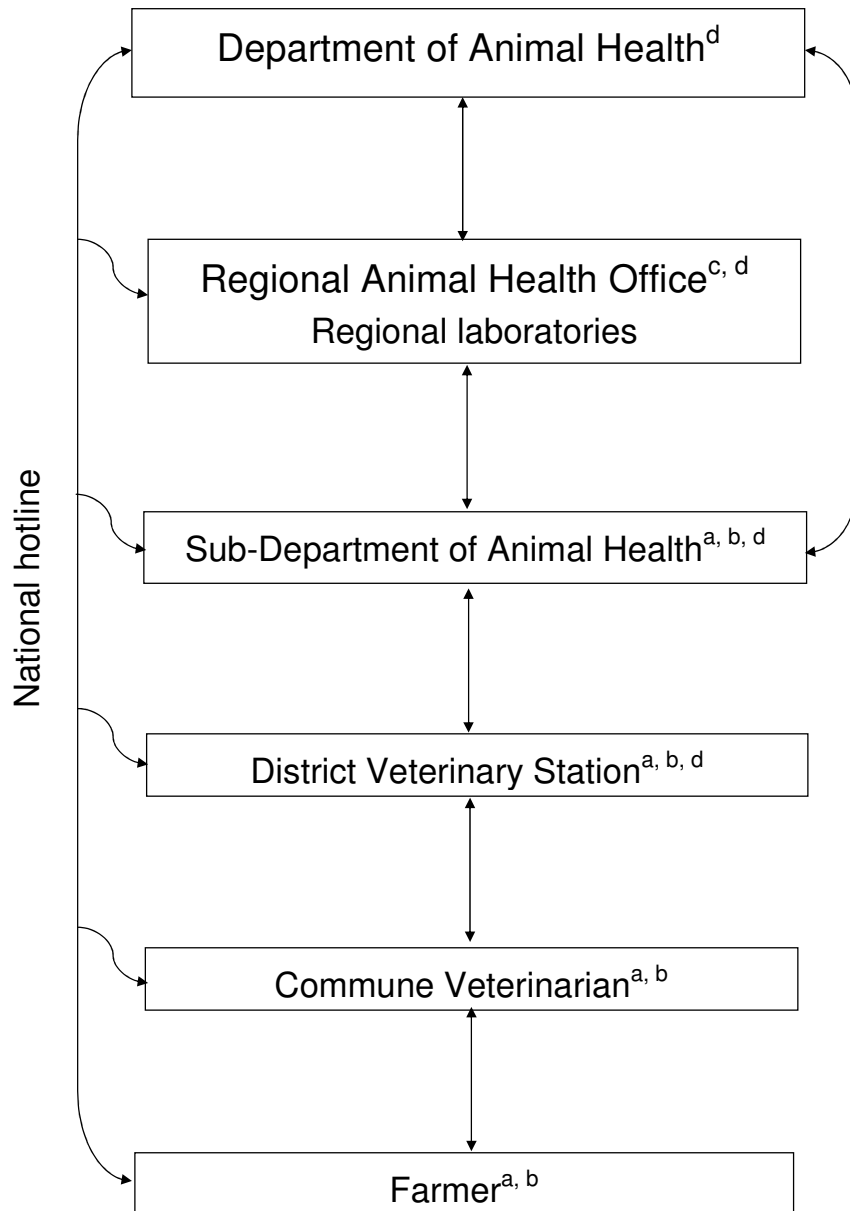
In Vietnam disease reporting methods, similar to other countries, can be either active or passive. In active surveillance, veterinary authorities contact sources in the field and request specific data on a disease of interest. However, active surveillance strategy requires substantial resources that are not always available in a developing country like Vietnam. Therefore, passive surveillance tends to be the more commonly used approach. The Vietnamese animal health system is structured as follows: The Department of Animal Health (DAH) under the Ministry of Agriculture and Rural Development (MARD) coordinates national disease control policies, while the Regional Animal Health Offices ( $n = 7$ , Figure 2.4) manage their particular territories. Each province ( $n = 64$ ) has its own

<sup>3</sup><http://www.who.int/zoonoses/outbreaks/glews/en/index.html>

Sub-Department of Animal Health (SDAH). There are more than 600 District Veterinary Stations (DVS) across the country. Outbreak data are mainly recorded through a routine reporting system which has, at its base, the village (Figure 2.5). Whenever a farmer recognises sick or dead poultry in a flock, this event is reported to a village Animal Health Worker (AHW) who is also a member of commune veterinarian team. A commune veterinarian visits the flock to inspect the incident. Suspected cases are reported to a DVS and this triggers an outbreak investigation. A veterinarian from the DVS visits the field for recording the incident, taking samples, sending samples to an appropriate laboratory, and notifying superiors. A hotline has been set up for reporting of suspected HPAI. The line is free of charge for callers. Improved information sharing protocols are also used to facilitate accurate and timely release of information. The emergency reporting system uses telephones and facsimiles. In addition, the FAO-provided Transboundary Animal Disease Information System (TADinfo, web-based version) has been customised to facilitate HPAI reporting in Vietnam. All data are sent to the central site for processing and evaluation of disease. The information is then shared with regional and provincial veterinary offices. In summary it has been recognised that a co-ordination of reporting activities could improve information flow during HPAI H5N1 outbreaks in Vietnam. Animal Health Workers play a critical part in both active and passive surveillance systems.



**Figure 2.4:** Choropleth map showing the areas administered by each of the Regional Animal Health Offices (RAHO,  $n = 7$ ) in Vietnam. The crosses indicate the location of the seven RAHO laboratories.



**Figure 2.5:** Flow diagram showing the information flow for routine reporting of HPAI in Vietnam. Bidirectional arrows demonstrate flows of reporting and feedback between institutions and individuals. Potential sources of bias at the various levels are indicated. Key: (a) bias in disease recognition, (b) bias in disease reporting, (c) diagnostic bias, and (d) bias arising from incorrect analysis.

### **Laboratory system**

The clinical signs of HPAI vary by virus strain and species affected. It is also important to differentiate HPAI from other poultry diseases such as fowl cholera and Newcastle disease (OIE 2002). Therefore, laboratory testing is required for definitive diagnosis of HPAI infection. Prior to 2004, the national veterinary laboratory capacity for HPAI diagnosis was poorly developed and resourced in Vietnam. After the first occurrence of HPAI H5N1 outbreaks during 2003 – 2004, the country received substantial contributions from international agencies to improve the capacity of national laboratories. National and regional laboratories are now capable of performing H5-gene based RRT-PCR (Real time Reverse Transcriptase Polymerase Chain Reaction) to detect H5 virus from swab samples (Table 2.2). In contrast to Indonesia where AI can be diagnosed in the field by trained teams using rapid tests, in Vietnam AI virus can only be definitively tested in the laboratory. The national laboratory network (LabNet) has been established to enable sharing information between central and regional laboratories. The country also has several provincial laboratories capable of HPAI diagnosis. In order to be fully confident that surveillance reflects the true situation of HPAI, sampling strategies and laboratory quality assurance are critical issues in the Vietnamese HPAI surveillance programme. In addition, not only positive results from laboratories are present for outbreak confirmation, but negative results should also be present to support the absence of HPAI infection. In some circumstances, collection and testing of samples from flocks in areas surrounding confirmed HPAI outbreaks can be conducted to compare the performance of passive versus active surveillance.

### **Surveillance programmes**

During 2004, because the country was overwhelmed by the number of HPAI H5N1 outbreaks and all resources were prioritised into controlling the spread of disease, there was little or no structured surveillance and investigation programmes conducted in Vietnam. Since 2004, due to widespread disease in poultry and the occurrence of human infection, national and international efforts have been applied to improve the capacity for disease surveillance and laboratory diagnosis. As consequence, the HPAI surveillance system in Vietnam has been improved. Surveillance is essential to detect new incursions and identify remaining foci of disease. Although the veterinary infrastructure has been improved

**Table 2.2:** National and regional laboratories capable to perform H5-gene based RRT-PCR in Vietnam.

Laboratory	Location
National Centre for Veterinary Diagnosis (NCDV)	Ha Noi, north
National Institute of Veterinary Research (NIVR)	Ha Noi, north
Regional Centre for Veterinary Diagnosis No. 1 <sup>a</sup>	Ha Noi, north
Regional Centre for Veterinary Diagnosis No. 2	Hai Phong, north
Regional Centre for Veterinary Diagnosis No. 3	Nghe An, centre
Regional Centre for Veterinary Diagnosis No. 4	Da Nang, centre
Sub-Institute of Veterinary Research (SIVR)	Khanh Hoa, centre
Regional Centre for Veterinary Diagnosis No. 6	Ho Chi Minh City, south
Regional Centre for Veterinary Diagnosis No. 7	Can Tho, south

<sup>a</sup> The Regional Centre for Veterinary Diagnosis belongs to the corresponding Regional Animal Health Office (RAHO Nos. 1 – 7). The Regional Centre for Veterinary Diagnosis has not been established for RAHO No. 5 in the Central highlands.

in recent years, there are still concerns about the capacity to detect and respond to HPAI H5N1 outbreaks when they occur.

Routine surveillance is mainly based on recognition of clinical signs of HPAI infection. When a dead chicken is found, samples are collected as soon as possible and dispatched for laboratory examination (DAH 2005*b*). Because species such as waterfowl can be infected and transmit HPAI viruses without showing obvious clinical signs, this approach of surveillance alone cannot assure that HPAI infection is always detected. Furthermore, the sensitivity of routine surveillance is also influenced by the willingness of farmers and field personnel to report the presence of disease. In addition, the linkage between provincial veterinary authorities and AHWs at the commune or village level may influence the flow of information on HPAI. In order to detect all cases of HPAI H5N1 infection in areas where the virus has been shown to circulate silently at low levels (Chen et al. 2006), especially in domestic duck flocks, sufficient resources and required to conduct targeted surveillance programmes.

During 2005 – 2006, massive surveillance programmes were conducted to assess efficiency of vaccination and detect evidence of virus circulation in chickens, ducks, and poultry in live bird markets. A sero-monitoring programme was carried out to assess the efficacy of vaccination. Along with passive surveillance programmes based on farmers and AHWs reporting suspected signs of HPAI, sentinel birds and unvaccinated waterfowl were monitored in high risk areas (that is, areas with a relatively high density of



poultry that had experienced previous outbreaks). Samples were also taken from selected live bird markets to test for evidence of HPAI virus (Taylor & Do 2007). Serology and swab sampling was used to detect evidence of H5N1 infection. Serology may increase the sensitivity of HPAI surveillance programmes to detect evidence of infection, because antibodies may persist for a longer time after infection than the duration of virus excretion. Therefore, it is easier to find antibodies by random sampling within an infected population. The prevalence of virus circulation at any one time may be lower than the prevalence of sero-positive poultry. In some circumstances, antibodies may not indicate the true prevalence of HPAI infection, due to past infection or as a result of vaccination. Swab sampling can be used to detect evidence reflecting that field virus is circulating.

It has been demonstrated that numbers of HPAI H5N1-infected flocks detected through active surveillance programmes are substantially higher than those detected by passive surveillance. For instance, there was serological evidence that HPAI H5N1 virus was circulating among unvaccinated waterfowl during 2006 in the south of Vietnam, but no clinical outbreaks were reported in this area during that time (Taylor & Do 2007). This indicates that the levels of detection through passive surveillance underestimate the true level of occurrence of H5N1 infection in the country and passive surveillance based on observation of clinical signs alone might have not been effective. Therefore, it is essential to implement appropriate active surveillance in order to find foci of HPAI H5N1 infection.

### **Constraints of the current surveillance system**

Though the surveillance capability for HPAI in Vietnam has considerably improved during recent years, and active surveillance programmes have been initiated to monitor the effectiveness of vaccination campaigns and virus circulation levels, the current surveillance system still faces some constraints. The system is mainly based on passive rather than active forms of surveillance. Approaches based on the observation of clinical signs, which relies on farmers and AHWs noticing suspected signs of HPAI, still plays a key role. This requires human resources responsible for field surveillance and monitoring at the village level. Inefficient payment mechanisms for compensation may discourage farmers from reporting suspicious cases. In some circumstances, if farmers and field AHWs are not provided with feedback for what they have reported to veterinary authorities, they may be less willing to contribute to future surveillance programmes. In passive surveillance sys-

tems, underreporting also occurs as a result of time and lack of interest by field staff and farmers. In addition, data supplied by laboratories often lack sufficient background data on tested flocks. There are many difficulties for effective surveillance system in a country where most households keep backyard poultry and the flow of animal health information is mainly by hard copy at almost all steps in the reporting system. When an outbreak affects village poultry, reporting may be delayed and the disease may have already spread widely before control activities can be implemented. The current surveillance system is facing some constraints due to the reporting methodology and data management, or in some circumstances, the stakeholders are not willing to report the information related to HPAI. These deficiencies may cause uncertainty over the validity and limit the depth of epidemiological analyses that are able to be undertaken.

The above-mentioned constraints can only be handled by improving detection and reporting under the routine animal diseases information data flow or implementing targeted active surveillance programmes. It is important to enhance communication strategies to allow a sufficient response to disease events detected through the surveillance system. Training is necessary to enable commune veterinarians to screen reports when a notification is made. In addition, district veterinarians should be able to perform basic data analysis and propagate feedback to (local) stakeholders to keep them motivated. Whether active or passive surveillance approaches are applied, it depends on the active involvement of the community including livestock owners and animal health workers. In order to strengthen the link between the official veterinary services and the key informants of the animal health sector, participatory approaches appear to be able to provide some value.

### **2.5.3 Participatory approaches for HPAI surveillance**

#### **Overview of participatory epidemiology**

Participatory epidemiology refers to the involvement of particular group of people taking part in epidemiological research and disease surveillance. Participatory methods overcome some of the limitations of conventional epidemiological methods in animal disease surveillance (Jost et al. 2007). While conventional methods tend to involve limited interactions with local people and research topics are identified by researchers rather than communities, participatory methods accomplish a proactive involvement of local people

in all stages of the research process (Cornwall & Jewkes 1995). Conventional research aims to gather information rather than involving local people in devising research procedures and in presentation of outcomes. Lack of veterinary services is a challenge in rural and remote areas, particularly in developing countries. Therefore, it is difficult to gather information using conventional approaches which are often time-consuming and costly to implement. Participatory approaches, also known as community-based approaches (Catley et al. 2002), emphasise the importance of taking community members (that is, those most directly affected) into account and giving them the chance to communicate and transfer their knowledge about epidemiological related information to research personnel. In participatory research local people participate more actively; they have the option of seeking technical assistance according to their own perception of need rather than according to external assessment by professionals. In summary, participatory approaches ensure that local people are actively involved in defining problems, proposing solutions and implementing applications.

### **Applications of participatory epidemiology**

Application of a participatory approach that involves local people as participants may ultimately enhance the effectiveness of epidemiological research. All stakeholders should be involved right from the start of work using participatory approaches (Catley et al. 2002). Exploring the local knowledge and perception of local people is a key strength of this approach where researchers and local people work together as colleagues within a mutual learning process. However, degrees of participation may influence the effectiveness of participatory research. Local people may have little or no confidence in what they know and look to researchers for direction. Therefore, it is essential to engage people into exercises, analyses, and discussions actively. Using participatory methods, data should not be presented in complicated formats that are difficult to follow. Graphs and simple maps are the best way to present information during group discussions. Participatory approaches should be flexible and able to adapt to new issues raised during discussions.

Participatory approach has been widely applied in health research in developed countries. Recently, it has been increasingly used as a surveillance tool for animal diseases such as rinderpest, foot-and-mouth disease, peste des petits ruminants and HPAI in developing countries in Africa and Asia (Jost et al. 2007). Community-based approaches have also

been applying in many animal health programmes in African, Asian, and Latin American countries (Catley et al. 2002, Normile 2007). Many developing countries are faced with time delays and poor data quality of disease surveillance programmes. Data obtained through routine surveillance often include the most essential information such as number of cases. In many instances, additional information such as the size of the population at risk, geographic location of cases, time of onset, and circumstances leading to the disease onset can be sought by adopting a participatory approach. An important principle in participatory approach is to obtain what community members already know and are doing, aiming to make the best use of local husbandry knowledge and skills. Since January 2006, the Indonesian veterinary service has implemented the participatory disease surveillance and response (PDSR) approach to HPAI (Azhar et al. 2010). Over two thousand PDSR officers, who are employees of the district animal health services in Indonesia, have been trained to conduct a surveillance programme focusing on villages. This approach allows PDSR officers to work with all poultry farmers, traders, and community leaders within the village. PDSR officers are provided with influenza A rapid antigen detection tests and personal protective equipment for their field work. Up to March 2009, the PDSR programme had been operational in 76% (341 of 448) of districts in Indonesia with the participation of approximately 2.5 million community members in PDSR activities (Azhar et al. 2010). At the beginning of the programme the extent of HPAI infection in Indonesia was not known. Within the first year of operation the PDSR programme detected 800 HPAI cases in poultry (Jost et al. 2007). In general, participatory disease surveillance has enhanced the sensitivity of the national surveillance system and made an important contribution towards controlling HPAI in Indonesia.

### **Participatory approach for HPAI surveillance in Vietnam**

The aims of any HPAI surveillance programme are to: (1) prevent and control the spread of disease, (2) detect and respond to disease outbreaks, and (3) identify susceptible subgroups of the population at risk. In Vietnam, a HPAI surveillance system that requires the participation of many stakeholders will only be effective and useful when a vertical link at the community level is established in order to enable rapid reporting based on quick detection of disease. Accurate and effective surveillance for poultry outbreaks and human infections of HPAI H5N1 in Vietnam is challenging because it depends on the willing-

ness of stakeholders to report the disease (Desvaux et al. 2008). Common stakeholders in community-based animal health services in Vietnam include livestock owners who may keep a few chickens in their backyard or thousands of chickens in intensive commercial farms, and veterinary personnel which includes government veterinary staff at local and central levels, private veterinarians, and veterinary drug traders.

In Vietnam, it is proposed that AHWs could use a participatory approach to work with communities to determine and work out solutions to local animal health problems. It is important that survey findings using a participatory approach should be reported back to communities promptly. This allows community members to see that their contributions are important, and they have ownership of the work. These advantages overcome some of the problems in conventional surveys in which data are taken away from communities and analysed by researchers only and community members do not always receive a copy of research results (Catley et al. 2002). Several projects have been implemented by Agronomes at Vétérinaires Sans Frontières (AVSF) and CARE (Cooperative for Assistance and Relief Everywhere) Vietnam to strengthen HPAI surveillance system in Vietnam using commune-based surveillance approaches (Desvaux et al. 2008). In these projects a network of volunteers at the village level has been established. The village volunteers are trained to look out for possible signs in village poultry and to report trained AHWs and community leaders of suspected cases of HPAI. Monthly meetings are organised for volunteers to submit reports, share lessons, and receive updated information and technical support. These projects have encouraged the participation of the private sector that potentially plays an important role in outbreak investigation, detection, reporting and control of HPAI outbreaks. Currently, a toll-free hotline has also proved to be useful tools for the reporting of suspected HPAI outbreaks throughout the country.

There are many potential sources of animal disease information in a community. Although laboratory confirmation may be lacking, livestock owners and AHWs are often good at describing clinical signs and have excellent local knowledge of animal diseases. However, their information may not be well integrated with that of the government services. Local AHWs, who act as the 'eyes and ears' of a HPAI surveillance system at the grass root level, play an essential role in reinforcing links between communities and national surveillance and response systems. A network of AHWs should be established to improve the effectiveness of a national HPAI surveillance system. Because poultry owners

and AHWs are mainly concerned with clinical disease and disease terminology may vary between communities, it is essential to prepare standard case definitions and train various groups that play different roles in community-based approaches for HPAI surveillance. It is also essential to involve animal traders, drug and feed suppliers in disease surveillance programmes because they can play an important role in communication between different livestock sectors. Surveillance and reporting of HPAI at the community level needs to consider the community context. It is essential to improve an extensive network of all stakeholders involved in animal disease surveillance and control at the central as well as at local levels.

## 2.6 Conclusion

This chapter has reviewed various aspects related to AI, the emergence of HPAI, disease control and prevention, and surveillance approaches for HPAI. In addition, this review has examined challenges and considered some recommendations to improve current HPAI surveillance strategies in Vietnam.

The recent HPAI H5N1 epidemic in Asia is a crisis of global importance. Some countries are now endemically infected with HPAI H5N1 viruses. To prevent a human pandemic of HPAI H5N1 vigilance and rigorous enforcement of control, prevention, and elimination measures must be applied to poultry populations. Approaches for disease control and eradication require a sustained long term strategy according to epidemiological, economic, political and social factors. The most appropriate strategy for disease control and prevention must rely on a thorough understanding of the epidemiology of the disease. In countries where surveillance systems are based principally on owner reporting, several methods can be used to improve the level of awareness of H5N1, which in turn influences the likelihood of detection of disease and reporting to authorities. Reporting may be improved by ensuring that adequate funding is available to reduce the delay in providing compensation payments to affected flocks owners. Detection may also be enhanced by targeted surveillance specifically designed to detect H5N1 viruses, particularly where silent transmission may occur — such as in extensively managed free range duck flocks which are grazed in paddy fields (Taylor & Do 2007), live bird markets (Nguyen et al. 2005), and vaccinated poultry flocks (Savill et al. 2006). Furthermore, it is important

to conduct adequate investigations of both human and poultry cases to identify potential sources of infection and risk factors. Finally, ongoing education campaigns and training courses are important to increase awareness of risky behaviors in humans and to improve hygiene practices.

Vietnam has made remarkable progress in managing HPAI H5N1 in poultry and humans since 2004. However, it is necessary to move from the current control programme of extensive and costly vaccination to restricted vaccination targeted at high risk areas. This approach requires extensive and reliable surveillance strategies, rapid and effective outbreak response. It is important to improve detection and response to HPAI outbreaks, facilitate HPAI prevention and control activities by communities and the government, and increase the overall understanding of the epidemiology of HPAI in the country.





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## The association between human cases and poultry outbreaks of highly pathogenic avian influenza in Vietnam from 2003 to 2007: A nationwide study

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**Abstract** – This study quantifies the spatio-temporal association between outbreaks of HPAI H5N1 in domestic poultry ( $n = 3,050$ ) and human cases ( $n = 99$ ) in Vietnam during 2003 – 2007, using rare events logistic regression. After adjusting for the effect of known confounders, the odds of a human case being reported to authorities increased by a factor of 6.15 (95% CI 3.33 – 11.38) and 2.48 (95% CI 1.20 – 5.13) if poultry outbreaks were reported in the same district one week and four weeks later, respectively. When jointly considering poultry outbreaks in the same and neighbouring districts, occurrence of poultry outbreaks in the same week, one week later, and four weeks later increased the odds of a human case by a factor of 2.75 (95% CI 1.43 – 5.30), 2.56 (95% CI 1.31 – 5.00), and 2.70 (95% CI 1.56 – 4.66), respectively. Our study found evidence of different levels of association between human cases and poultry outbreaks in the north and the south of the country. When considering the 9 week interval extending from four weeks before to four weeks after the week of reporting a human case, in the south poultry outbreaks were recorded in 58% of cases in the same district and 83% of cases in either the same or neighbouring districts, whereas in the north the equivalent results were only 23% and 42%. The strength of the association between human and poultry cases declined over the study period. We conclude that owner reporting of clinical disease in poultry needs to be enhanced by targeted agent-specific surveillance integrated with preventive and other measures, if human exposure is to be minimised.

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### 3.1 Introduction

Since late 2003, outbreaks of HPAI H5N1 have occurred in more than 60 countries in Asia, Europe, and Africa, resulting in important economic loss arising from premature death and culling of domestic poultry (OIE 2009*b*). More importantly, sporadic human infections have raised concerns of a future human pandemic. Up to February 2009, 407 confirmed cases of HPAI H5N1 infections in humans have been reported in 15 countries, 254 of which have been fatal (WHO 2009). After Indonesia ( $n = 141$ ), Vietnam has reported the second highest number of human cases ( $n = 108$ ) with 52 fatalities. To date, limited human-to-human transmission of disease has occurred, and only a few family clusters have been identified in Indonesia (Yang et al. 2007), Thailand (Ungchusak et al. 2005), and Vietnam (Hien et al. 2004). Repeated introduction of H5N1 virus into humans provides increased opportunities for viral reassortment, potentially increasing the likelihood of pandemic flu in the human population (Thomas & Noppenberger 2007).

Factors associated with human cases include direct and indirect exposure to infected poultry. Pigs are not considered an important intermediate host in the transmission of HPAI H5N1 to humans (Van Reeth 2007) and antibodies to H5N1 virus were only found in 0.25% of 3,175 pigs tested in Vietnam in 2004 (Choi et al. 2005). The risk of bird-to-human transmission of HPAI may vary depending on poultry handling practices in affected countries. Results from a recent survey indicate that exposure of people to backyard poultry was 100 – 700 times greater in Vietnam than in Hong Kong (Fielding et al. 2007). Risky practices in Vietnam include: (1) close physical contact between humans and poultry, (2) live bird markets where poultry are selected alive and killed in front of the consumer, and (3) marketing practices where, in some circumstances, sick and dead poultry may be offered for sale. An intensive nationwide educational campaign was conducted around these high-risk behaviours (Chen, Chen, Dai & Sun 2007), which may be one reason for the reduced occurrence of human cases in Vietnam since 2006.

In Vietnam, data for HPAI H5N1 outbreaks in poultry are mainly derived from a system of passive surveillance, which relies on the reporting of suspected cases of HPAI in domestic poultry. Given the nature of poultry farming practices in Vietnam, it has been proposed that the presence of HPAI H5N1 infection in domestic poultry could provide evidence of an emerging, increased risk of disease in the human population (Halliday et al. 2007).

Several studies have been published describing clinical, epidemiological, and/or virological findings for human cases in Indonesia (Sedyaningsih et al. 2007), Cambodia (Buchy et al. 2007), and China (Yu et al. 2007). Furthermore, studies have been conducted to investigate risk factors for H5N1 infection of humans in Vietnam (Dinh et al. 2006) as well as live poultry exposures and risk perceptions in a number of Asian countries (Olsen et al. 2005, Barennes et al. 2007, Fielding et al. 2007). However, at the time of writing no investigations have addressed the spatial and temporal relationship between H5N1 outbreaks in poultry and members of the human population. That is, do outbreaks of H5N1 in domestic poultry increase the likelihood of subsequent diagnoses in humans, or does the presence of a human diagnosis precede the reporting of poultry cases, due to increased awareness of disease and consequently an increased level of surveillance? To address this question we analysed retrospective surveillance data to describe the association between poultry outbreaks and human cases in space and time.

## 3.2 Materials and methods

### 3.2.1 Study population

The study area was the Socialist Republic of Vietnam. Digital maps of the 64 provinces, 661 districts, and 10,805 communes in Vietnam were obtained from the Ministry of Natural Resources and Environment. The unit of interest was the district, which occupies a median land area of 370 square kilometres (minimum: 2 square kilometres, maximum: 3,662 square kilometres). An estimate of the population of humans within each district was obtained from the 1999 census conducted by the General Statistics Office of Vietnam (General Statistics Office of Vietnam 1999). Human population density within each district was calculated and expressed as the number of humans per square kilometre. The period of interest was from 8 December 2003 (the date on which the first human case was reported) to 26 August 2007 (a total of 194 weeks).

### 3.2.2 Data sources and case definition

Details of the 100 human cases of H5N1 that were diagnosed throughout the study period were obtained from the Ministry of Health. Details for each case included the district

(and commune) of residence, travel history, and the date of onset of symptoms, the date of hospitalisation, and the date of recovery or death. The date of onset was based on the onset of symptoms. The number of days between the date of onset of symptoms and date of hospitalisation was calculated for all cases with valid data. Where the date of onset of symptoms was missing ( $n = 3$ ), an onset date was estimated as the date of hospitalisation less the median onset of symptoms to hospitalisation interval (in days).

Data on poultry outbreaks were obtained from the DAH. From 12 December 2003 to 29 February 2004, outbreaks were identified on the basis of poultry showing characteristic clinical signs of HPAI. Samples were taken from selected outbreaks for laboratory confirmation at the Centers for Disease Control and Prevention (Atlanta, USA) and the Australian Animal Health Laboratory (Geelong, Australia). From 1 March 2004, the presence of HPAI H5 virus was confirmed by PCR for all reported outbreaks by the Veterinary Regional Laboratories and the National Centre for Veterinary Diagnosis in Vietnam. Details of recorded poultry outbreaks included the date of onset of clinical signs and location (commune and/or district) in which the outbreak occurred. Outbreaks that occurred in the same commune (or district if commune information was unavailable) within seven days of an initial outbreak were treated in these analyses as a single outbreak.

### **3.2.3 Analytical methods**

Human cases and poultry outbreaks were stratified into four periods starting on 1 September and ending on 31 August in the following calendar year within the range of the study period. These date ranges were chosen to cover the date range of the distinct epidemics of HPAI H5N1 in both poultry and humans during each 12-month period.

#### **Associations in space and time**

We calculated the number of poultry outbreaks at the level of commune and district, stratified by period. A series of choropleth maps were constructed to show the number of poultry outbreaks per district within each period. The point locations of human cases of H5N1 were superimposed on these maps to provide a qualitative description of the spatial association between human cases and poultry outbreaks. We investigated the temporal association between human cases and poultry outbreaks by plotting the daily number of

human cases and poultry outbreaks that were recorded at the commune level as a function of calendar time.

### Rare-events logistic regression model

A logistic regression approach was used to quantify the association between poultry outbreaks and human cases. For these analyses the dependent variable  $Y_{ij}$  was a dichotomous response indicating whether or not at least one human case occurred in the  $j$ th district in the  $i$ th week of the study period. Only districts in which a human case was reported were included in the analysis ( $n = 67$ ) since our purpose was not to predict the overall incidence rate of human cases but to investigate the relationship between incidence and possible predictors.<sup>1</sup> Due to the large number of combinations of districts and weeks ( $n = 12,998$ ), the fraction of district-weeks that experienced a human case of disease was small. King & Zeng (2001b) showed that, in rare event data, ordinary logistic regression analysis leads to biased estimates of covariate effects.<sup>2</sup> A proposed method to adjust coefficient estimates for rare-events bias was implemented using the *zelig* package in R (Ihaka & Gentleman 1996, Imai et al. 2007, King & Zeng 2001b). Using this approach, coefficients are corrected for rare-events bias ( $\tilde{\beta}$ ) as follows:

$$\tilde{\beta} = \hat{\beta} - bias(\hat{\beta}) \quad (3.1)$$

The bias term is:

$$bias(\hat{\beta}) = (X'WX)^{-1}X'W\xi \quad (3.2)$$

where, in Equation 7.2  $\xi_{ij} = 0.5Q_{ijij}[(1 + \omega_1)\hat{\pi} - \omega_1]$ ,  $Q_{ijij}$  are the diagonal elements of  $Q = X(X'WX)^{-1}X'$ ,  $W = \text{diag}\{\hat{\pi}_{ij}(1 - \hat{\pi}_{ij})\}\omega_{ij}$ ,  $\omega_{ij} = \omega_1 Y_{ij} + \omega_0(1 - Y_{ij})$ ,  $X$  is the explanatory variable, and  $\omega_0$  and  $\omega_1$  represents the fraction of zeros and ones in the sample relative to the fraction in the population. This method is implemented in the `relogit` function in the *zelig* package. One of the key questions in the epidemiology of

<sup>1</sup>Including all 661 districts would have caused more rare-events bias (0.07% versus 0.7% of positive district-weeks) and including all of the negative districts was considered to add little value to the current analyses.

<sup>2</sup>The data were analysed using ordinary logistic regression. Results (not presented) showed that point estimates of the coefficients did not change. However, the magnitude of the standard errors of the coefficients from the ordinary logistic regression were, in general, slightly larger than those using the rare-events approach.

HPAI in Vietnam is whether passive surveillance of poultry outbreaks adequately assesses the risk of H5N1 exposure of humans. To assess this hypothesis, we first created two dichotomous variables: (1) whether or not at least one poultry outbreak occurred in a given week in the same district as the human case ( $\text{Poultry}_{\text{Distr}}$ ), and (2) whether or not at least one poultry outbreak occurred in the same or directly adjacent districts as the human case ( $\text{Poultry}_{\text{Neighb}}$ ). Next, both  $\text{Poultry}_{\text{Distr}}$  and  $\text{Poultry}_{\text{Neighb}}$  were lagged by  $x$  weeks, where  $x = \{-4, -3, \dots, +4\}$ , to capture the temporal association, if any, between poultry outbreaks and human cases.

Two models were constructed using either weekly lags of  $\text{Poultry}_{\text{Distr}}$  (Model 1) or  $\text{Poultry}_{\text{Neighb}}$  (Model 2) as predictors. Other fixed effects tested in the model included period, region, and human population density at the district level. We investigated interaction terms between explanatory variables. For modeling purposes, we merged periods 3 and 4 (from 1 September 2005 to 26 August 2007) since only one human case was reported in period 3. We grouped the eight geographic regions of Vietnam into two larger regions that is, the north (Red River Delta, Northeast, Northwest, North Central Coast) and the south (South Central Coast, Central Highlands, Southeast, Mekong River Delta) (Figure 3.1). Covariates were selected using backward elimination with a significance threshold of 0.05. We assessed model fit using the Akaike Information Criterion (AIC) score.

To account for the clustering of cases within districts, we derived standard errors for the estimated regression coefficients using the ‘delete-a-group’ jackknife (Lipsitz et al. 1994), a robust method that gives consistent estimates in the presence of within-group correlation. The jackknife coefficient estimate,  $\tilde{\beta}_{-k}$ , was obtained by deleting the  $k$ th district, and refitting the logistic regression model to the resulting dataset. The jackknife variance is then given by

$$V_J(\tilde{\beta}) = \frac{n}{n-1} \sum_{k=1}^n (\tilde{\beta}_{-k} - \tilde{\beta})^2 \quad (3.3)$$

A less computationally-intensive approximate answer could instead be found from the output of the original model, by performing one step of the Newton-Raphson algorithm using the estimate from `relogit` as the starting value:

$$\tilde{\beta}_{-k} = \tilde{\beta} + \left[ \sum_{j=1, j \neq k}^n I_j(\tilde{\beta}) \right]^{-1} \sum_{j=1, j \neq k}^n u_j(\tilde{\beta}) \quad (3.4)$$

where, in Equation 7.4  $u_j(\tilde{\beta})$  is the contribution to the score vector from the  $j$ th district and  $\sum_{j=1, j \neq k}^n I_j(\tilde{\beta}) = -\sum_{j=1, j \neq k}^n [du_j(\tilde{\beta})/d\beta]$  is the information matrix.

An advantage of the ‘delete-a-group’ jackknife is that model diagnostics can be performed to test for the influence of individual districts on the estimated coefficients. Frequency distributions of  $\tilde{\beta}_{-k}$  were inspected to identify districts having an influential effect on these coefficient estimates.

For each human case included in the models, we created a binary variable indicating whether there was a poultry outbreak in the same district ( $\text{DISTR}_{9\text{weeks}}$ ) or in the same and neighbouring districts ( $\text{NEIGHB}_{9\text{weeks}}$ ) in the period from four weeks prior to four weeks after the week of onset of clinical signs of a human case. We tested for the effect of district size, period, and region on  $\text{DISTR}_{9\text{weeks}}$  and  $\text{NEIGHB}_{9\text{weeks}}$  using a two-sided Fisher’s Exact test. We assessed whether frequency of  $\text{DISTR}_{9\text{weeks}}$  and  $\text{NEIGHB}_{9\text{weeks}}$  varied across ordered categories of period using the exact Cochran-Armitage test for trend (Agresti 2002).

Data were manipulated using Microsoft Excel and Microsoft Access. Epidemic curves and statistical analyses were conducted with R version 2.7.0 (R Development Core Team, Vienna, Austria), and SAS 9.1 (SAS Institute, Inc., Cary, NC). Manipulation of spatial data was carried out using ArcView GIS 3.2a (ESRI Inc., Redlands, California, USA).

### 3.3 Results

Ninety-nine human cases were used for analysis; one case that occurred in 2005 was omitted because no details were available concerning the district of residence. The median time between onset of symptoms and hospitalization was 4 days (minimum: 0 days, maximum: 34 days). Sixty-eight *percent* of the total number of human cases were under 30 years of age at the time of diagnosis. Epidemiological information indicated a recent history of travel in 4% of cases. In total, 3,050 poultry outbreaks were reported in 433 districts. Dates were not recorded for 91 poultry outbreaks during the 2003 – 2004 epidemic.

Figure 3.2 shows the spatial distribution of districts reporting human cases and poultry outbreaks stratified by period. Both human cases and poultry outbreaks were clustered in the RRD in the north and in the MRD in the south of Vietnam. Reported human cases in periods 3 ( $n = 1$ ) and 4 ( $n = 7$ ) were restricted to the north.

Epidemic curves of HPAI H5N1 human cases and poultry outbreaks (Figure 3.3) show a temporal association between human cases and poultry outbreaks. The onset of epidemics in humans was at a similar time or slightly earlier than the start of poultry epidemics. Apart from the second epidemic wave in 2007, poultry outbreaks occurred between November and April with only sporadic outbreaks in between. Human cases did not show a clear seasonal pattern.

For the multivariate logistic regression, we excluded ten human cases as they were considered to be infected through the same source of exposure ('family clusters'). Furthermore, five cases were lost to analysis as they occurred in the same week and district as another case, resulting in 84 district-weeks with at least one human case. Repeated human cases occurred in 13 districts. The maximum number of cases per district was four. The median time between repeated cases was three weeks (Interquartile range [IQR] 1 – 17 weeks). We present two models: the first where the probability of an outbreak in humans at the district level was dependent on the presence of poultry outbreaks in the same district up to four weeks before and four weeks after the date of onset of symptoms in the index human case (Table 3.1). For the second model, the probability of an outbreak of disease in humans was dependent on the presence of poultry outbreaks in the same district or its neighbours during the same time frame (Table 3.2). After adjusting for the effect of period, the odds of a human case increased by a factor of 6.15 (95% CI 3.33 – 11.38) and 2.48 (95% CI 1.20 – 5.13) if poultry outbreaks were reported in the same district one week and four weeks later, respectively (Table 3.2). In Table 3.2, poultry outbreaks in the same week, one week later, and four weeks later increased the odds of a report of a human case in the same or a neighbouring district by a factor of 2.75 (95% CI 1.43 – 5.30), 2.56 (95% CI 1.31 – 5.00), and 2.70 (95% CI 1.56 – 4.66), respectively.

During a period of four weeks prior to four weeks after the reporting of a human case, of the 84 district-weeks with at least one human case of disease, poultry outbreaks were present in 33% (in the same district) and 54% (in either the same or contiguous neighbouring districts) of cases. The proportion of positive district-weeks for human cases



showing an association with poultry outbreaks was higher in the south than in the north when considering only the same district (58% and 23% respectively,  $\chi^2 = 9.5$ ,  $df = 1$ ,  $P = 0.004$ ), or considering the same and neighbouring districts (83% and 42% respectively,  $\chi^2 = 12.0$ ,  $df = 1$ ,  $P < 0.001$ ). The proportion of district-weeks with a human case that showed an association with poultry outbreaks decreased over the three periods, when considering poultry outbreaks either in the same ( $Z = -3.02$ ,  $P = 0.003$ ) or in the same and neighbouring districts ( $Z = -2.40$ ,  $P = 0.025$ ).

**Table 3.1:** Estimates from the logistic regression model for rare events for association between human cases and poultry outbreaks of HPAI H5N1 in the same district for districts with at least one human case ( $n_h = 67$ ).

Variable	Beta	SE <sup>a</sup>	P-value	OR (95% CI)
Intercept	-4.84	0.17	< 0.001	
Poultry outbreak reported one week after the week of reporting a human case	1.82	0.31	< 0.001	6.15 (3.33 – 11.38)
Poultry outbreak reported four weeks after the week of reporting a human case	0.91	0.37	0.018	2.48 (1.20 – 5.13)
Period:				
8 December 2003 to 31 August 2004	Reference			
1 September 2004 to 31 August 2005	0.49	0.27	0.055	1.63 (0.97 – 2.75)
1 September 2005 to 26 August 2007	-1.93	0.36	< 0.001	0.14 (0.07 – 0.29)

The observation period was 194 weeks. The proportion of district-weeks with human cases over 12,998 district-weeks was 0.0065.

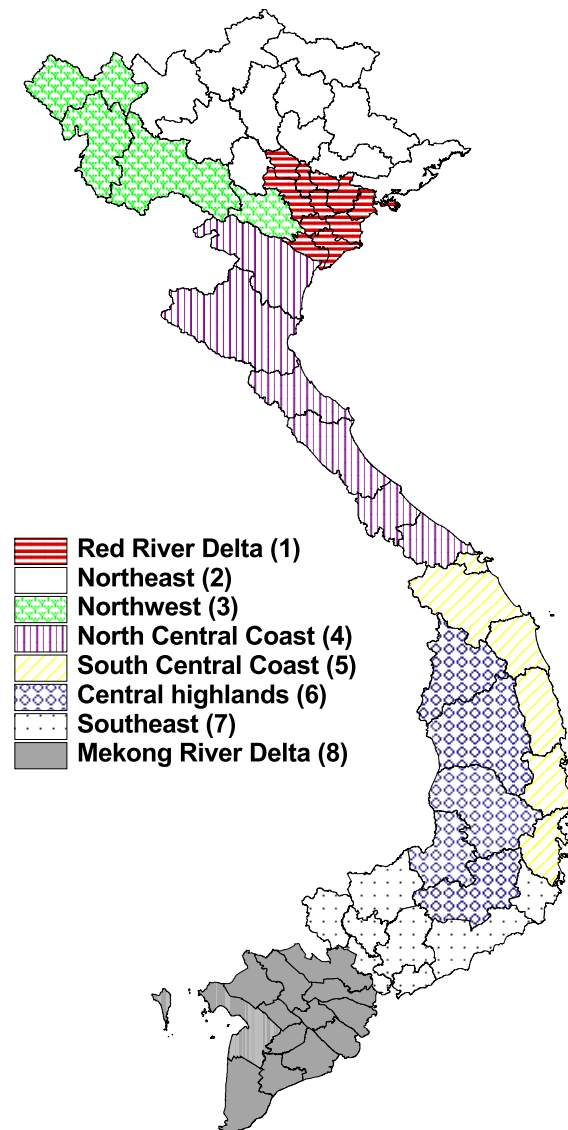
<sup>a</sup> Standard error derived from the 'delete-a-group' jackknife.

**Table 3.2:** Estimates from the logistic regression model for rare events for association between human cases and poultry outbreaks of HPAI H5N1 in the same or neighbouring districts for districts with at least one human case ( $n_h = 67$ ).

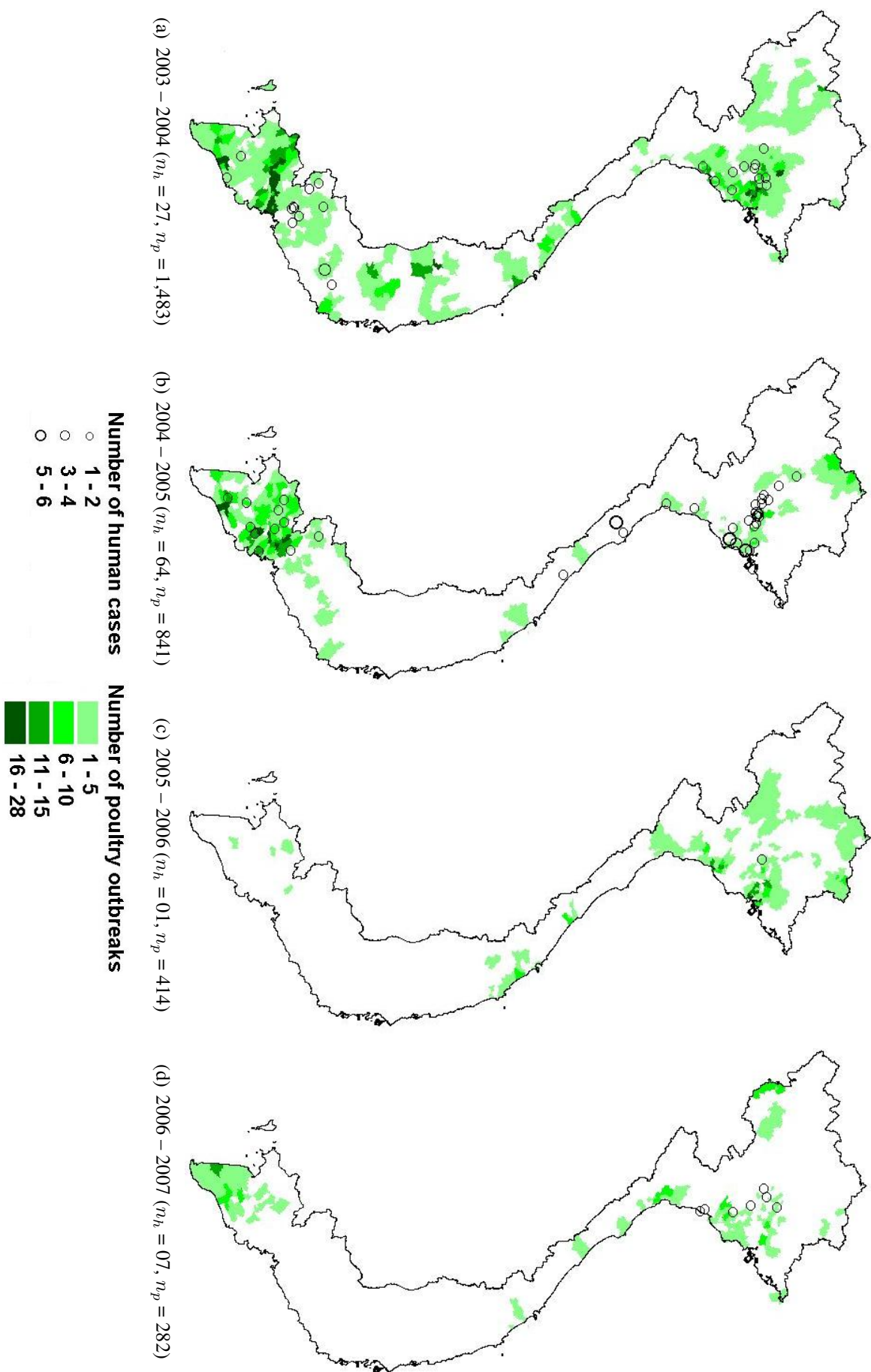
Variable	Beta	SE <sup>a</sup>	P-value	OR (95% CI)
Intercept	-4.95	0.17	< 0.001	
Poultry outbreak reported in same week of the week of reporting a human case	1.01	0.33	0.008	2.75 (1.43 – 5.30)
Poultry outbreak reported one week after the week of reporting a human case	0.94	0.34	0.025	2.56 (1.31 – 5.00)
Poultry outbreak reported four weeks after the week of reporting a human case	0.99	0.28	0.001	2.70 (1.56 – 4.66)
Period:				
8 December 2003 to 31 August 2004	Reference			
1 September 2004 to 31 August 2005	0.58	0.27	0.030	1.79 (1.06 – 3.01)
1 September 2005 to 26 August 2007	-1.78	0.35	< 0.001	0.17 (0.09 – 0.33)
Region:				
North	Reference			
South	-0.71	0.15	0.006	0.49 (0.36 – 0.66)

The observation period was 194 weeks. The proportion of district-weeks with human cases over 12,998 district-weeks was 0.0065.

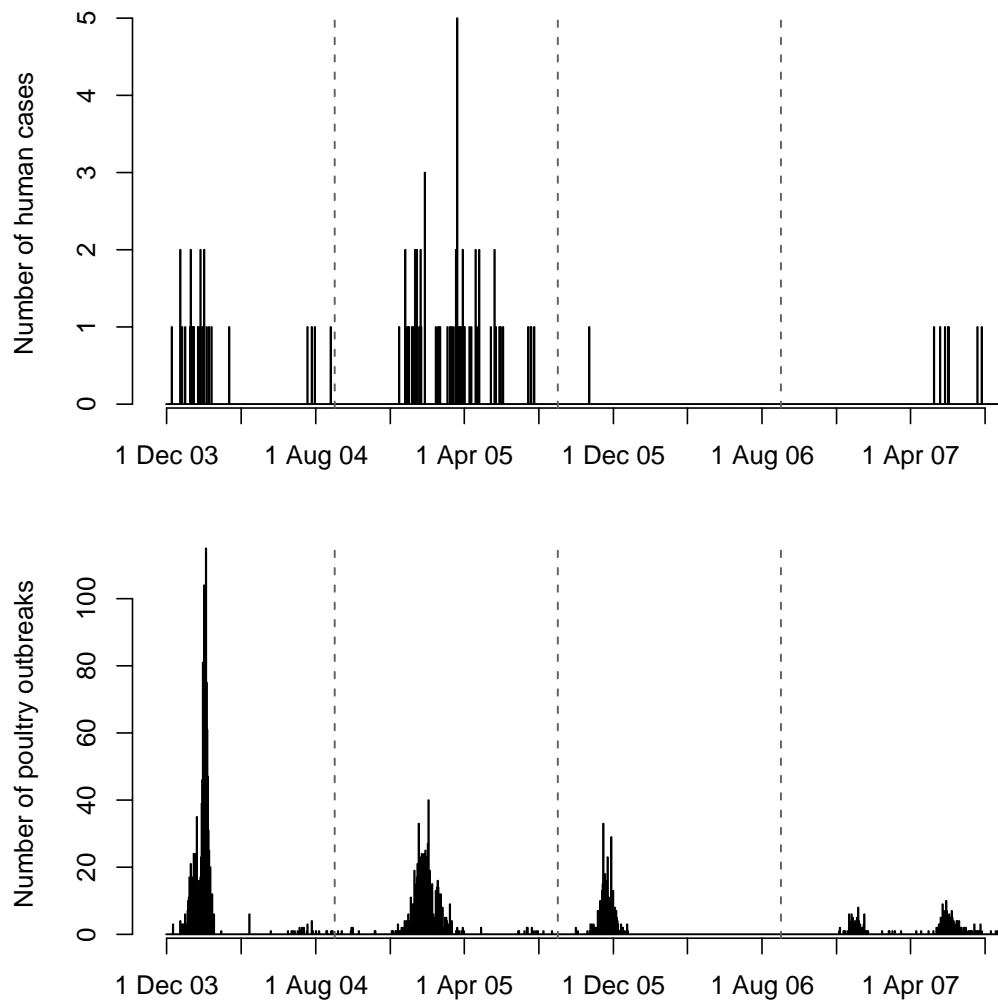
<sup>a</sup> Standard error derived from the 'delete-a-group' jackknife.



**Figure 3.1:** Locations of the eight geographic regions in Vietnam. Median district size (minimum to maximum) of regions 1 to 8: Region 1: 119 square kilometres (5 – 452 square kilometres), region 2: 579 square kilometres (30 – 2,071 square kilometres), region 3: 840 square kilometres (64 – 3,663 square kilometres), region 4: 487 square kilometres (2 – 2,822 square kilometres), region 5: 456 square kilometres (9 – 1,847 square kilometres), region 6: 914 square kilometres (200 – 2,409 square kilometres), region 7: 260 square kilometres (4 – 1,872 square kilometres), region 8: 332 square kilometres (26 – 1,044 square kilometres)



**Figure 3.2:** Maps showing the number of reported poultry outbreaks (green monochromatic) and human cases (grey cycle) of HPAI H5N1 per district in Vietnam between 8 December 2003 and 26 August 2007 stratified by period. Each period started on 1 September and ended on 31 August the following year. The number of human cases ( $n_h$ ) and poultry outbreaks ( $n_p$ ) is shown.



**Figure 3.3:** Epidemic curves showing the daily number of human cases (top,  $n = 99$ ) and poultry outbreaks (bottom,  $n = 2,959$ ) of HPAI H5N1 recorded in Vietnam between 8 December 2003 and 26 August 2007. Vertical lines mark the four periods.

### 3.4 Discussion

In Vietnam details of outbreaks of HPAI H5N1 in poultry are collected through a system of passive surveillance which relies on the observation of clinical signs and the preparedness of poultry keepers to report the presence of disease to animal health authorities. Subclinical infections and underreporting are factors affecting the sensitivity of a passive surveillance system (Salman 2003). Investigating the association between poultry outbreaks and human cases may provide an indication as to what extent passive surveillance of clinical disease in poultry captures the risk of human exposure to H5N1 virus. If human cases precede poultry outbreaks then it is likely that outbreaks in poultry are going undetected until the presence of disease in humans prompts a change in passive surveillance intensity. This temporal relationship, if identified, provides indirect evidence of underreporting of HPAI H5N1 poultry outbreaks in Vietnam. At larger spatial scales there is spatial and temporal association between poultry outbreaks and human cases of H5N1 disease (Figures 3.2 and 3.3), but it is desirable to determine whether this relationship also holds at local spatial scales.

Rare-events logistic regression was used to quantify the spatio-temporal association between poultry outbreaks and human cases at local scale. Rare-events logistic regression has been previously used in political science (King & Zeng 2001*a*), fraud detection (Bolton & Hand 2002), criminology (Piquero et al. 2005), and geomorphology (Van Den Eeckhaut et al. 2006, Vanwalleghem et al. 2008). To the best of our knowledge, this is the first use of the technique in epidemiology.

For modeling purposes, we decided to use human cases as the outcome and poultry outbreaks over different weekly lags as predictors. Our rationale for this approach was as follows. First, HPAI H5N1 is transmitted directly from poultry to humans. Secondly, the likelihood of reporting of disease was greater in humans than in poultry, allowing us to be confident that the spatio-temporal distribution of cases comprising the study outcome was free of selection or misclassification bias. Poultry outbreaks were lagged over four weeks in each direction from the week of the human case to capture temporal associations within a month either side of the occurrence of a human case. We constructed separate models using poultry outbreaks either in the same district as the human case (Model 1) or in the same and neighbouring districts (Model 2). Including poultry outbreaks in neighbouring

districts in Model 2 allows for the fact that the exact location of the human case within the district is unknown and also that district boundaries provide no natural boundary for either the virus or human activities.

Our main finding was a significant association between poultry outbreaks and human cases in particular week lags. However, contrary to the expected biological transmission pathway, poultry outbreaks in the same district were typically reported one week or four weeks after the occurrence of a human case in Model 1 (Table 3.1). In addition, the presence of poultry outbreaks in either the same or neighbouring districts in the same week as the human case was significant in Model 2 (Table 3.2). Several reasons may explain why poultry outbreaks were reported after confirmations of human cases. First, it may have been caused by delayed detection of clinical signs in poultry flocks. Whilst the average incubation period for H5N1 lasts for only a few days in both humans (WHO 2006) and individual birds (OIE 2002, Saif 2003), detection of increased mortality in poultry flocks may take longer, especially in smaller extensively managed flocks of the type kept in Vietnam. Sims et al. (2003) reported results from unpublished data from Hong Kong indicating that mortality in a flock of birds may not show a marked increase for a week or more after exposure to H5N1 virus. Ducks are the principal poultry species in the HPAI-affected areas of Vietnam, and expression of disease may take longer in ducks than chickens, at least for some strains of H5N1 virus. Therefore, it is possible that clinical signs of H5N1 infection are detected later in poultry than in humans. The fact that detection of a poultry outbreak is increased at one or four weeks after a human case but not in the intervening period, may reflect the clinical expression of a first and a second wave of disease in the local area.

Another possible explanation is enhanced detection and reporting of poultry outbreaks by poultry owners after they become aware of a local human case. In addition, animal health authorities may increase their efforts to detect H5N1 infection in poultry in those districts in which a case of human disease has been identified. For instance, Normile (2007) provided anecdotal reference that Indonesian authorities increased their control efforts after reporting of a human case.

Our study provides evidence to support the hypothesis that variation in reporting effectiveness influences the degree of association between human cases and poultry outbreaks. When considering the 9-week interval extending from four weeks prior to four weeks after

the week of reporting a human case, our results show that for the whole country poultry outbreaks were recorded in 33% (in the same district) and 54% (in either the same or neighbouring districts). However, there was a significant difference between the southern half of the country, where the two figures were 58% and 83%, and the northern half where the equivalent values were 23% and 42%. Since it is more difficult to detect disease in ducks and they are at much higher density in the south, this difference cannot be explained by epidemiological factors, and appears to be due to much lower detection levels in the north. This is supported by the finding that the degree of association between poultry outbreaks and human cases has declined as the epidemic has progressed, and the early high level of concern among farmers has decreased to some degree. It appears that underreporting of poultry outbreaks still exists in Vietnam and that intensified surveillance is effective in detecting additional outbreaks.

Human cases would be expected in the absence of poultry outbreaks if humans became infected whilst travelling to areas beyond neighbouring districts. This was unlikely to have been an issue in these analyses since only four human cases (4%) had a history of travel prior to the onset of symptoms. However, movement of infected poultry or contaminated poultry products from areas outside the neighbouring districts may explain the lack of association between the location of some human cases and any nearby poultry outbreaks.

Another factor which may reduce the predictive value of poultry cases is the sub-clinical circulation of H5N1 (Sturm-Ramirez et al. 2005). It is unclear whether clinically healthy ducks excrete sufficient virus to cause human infection. However, consumption of raw duck blood dishes was linked to some human cases in Vietnam (WHO 2005). Hence, ducks may act as a silent source of infection for humans, particularly where risky food handling and consumption behaviors are prevalent. Live bird markets may facilitate silent spread of HPAI viruses, which contributes to the perpetuation of AI viruses and transmission to other avian species (Seo & Webster 2001, Shortridge et al. 1998) and possibly to humans (Mounts et al. 1999, Sims et al. 2003, Yu et al. 2007). Additionally, H5N1 infection may not be detected if infected birds are sold during the incubation period. Alternatively, unusually high mortality may be detected but not reported to authorities. These factors may be potential causes for humans being infected through live bird markets, without disease being reported in poultry. Further investigations are needed to assess



the extent to which live bird markets act as a source of infection to humans. Measures such as weekly rest days (Kung et al. 2003, Lau et al. 2007) or barriers between live birds and customers (Mumford et al. 2007) would be logical strategies to mitigate these risks.

Vaccination may reduce the amount of virus shedding and thus the potential for human exposure. Capua & Marangon (2006) suggested that the absence of human cases in Vietnam in 2006 was an indication that the nationwide vaccination campaign, which has been conducted twice a year since September 2005, was effective in preventing human infections. However, the recrudescence of human infections in the north of Vietnam in 2007 suggests the need to monitor vaccine protection and adjust vaccine formulation to take account of the emergence of new variants of H5N1 virus, such as the Fujian-like sublineage of H5N1 identified in 2008 (Le et al. 2008). Incomplete protection at the flock level may enhance the potential for selection of variants that exhibit antigenic drift (Lee et al. 2004). Savill et al. (2006) showed that if vaccination coverage is only sufficient to induce partial flock immunity, mortality of infected flocks may not rise above typical levels, yet virus shedding and transmission still occurs. Surveys to monitor the efficacy of vaccination programmes would allow assessment of the adequacy of vaccination programmes to be more evidence based.

### 3.5 Conclusions

Our results show that for Vietnam the occurrence of clinical disease in poultry is not a useful predictor of subsequent human cases in the same locality. Rather, the presence of a case of H5N1 in humans was associated with an increase in the likelihood of disease being detected in poultry one and four weeks later. Our results indicate that reliance on owner reporting of clinical disease produced by H5N1 virus has resulted in under-reporting of poultry outbreaks in Vietnam, with detection effectiveness enhanced by the presence of disease in humans. We recommend additional measures to complement owner reporting, to minimise human exposure. These include the provision of adequate compensation of poultry owners for losses due to reported H5N1 infections, active surveillance to detect circulating virus in at-risk areas, surveys to monitor the efficacy of HPAI H5N1 vaccination campaigns, and ongoing education campaigns to increase awareness of human risky behaviors. Furthermore, it is important to conduct adequate epidemiological investiga-

tions of both human cases and poultry outbreaks to provide a sound basis for adjusting local control strategies.

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## The spatio-temporal epidemiology of highly pathogenic avian influenza outbreaks in the two deltas of Vietnam during 2003 – 2007

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**Abstract** – Outbreaks of HPAI subtype H5N1 have occurred in Vietnam as a series of epidemic waves since December 2003. We describe the spatial and temporal patterns of the HPAI H5N1 epidemics in the Red River Delta in the north (785 outbreaks in 606 communes) and the Mekong River Delta in the south of Vietnam (1,313 outbreaks in 837 communes), where the epidemics were concentrated. Throughout the study period the percentage of outbreaks affecting ducks increased steadily to a peak of 78% during the 2006 – 2007 epidemic in both deltas. Five of the seven epidemic waves occurred in the period of active poultry population buildup immediately prior to the Vietnamese New Year (Tết festival). Recorded outbreaks were clustered in space and time within both deltas, consistent with infection transmission occurring via a combination of local and long-distance spread. Our analyses demonstrate that the epidemiology of HPAI in Vietnam has changed over the 4-year study period, with outbreaks now occurring in the warmer months of the year and ducks featuring more prominently as affected species. To determine the relative importance of local and long-distance spread on infection transmission, precise details of outbreak location, date of onset of clinical signs, and size and composition of the poultry population at risk need to be recorded during future outbreak responses.

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## 4.1 Introduction

Outbreaks of HPAI H5N1 have occurred in Vietnam since December 2003. During the initial epidemic wave in 2003 – 2004, eradication was attempted using restriction of animal movement and pre-emptive culling of 44 million poultry (To et al. 2007). Despite these efforts further outbreaks occurred in 2004 and 2005, and after an extended period free of cases, new outbreaks occurred between December 2006 and July 2007. Up to July 2007, 59 of the 64 provinces of Vietnam had experienced outbreaks. During this time, Vietnam had poultry outbreaks and 100 human cases of HPAI H5N1 occurred (46 of which were fatal) (WHO 2007).

Since September 2005, chickens and ducks (>14 days old) have been vaccinated for HPAI H5N1 using inactivated H5N1 vaccine (for chickens and ducks) and H5N2 vaccine (for chickens only). Vaccination campaigns targeted high-risk areas identified by criteria such as the disease situation, human and poultry densities, and extent of virus circulation. The Vietnamese government also enforced a national ban on waterfowl hatching from February 2005 to February 2007 to reduce the influence of waterfowl as a reservoir of disease.

We focused on the RRD and the MRD, which were previously identified as high-risk areas during the initial epidemics (Lockhart et al. 2006, Pfeiffer et al. 2007, Taylor & Do 2007). The RRD has a high density of chickens, whereas the MRD is the area with the highest duck density. Duck production is an essential element of agricultural production in these areas. Scavenging duck flocks (known in Vietnam as ‘field running ducks’) form an important part of the traditional integrated farming system in both deltas, especially in the MRD (Men 2007, Gilbert et al. 2006) reported a strong association between duck raising, rice crops and HPAI outbreaks in Southeast Asian countries. All previous studies have used only the data for 2004 and 2005 epidemics at the national level. Because the infection is still spreading in the country, it is crucial to provide better insight in the infection dynamics in the two deltas using all available outbreak data.

Our objective was to describe the spatial and temporal patterns of the HPAI H5N1 epidemics in the two deltas of Vietnam. Findings from this study can then be used to direct future research into HPAI epidemiology and serve as a starting point for devising more effective programmes to control the disease in the country.

## 4.2 Materials and methods

### 4.2.1 Study area and study population

The study focused on the two main river deltas in Vietnam (Figure 4.1). The disease was first identified in the two deltas, before spreading throughout the country during the 2003 – 2004 epidemic (DAH 2005*b*). Vietnam's 64 provinces are grouped into eight geographical regions. The RRD and the MRD are the predominant rice-growing areas with dense river systems. The two deltas held 46% of the national poultry population in 2003 (General Statistics Office of Vietnam 2007). The RRD occupies a land area of 14,777 square kilometres and is comprised of 2,236 communes, 111 districts, and 11 provinces. The MRD (40,186 square kilometres) is comprised of 1,518 communes, 117 districts, and 13 provinces. The period of interest was from 12 December 2003 to 31 July 2007. The unit of observation was the commune, which is typically comprised of five to ten villages.

### 4.2.2 Data sources and case definition

From 12 December 2003 to 29 February 2004, we used data obtained from a retrospective survey that was conducted DAH in collaboration with the Food and Agriculture Organization (FAO), as described by Lockhart et al. (2006). From 1 March 2004 to 31 July 2007, routine surveillance data from DAH were used.

We used the date and location of commune outbreaks. The recorded date of an outbreak was based on the earliest date that poultry showed clinical signs. Outbreaks in the same commune within 7 days of an initial outbreak were considered as a single outbreak. A commune was considered HPAI-positive if it had at least one recorded HPAI H5N1 outbreak within the period of interest.

In the 2003 – 2004 epidemic, case communes were identified on the basis of poultry showing typical clinical signs of the disease. Samples were taken from selected outbreaks for laboratory confirmation at the Centers for Disease Control and Prevention (Atlanta, USA) and the Australian Animal Health Laboratory (Geelong, Australia). In later epidemics, the presence of HPAI H5N1 was confirmed for all reported outbreaks by the Veterinary Regional Laboratories and the National Centre for Veterinary Diagnosis in Vietnam.

### 4.2.3 Analytical methods

We used several techniques to investigate temporal, spatial, and spatio-temporal patterns of HPAI outbreaks among the 3,754 communes of the two deltas. Dates were not recorded for 90 outbreaks in Hai Duong province (RRD) during the 2003 – 2004 epidemic. These outbreaks were included only in the spatial analyses.

Outbreaks were stratified into four periods, each starting on 1 August and ending on 31 July of the following calendar year. This classification was used to avoid splitting the peak transmission period that occurs from December to March (Park & Glass 2007). The 2003 – 2004 period started on 12 December 2003, when the first outbreak was recorded in Vietnam.

#### **Affected species**

For each period, we calculated the percentage of commune outbreaks affecting different bird species, with exact binomial 95% confidence intervals. We distinguished between outbreaks in chickens, ducks, muscovies, and quails.

#### **Temporal analyses**

For each study area, we plotted the total number of infected communes identified per day as a function of time as epidemic curves. Durations of HPAI vaccination campaigns and dates of New Year (Têt) festivals were superimposed on these plots to identify potential links between these activities and outbreaks.

#### **Spatial analyses**

A map of administrative units was obtained from the Ministry of Natural Resources and Environment. Centroid coordinates for each commune were calculated using ArcView 3.2a (Environmental Systems Research Institute Inc., Redlands, CA, USA). For our spatial analyses, we did not distinguish repeated outbreaks in the same commune within a given period.

Kernel smoothing techniques were used to describe the spatial features of HPAI outbreaks. For each period, two density surfaces representing the number of communes per

square kilometer were constructed. The first surface was based on all infected communes that were identified in each period, and the second on communes at risk at the start of the period. The ratio of kernel density of HPAI H5N1-positive communes to density of all communes created a surface showing the proportion of communes per square kilometer which were HPAI-infected during each period. Considering differences in commune sizes between the two deltas, a fixed bandwidth of 10 kilometres was chosen for the RRD and 15 kilometres for the MRD. The selection of bandwidth aimed to compromise between detail and smoothness in exploring the spatial distribution of HPAI-infected communes (Venables & Ripley 2002). To account for edge effects (Lawson 2001), we computed kernel density surfaces on the basis of communes located in both the study area and neighbouring provinces (guard area). Only the density surfaces for the study area were reported (Lawson 2006).

### Spatio-temporal analyses

Detection of disease clusters was conducted using the space-time scan statistic (Kulldorff et al. 1998), based on a Poisson model using the daily infection status of commune as the outcome. All communes present at the start of each period were considered as the population at risk. Time and space windows were set at 21 days and 50% of all communes at risk. The scan window moved over the study area and duration of interest. Numbers of observed and expected infected communes were counted for each window. Disease clusters were identified when there was an excess of observed infected communes.

To identify whether or not the spatial distribution of HPAI-positive communes showed a pattern consistent with an infection transmission process that had features of local spread (that is, spread of infection over 0- to 10- kilometres distances when there is no clear linkage other than geographical proximity (Sanson et al. 2006)), we used the K-function,  $K(s)$  (Diggle et al. 2007). K-functions were computed for infected communes,  $K_{case}(s)$ , and all communes,  $K_{pop}(s)$ . The observed difference function,  $D(s) = K_{case}(s) - K_{pop}(s)$ , was considered a measure of the additional aggregation of infected communes over all communes. To account for broad-scale spatial trend (first order effects), we computed for each period the K-function for communes within the most likely spatio-temporal cluster identified using the spatial scan statistic. For each cluster, the observed difference function was computed using 99 Monte Carlo permutations. For

each permutation communes were randomly assigned a HPAI-positive label and  $D(s)$  re-computed on each occasion. If significant spatial autocorrelation were present within the cluster then the plot of the observed  $D(s)$  as a function of distance would lie outside of the range of the upper and lower Monte Carlo simulation envelopes.

Data were manipulated using Microsoft Excel and Microsoft Access. Statistical analyses were conducted using R version 2.5.0 (R Development Core Team 2007), and ArcView 3.2a (Environmental Systems Research Institute Inc., Redlands, CA, USA).

### 4.3 Results

In total, 785 outbreaks were reported in 606 communes of the RRD and 1,313 outbreaks were reported in 837 communes of the MRD. Within the same period, the median time between repeated outbreaks in the same commune was 14 days (IQR 11 – 18 days) in the RRD and 19 days (IQR 12 – 28 days) in the MRD.

#### Affected species

From 2003 – 2004 to 2006 – 2007, the percentage of outbreaks that included ducks increased from 11% to 78% in the RRD, and from 26% to 78% in the MRD (Tables 4.1 and 4.2). Outbreaks in muscovies were almost exclusively reported in 2006 – 2007. Quail outbreaks were only reported in the second and third periods, with a significantly higher percentage of outbreaks affecting quails in the RRD compared to the MRD. During the study period, there was only one goose outbreak recorded. This occurred in 2003 – 2004 in the MRD, and was included with muscovies.

#### Temporal analyses

During 2003 – 2004 and 2004 – 2005, the highest frequency of outbreaks was recorded between December and March in both deltas (Figure 4.2). In the RRD, reported outbreaks occurred earlier in 2005 – 2006 (October to December) and later in 2006 – 2007 (May to June), while outbreaks remained seasonal in the MRD. The third epidemic reached its peak in November 2005, soon after implementation of the first round of vaccination.



### Spatio-temporal analyses

Figures 4.3 and 4.4 show the spatial distribution of infected and non-infected communes and kernel density estimates of the proportion of infected communes per square kilometre for the RRD and MRD, respectively. Over the 4 years, the number of infected communes was 428, 83, 152, and 82 in the RRD, and 558, 430, 12, and 90 in the MRD.

Figure 4.3 shows the spatio-temporal clusters of HPAI H5N1 infection for the RRD, as identified by the space-time scan statistic. The most likely spatio-temporal cluster was in the north and northeast of the delta in the first three periods. In the third period, a secondary cluster was present in the southwest, occurring earlier than the most likely cluster in this period.

Figure 4.4 shows the identified spatio-temporal clusters for the MRD. Areas with high density of outbreaks occurred throughout the MRD in 2003 – 2004 and 2004 – 2005, whereas outbreaks were predominantly located in the southern part of the MRD in 2006 – 2007. The time at which clusters occurred was closely related to the date of the Têt festivals in 2003 – 2004 and 2004 – 2005, whereas it ended 36 days prior to the Têt festival in 2006 – 2007 (Figures 4.2, 4.3, and 4.4). Plots of the observed K-function difference function,  $D(s)$ , showed significant spatial autocorrelation of HPAI H5N1-infected communes in the first period in both deltas, and in the second period in the MRD (Figures 4.5 and 4.6).

**Table 4.1:** Percentage (and 95% CI) of HPAI H5N1 outbreaks that affected chickens, ducks, muscovies, and quails in the Red River Delta stratified by period. One outbreak could affect more than one poultry species.

Species	2003 – 2004 <sup>a</sup> ( <i>n</i> = 429)	2004 – 2005 <sup>b</sup> ( <i>n</i> = 90)	2005 – 2006 <sup>b</sup> ( <i>n</i> = 178)	2006 – 2007 <sup>b</sup> ( <i>n</i> = 88)
Chicken	61 (56 – 66)	93 (86 – 98)	46 (39 – 54)	42 (32 – 53)
Duck	11 (8 – 14)	36 (26 – 46)	26 (20 – 33)	78 (68 – 87)
Muscovy	2 (1 – 4)	0 (0 – 4)	0 (0 – 2)	22 (14 – 32)
Quail	4 (2 – 6)	81 (72 – 89)	55 (48 – 63)	0 (0 – 4)
Unknown	44 (39 – 49)	0 (0 – 4)	4 (2 – 8)	3 (1 – 10)

*n*: Number of commune level outbreaks.

<sup>a</sup> The period started on 12 December 2003 — when the first outbreak was recorded in Vietnam — and ended on 31 July 2004.

<sup>b</sup> Each period started on 1 August and ended on 31 July of the next calendar year.

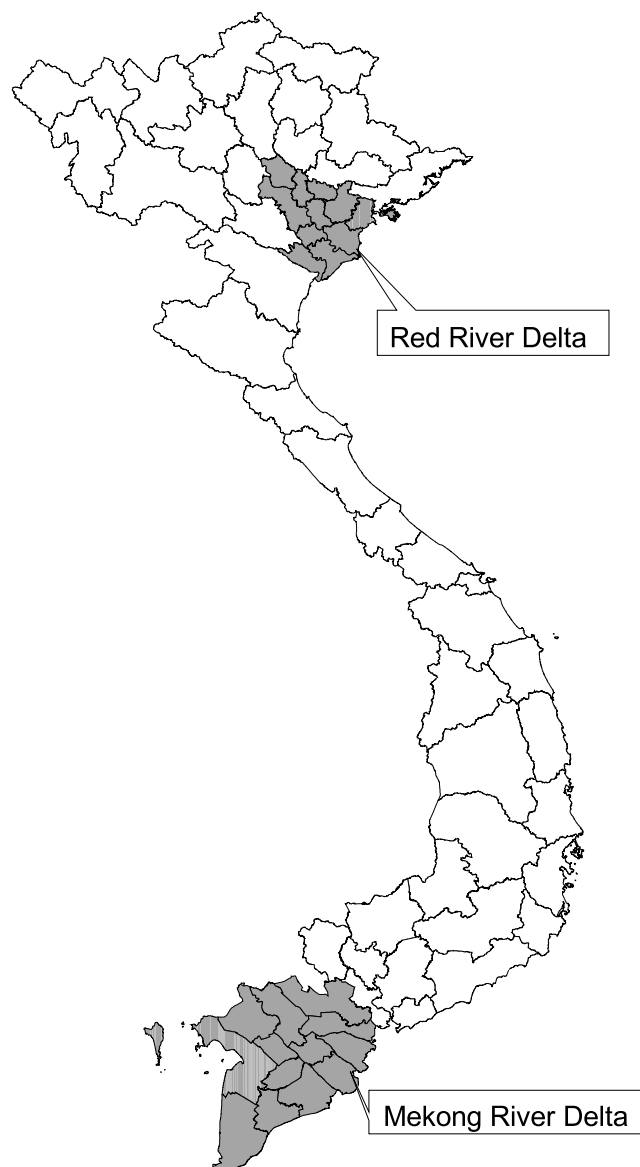
**Table 4.2:** Percentage (and 95% CI) of HPAI H5N1 outbreaks that affected chickens, ducks, muscovies, and quails in the Mekong River Delta stratified by period. One outbreak could affect more than one poultry species.

Species	2003 – 2004 <sup>a</sup> ( <i>n</i> = 571)	2004 – 2005 <sup>b</sup> ( <i>n</i> = 624)	2005 – 2006 <sup>b</sup> ( <i>n</i> = 12)	2006 – 2007 <sup>b</sup> ( <i>n</i> = 106)
Chicken	71 (68 – 75)	78 (75 – 81)	83 (52 – 98)	19 (12 – 28)
Duck	26 (23 – 30)	71 (67 – 75)	0 (0 – 26)	78 (69 – 86)
Muscovy	0.4 (0 – 1)	0 (0 – 1)	0 (0 – 26)	17 (10 – 26)
Quail	3 (2 – 5)	41 (37 – 45)	17 (2 – 48)	0 (0 – 3)
Unknown	13 (11 – 16)	4 (2 – 5)	8 (0 – 38)	0 (0 – 3)

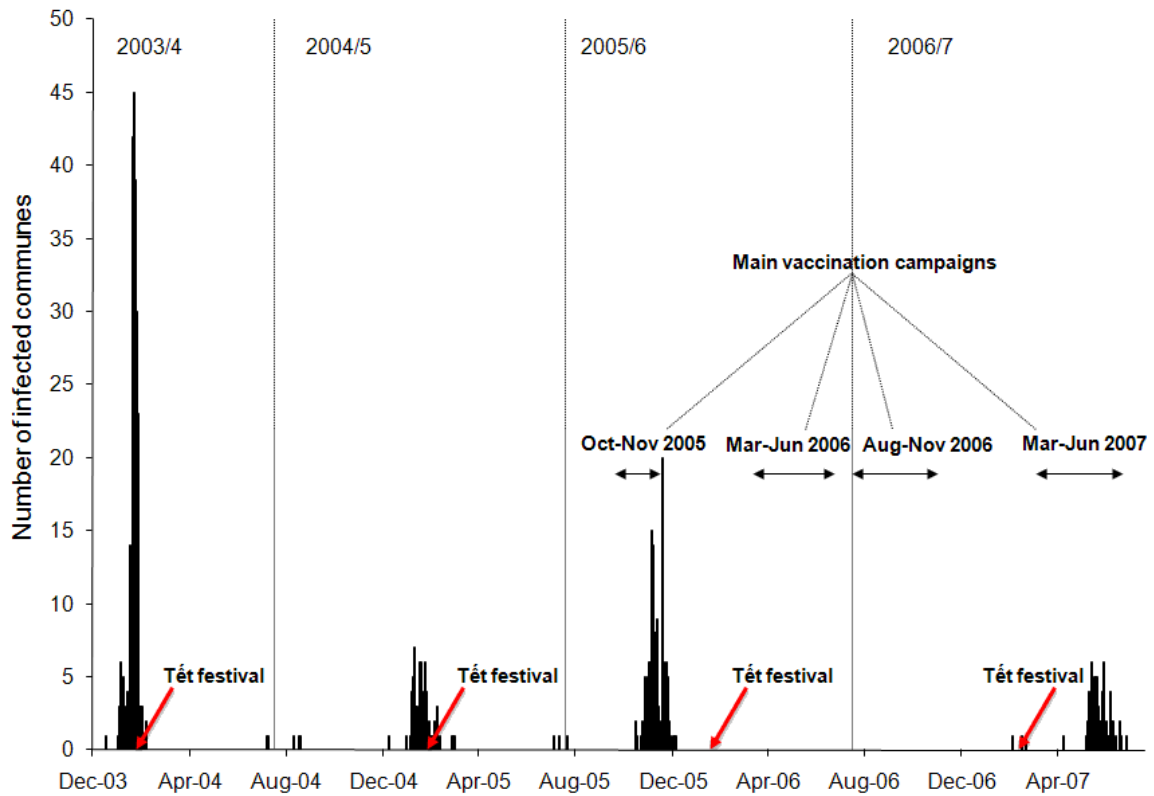
*n*: Number of commune level outbreaks.

<sup>a</sup> The period started on 12 December 2003 — when the first outbreak was recorded in Vietnam — and ended on 31 July 2004.

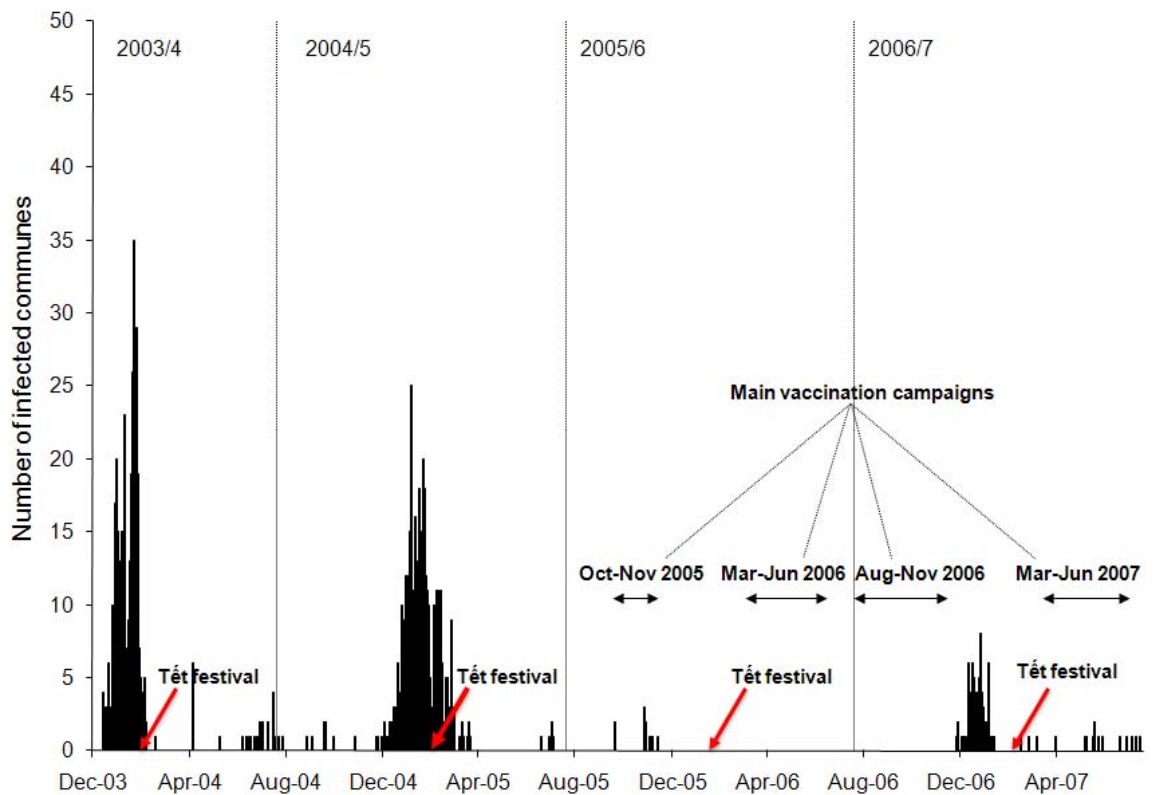
<sup>b</sup> Each period started on 1 August and ended on 31 July of the next calendar year.



**Figure 4.1:** Map of Vietnam showing the location of the Red and Mekong River Deltas (shaded). Median size of communes is 5.9 square kilometres (IQR 4.1 – 7.9 square kilometres) in the Red River Delta, and 20.1 square kilometres (IQR 11.6 – 34.0 square kilometres) in the Mekong River Delta.

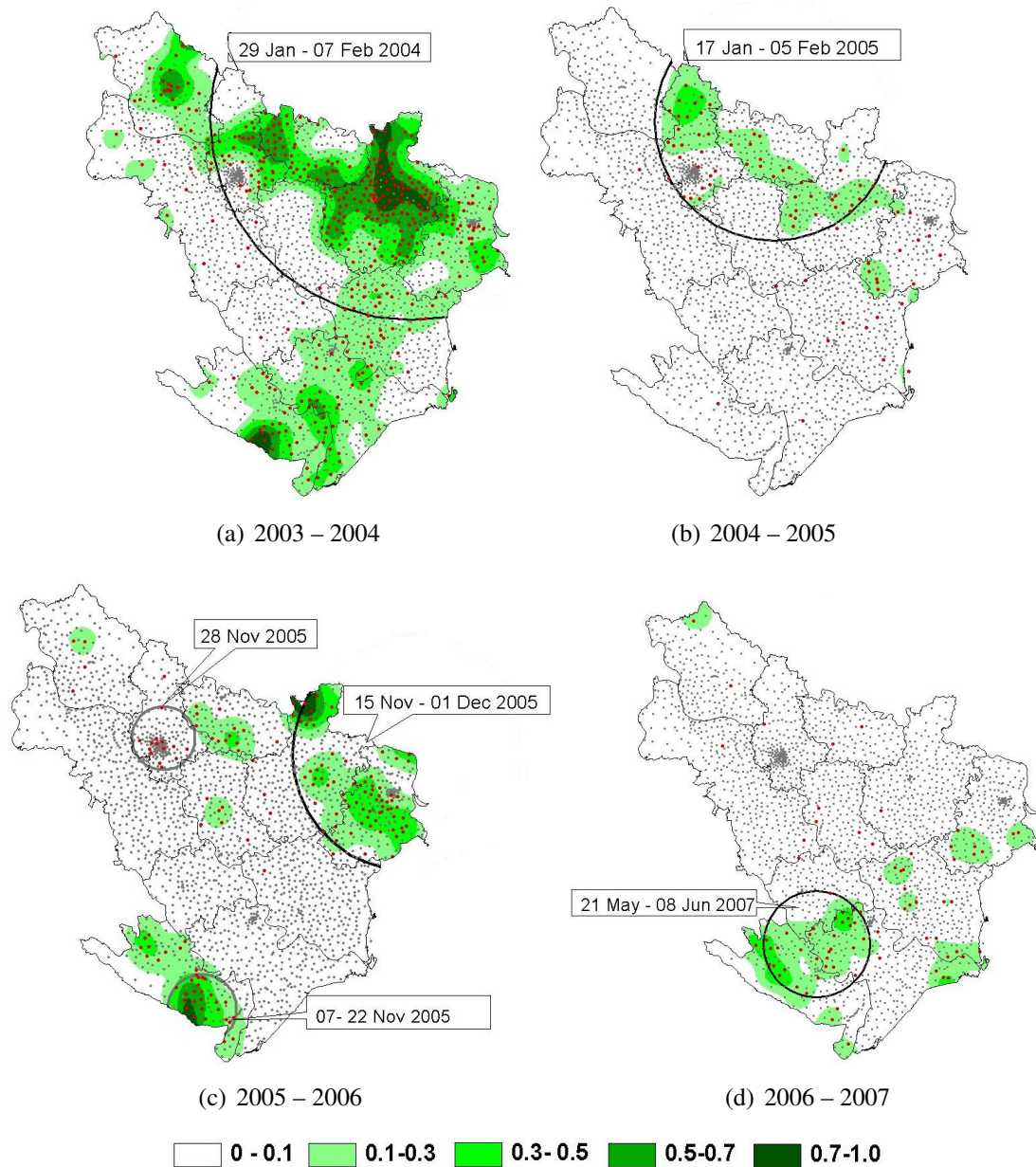


(a) Red River Delta

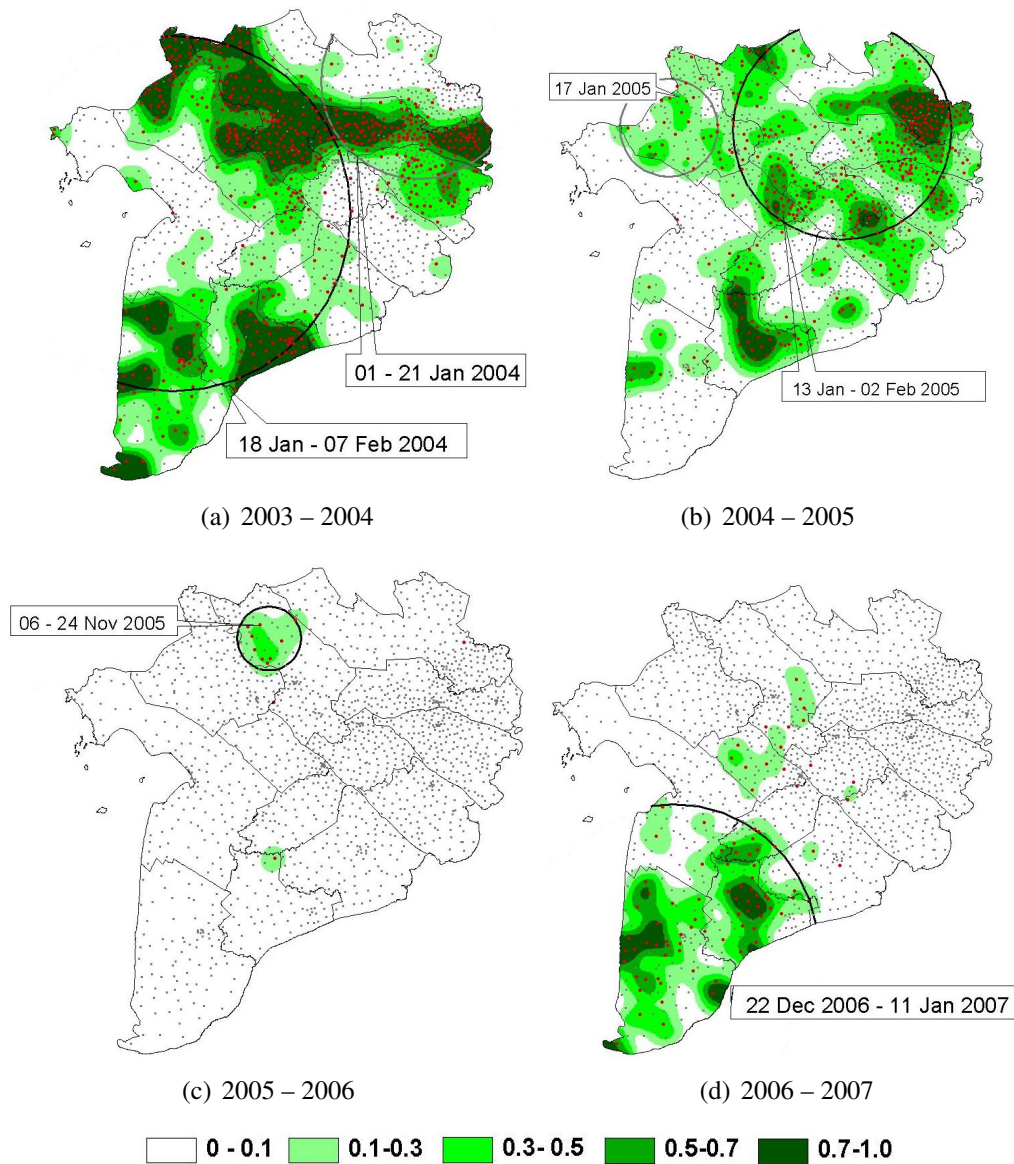


(b) Mekong River Delta

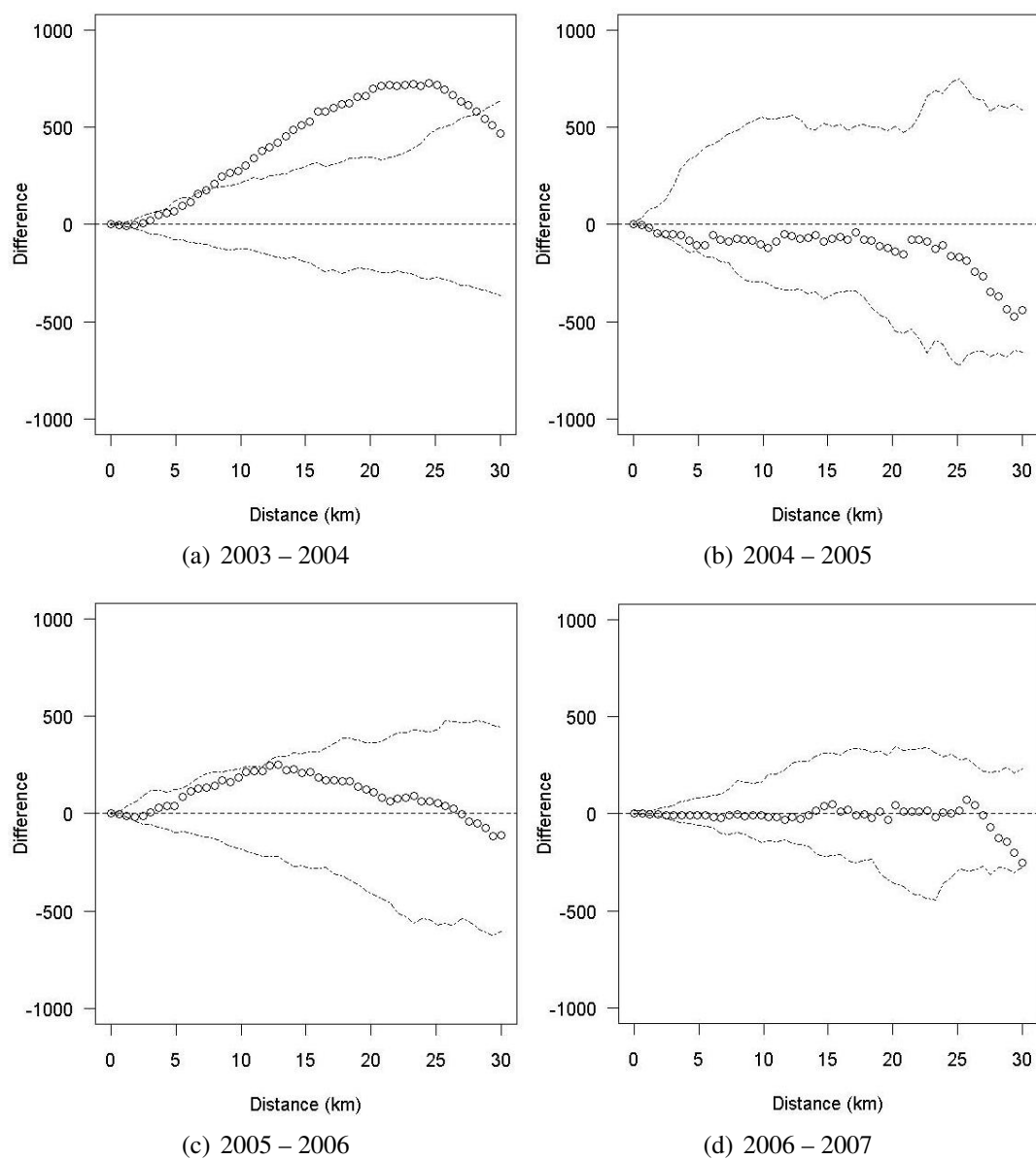
**Figure 4.2:** Epidemic curves showing the daily number of HPAI H5N1-infected communes in (a) the Red River Delta, and (b) the Mekong River Delta. Vertical lines mark the four periods described in the text. The Têt festival was on 9 – 11 February 2004, 11 – 13 February 2005, 29 – 31 January 2006, and 16 – 18 February 2007.



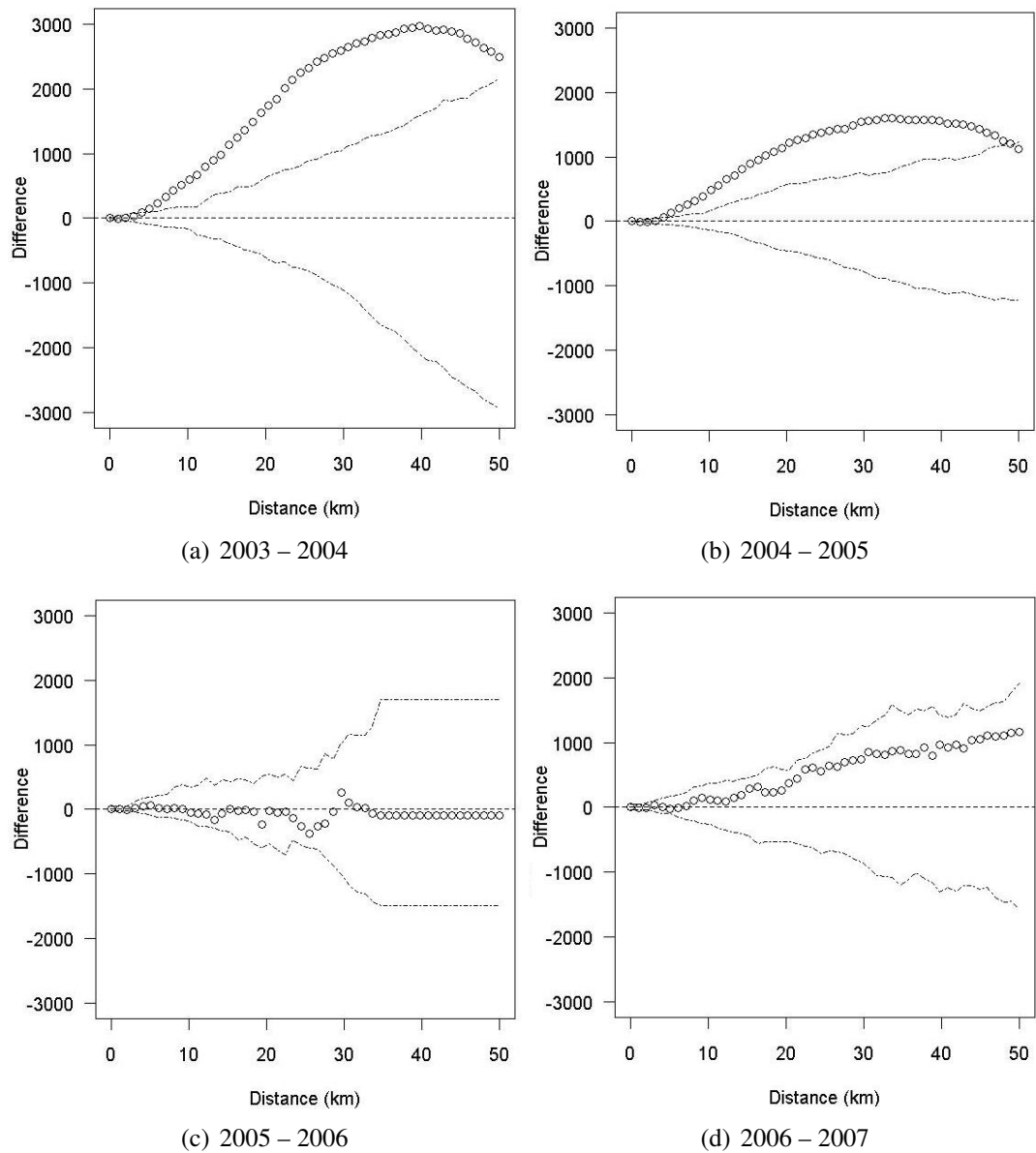
**Figure 4.3:** Maps of the Red River Delta showing: locations of HPAI H5N1-infected (red dot) and non-infected communes (gray dot), kernel density of the proportion of infected communes per square kilometre (green monochromatic), and areas with the result from the spatial scan statistic ( $P < 0.001$ ) showing the most likely cluster (dark circle) and secondary cluster (gray circle) of infected communes. The time frame of each cluster is also shown.



**Figure 4.4:** Maps of the Mekong River Delta showing: locations of HPAI H5N1-infected (red dot) and non-infected communes (gray dot), kernel density of the proportion of infected communes per square kilometre (green monochromatic), and areas with the result from the spatial scan statistic ( $P < 0.001$ ) showing the most likely cluster (dark circle) and secondary cluster (gray circle) of infected communes. The time frame of each cluster is also shown.



**Figure 4.5:** The spatial autocorrelation function difference of HPAI H5N1-infected communes relative to all communes within the most likely spatio-temporal cluster in the Red River Delta.



**Figure 4.6:** The spatial autocorrelation function difference of HPAI H5N1-infected communes relative to all communes within the most likely spatio-temporal cluster in the Mekong River Delta.



## 4.4 Discussion

### 4.4.1 Affected species

The progressively increasing percentage of outbreaks affecting ducks over the 4-year period is consistent with findings from studies reporting the emergence of H5N1 viruses in China, Indonesia, Thailand, and Vietnam during the 2004 outbreaks, which showed greater virulence of H5N1 virus strains in ducks (Sturm-Ramirez et al. 2005, Vascellari et al. 2007). A ban on hatching of ducks is likely to have influenced duck-related activities throughout the study period. For instance, bans may have prompted farmers to purchase unvaccinated ducklings from sources that were distant from their home village. Several studies and support activities have been carried out to define the epidemiology of HPAI H5N1 in Vietnam and aid its control. However, the role of field running ducks in maintaining and spreading the infection is not currently the subject of any substantial investigation. There remain important issues about mechanisms of transmission and maintenance of H5N1 infection in different poultry species, which need to be addressed in further epidemiological studies and future outbreak investigations.

### 4.4.2 Temporal patterns

The temporal pattern of HPAI H5N1 changed over the course of the 4-year study period in the RRD, but remained seasonal in the MRD — except for 2005 – 2006 when the number of outbreaks in the MRD was negligible (Figure 4.2). In the first two periods, epidemic waves occurred between December and March in both deltas. This calendar time, identified as the high-risk period for spread of HPAI virus in Vietnam (Pfeiffer et al. 2007) and other countries in Southeast Asia (Park & Glass 2007), may be explained by several factors. First, December to March coincides with both the Têt festival (January or February) and the wedding season in Vietnam (October to April). Both festivals are associated with a substantial increase in poultry consumption. Thus, large numbers of birds are produced and sold during this period, which is likely to enhance infection transmission. Secondly, the main rice harvest normally occurs between December and February. During this time, field running ducks are often moved between rice fields to scavenge feed (Men 2007), which might have contributed to infection spread (Gilbert et al. 2006). Thirdly, it has

been suggested that migratory waterfowl are also a potential source of virus for HPAI outbreaks in Southeast Asia during December – March (Park & Glass 2007). Phylogenetic analysis (Smith et al. 2006) and epidemiological investigations (Vannier 2007) indicate that within-country infection transmission is predominantly driven by poultry movements rather than through repeated introduction by wild birds. Lastly, it has been suggested that lower temperatures enhance the persistence of AI viruses (FAO 2004a, Shortridge et al. 1998). The average temperature between December and March ranges from 15 to 20°C in the north and from 25 to 28°C in the south of Vietnam (General Statistics Office of Vietnam 2007). While lower temperatures might have facilitated the incidence and spread of the infection during the winter epidemics in the RRD, temperature patterns alone do not adequately explain the spread of the infection at the same time in the MRD.

The fourth epidemic in the RRD was the first HPAI H5N1 epidemic in Vietnam to start in summer (May to June 2007) when temperatures range from 27 to 30°C (General Statistics Office of Vietnam 2007). Outbreaks in the summer have also been reported recently in China (Park & Glass 2007) and Germany (Anonymous 2007a). It is currently not understood which factors might have changed to facilitate outbreaks now in the summer months as well as in the cooler seasons. As H5N1 viruses have become endemic in the Southeast Asian region (Li et al. 2004), the virus genotype, the transmission patterns and the role of various species might all have involved, resulting in new patterns of infection developing.

The temporal pattern of HPAI H5N1 outbreaks might also have been influenced by disease control activities. The absence of HPAI H5N1 outbreaks from January to November 2006 might reflect the effectiveness of the mass-vaccination campaigns that were carried out in both deltas. Post-vaccination surveys in 2006 showed that H5N1 virus still circulated in unvaccinated waterfowl, especially duck flocks in the MRD (Taylor & Do 2007), but the vaccination coverage might have prevented clinical outbreaks of H5N1 until immunity levels dropped. Despite the ban on duck hatching, ducklings were still hatched illegally over that period, because ducks found to be infected in the MRD during 2006 – 2007 were mainly < 3 months old and unvaccinated (DAH 2007).

### 4.4.3 Spatio-temporal patterns

The results of our space-time analyses clearly show that the HPAI H5N1 outbreaks were clustered in space and time in both deltas during the four periods (Figures 4.3 and 4.4). The K-function analyses indicate that local spread of infection was present during the first period in both deltas and during the second period in the MRD (Figures 4.5 and 4.6). Based on these analyses we hypothesize that control activities were successful in reducing local spread of infection, but did not prevent long-distance movement spread during the later periods. Long-distance movement of birds was a likely explanation for at least some of the disease clustering in both deltas (Gilbert et al. 2006). In the RRD, there was evidence of transition from local spread in early situations to longer distance spread in the third period, because three disease clusters occurred concurrently in different areas during November 2005. Determining the relative contribution of local versus long-distance spread as factors influencing the infection transmission under field conditions would allow strategies to enhance the effectiveness of disease control to be more evidence based. For instance, if local spread of virus were identified as being relatively unimportant, outbreak responses should strictly enforce movement controls. If, on the other hand, local spread were found to be important, outbreak responses should emphasise (as in the case of foot-and-mouth disease) rapid identification and stamping out of infected places in addition to movement controls. It was not possible, using the available data, to distinguish outbreaks arising from local and long-distance spread and therefore to comment on the relative importance of the two spread mechanisms. We recommend that data collection during future outbreaks of HPAI should define the village as the epidemiological unit of interest and ensure that the location, date of onset of clinical signs, and size and composition of the poultry population at risk is recorded in affected villages. Sufficient numbers of trained personnel need to be available to conduct forward- and backward-tracing activities, as routinely used in many countries during foot-and-mouth disease and classical swine fever outbreaks (Schnurrenberger et al. 1987).

Visual examination of the human and poultry-density maps for the two areas (not presented) suggests that the high-density areas of HPAI-infected communes coincide with high human and poultry densities in the RRD and the northern region of the MRD. Risky behaviors by people might have facilitated the spread of infection within and between villages in these areas, where hygiene measures are generally poor and poultry movements

are extensive (Capua 2007). However in the MRD, a high density of HPAI-infected communes also occurred in the southern part, where human and poultry densities are relatively low. Field running duck flocks that are moved over long distances within the region are likely to have contributed to infection spread in this area (Gilbert et al. 2006, Men 2007), although these birds comprise only a small proportion of the duck population.

Some issues might have influenced the findings of this study. Firstly, for 2003 – 2004 retrospective data collection was used to provide missing details. For this period recall bias may have influenced our results, and for the entire period underreporting and undiagnosed subclinical infection might have been influential. Secondly, the number of poultry affected and the size of the population at risk were both uncertain in each outbreak. Therefore, it was impossible to use poultry-density data in the analyses. As a compromise we used commune as the unit of interest. The use of communes as the basis for representing outbreak location might have induced a form of measurement bias on account of communes differing widely in size and shape between the two areas (Jacquez & Jacquez 1999). Thirdly, outbreak data were not recorded at the village level which meant that considering repeated outbreaks in the same commune within 7 days as a single outbreak might not accurately record infection spread, because the outbreaks might have occurred in different villages. It was a practical solution to the limitations of the data, and relied on the incubation period of the disease as a basis for setting the minimum interval between outbreaks.

## **4.5 Conclusions**

Our analyses show that, consistent with studies conducted in other countries, the epidemiology of HPAI H5N1 infection in Vietnam is undergoing change. The percentage of outbreaks of HPAI which affect ducks has increased over time. The role of ducks in the transmission and maintenance of H5N1 in countries where the disease is endemic needs further investigation. In the RRD, the temporal pattern of outbreaks changed over the course of the study period, with later outbreaks occurring during both winter and summer months. In contrast, outbreaks remained concentrated between December and March in the MRD. Our spatio-temporal analyses show that outbreaks were clustered in both space and time, and indicate that infection transmission occurs by a combination of local

and long-distance spread. To determine the relative importance of these effects on infection transmission, precise details of the location of disease-positive villages, date of onset of clinical signs, and size and composition of the poultry population at risk need to be recorded during future outbreak responses.



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## A description of the management of itinerant grazing ducks in the Mekong River Delta of Vietnam

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**Abstract** – A cross-sectional survey of management practices of itinerant grazing ducks was conducted during March 2008 in four districts of Can Tho and Bac Lieu provinces in the Mekong River Delta, Vietnam. The objective was to characterise the demographic structure of the itinerant grazing duck population and the management practices which might be related to the continuing outbreaks of HPAI H5N1 in this region. Broiler duck flocks were owned by 55% of survey respondents, and layer flocks by 53%. Greater than 80% of ducks within 93% of villages and 99% of duck flocks were reported to have been vaccinated against HPAI H5N1, whereas only 19% of villages and 60% of chicken flocks had greater than 80% of chickens vaccinated. Fifty-nine percent of duck owners moved their ducks outside their home communes, whilst 37% and 28% of owners moved their ducks outside their home district and province, respectively. Larger flocks were more likely to be run outside their home district compared with smaller flocks. After adjusting for the effect of flock production type and district, the odds of an out-of-district field running duck flock movement was increased by a factor of 7.24 (95% CI 2.89 – 19.24) for households with flocks of more than 800 ducks, compared with flocks of less than 250. Most households sold ducks to traders (72%) or to neighbours (33%), whereas less than 20% sold their birds through markets. The findings of this study suggest that surveillance strategies for field running duck flocks should focus on layer flocks as well as larger flocks as they are more likely to be moved outside of their home district, facilitating long-distance disease spread.

Minh, P. Q., Stevenson, M. A., Morris, R. S., Schauer, B., Quy, T. D. (2010). A description of the management of itinerant grazing ducks in the Mekong River Delta of Vietnam. *Preventive Veterinary Medicine*, **94**, pp. 101 – 107.

## 5.1 Introduction

Waterfowl, including ducks, have been implicated in the maintenance and spread of AI infections in domestic and wild bird populations. Tiensin et al. (2005) demonstrated that free-grazing ducks played an important role in maintenance and transmission of infections during the second wave of the HPAI epidemic in Thailand during 2004, concluding that controls measures were difficult to apply due to the way free-ranging ducks are managed. Free-ranging duck flocks were also suspected as a source of an HPAI H5N1 outbreak in chickens which lead to human infection in Thailand in October 2005 (Songserm et al. 2006). Findings from an experimental study conducted by Keawcharoen et al. (2008) demonstrated that some wild duck species can act as a long-distance vector of H5N1 virus. Wild ducks can contribute to the perpetuation of AI viruses and facilitate spread of disease to domestic ducks and other avian species. Results from a cross-sectional virological study conducted in Vietnam during 2001 showed that avian influenza A viruses of the H4N6, H5N2, and H9N3 subtypes were present in healthy ducks presented for sale in live bird markets in Hanoi (Nguyen et al. 2005). Results from an experiment conducted by Sturm-Ramirez et al. (2005) showed that HPAI H5N1 viruses causing minimal signs of disease in ducks can spread silently and efficiently among domestic and wild ducks in Asia. Since December 2006, HPAI outbreaks in Vietnam have mainly involved ducks (DAH 2007, Minh et al. 2009) and it is suspected that the management of duck flocks may influence HPAI H5N1 transmission dynamics among poultry in Vietnam.

There are approximately 60 million domestic ducks distributed throughout Vietnam (Men 2007). An estimate of the size of the duck population in the MRD is approximately 18 million. Ducks represent about 60% of domestic poultry in the delta, and the duck population in the delta makes up 30% of the total duck population in Vietnam. Although itinerant grazing duck (known as field running duck, FRD) flocks represent only about 10% of the total number of duck flocks, they form 75 percent of the total duck population in Vietnam (Men 2007, Agronomes et Vétérinaires sans Frontières 2006). The remainder are raised in small flocks under various confinement systems. In this paper we use the term field running ducks (vịt chạy đồng) to refer to ducks that scavenge for feed in rice fields. The integrated rice-duck farming system is common in the MRD, which is the main area of rice production in Vietnam. FRD flocks form a key part of an efficient traditional



integrated farming system, where ducks scavenge in rice fields following harvest to glean grains that fall to the ground during harvesting. A feature of FRD management, which has implications for the epidemiology of AI, is that flocks can move within or between districts and/or provinces throughout the yearly production cycle to exploit readily available feed resources. Young ducks (< 3 months of age) scavenge for insects and snails during rice growing periods as part of an integrated pest management system (Teo 2001). Adult ducks (> 3 months of age) are moved to rice fields after harvest to scavenge for leftover rice grains. Ducks can be walked or moved by boats and trucks to scavenging locations (Henning, Henning, Vu, Yulianto & Meers 2009). Due to the presence of feed, post harvest rice fields may act as a meeting point for wild birds and domestic poultry (Gilbert et al. 2007), facilitating the spread of HPAI H5N1 viruses. In Vietnam, duck farming systems have recently been described in the Red River Delta (Agronomes et Vétérinaires sans Frontières 2006) and the MRD (Men 2007). However, demographic and movement patterns of FRD flocks have not been well defined from a disease management perspective. Henning, Henning, Vu, Yulianto & Meers (2009) described management practices of moving duck flocks that travelled distances of more than 10 kilometres in the MRD, concluding that the moving duck production system may pose a risk for AI transmission. We conducted a cross-sectional interview survey in two provinces of the MRD to describe details of FRD flocks and their management in relation to AI transmission. Acknowledging the importance of FRDs in transmission of HPAI H5N1 in the country, our aim was to provide information about management systems and practices that might be used for future studies identifying risk factors for the disease transmission associated with FRD farming practices in the delta. A secondary objective was to use the survey data to identify characteristics of flocks (e.g. size, production type) that render them more likely to be run outside of their district of origin, and therefore potentially increase the risk of spreading infectious diseases such as AI to new locations.

## 5.2 Materials and methods

A cross-sectional survey was conducted in March 2008 in two districts of Can Tho province (Vinh Thanh and Co Do) and two districts of Bac Lieu province (Phuoc Long and Vinh Loi) in the MRD of Vietnam (Figure 5.1). The four districts were purposively selected

as ongoing surveillance data showed that these districts had a history of HPAI outbreaks and they derived their main income from integrated duck-rice production (Tran D. Quy, personal communication).

Thirty villages were selected at random from the two selected districts in each province. Villages were selected from a list of villages that were known to keep FRDs. This information was obtained from the provincial offices of the Department of Animal Health and validated during a training workshop held in Can Tho province on 20 March 2008. The thirty villages included in the survey accounted for 17% and 37% of villages that kept FRDs in the selected districts of Can Tho and Bac Lieu provinces, respectively. In each village, an interviewer obtained a list of households that kept FRDs and randomly selected five households from that list using a table of random numbers. The headman ( $n = 60$ ) from each village as well as five households from each village ( $n = 300$ ) were selected for interview.

Two questionnaires were developed (Appendix A). One questionnaire targeted the village headman, the other targeted individual households. The village headman and household questionnaire included 36 and 50 questions, respectively. Both questionnaires included sections on general information, poultry production, aspects of FRD management, biosecurity (for example the frequency and amount of contact a flock was permitted to have with other poultry and/or traders) and attitudes to AI. The questionnaires were tested in two pilot villages in March 2008 and subsequently refined. Sixteen trained district and provincial veterinarians conducted the interviews. All contacted respondents agreed to participate in the survey.

Duck flock sizes were stratified into quartiles. Survey respondents were requested to express the timing of events at the lunar month level (the lunar calendar being the most commonly used measure of date in Vietnam). These were then converted to solar months at the time of data entry, with a solar month being approximately 30 days prior to the corresponding lunar month. Throughout this paper time is expressed in the solar calendar format. Movements of ducks were classified into two categories: those running only within their home district and those running outside. A district in the MRD occupies a median land area of 332 square kilometres (minimum: 26 square kilometres, maximum: 1,044 square kilometres). Respondents were asked to describe movements of ducks relative to administrative boundaries rather than physical distance, since these terms were the

most common means for describing the extent of movement events throughout the delta. Survey data were entered into a customized Microsoft Access database (Microsoft, Redmond, USA). The data were proofread against the original data on the questionnaires to correct data entry errors. Descriptive statistics were carried out to summarise the main features of the data. Univariate analyses were undertaken to select a set of variables for multivariable modeling. All variables associated with the presence or absence of an out-of-district movement event at an alpha level less than 0.20 were selected for statistical modeling.

A multivariable logistic regression model was used to identify characteristics associated with whether or not a duck flock was likely to be run outside its home district. We used a backward elimination approach to select variables that best explained the odds of a duck flock being run outside its home district. The significance of categorical variables was tested using the Wald test (Agresti 2002, Polit 1996). A series of multivariable logistic regression models was conducted to test and remove variables that were not statistically significant from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all the variables were significant at an alpha level of less than 0.05. The results of the final model are reported in terms of adjusted odds ratios for each explanatory variable. A Receiver Operating Characteristic (ROC) curve was constructed to quantify the predictive accuracy of the logistic model. The area under the ROC curve, which ranges from zero to one, provided a measure of the model's ability to discriminate between the presence and absence of an out-of-district event. The greater the area under the ROC curve the better the model's discriminatory power (Hosmer & Lemeshow 2000). Statistical analyses were performed using R version 2.7.0 (R Development Core Team, Vienna, Austria).

## 5.3 Results

### 5.3.1 Village information

Details of the surveyed villages are provided in Table 5.1. All villages grew rice in two or three cycles per year. Apart from ducks, all villages kept chickens and muscovies. Other poultry species were also raised in villages (geese: 78% of villages, fighting cocks:

68%, pigeons: 39%, pet birds: 12%, and quails: 2%). Ninety-three *percent* of villages had more than 80% of their ducks vaccinated against HPAI. Only 19% of villages had a vaccination coverage of greater than 80% for chickens (coverage of 60% – 80%: 54% of villages; coverage of < 60%: 27% of villages). Eighty-six *percent* of villages were visited by duck herders from outside.

### 5.3.2 Household information

The percentage of missing data for the household questionnaires was less than 5% for all of the reported variables. Seventy-three *percent* of surveyed households kept poultry species other than ducks (chickens: 68%, muscovies: 23%, geese: 8%, fighting cocks: 3%, and pigeons: 2%). Only one surveyed household kept pet birds. Quails were not recorded in any household. Eighty-six *percent* of households growing rice (244 of 300) stated that they raised ducks in their rice fields during the rice growing periods. Of this group 43% (105 of 244) ran ducks into the fields after harvest.

#### Field running duck flocks

The median size of duck flocks was 400 (IQR 250 – 800 ducks). The percentage of households keeping broiler and layer duck flocks was 55% and 53%, respectively. Only 8% of households kept both broiler and layer flocks. The percentage of households keeping only broiler flocks was lower in Can Tho (25%) than in Bac Lieu (69%), whilst the percentage of layer flocks was greater in Can Tho (63%) than in Bac Lieu (26%). Replacement birds were mostly purchased as ducklings (84% of households), but also as adult ducks (29%). Only 2% of surveyed households indicated that they purchased eggs that were ready to hatch.

Fifty *percent* of households owning their own rice fields indicated that FRD flocks originating from other villages visited their rice fields. Ducks were moved outside their home village throughout the year in the four districts. Movement patterns and percentage of movement events differed between the four districts throughout the year (Figure 5.2). Table 5.2 shows that 70% of households moved ducks outside their home village. Fifty-nine *percent* of duck owners moved their ducks outside their home communes, whilst 37% and 28% of owners moved their ducks outside the district and province, respectively. The

median length of time that ducks spent away from their home village was 4 months per year (IQR 2 – 6 months). There was no difference in the median duration of movement for boiler and layer flocks.

Table 5.3 shows the results of the logistic regression model. The area under the ROC curve for the model was 0.81, indicating that the model had moderate to good discriminatory ability. Larger FRD flocks were more likely to be run outside of their home district compared with smaller flocks. After adjusting for the effect of flock production type and district, the odds of a flock making an out-of-district movement was increased by a factor of 7.24 (95% CI 2.89 – 19.24) for households with flocks comprised of more than 800 ducks, compared with flocks of less than 250. After adjusting for the effect of flock size and district, the odds of a flock making an out-of-district movement increased by a factor of 5.57 (95% CI 2.86 – 11.35) for households keeping layer flocks only, compared with those with broiler flocks only.

### **Biosecurity and avian influenza prevention**

Twenty-three *percent* of households that kept poultry species other than ducks allowed mixing to occur. Most households sold ducks from the farm gate to traders (72%) or neighbours (33%), while less than 20% of households sold their ducks at markets. Fifteen *percent* of households stated that poultry were sold during the period when ducks were moved from one location to another (during field running). Seventy-five *percent* of households reported that traders were allowed to enter their poultry raising areas to purchase ducks.

Survey results indicate that most farmers acted responsibly when their poultry were sick or unusually high mortalities occurred in their flocks. For 60% of households raising chickens, HPAI vaccination coverage for chicken flocks was less than 80%. In contrast, 99% of the surveyed households indicated that at least 80% of their ducks were vaccinated. Ninety-four *percent* of households stated that they had HPAI vaccination certificates for their poultry. Eighty-two *percent* of households reported that they ranked the importance of AI at a very high level.

**Table 5.1:** Summary information obtained from the questionnaire for surveyed villages. (Source: village headmen,  $n = 60$ ).

Variable	Median	IQR <sup>a</sup>	Missing <sup>b</sup>
Village size (ha)	293	219 – 408	2
Percentage of village area used for rice production	87	80 – 91	3
Number of households per village	272	212 – 344	4
Number of households keeping poultry per village	145	72 – 254	9
Number of households keeping field running ducks per village	13	9 – 20	13
Number of ducks per village	5,000	3,480 – 7,000	8
Number of muscovies per village	138	98 – 255	8
Number of chickens per village	657	400 – 1,500	9

<sup>a</sup> Interquartile range.

<sup>b</sup> Information was not recorded in the questionnaire or village headmen did not know.

**Table 5.2:** Percentage (and 95% CI) of field running duck flocks that run outside specific administrative boundaries.

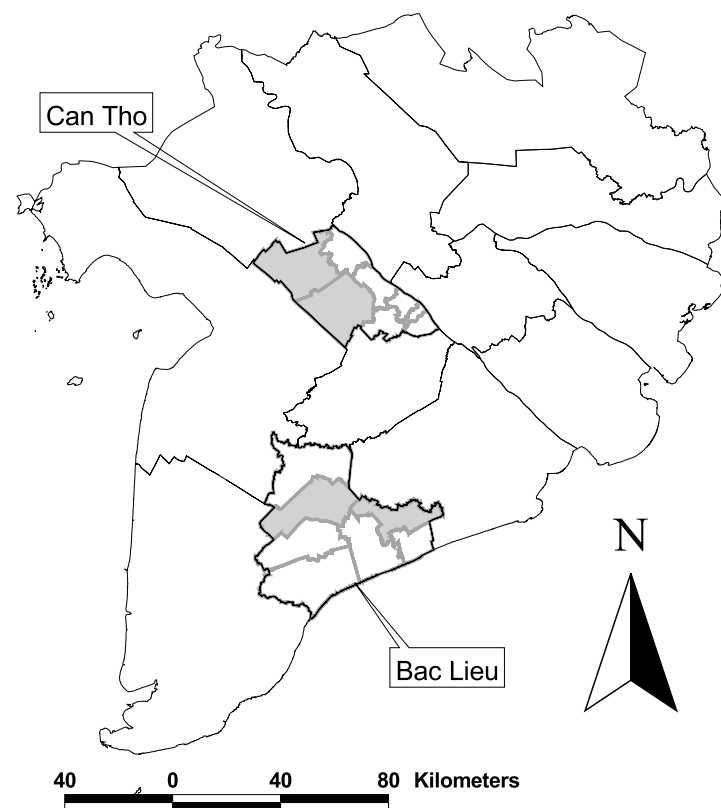
Areas of running ducks	Bac Lieu ( $n = 150$ )	Can Tho ( $n = 150$ )	Total ( $n = 300$ )
Outside the home village	56 (48 – 64)	85 (78 – 90)	70 (65 – 76)
Outside the home commune	47 (39 – 56)	70 (62 – 77)	59 (53 – 64)
Outside the home district	27 (20 – 35)	47 (39 – 56)	37 (32 – 43)
Outside the home province	17 (12 – 24)	39 (31 – 47)	28 (23 – 34)

**Table 5.3:** Regression coefficients and standard errors from a logistic regression model of factors associated with out-of-district movement events made by field running duck flocks in the Mekong River Delta of Vietnam.

Variable	Out-of-district movement		Regression coefficient (SE) <sup>a</sup>	OR (95% CI)
	Yes ( <i>n</i> = 112)	No ( <i>n</i> = 188)		
Flock size:				
< 250 ducks	11	64		Reference
250 – 400 ducks	15	43	0.49 (0.49)	1.63 (0.63 – 4.32)
400 – 800 ducks	36	57	1.91 (0.44)	2.49 (1.07 – 6.11)
> 800 ducks	50	24	1.98 (0.48)	7.24 (2.89 – 19.24) <sup>b</sup>
Production type:				
Broiler only	25	116		Reference
Layer only	71	62	1.72 (0.35)	5.57 (2.86 – 11.35)
Broiler and layer	16	10	1.62 (0.52)	5.04 (1.86 – 14.34)
District:				
Vinh Loi	8	62		Reference
Co Do	50	50	0.76 (0.49)	2.14 (0.83 – 5.92)
Vinh Thanh	21	29	0.42 (0.55)	1.52 (0.52 – 4.60)
Phuoc Long	33	47	1.70 (0.49)	5.49 (2.17 – 15.29)

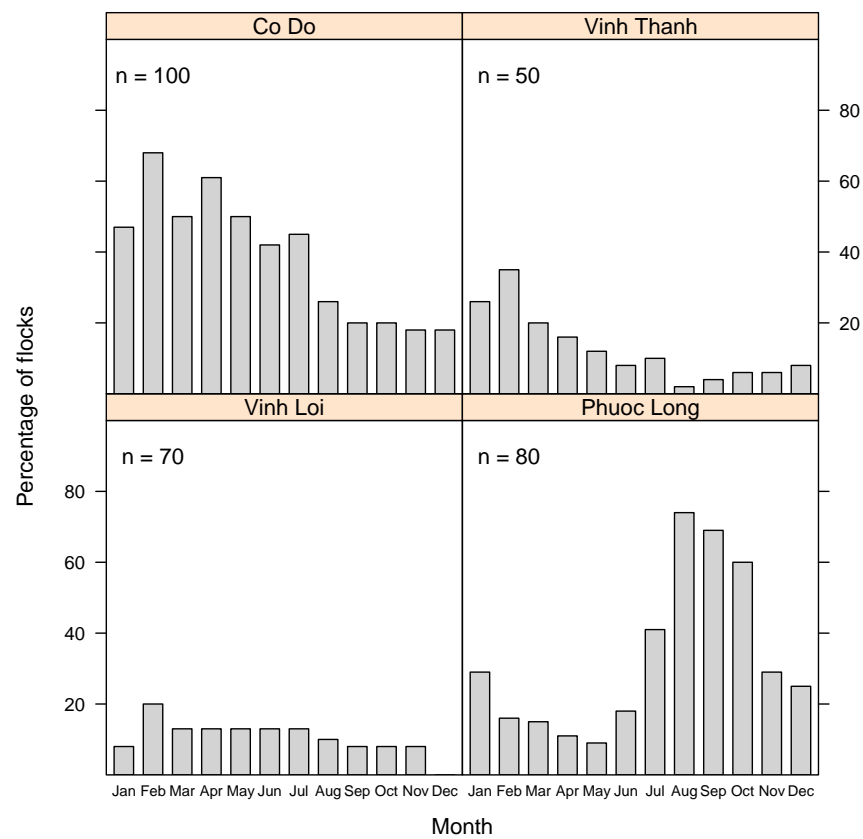
<sup>a</sup> Standard error of regression coefficient.

<sup>b</sup> Interpretation: compared with flocks comprised of less than 250 ducks, the odds of an out-of-district movement for flocks comprised of more than 800 ducks was increased by a factor of 7.24 (95% CI 2.89 – 19.24).



**Figure 5.1:** Map showing boundaries of provinces in the Mekong River Delta (black lines), district boundaries in the two surveyed provinces (grey lines), and locations of the four surveyed districts (shaded).





**Figure 5.2:** Bar plots showing the percentage of field running duck flocks that run outside the home village per month in the four surveyed districts. The number of surveyed households in each district ( $n$ ) is shown in each panel.

## 5.4 Discussion

This paper provides a better understanding of the demographic structure and the management practices of FRD flocks in the MRD. Findings from this survey may be used to generate hypotheses for future studies on AI transmission in this area of Vietnam.

Our findings show that the majority of FRD flocks (59%) are moved outside their home commune, but within the same district (typically 10 to 15 communes). However, 37% of duck flocks are moved over larger geographical areas. The FAO guidelines on AI control and prevention have recommended different strategies for each of four poultry production sectors: (1) industrial integrated systems with high levels of biosecurity, (2) commercial poultry production systems with moderate to high biosecurity, (3) commercial poultry production systems with low to minimal biosecurity, and (4) village or backyard production systems with minimal biosecurity (FAO 2004a). In Vietnam, a fifth sector may be defined for FRD flocks that often move over large distances beyond their home village, and potentially make contact with other flocks, both free ranging and confined, in different villages they pass through. Therefore, a separate surveillance strategy may be required for this particular sector from that used for sedentary poultry flocks. The village level data shows that surveyed villages grew two or three cycles of rice per year and poultry were kept in the majority of households (Table 5.1). Ducks often roam around rice fields in home villages to scavenge for food, particularly leftover rice after harvest. When there is no leftover rice in the village, FRDs are typically moved to other villages within or outside their home district (Table 5.2). Duck owners may rent rice fields in advance and move their ducks in a planned transhumant rotation during rice harvest (Men 2007). Given that the number and duration of rice cycles vary between localities in the region, movement patterns of FRD flocks are likely to be influenced by the timing of rice harvest. This variation depends on whether rice fields are located in the upper, central, or coastal areas of the delta, as well as the availability of water resources (Sakamoto et al. 2005). A study using satellite data in Thailand indicated that there is a strong association between rice crop intensity and free grazing duck density (Gilbert et al. 2007). If the timing of rice cycles can be assessed through satellite images and the relation between rice cycle and duck movement is relatively consistent, this information might be used to refine risk-based surveillance systems for HPAI in this area of Vietnam.

Our analyses indicate that larger FRD flocks and layer FRD flocks are more likely to be moved outside of their home district (Table 5.3), potentially increasing the likelihood of HPAI spread if these flocks were infected. Findings from this survey support findings from a previous study in the MRD demonstrating that there is local clustering of HPAI H5N1 outbreaks at the commune level, with evidence of a combination of local and long-distance spread of disease (Minh et al. 2009). While within-district movement may have contributed to local spread of infection, out-of-district movements by poultry may have facilitated the spread of the disease over larger distances. In the MRD, inter-provincial movements of FRD flocks have been known to occur from November to March (Men 2007). Our findings show that out-of-village movements of FRD flocks take place all year round, and calendar months with the higher percentage of movement events vary by district (Table 5.2). This pattern of movement may depend on the time of rice harvest which varies between localities. We propose that veterinary authorities should not only focus on animals but also look at local agricultural practices to identify risk periods and geographical areas where mixing of poultry occurs. In the MRD, almost all FRD flock owners sold their birds either locally or through traders, so these traders may be important in transmission and should be included in surveillance activities. As a consequence, surveillance strategies should be designed acknowledging the influence of these practices in relation to movement patterns of FRD flocks.

Our survey identified several characteristics of FRD households, such as purchase of adult ducks, the freedom of traders to come in contact with flocks, and mixing of poultry species that potentially render them more likely to be a risk for HPAI transmission. On the other hand, some characteristics may reduce the risk of HPAI maintenance and transmission such as the reportedly high percentage of HPAI vaccine coverage amongst duck flocks. Most households were uniformly high in their reported duck vaccination coverage (> 80%). Vaccine coverage was more adequate in ducks than chickens which might have been influenced by disease surveillance and control strategies. Although this finding was not verified by serological testing, it is likely that vaccination of ducks is more common as vaccination certificates are required for FRD movement events (Anonymous 2007b).

Whereas the internal validity of this study — particularly in relation to the size and management of flocks — is likely to be high, the ability to generalise our findings to other parts of Vietnam should be made with caution due to regional differences in rice growing

activities and flock management. Selection bias may have occurred in this study since selection of households was performed once field staff arrived in villages. Obsequiousness bias might have occurred due to respondents changing their responses in a way to suit the expectations of interviewers who were all employees of the district/provisional veterinary service (Gerstman 2003). Some results obtained from interviews, such as opinions about the importance of HPAI, might reflect politically correct responses and not necessarily field reality.<sup>1</sup> An additional issue, in terms of the movement patterns of FRD flocks, is that out-of-district movements for flocks in villages in communes contiguous with a district border may not have involved large travel distances (Figure 5.1).

This study has provided useful details of the management of FRD flocks in the MRD. However, it does not provide definitive information about the role that FRDs play in the spread of HPAI infection in Vietnam. These issues are being further investigated in a case-control study and a longitudinal study in the MRD.

## 5.5 Conclusions

The findings reported in this study support the hypothesis that FRD flocks may play an important role in facilitating the spread of HPAI infection in the MRD. A separate surveillance strategy may be required for FRD flocks from that used for sedentary poultry flocks. Surveillance strategies for this particular sector should focus on layer flocks as well as larger flocks as they are more likely to be moved outside of their home district, facilitating long-distance disease spread.

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<sup>1</sup>We do not believe that recall bias was an issue in this study because the surveyed respondents were asked to provide details of their *usual* activities and practices as distinct from details of *specific events* such as disease outbreak details.

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## Risk factors associated with outbreaks of highly pathogenic avian influenza H5N1 in the Mekong River Delta of Vietnam

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**Abstract** – A matched case-control study was conducted to identify risk factors associated with outbreaks of HPAI H5N1 in ten provinces of the Mekong River Delta of Vietnam between 1 January and 30 September 2009. Forty households with poultry showing clinical signs of disease and identified as H5N1 positive by RRT-PCR were enrolled in the study as cases. For each case, four control households matched on location were selected from the same commune. Swab samples were collected to detect H5N1 virus in poultry of control households using RRT-PCR. Ten *percent* of control households were H5N1-positive and were replaced. Factors associated with the presence of disease at the household level included those with larger flock sizes (OR = 3.33; 95% CI 1.75 – 6.35), those that raised itinerant grazing ducks (OR = 4.68; 95% CI 1.12 – 19.60), those where poultry had not been vaccinated against H5N1 (OR = 3.90; 95% CI 1.27 – 12.02), and those that had not received visits from paraveterinarians during the previous month (OR = 5.58; 95% CI 1.60 – 19.44). Our analyses identified no risk factors for HPAI at the village level. This study provides evidence that disease control strategies for HPAI should emphasize the reduction of household-level, rather than village-level, risks for disease.

Minh, P. Q., Stevenson, M. A., Morris, R. S., Schauer, B. (2010). Risk factors associated with outbreaks of highly pathogenic avian influenza H5N1 in the Mekong River Delta of Vietnam. Submitted to *Preventive Veterinary Medicine*.

## 6.1 Introduction

HPAI was first reported in Southeast Asia in late 2003 (FAO 2007*b*). The disease has resulted in high economic losses due to death and culling of poultry. Sporadic human cases of HPAI H5N1 have raised concerns of a human influenza pandemic, particularly due to the high case fatality in humans with HPAI H5N1 illness. Up to December 2009, 467 human cases have been reported worldwide, of which 282 (60%) have been fatal (WHO 2010). Factors associated with human cases of HPAI H5N1 include direct and indirect exposure to infected poultry (Zhou et al. 2009). Current knowledge indicates that the most important element in limiting opportunities for exposure of humans to the virus is to control HPAI in domestic poultry.

Although several epidemic waves have occurred in Indonesia, Thailand and Vietnam since 2004 (Gilbert et al. 2008), few studies have identified risk factors associated with HPAI H5N1 in individual poultry flocks. A case-control study to identify risk factors for outbreaks of HPAI H5N1 between December 2006 and January 2007 in the MRD of Vietnam, concluded that incomplete vaccination status (flocks within households receiving either no or only a single dose of vaccine), visits of people to farms, and sharing of scavenging areas increased the risk of a household-level outbreak of HPAI H5N1 (Henning, Henning, Vu, Yulianto & Meers 2009). Despite extensive control measures including animal movement restrictions, destruction of affected flocks, and vaccination of chickens and ducks against HPAI H5N1, outbreaks continue to occur in Vietnam, particularly in the MRD.

The MRD is a main area for poultry production in Vietnam. Poultry production in this region is characterised by village or backyard system with relatively low levels of biosecurity. Poultry and poultry products are mainly sold and consumed locally (Agronomes et Vétérinaires sans Fontières 2006). Controlling HPAI H5N1 is challenging in this area where poultry are kept in relatively small flocks within households and the virus circulates persistently. For this reason disease control and prevention strategies need to be underpinned by a comprehensive understanding of risk factors influencing the maintenance and spread of disease.

The objective of this study was to identify household- and village-level risk factors for HPAI H5N1 outbreaks in the Mekong River Delta of Vietnam. A matched case-control design was used, with households and villages recruited into the study between 1 January

and 30 September 2009, to determine risk factors that could be targeted in intervention strategies.

## 6.2 Materials and methods

In Vietnam, surveillance for HPAI H5N1 relies on the owners of poultry recognizing the presence of sick or dead poultry in their flocks and reporting the details of these events to commune veterinarians (known in Vietnam as paraveterinarians). When signs are reported commune veterinarians visit the flock to inspect and verify case details. Suspected case flocks are then reported to district veterinarians, triggering an outbreak investigation. Veterinarians from the district veterinary office visit the affected farm or household to record details of the incident, take swab samples, and submit those samples to an appropriate laboratory to confirm the presence of disease. Following disease confirmation by RRT-PCR, disease control managers at the provincial, regional and national levels are notified.

### 6.2.1 Study design

A matched case-control study was carried out in the south of Vietnam. The area of interest comprised ten provinces in the MRD. The source population for this study comprised all households located within the boundaries of the ten provinces that comprised the study area. The eligible population comprised all households that kept at least one of the following poultry species: chickens, ducks, muscovies, geese, and quails.

### 6.2.2 Definition and selection of cases and controls

Cases were those households with poultry that showed clinical signs of disease between 1 January and 30 September 2009. The criteria prescribed by DAH (2004) was used to define poultry as HPAI-positive, that is two or more of the following clinical signs had to be present: acute reduction in feed intake, ocular and nasal discharge, coughing, cyanosis of unfeathered skin, swelling of the sinuses and head, depression, nervous signs, diarrhea, an acute decline in egg production, and/or high mortality (DAH 2004). Because it may be

difficult to distinguish clinical signs of HPAI H5N1 from other poultry diseases such as Newcastle disease which is often reported in many provinces of Vietnam (DAH 2009a), virus detection by RRT-PCR provided a definitive diagnosis of HPAI H5N1 outbreaks (OIE 2002). Samples from the trachea, lungs, and brain were collected from three to five affected poultry for each case household (Tran D. Quy, personal communication). These samples were processed as a pool for identification of the agent. Case households were identified via the routine HPAI surveillance programme operational within Vietnam during 2009. Samples were tested by the Regional Diagnostic Centre in the Regional Animal Health Office No.7, Can Tho, Vietnam.

Controls were defined as households that had poultry not showing clinical signs of disease, tested by RRT-PCR and found to be H5N1 negative. For each case, four controls matched on location (within the same commune as the case) were selected as two pairs. The first pair comprised two controls from the same village as the corresponding case. The second pair comprised two controls from two different, unaffected villages in the same commune as the case household. The intention here was to determine risk factors related to HPAI H5N1 at the village level that may distinguish cases from the same and different villages (Dejong et al. 1974). For each village a sampling frame to allow selection of controls existed as a list of all households with poultry compiled by the village headman. In Vietnam it is usual for the village headman to keep a list of all households present, listing details of the type and number of livestock present on each. Households were selected from these lists using a table of random numbers. The table of random numbers was a compilation of five-digit numbers with frequency and sequence of occurrence having been determined entirely by chance. Each poultry household in a village was assigned a unique number (1, 2, ...,  $n$ ). The interviewer chose an arbitrary starting number on the table and read subsequent numbers either horizontally or vertically until reaching the first number appearing in the household list. If a village had less than 100 households with poultry, random numbers of two digits were required, and three digits otherwise ( $n < 1,000$ ). The second household was a consecutive number obtained by reading across the columns from left to right on each successive line in the table until the required number was defined. Information at the village level was obtained for villages with case households and villages that comprised the second pair of control villages. Flocks from candidate control households were tested for H5N1 by RRT-PCR to ensure



that they were without detectable infection at the time the study was carried out.

### 6.2.3 Sample collection of control flocks

Because of sub-clinical circulation of H5N1 virus, especially in waterfowl (Chen et al. 2004, Sturm-Ramirez et al. 2005, Taylor & Do 2007) and vaccinated flocks (Savill et al. 2006), verification of the infection status of control flocks was necessary to avoid errors that may arise if selected control flocks were infected with H5N1, without showing clinical signs during the date of interviewing. Oropharyngeal swab samples were collected from twenty birds owned by each control household, to be 95% certain of detecting HPAI H5N1 if it was present in at least 14% of the poultry population (De Blas et al. 2000). Individual bird samples were pooled into groups of five for control households in the same village and into groups of ten for control households in neighbouring villages. Pooling of five samples is a standard method to reduce laboratory costs with little effect on detection sensitivity (FAO 2004*b*). If poultry from a control household tested H5-positive, N1 RRT-PCR was performed to confirm the presence of H5N1 virus. A control flock was considered to be positive and replaced when at least one pooled sample was H5N1 positive by RRT-PCR. Oropharyngeal swab samples were also collected from all replacement households and tested for H5N1.

### 6.2.4 Questionnaires

Two questionnaires were developed in Vietnamese. The first targeted individual poultry households, whereas the other targeted village headmen. The household questionnaire comprised 43 questions including a wide range of items with four main sections on: (1) general information, (2) poultry production and management practices, (3) duck production and management practices, and (4) biosecurity measures. The same questionnaire was used for case and control households. Six additional questions were added at the end of the questionnaire for case households to obtain further information about affected flocks such as the date clinical signs were first observed in infected poultry, species affected, and the flock owner's opinion on possible sources of infection. This information was only used for descriptive analyses of outbreaks. All questions relevant to events and practices during the previous month referred to the month before the date of the interview.

The village headman questionnaire included 16 questions that solicited details of village characteristics (such as village area, rice area, human population, poultry population, whether or not field running ducks — which grazed in rice fields — from other villages had been observed in the previous month, and an estimate of the vaccine coverage for current village chickens and ducks).

Both questionnaires were tested in two pilot communes in September 2008 and subsequently refined (Appendix B).

### **6.2.5 Data collection**

Questionnaires were administered to owners of case and control households using face-to-face interviews. All interviews were conducted by five regional veterinarians who had been trained in delivery of standardised interviews. For the household questionnaire either the poultry owner or those directly involved in the day-to-day management of birds were interviewed. For the village questionnaire the village headman was interviewed. Repeat visits were made if the required individuals were not at home. Poultry owners and village headmen were asked if they were willing to participate in the study. All questionnaires were administered within seven days after confirmation of the index case of each outbreak. Investigation teams were made up of regional, district and commune veterinarians. District and commune veterinarians took swab samples from controls under the supervision of regional staff. Pooled samples were transferred to the Regional Diagnostic Centre in Can Tho. If a control flock was found to be H5N1-positive, field staff returned to the affected commune and identified a replacement household. The same interviewer collected information from both case and control households in each commune outbreak. Data were entered into the customized relational database (Microsoft Office Access 2003, Redmond, USA).

### **6.2.6 Data analysis**

The data set comprised records for 40 case and 159 control households. A flock was considered to be vaccinated if a vaccination event was recorded within a period of two weeks to six months prior to the date of interview. This restriction was applied to provide a

reasonable estimate of immune status, given that it takes approximately 14 days to develop immunity post vaccination (FAO 2004a, Anonymous 2006). The data were analysed using bivariate and multivariable logistic regression techniques respecting the matched nature of the data. The variable defining flock size was log transformed prior to analysis. Statistical analyses were conducted in R version 2.10.0 (The R Foundation for Statistical Computing, 2009).

Correlations between continuous candidate explanatory variables were assessed using Pearson's correlation coefficients. If explanatory variables were found to be correlated, only that allowed the prediction of the other considered more important in biological processes were included in the model building process (Armitage et al. 2001). Bivariate analyses were undertaken to select a set of variables for multivariable modeling. All explanatory variables associated with the presence of disease at  $P < 0.30$  were selected for statistical modeling. Conditional logistic regression was applied using backward variable elimination to identify explanatory variables that were statistically significant at  $P < 0.05$ . Adjusted odds ratios were obtained to quantify the strength of association between each of the explanatory variables and the outcome. Acknowledging that the strength of the association between each of the explanatory variables and the outcome may have changed depending on which variables were included in the model, we forced variables that were not significant at  $P < 0.30$  in the bivariate analyses but considered important risks for disease into the model. The effect of potential confounders and interactions of risk factors was also assessed.

A simulation-based approach was used to perform *post hoc* power calculations (Self & Mauritsen 1988, Armitage et al. 2001). The expected probability of a household being disease-positive,  $\Theta_i$ , was calculated based on the results of the regression analyses. We conducted 1000 simulations wherein, for each simulation, the disease status for each household was estimated by taking a single draw from a binomial distribution with probability  $\Theta_i$ . The simulated household-level disease status values were then used as the outcome variable in the regression analysis. The P-value for each of the explanatory variables was noted for each simulation. The proportion of data sets in which the P-value of the explanatory variable of interest was less than 0.05 represented an estimate of the power of the study to detect a true effect corresponding to the observed regression coefficient.

Predictive accuracy of the logistic model was quantified using a ROC curve. The area

under the ROC curve provided a measure of the model's ability to discriminate between the presence and absence of disease (Hosmer & Lemeshow 2000).

## 6.3 Results

### 6.3.1 Descriptive analyses

Between 1 January and 30 September 2009, a total of 42 outbreaks of HPAI H5N1 were confirmed in six provinces of the study area, of which 40 were investigated resulting in an initial number of 40 case households and 160 candidate control households. Two outbreaks were not investigated due to either interviewers or field staff being inadequately informed. All of the identified case and control household owners as well as village headmen agreed to participate in the study. Apart from the variable identifying whether or not poultry had contact with other poultry in the same village during the previous month (7% missing), the percentage of missing data for the household questionnaires was less than 5% for all variables. Households with missing data were excluded from analyses. The median duration from first clinical signs observed to the laboratory confirmation of an outbreak was 2 days (IQR 3 – 12 days). The median duration from first clinical signs observed to interviews conducted was 8 days (IQR 7 – 14 days). The median duration from the confirmation of an outbreak to interviews was 3 days (IQR 2 – 4 days). For each outbreak, all interviews of poultry owners (one case and four control households) and village headmen (one case and two control villages) were completed by one interviewer within two days of investigation. For households where poultry were vaccinated against HPAI H5N1, 82% of households raising chickens indicated that greater than 80% of their chickens were vaccinated, while the same level of vaccine coverage was recorded in 94% of households that owned ducks. The date of the most recent vaccination was recorded for 76% of vaccinated households. The median size of poultry flocks was 150 (IQR 50 – 400).

H5N1 infected ducks were recorded in 83% (33 of 40) of case households, while H5N1 infected chickens were recorded in 38% (15 of 40) of case households. Infected muscovies were recorded in 9% of case households. One outbreak involved geese. Twenty percent of households had both infected ducks and chickens. Only duck flocks up to three

months of age were infected with H5N1, whilst chicken flocks of all ages were infected. All of the 40 investigated case flocks were confirmed as H5N1-positive by RRT-PCR. Oropharyngeal swab samples from 16 control flocks (10%) were H5N1-positive by RRT-PCR. An additional fifteen control households were selected to replace the positive controls (one could not be replaced as all poultry in the village had been destroyed as a consequence of disease control measures). None of the replacement households tested H5N1-positive. The median duration from the date of interviewing a positive control to the date of interviewing a corresponding replacement control was 9 days (IQR 9 – 10 days). Because information on clinical signs of positive controls was not fully accessed (mortality details were recorded in only six flocks) and these did not meet the definition of case and control households, all positive controls were excluded from the study. The final data set comprised 40 cases and 159 controls. Village-level details were recorded for 40 case and 80 control villages.

### 6.3.2 Bivariate analyses

Forty-eight putative risk factors at the household level were assessed in the bivariate analyses. The results of bivariate analyses for two different pairs of controls, in the same and different villages, were substantially identical; therefore we present results of data analyses for the combined group of four controls per case.

Table 6.1 shows the relationship between HPAI H5N1 outbreaks and potential risk factors that were significant at  $P < 0.30$ . At the household level factors associated with an increase in the odds of disease ( $P < 0.05$ ) included those households with larger flock sizes, those that purchased supplementary feed, those where poultry had come into contact with poultry from other villages during the month prior to the outbreak, those that did not receive visits from a paraveterinarians during the month prior to the outbreak, those that raised field running ducks, those where poultry had not been vaccinated against H5N1, and households where poultry owners did not think their poultry could be affected by HPAI.

Each of putative risk factors at the village level was assessed using bivariate techniques, but none fulfilled the criteria for inclusion in the multivariable logistic regression model.

### 6.3.3 Multivariable analyses

Table 6.2 presents results of the multivariable model of household level HPAI H5N1 risk. Factors associated with the presence of disease included those households with larger flock sizes, those that raised field running ducks in the month prior to the outbreak, those where poultry had not been not vaccinated against H5N1, and those that did not receive visits from paraveterinarians during the month prior to the outbreak. Poultry species kept in each household and the presence or absence of ducks were forced into the model. None were significant risk factors. All possible interactions between variables were tested in the model, none were significant.

Power calculations showed that the data set was of sufficient size to be 99% (flock size), 59% (field running ducks), 81% (vaccination status), and 84% (paraveterinarian visits) certain of detecting a significant association between these variables and the outcome if they were actually present. The area under the ROC curve was 0.88 indicating that the model had good discriminatory ability in terms of predicting household level HPAI H5N1 status.

**Table 6.1:** Results of bivariate analyses of potential risk factors associated with outbreaks of HPAI H5N1 significant with P-value < 0.30 in the Mekong River Delta of Vietnam.

Variable	Number of cases (n = 40)	Number of controls (n = 159)	OR (95% CI)	P-value
Number of poultry per household in the previous month <sup>a</sup> :				
	40	159	1.81 (1.34 – 2.51)	< 0.001
Levels of contribution of poultry production to household's income:				
Low (<75%)	28	130	Reference	
High (>75%)	10	23	2.02 (0.84 – 4.63)	0.104
Missing	2	6		
Sale of live poultry (purpose of raising poultry):				
Absent	8	50	Reference	
Present	29	124	1.74 (0.77 – 4.34)	0.201
Missing	3	5		
Purchase of supplementary feed for poultry:				
Absent	9	70	Reference	
Present	31	89	2.71 (1.25 – 6.38)	0.015
Household poultry had contact with poultry from other villages in the previous month:				
Absent	27	144	Reference	
Present	9	8	6.00 (2.12 – 17.36)	< 0.001
Missing	4	7		
Presence of paraveterinarians in the previous month:				
Absent	21	38	3.46 (1.69 – 7.17)	< 0.001
Present	19	119	Reference	
Missing	0	2		
Field running ducks raised in the previous month:				
Absent	22	120	Reference	
Present	18	39	2.52 (1.22 – 5.18)	0.012
Ducks ran outside the village in the previous month:				
Absent	33	149	Reference	
Present	7	10	3.16 (1.08 – 8.85)	0.030
H5N1 vaccination status of flock:				
Absent	34	97	3.56 (1.51 – 9.87) <sup>b</sup>	0.007
Present	6	61	Reference	
Missing	0	1		
Poultry owners had ever thought their poultry could be affected by HPAI:				
Absent	27	83	2.19 (1.04 – 4.89)	0.046
Present	11	74	Reference	
Missing	2	2		

<sup>a</sup> Log transformed.

<sup>b</sup> Interpretation: Compared with households where poultry were vaccinated, the odds of HPAI H5N1 in households without vaccination was increased by a factor of 3.56 (95% CI 1.51 – 9.87).

**Table 6.2:** Results of the multivariable logistic regression for risk factors associated with outbreaks of HPAI H5N1 in the Mekong River Delta of Vietnam.

Variable	Regression coefficient (SE) <sup>a</sup>	Wald test	P-value	OR (95% CI)
Number of poultry per household in the previous month <sup>b</sup> :				
	1.200 (0.33)	3.65	< 0.001	3.33 (1.75 – 6.35)
Field running ducks per household in the previous month:				
Absent				Reference
Present	1.54 (0.73)	2.11	0.035	4.68 (1.12 – 19.60)
H5N1 vaccination status of flock:				
Absent	1.36 (0.57)	2.37	0.018	3.90 (1.27 – 12.02)
Present				Reference
Presence of paraveterinarians in the previous month:				
Absent	1.72 (0.64)	2.70	0.007	5.58 (1.60 – 19.44)
Present				Reference

<sup>a</sup> Standard error of regression coefficient.

<sup>b</sup> Log transformed.



## 6.4 Discussion

This study has identified household-level risk factors for HPAI H5N1, supporting findings from previous epidemiological studies in Vietnam. Case households were more likely to have large flocks, to have not received visits from paraveterinarians during the month prior to the outbreak, to raise field running ducks, and to have poultry which were not vaccinated against H5N1. The consistency of risk factors identified in this study and those identified in previous work (Otte et al. 2008, Henning, Henning, Vu, Yulianto & Meers 2009) strengthens the evidence that these factors are truly associated with the presence of disease, providing useful information for the design of control activities. Our analyses did not identify risk factors at the village level. This suggests one of two things. Firstly, the exposure variables operating at the village level were not the most relevant ones to distinguish between case and control village status. Secondly, risk factors for HPAI in the study area were driven by characteristics operating at the household rather than the village level. The second explanation is considered to be more likely, and implies that disease control strategies need to be focused on the activities of individual household owners.

Our analyses show that larger flock sizes are associated with an increased risk of disease. This finding is consistent with analyses conducted by Otte et al. (2008) using routinely collected outbreak data from the 2004 – 2007 epidemic waves in Vietnam. What is not clear from our work and the work of Otte et al. (2008) is whether it is flock size itself or the way large flocks are managed that drives disease risk. Another question is whether large flocks are at greater risk of being infected or detected or both. In this study, putatively uninfected control flocks that were then confirmed to be H5N1 positive by laboratory RRT-PCR confirmation appeared in all quartiles of flock size. This provides limited evidence that, in this study at least, the likelihood of detection was similar across flocks of different size. It is also noteworthy that no commercial poultry flocks were included in this study, and so the term ‘large flock’ refers to large numbers of household poultry free-ranging and with generally low levels of biosecurity. The findings of this study suggest that poultry from households raising field running ducks have an increased risk for HPAI. Our recent survey on the management of field running duck flocks in the MRD indicated that larger flocks are more likely to be moved over larger distances (Minh, Stevenson, Morris, Schauer & Quy 2010). We hypothesize that in this situation free-ranging poultry

have a greater opportunity to make contact with wild birds and other domestic poultry, increasing the risk of being infected and disseminating HPAI.

Our findings reaffirm the observation that vaccination plays an important part in HPAI H5N1 control and prevention in Vietnam. Unvaccinated flocks were more likely to be infected with H5N1. This is consistent with findings from a case-control study conducted in the MRD in 2007 (Henning, Henning, Vu, Yulianto & Meers 2009). The aims of our study were not to describe the efficacy of vaccination coverage in this area. Rather, we determined vaccination status based on records provided by the household owner. For households with poultry that were vaccinated but the date of last vaccination was not recorded, we inferred a vaccination date basing on records for other households in the same village and/or commune. This was thought to be an appropriate strategy since vaccination campaigns are carried out in Vietnam, with all household flocks within a village being vaccinated within a relatively short period of time. Almost all households reported vaccine coverage of greater than 80% of chickens and ducks, suggesting a positive impact of vaccination protocols on control of H5N1 outbreaks in this region.

This study also demonstrates that those households that did not receive a visit from a paraveterinarian in the previous month had an increased risk for HPAI. It is unlikely that this association is causal, rather regular presence of a paraveterinarian would be an indicator variable for good flock management since paraveterinarians provide poultry owners with information on the local disease situation and supervise disease prevention activities (Catley 2005). Further studies are needed to provide more precise details of the way paraveterinarians reduce the risk of disease.

The finding of 10% putatively uninfected control flocks testing H5N1 positive indicates that verification of control status is important when conducting a study of this type. It also indicates that disease may be more widespread in outbreak areas than indicated by passive surveillance. Without sample collection, the results of this study would have been biased due to misclassification of case flocks as controls. To investigate this issue further we tested the effect of misclassification on the study outcome. Including the H5N1-positive controls as true controls did not, on this occasion, alter our overall conclusions. It however did influence the strength of the association between the outcome and each of the explanatory variables. When positive flocks were misclassified as controls, the odds ratios for each explanatory variable were biased towards an odds ratio of 1.00. Testing of

flocks in areas surrounding newly detected outbreaks may be considered as a form of active surveillance for disease, thus increasing the probability of detecting HPAI outbreaks that may not have been detected through a passive surveillance programme.

In Vietnam HPAI surveillance relies on a passive reporting system. This requires poultry owners to report the presence of sick or dead poultry to animal health authorities. Poultry owners may be reluctant to report or may not know the signs of HPAI, potentially resulting in missed cases of HPAI during the study period. We were unable to quantify the level of underreporting in this study. While differential misclassification of case and control status may have been minimised in this study by laboratory confirmation of H5N1 virus, there are two sources of information bias inherent in a study of this type: recall bias and obsequiousness bias. Recall bias is often a problem when conducting case-control studies. It may have been difficult for poultry owners to remember information related to their flocks if the information was obtained after a long period of time since outbreaks of HPAI H5N1 were confirmed. Nevertheless, we believe the effect of recall bias in this study was small due to exposure details being solicited from case and control household owners within a median of 8 days (IQR 7 – 14 days) from the date clinical signs of disease were observed. This strategy was adopted to ensure that case and control households were similar in regard to the information reported on exposure to past potential risk factors (Schlesselman & Stolley 1982). Obsequiousness bias relates to the possibility that household owners and village headmen changed their responses in a way to suit the expectations of interviewers who were employees of the government veterinary service. The effect of this bias on our results is difficult to quantify, although if it was present then it would have been unlikely that a relatively high proportion of household owners (110 of 195, 56%, Table 6.1) would state that they did not think their poultry could be affected by HPAI.

## 6.5 Conclusions

Our study has demonstrated that larger flock sizes, field running ducks, the absence of vaccination, and the absence of paraveterinarian visits in the previous month increased the risk of disease in poultry owning households in the MRD of Vietnam. Our findings provide limited evidence to indicate that disease control strategies should emphasise the reduction of household-level, rather than village-level, risks for disease. Active surveil-

lance should be strengthened (see discussion in page 129) to detect subclinical disease, particularly in areas surrounding newly detected outbreaks.

## **Spatio-temporal analyses of highly pathogenic avian influenza H5N1 outbreaks in the Mekong River Delta, Vietnam, 2009**

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**Abstract** – This paper presents the results of spatio-temporal analyses and epidemic modelling of HPAI H5N1 outbreaks that occurred in four provinces of the Mekong River Delta, Vietnam between January and March 2009. Significant spatio-temporal interaction of disease risk was observed within a distance of 10 km and 12 days following the detected onset of clinical signs. We estimate that the household-to-household infection rate within a commune was approximately 50 times greater than the household-to-household infection rate between communes. Our findings show that the predominant mechanism of infection transfer was local spread. A comparison of disease control procedures and veterinary capacity in communes with relatively high and low infection rates should help to identify procedures essential for effective outbreak management in this area of Vietnam.

Minh, P. Q., Stevenson, M. A., Jewell, C., French, N., Schauer, B. (2011). Spatio-temporal analyses of highly pathogenic avian influenza H5N1 outbreaks in the Mekong River Delta, Vietnam, 2009. *Spatial and Spatio-temporal Epidemiology*, **2**, pp. 49 – 57.

## 7.1 Introduction

HPAI H5N1 is a contagious disease of poultry characterised by high mortality and a wide host range (OIE 2002, Swayne 2008). Since 2003 outbreaks of HPAI have been common in many countries throughout Asia. Although HPAI outbreaks have been brought under control in many countries, eradication has not been achieved and in some areas the disease has become endemic. Control and prevention of the disease requires a proactive approach, particularly in countries where poultry are kept under backyard and free-ranging systems (Tiensin et al. 2005). Despite massive control efforts involving movement restrictions, culling of affected stock and vaccination, outbreaks of HPAI H5N1 continue to occur in Vietnam (DAH 2009a).

Poultry production in Vietnam is characterized by small individually-owned flocks. Poultry movement in Vietnam is complex (compared with other Asian countries) involving large numbers of small holders moving stock to and from traders, neighbors and local markets. Movement patterns are influenced by the type of poultry product being traded and where they originate from. For instance, poultry products (meat and eggs) are mainly sold in local markets in lowland areas, whereas farm-gate sales are characteristic in mid-land and highland areas. Broilers and eggs can be sold to traders at farm gates and local markets (Tung 2006). Bicycles and motorbikes are commonly used to transport poultry and poultry product to and from markets due to the ease with which they can move through narrow and congested road systems. An additional feature, with particular relevance to the epidemiology of HPAI, relates to the use of field running ducks, that is ducks moved from one location to another in the major rice growing areas (particularly in the south of the country) for the purpose of grazing on insects, snails and leftover rice grains. In addition to the accepted transmission mechanisms (local spread from infected flocks and long distance spread via movement of birds) the control of HPAI in Vietnam is complicated by the involvement of field running duck movements (Henning, Henning, Vu, Yulianto & Meers 2009). Selection of disease control measures should rely on a good understanding of the main transmission pathways of HPAI. Surveillance systems should target identification of important local and long-distant transmission pathways for HPAI in this area.

In Vietnam a number of spatial and spatio-temporal analyses of HPAI H5N1 outbreaks

have been conducted to characterize the geographic distribution and quantify the spatial and temporal spread of HPAI H5N1 outbreaks at the commune level (Pfeiffer et al. 2007, Henning, Pfeiffer & Vu 2009, Minh et al. 2009). An inter-commune transmission model was developed by Walker et al. (2010) to quantify the spatial and temporal dynamics of the spread of HPAI H5N1 infection, indicating a substantial decrease in the transmissibility of infection between communes after vaccination. In all previous studies, in the absence of detailed data defining flock location, commune centroids have been used to represent the point location of outbreaks. This represents a simplification of the data that has the potential to obscure some of the more subtle features of disease spread mechanisms.

The aim of this study was to provide insight into the spatio-temporal patterns of HPAI H5N1 outbreaks in the MRD and to quantify between-household transmission characteristics. In contrast to earlier epidemiological studies of HPAI H5N1 in Vietnam, the observational unit for our descriptive analyses was the individual household. Findings from this work should help to better understand the spread mechanisms of HPAI H5N1 in Vietnam.

## **7.2 Materials and methods**

### **7.2.1 Study area and study period**

The geographical area of interest for this study comprised the 337 communes within four provinces (Soc Trang, Hau Giang, Bac Lieu and Ca Mau) in the MRD of Vietnam (Figure 7.1). Poultry were kept by 269,755 households in the study area. The median number of households keeping poultry per commune was 414 (IQR: 199-978 households) (DAH 2009*b*). Between 1 January and 12 March 2009 inclusive, thirty-eight commune-level outbreaks of HPAI H5N1 were recorded in five provinces in the MRD. Of these, thirty-seven commune-level outbreaks (97%) occurred in the four contiguous provinces defined as the study area.

### **7.2.2 Outbreak definition**

In this study, a household-level outbreak was defined as both an index case household and subsequent households where poultry showed clinical signs of HPAI H5N1 during the

outbreak period. In Vietnam, a commune-level outbreak of HPAI H5N1 was defined as a commune that had at least one household with poultry showing clinical signs of disease and laboratory confirmation of HPAI H5N1 using RRT-PCR. The first diagnosed household in a commune was defined as the index case for that commune. Subsequent flocks in the same commune showing typical clinical signs of disease, as prescribed by the Department of Animal Health of Vietnam (DAH 2004), were considered to be HPAI H5N1 infected and culled without laboratory diagnosis. It is thought that more than 90% of samples collected from HPAI suspected poultry in the MRD are subsequently diagnosed as being positive with H5N1 using RRT-PCR (Tran D. Quy, personal communication). A commune was declared free of disease 21 days after the last clinically affected poultry were destroyed (MARD 2007, DAH 2008). The period from the detection of the index household outbreak to the declaration of disease freedom was defined as the outbreak period.

### 7.2.3 Data collection

In Vietnam, the routine HPAI H5N1 surveillance program relies on poultry farmers recognizing sick or dead poultry in a flock and then reporting the details of these events to commune veterinarians. In 2009 a case-control study was conducted to identify risk factors associated with outbreaks of HPAI H5N1 in the MRD. In this study, the index household in each commune, that is the first case ever recorded and diagnosed to be H5N1 positive in the commune, was recruited as the case and controls were defined as households that had poultry not showing clinical signs of disease and tested by RRT-PCR to be H5N1 negative. After the case-control study, commune veterinarians were asked to record all subsequently affected households in their respective communes. Data were gathered on all flocks with typical clinical signs of HPAI H5N1 within the same commune outbreak as the index case in our previous case-control study, allowing us to document entire commune outbreaks. In Vietnam, poultry owners, who have poultry destroyed as a result of HPAI control, are given a compensation of 70% of poultry market value (Anonymous 2008). Coordinates were recorded for each affected household by a veterinarian from the Regional Office of Animal Health No.7. Data gathered from commune veterinarians included the village in which each household was located, the species kept by the household, the number of birds of each species affected, the date on which HPAI signs



in poultry were first observed, the number of poultry destroyed, dates of destruction, and vaccination history details. These details were entered into relational database the regional animal health office. At the time of transfer of details from paper records to the database a series of checks were made to ensure that each data point was biologically plausible. Field veterinary staff were then contacted to provide clarification and corrected details, if necessary. In this study, data on the number of poultry households in each commune were gathered by the Sub-Departments of Animal Health (SDAHs) in the four provinces. The data were provided by the Department of Animal Health of Vietnam (DAH 2009b).

#### 7.2.4 Data analysis

The interval from the date of onset of clinical signs to date of destruction was calculated for each infected household and plotted as a frequency histogram. A lognormal distribution was fitted to these data, allowing us to estimate the distribution mean and standard deviation of this interval. For those infected households without a date of onset of signs recorded (39 of 164), an estimate was obtained by taking a random draw from a lognormal distribution with mean and standard deviation calculated earlier, and subtracting that value from the date of destruction which was present for all case households. Sensitivity of our results to the choice of mean and standard deviation for the lognormal distribution was tested by varying each parameter within a range of biological plausible values and re-running our analyses based on these altered values. Insensitivity of our results to these changes provided reassurance of the robustness of the mean and standard deviation estimates used.

Locations of HPAI infected households with coordinates recorded were plotted using Google Earth (version 5.1.3533.1731) (Google Inc. 2010). Satellite images of outbreak locations were captured at the village level and saved as georeferencing images. These images were then loaded into a Geographic Information System, allowing us to create digital maps of household locations and village boundaries (Gibin et al. 2008). For HPAI infected households with missing coordinates (32 of 164), location details were estimated by selecting random points within the boundaries of the village of interest using the *spatstat* package in R (Baddeley & Turner 2005). The approach here varied depending on the geography of each village. For those villages where the distribution of households was

characteristic of ribbon development (i.e. households located along a major roadway), a point was chosen at random along the appropriate roadway. For villages showing a clustered settlement pattern (i.e. households positioned around a central location) a point was chosen at random within the defined village boundaries.

We used the space-time K-function to describe the spatio-temporal interaction of infection risk. A Bayesian approach was used to quantify the disease transmission rates of HPAI H5N1 within and between communes.

### **Spatio-temporal interaction**

The space-time K-function  $K(s, t)$  was computed to determine the magnitude of spatio-temporal interaction of HPAI H5N1 outbreaks (Diggle et al. 1995). The increase in the number of infected households within specified spatial ( $s$ ) and temporal ( $t$ ) separations of an arbitrarily selected infected household,  $D(s, t)$ , was calculated as the observed cumulative number of infected households minus the expected number of infected households if no space-time interaction was present. The proportional increase in risk attributable to space-time interaction,  $D_0(s, t)$ , was computed as the ratio of  $D(s, t)$  to  $K_S(s)K_T(t)$  where  $K_S(s)$  defined the K-function in space and  $K_T(t)$  the K-function in time. A value of  $D_0(s, t)$  equal to 1 indicates that the risk of HPAI H5N1 was 100% greater than that expected under the assumption that space-time interaction did not exist (Diggle et al. 1995). The significance of the observed values of  $D(s, t)$  was estimated by performing 99 Monte Carlo permutations in which each of 164 outbreaks were re-labeled with the observed 164 event times. A total of 99 estimates of  $D(s, t)$  was obtained and the observed sum of  $D(s, t)$  over all  $s$  and  $t$  was then compared with the empirical frequency distribution of the 99 estimates of  $D(s, t)$ . An extreme value of the observed  $D(s, t)$  compared with this distribution provided evidence of significant space-time interaction. In this study, maximum distance and time separations of 20 kilometres and 15 days were used. These analyses were implemented in the SPLANCS package (Rowlingson & Diggle 2009) within R.

### **Full epidemic model**

A Bayesian SIR approach was used to quantify disease transmission rates of HPAI H5N1 within and between communes. The approach described here is similar to that used in

Jewell, Kypraios, Christley & Roberts (2009), Jewell, Kypraios, Neal & Roberts (2009), and Neal & Roberts (2004) and uses a continuous time inhomogenous Poisson process to model both the time to infection and time from infection to observation of clinical signs.

For disease transmission rate, the basic model assumes that the infectious pressure  $\lambda$  acting on a susceptible household in commune  $j$  at time  $t$  is given by:

$$\lambda_j(t) = \alpha + \beta I_j(t) + \rho \sum_{i \neq j} w_{i \sim j} I_i(t) \quad (7.1)$$

This model represents an epidemic process whereby individuals are homogeneously mixing within communes, but heterogeneously mixing (according to a Markov random field) between communes.  $I_j(t)$  returns the number of infected households at time  $t$  in commune  $j$  and  $I_i(t)$  the number of infected households at time  $t$  in commune  $i$ . The variable  $w_{i \sim j}$  is an indicator taking the value 1 if commune  $i$  borders commune  $j$  and zero otherwise. Parameter  $\beta$  represents the household-to-household infection rate within a commune and  $\rho$  represents the household-to-household infection rate between communes. Finally,  $\alpha$  represents a ‘background’ infection rate common to all individuals in the population.

Results (not shown) from the model presented in Equation 7.1 indicated a large amount of overdispersion in the dataset at the commune level. To account for this increased variability in the spatial distribution of cases relative to the underlying susceptible population, we included a random effect term  $\eta_j$  for the  $j$ th commune representing a measure of commune-level infectivity relative to the population average as estimated by  $\beta$  and  $\rho$ . This gives the following form for the instantaneous infectious pressure:

$$\lambda_j(t) = \alpha + \beta I_j(t) \eta_j + \rho \sum_{i \neq j} w_{i \sim j} I_i(t) \eta_i \quad (7.2)$$

Where

$$\eta \sim \logNormal(0, 1/\omega) \quad (7.3)$$

The parameter  $\omega$  allows for commune-level heterogeneity in disease transmission, noting that  $\exp(0) = 1$ , giving the required unit mean in Equation 7.2.

The latent period (i.e. infection ( $I$ ) to observation ( $O$ ) time, measured in days)  $D$ , such

that  $d_k = O_k - I_k$  for individual household  $k$ , was Gamma distributed with a fixed shape parameter equal to 6 days, and unknown rate  $\gamma$  such that

$$D \sim \text{Gamma}(6, \gamma) \quad (7.4)$$

The value for the shape parameter was given such that the mean infectious period (given the prior mean for the rate parameter — see below) was equal to 6 days, in accordance with Stegeman & Bouma (2004). Prior distributions for  $\alpha, \beta, \rho, \gamma$ , and  $\omega$  were chosen as a Gamma prior because this is a convenient flexible distribution that has support (i.e. ‘allowed values’) greater than or equal to 0.

$$\begin{aligned} \alpha &\sim \text{Gamma}(0.1, 0.1) \\ \beta &\sim \text{Gamma}(0.1, 0.1) \\ \rho &\sim \text{Gamma}(1, 1) \\ \gamma &\sim \text{Gamma}(1, 1) \end{aligned} \quad (7.5)$$

A diagram of the structure of this model, in context of the SIR (susceptible, infected, removed) framework, is shown in Figure 7.2.

Using a custom Markov chain Monte Carlo (MCMC) algorithm written in C++ (see Jewell, Kypraios, Neal & Roberts (2009)) for details) using partial non-centering for both  $\gamma$  and  $\omega$  to improve mixing (Neal & Roberts 2004), the joint posterior distribution for  $\alpha, \beta, \rho, \gamma, 1/\omega$  and  $D$  was estimated. A total of 1,000,000 samples were obtained from the posterior distribution, with the first 5000 discarded as burn-in. Convergence was assessed visually, and autocorrelation was found to be low after thinning every 200 iterations (Kypraios 2007). An analysis of prior sensitivity was performed, varying both the prior means and variances.

To investigate commune-level epidemic risk, and quantify model fit, the Bayesian predictive distribution of the epidemic was used to study likely outbreak scenarios given the observed data (Bernardo & Smith 1994). We use established continuous time stochastic simulation methodology using the epidemic model as specified above, where each simulation was run using a sample from the joint posterior density (Gillespie 1976). The resulting collection of simulation results thus represents a distribution over the possible

epidemic outcomes, given our observation of the original epidemic.

## 7.3 Results

Dates of onset of clinical signs of disease were recorded for 76% (125 of 164) of household outbreaks. Dates of destruction were recorded for 98% (161 of 164) of outbreaks. The duration from first detection of clinical signs to destruction was available for 122 outbreaks. Median duration was 2 days (IQR 1 – 4 days). Coordinates were provided for 81% (132 of 164) of affected households.

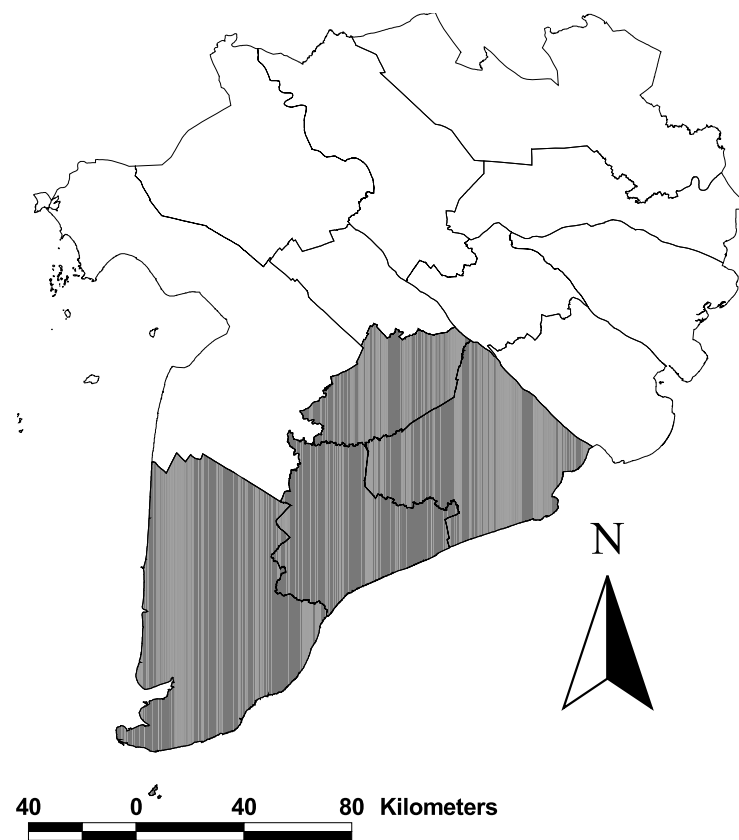
### 7.3.1 Spatio-temporal interaction

Spatio-temporal interaction between outbreaks was present in the study area (Figure 7.3).  $D_0$  ranged from 0.07 to 4.38. In Figure 7.3 values of  $D_0(s, t) > 1.0$  show the distance and time separations from an arbitrarily-selected outbreak where there was a 100% increase in HPAI H5N1 risk attributable to space-time interaction. The highest space-time interaction was observed within a distance of 7 kilometres and 3 days following the detected onset of clinical signs. The P-value computed on the basis of the 99 Monte Carlo permutations was 0.01.

### 7.3.2 Full epidemic model

Figure 7.4 shows the probability density function of posterior values of transmission parameters. The results show that the household-to-household infection rate within the commune ( $\beta$ ) was approximately 50 times higher than the household-to-household infection rate between communes ( $\rho$ ). The posterior mean of household-to-household infection rate was  $5.44 \times 10^{-5}$  infections per individual per day (95% highest posterior density region [HPD]  $3.14 \times 10^{-5}$ ,  $8.33 \times 10^{-5}$ ) for households in the same commune and  $1.07 \times 10^{-6}$  infections per individual per day (95% HPD  $2.69 \times 10^{-7}$ ,  $2.09 \times 10^{-6}$ ) for households in different communes. The posterior median value was  $1.37 \times 10^{-6}$  infections per individual per day (95% HPD  $9.14 \times 10^{-7}$ ,  $1.95 \times 10^{-6}$ ) for the background infection rate ( $\alpha$ ), 1.76 (95% HPD 0.47, 2.66) for the variance of commune-level random effect ( $1/\omega$ ), and

2.33 (95% HPD 1.59, 3.21) for the scale parameter of ( $\gamma$ ) of the Gamma distribution of the latent period. In this study area, findings from the model show that the distribution of time from infection to observation of clinical signs ( $\overline{O - I}$ ) ranged from 1.5 to 4 days. The model was found to be insensitive for all reasonably weak specifications of the priors. Figure 7.5 shows the mean of the Bayesian predictive distribution for commune-level epidemic size, conditional on the epidemic starting in commune 3 (the supposed origin of the epidemic in our dataset). This serves both as a predictive measure of risk should another epidemic occur, and also as a check for model fit. In Figure 7.6 we plot the posterior mean of the commune-level random effect, showing several apparent outliers at the commune level.

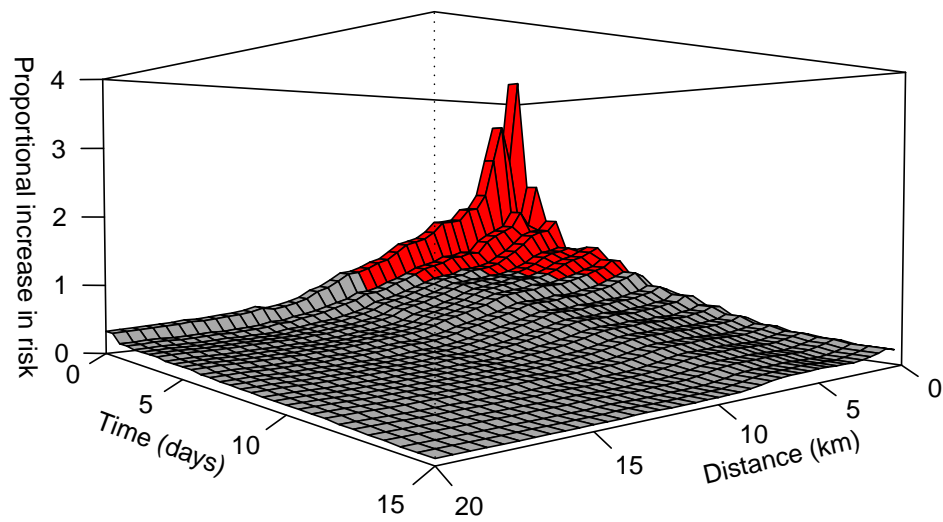


**Figure 7.1:** Map showing boundaries of provinces ( $n = 13$ ) in the Mekong River Delta and locations of the four studied provinces (shaded).

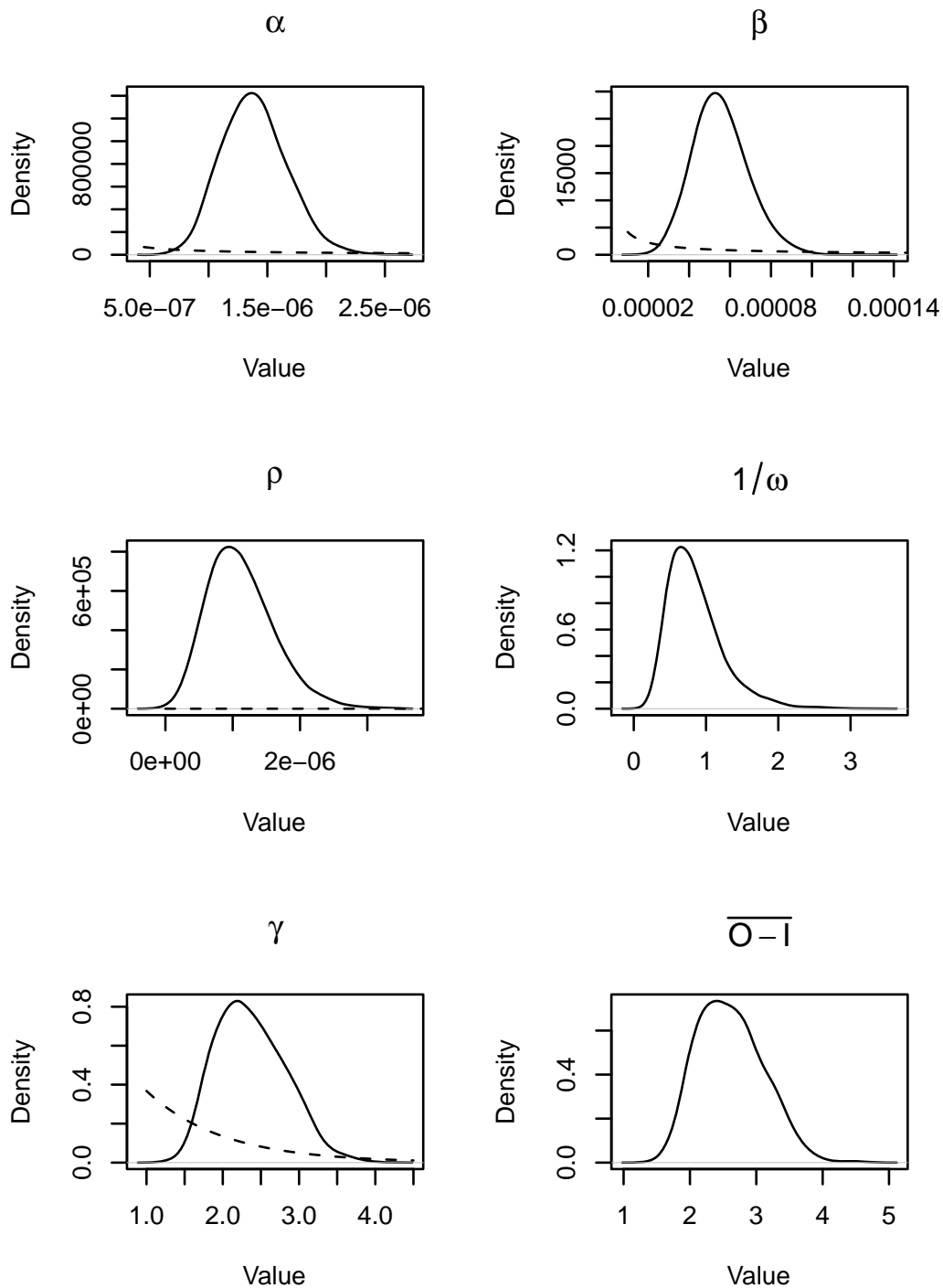


**Figure 7.2:** Diagram showing the structure of the SIR model with specific states (susceptible, infected, removed) and transmission parameters.

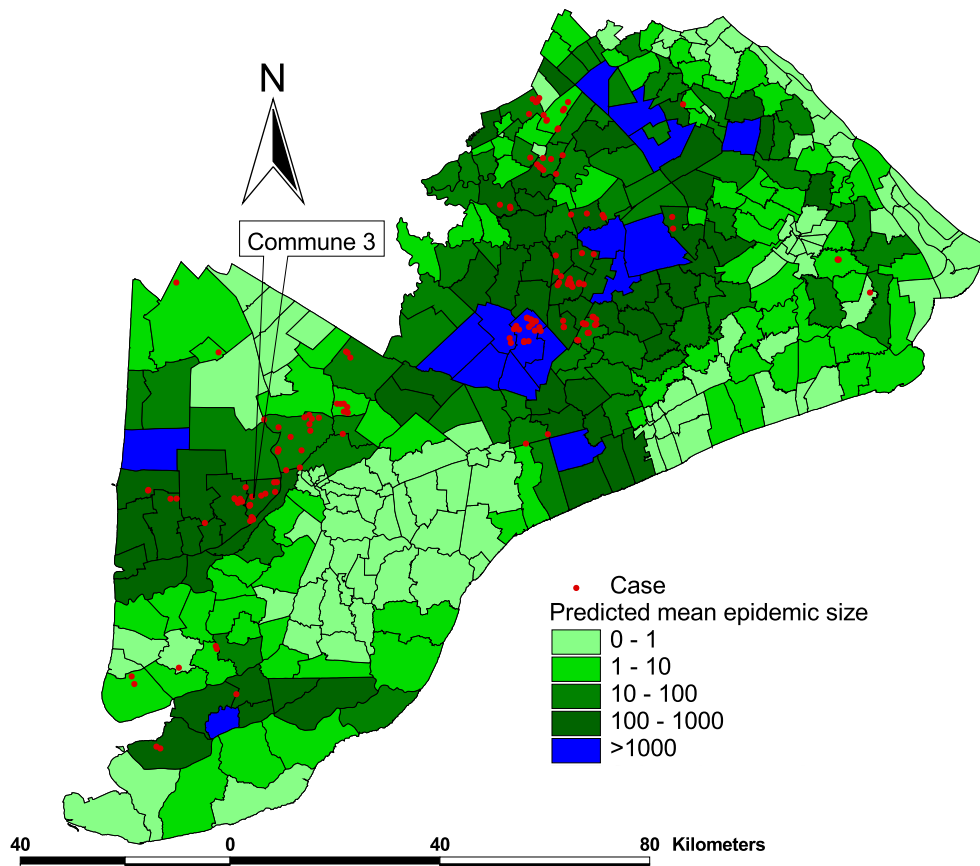




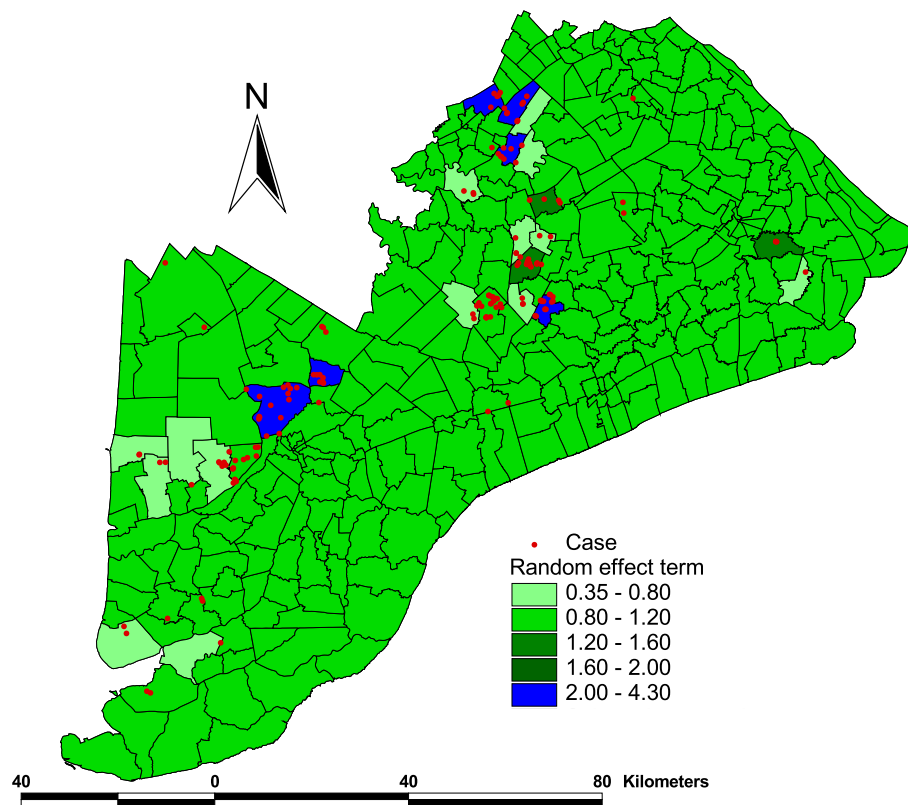
**Figure 7.3:** Spatio-temporal interaction of HPAI H5N1 risk among household-level outbreaks ( $n = 164$ ) in four provinces of the Mekong River Delta, Vietnam between 1 January and 12 March 2009. The red shaded area shows the distance-time separations where the proportional increase in risk attributable to space-time interaction was greater than one.



**Figure 7.4:** Graphs showing the probability density functions of the background rate of infection ( $\alpha$ ), the household-to-household infection rate within the commune ( $\beta$ ), the household-to-household infection rate between communes ( $\rho$ ), the commune-level random effect ( $1/\omega$ ), the rate parameter of the Gamma distribution of the time from infection to observation of the clinical signs ( $\gamma$ ), and the time from infection to observation of the clinical signs ( $\overline{O-I}$ ). Dotted lines indicate the prior distributions for the parameters. A value of  $\beta = 0.00005$  means that the rate of household-to-household infection transmission within the commune was 0.00005 infections per individual per day.



**Figure 7.5:** Spatio-temporal analyses of HPAI H5N1 outbreaks in the Mekong River Delta, Vietnam, January to March 2009. Map of the study area showing: (1) locations of HPAI H5N1-infected households ( $n = 164$ ), and (2) the mean predicted epidemic size in each commune ( $n = 337$ ). Boundaries of communes are shown as black lines. The estimated origin of the epidemic (commune 3) is indicated.



**Figure 7.6:** Spatio-temporal analyses of HPAI H5N1 outbreaks in the Mekong River Delta, Vietnam, January to March 2009. Map showing: (1) locations of HPAI H5N1-infected households ( $n = 164$ ), and (2) the posterior mean of the commune level random effects. The random effect allows spatial heterogeneity in infectivity, thus partially accounting for commune-level overdispersion in the data. Boundaries of communes are shown as black lines.

## 7.4 Discussion

The work presented in this paper aims to provide a concise overview of the course of HPAI H5N1 outbreaks in the MRD of Vietnam in early 2009. To the best of our knowledge this has been the first study in Vietnam to identify and quantify the level of spatio-temporal interaction and transmission characteristics of HPAI H5N1 outbreaks at the individual household level. Our analyses provide strong evidence of spatio-temporal interaction of infection (Figure 7.3) and strong indication that the household-to-household infection rate was much higher if poultry households were in the same commune, compared with households in different communes. These findings here have partly addressed a hypothesis generated in our previous study of HPAI transmission in the MRD, that disease transmission arises from a combination of local and long-distance spread (Minh et al. 2009) and, based on this set of outbreak data at least, that local spread is the more dominant of the two spread mechanisms.

Figure 7.3 shows that for households infected between 1 January and 12 March 2009 the risk contour of greater than 100% persisted at 7 km for 3 days after the detected onset of clinical signs, and then declined to 2 km by 5 days. The risk contour of greater than 10% persisted at 10 km for 12 days after onset of clinical signs date. These findings are useful from a disease control perspective in that they provide an indication of the length of time after onset of clinical signs that an infected household poses a risk to others. It is important that infected poultry can be destroyed quickly after the onset of clinical signs in order to reduce the disease transmission to a level where the epidemic cannot 'take off'. These findings stress the need to re-iterate training and awareness programs for farmers and animal health workers so that clinical signs are detected promptly once flocks become infected.

Our analyses indicate that the distance range over which an infected household poses a risk to others is up to 10 km. It is important to point out that this was an epidemic that was tightly controlled, so these distance and time ranges are likely to be smaller than for epidemics where the intensity of control measures is less. This may explain why local spread predominated during this period. Households with poultry that were culled may or may not have been infectious at the time of culling. It should be noted that the space-time K-function used the date of onset of clinical signs and did not take into ac-

count the incubation period of HPAI which is 2 to 5 days following the date of infection (OIE 2002). Because the destruction of poultry in detected households was carried out relatively quickly during this epidemic (median 2 days after detection) we speculate that a major factor contributing to disease spread was the presence of households where disease was either undetected or delayed. The number of infected households in the study area is likely to be greater than the number of detected households as the latter depends on stock owners recognizing the presence of clinical signs and reporting their findings to authorities. In our recent case-control study in the MRD, ten *percent* of control flocks did not show clinical signs of disease but subsequently tested positive for HPAI H5N1 using RRT-PCR (Minh, Stevenson, Morris & Schauer 2010). Failure to detect or delayed detection of disease in households in close proximity to detected households was a likely explanation for the length of time that detected households posed a risk to others. We propose that future outbreak investigations should apply active surveillance at specified space-time separations from identified disease-positive households, increasing the probability of detecting outbreaks that may not have come to the attention to animal health authorities through passive surveillance. It should be noted that the K-function analysis is purely descriptive, providing a summary estimate of the spatio-temporal interaction of infection across the entire study area. The full epidemic model provides the opportunity to investigate these issues in further detail, allowing us to identify specific locations within the study area where spatio-temporal interaction was greater than others.

The full epidemic model provides evidence that, in this study, household-to-household infection rate within the commune was approximately 50 times greater than the household-to-household infection rate between communes. These results support our findings from the case-control study of risk factors for HPAI H5N1 in the MRD, that HPAI H5N1 risk was predominantly driven by characteristics operating at the individual household level. Given the contagiousness of HPAI, the disease status of a household at a particular point in time depends on the status of nearby households. The strength of the full epidemic modelling approach applied here is that each household was not only considered to be dependent on its within-commune neighbors, but also on those in neighboring communes. In other words, the model has taken into account the localized clustering of infection risk observed in the data (Figure 7.5). It is noted, however, that these results are conditional on a discrete space model chosen to reflect the spatially aggregated susceptible popula-

tion data. Commune boundaries, of course, present no barrier to disease, and a preferable data set would therefore contain the precise locations of all susceptible households. Our findings provide additional evidence to indicate that disease control strategies in this area of Vietnam should emphasise the reduction of household-to-household transmission risk on a local scale when a HPAI H5N1 outbreak is detected. Although the background transmission parameter ( $\alpha$ ) is low, it should be noted that this is a per-household background rate of infection. Multiplying this by the total population of 269,755 households over the 10 week period yields a posterior median of 26 households infected by this background process. The background rate of infection accounts for infections that occur due to mechanisms that are not explicitly modeled by the SIR parameterisation. It may therefore indicate the presence of disease transmission due to long-range movement of poultry, as well as possible infection from undetected sources (e.g. wildlife or undisclosed infections).

Based on the full epidemic model, the time from infection to observation of clinical signs was around 2 to 3 days (Figure 7.4). Because the infection to observation time was not recorded directly during this outbreak, we took our prior mean infectious period to be 6 days, covering the time before the observation of clinical signs but during which poultry had been infected. The posterior distribution of infection to observation time ranged from 1.5 to 4 days, indicating that the observed epidemic data updated our prior distribution for infection to observation time. The full epidemic model assumes that once infected, a household immediately becomes maximally infectious which is not entirely biologically plausible. The inferred infectious period therefore represents a compromise between the true infection time and the time at which the household becomes infectious. A natural extension to this model would be an SEIR model which allows for an ‘exposed’ (i.e. infected but not yet infectious) state before progression to fully ‘infectious’. However, with current statistical methodology, this would require an extra assumption about the length of the exposed state. A benefit of this analysis is that the posteriors obtained here can be used as priors for analysis of the next outbreak. Unfortunately, it was not possible to assess the contribution of individual control measures reducing overall virus transmission from HPAI H5N1 affected households to unaffected households in this study. However, it is likely that the rapid destruction of HPAI H5N1 affected flocks played an important role in containing outbreaks in the MRD during the study period.

Figures 7.5 and 7.6 show the mean of the Bayesian predictive distribution for commune-

level epidemic size and the posterior mean of the commune-level random effect. The two largest sources of heterogeneity in disease risk are likely to be the within-commune population distribution, as well as the number of undetected cases. Given the assumption that a particular disease is infectious, it is important to know the size of the population at risk. Moreover, the accuracy of spatial disease risk measurement depends on knowing the spatial relationship of the infected to susceptible populations. This, therefore, highlights the need for careful governmental record keeping to support national livestock industries. In this study, data on HPAI H5N1 vaccination coverage were not available. For modelling purposes, we assumed all poultry households were susceptible to HPAI H5N1 during the epidemic. In the presence of vaccination, the effective size of the susceptible population is reduced; we therefore expect a downward bias on our transmission parameter estimates, since a large susceptible population requires less infectious pressure to observe the a given number of infections. In addition, the presence of underreporting might reduce the apparent number of infected households compared to what is expected from the model. Figures 7.5 and 7.6 are valuable because they identify communes that might have experienced high disease risk during the January – March 2009 MRD epidemic, and show several apparent outliers at the commune level. A useful thing to do would be to gather details of disease surveillance procedures and veterinary capacity in these communes, and compare them with those communes where the level of disease risk and estimated random effect terms from the model were somewhat less. This process should help to identify procedures essential for effective outbreak recognition and management.

Our findings were based on outbreak records obtained from commune veterinarians. Apart from the laboratory confirmation of HPAI H5N1 virus in the index case of each commune-level outbreak, the presence of subsequent cases was based on the observation of clinical signs of disease reported by poultry owners and paraveterinarians. These cases were not confirmed by RRT-PCR, but destroyed on suspicion of H5N1, so their true disease status was less well defined. Consideration should also be given to the sensitivity and specificity of H5N1 surveillance in Vietnam. It is difficult to assess the accuracy of passive surveillance for HPAI, or indeed any infectious disease of livestock. Poultry owners may be reluctant to report the presence of disease or may not be aware of the clinical signs of HPAI, leading to underreporting of cases. On the other hand, awareness activities conducted during the outbreak period may prompt poultry owners and field staff to identify



more suspects resulting in over reporting of cases. In addition, the prescribed compensation policy may have influenced the willingness of poultry owners to report the presence of clinical signs to veterinary authorities. This may depend on the satisfaction level of poultry owners with the amount of compensation provided. Missing information about detailed geographical location of infected households and date of onset of clinical signs were estimated for a proportion of the data (19% and 24%, respectively), and this issue may also have influenced our results. In this study, spatial data were only recorded for HPAI infected households. It would be extremely useful for future analyses if the spatial data are also available for all susceptible households.

## 7.5 Conclusions

Our analyses provide evidence of spatio-temporal interaction of HPAI H5N1 outbreaks at the household level, indicative of the contagiousness nature of this disease. The household-to-household infection rate was much greater within communes than between communes. These findings indicate that the predominant transmission mechanism for HPAI H5N1 in this study area was local (short distance) spread. We infer that an important factor driving local spread is failure to detect HPAI H5N1 infected households. Active surveillance should be applied in future outbreaks to increase the probability of detecting affected households that may not have come to the attention to animal health authorities through passive surveillance. Comparisons of disease control procedures and veterinary capacity in communes where the level of disease risk and the commune-level random effect terms differ should help to better understand the relative importance of individual procedures for effective outbreak recognition and management in this area of Vietnam.



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## General discussion

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Presented in this thesis are a series of epidemiologic studies of highly pathogenic avian influenza H5N1 in Vietnam. The studies have utilised data from the national surveillance system operational in Vietnam since 2003 and several field studies carried out in the Mekong River Delta in 2008 and 2009. The focus of the analyses presented in each study extends from the national to the local level. This ‘drill down’ strategy is a necessary approach for investigating complex animal health problems. While the analyses at the national level describe the overall situation of HPAI H5N1 outbreaks, findings at a higher level of spatial resolution identify specific patterns and differences in disease transmission that are specific to localised areas. This approach recognises that disease control options need to be adapted to respect the different poultry production systems, distributions of the population at risk, and movement of animals those exist throughout the country.

The studies presented in this thesis have increased our knowledge of the epidemiology of HPAI H5N1, the effectiveness of surveillance activities to detect disease and the advantages and disadvantages of the use of routine surveillance data as a means for assessing the true disease situation. Although the focus of this thesis has been on HPAI H5N1, it should be stressed that the epidemiological principles that underlie the analytical approaches are applicable to most, if not all, infectious diseases of livestock. In this respect, the lessons learnt here should assist Vietnamese animal health authorities to implement the necessary systems and infrastructure that will allow novel and emerging disease syndromes that are likely to emerge in future to be investigated promptly and efficiently.

The first two studies (Chapters 3 and 4) present investigations of the spatio-temporal patterns of HPAI H5N1 outbreaks at both the national and regional level using data routinely recorded by the Vietnamese Department of Animal Health. Chapter 3 examined the

spatio-temporal association between poultry outbreaks and human cases of HPAI H5N1 at the national level. This chapter provided information important for understanding determinants of disease at both the human and animal level. It also provided justification for promoting the improvement of HPAI surveillance in poultry since doing so would be likely to provide a benefit in terms of reducing the burden of disease in both animals and humans. Contrary to the expected biological transmission pathway, poultry outbreaks in the same district were typically reported one week or four weeks after the occurrence of a human case. When jointly considering poultry outbreaks in the same and neighbouring districts, the occurrence of poultry outbreaks was reported in the same week, one week later, and four weeks later. Although humans were identified as a sentinel for disease in poultry in this study, our findings showed that poultry outbreaks were identified close in space and time to human cases, a finding that provides evidence (albeit limited) that the routine disease recording systems in place in Vietnam provide a usable indicator of the true occurrence of disease in poultry. The conclusions from Chapter 3 were that surveillance should be improved so that HPAI H5N1 infections in poultry could be detected early enough to prevent virus from infecting humans in close proximity.

Chapter 4 described some of the temporal and spatial characteristics of HPAI outbreaks at the local level. The findings here indicate that the epidemiology of HPAI H5N1 in Vietnam had changed from 2003 to 2007 with greater proportion of ducks involved in later outbreaks. At the time of writing (August 2010) infection is still widespread in Vietnam, so these analyses provide useful insight into the course of the disease and the efficacy of control measures that have been applied in the recent past. The spread of HPAI H5N1 can take place over short distances between adjacent or nearby communes which typically results in local clustering of outbreaks. In addition, disease can spread over longer distances presumed to be the result of poultry movement from one location to another. If future studies can determine the relative contribution of factors influencing local versus long-distance spread under field conditions it would allow animal health authorities to fine tune control strategies. For example, if long-distance spread was found to be the most important driver of disease spread the emphasis of control activities could focus on the enforcement of movement restrictions in the event of an outbreak. On the other hand, if local spread were found to be important, outbreak responses should emphasise rapid identification and stamping out of infected places in preference to movement controls.

Chapters 5 and 6 presented two field studies conducted in the Mekong River Delta of Vietnam. Chapter 5 described the management of field running duck flocks in two provinces. The findings from this survey provide insights that add to the limited body of knowledge on free-range ducks in this area of Vietnam. Larger flocks and layer flocks were more likely to be moved over large distances and therefore likely be important in facilitating long-distance spread of HPAI H5N1 infection. This survey described the role that field running ducks may potentially play, however it cannot give an affirmative statement on the role they play in spreading HPAI H5N1. Nevertheless, the findings have provided essential information that would inform the design of a longitudinal study of the circulation of AI viruses in field running duck flocks and in-contact poultry in the Mekong. Chapter 6 presented the findings obtained from a case-control study on risk factors associated with HPAI H5N1 outbreaks in the Mekong. Larger poultry flocks, field running ducks, the absence of vaccination and an absence of visits by veterinarians in the month prior to the outbreak were identified as risk factors for disease. These findings make biological sense and were consistent with other studies presented in this thesis and also the work of others (e.g. Henning et al. 2009, Otte et al. 2008). This is also a useful study in that it identifies characteristics that are eminently suitable for targeting interventions. For example, analyses from the case-control study identified no risk factors for HPAI H5N1 at the village level. The inference here is that disease control programmes for HPAI H5N1 should emphasise the reduction of household-level, rather than village-level, risks for disease.

Chapter 7 quantified and characterised transmission patterns of HPAI H5N1 outbreaks in the Mekong River Delta during 2009 using Bayesian techniques. In this study we quantified the level of spatio-temporal interaction of disease risk at the localised level and disease transmission rates of HPAI H5N1 outbreaks at the household level. A key finding from this study was that household-to-household infection rate within communes was 50 times greater than the household-to-household infection rate between communes. The findings presented here suggest that failure to detect or delayed detection of disease in the vicinity of an infected household is a likely explanation for the length of time detected households pose a risk to others. Active surveillance should be carried out in future outbreaks to increase the probability of detecting outbreaks that may not have come to the attention of animal health authorities through passive surveillance.

## 8.1 The use of routine surveillance data

This thesis presents examples of how routine surveillance data can be used for epidemiological analyses. Information obtained from routine surveillance data can be used to direct further epidemiological studies (Thrusfield 2005). One of the main problems in undertaking epidemiological studies in animal populations is that data recorded by routine surveillance system are generally only available for cases, with limited or no information being available on the population at risk (Frerichs 1991, Stevenson et al. 2007). While data on the population at risk allows one to estimate disease incidence and undertake further in-depth epidemiological analyses, analysis of case data alone can provide useful insight, as demonstrated in Chapters 3, 4, and 7 of this thesis.

During the HPAI H5N1 epidemic the Vietnamese surveillance system implemented by the Department of Animal Health has recorded details of outbreaks at the commune, and recently household level. At the beginning of this study, there had been only one published paper analysing routinely recorded outbreak data of HPAI H5N1 in Vietnam. This paper described the spatial and temporal pattern of commune-level outbreaks of HPAI H5N1 in the country during 2004 – 2005 (Pfeiffer et al. 2007). This thesis presented a series of epidemiological studies utilising data from the national surveillance system operational in Vietnam from 2003 to 2009. Outbreak data have been recorded at both commune and household levels. The analyses presented here demonstrate examples of the use of routine surveillance data to better understand of HPAI H5N1 epidemiology and the current surveillance system, providing an opportunity to inform disease surveillance strategies. Routine surveillance data on both human cases and poultry outbreaks of HPAI H5N1 were analysed to examine to what extent passive surveillance of clinical disease in poultry captures the risk of human exposure to H5N1 virus (Chapter 3). In addition, Chapter 4 focused on the two river deltas, which were previously identified as high-risk areas during the initial epidemics. A problem arises when using routine surveillance data is that, in some circumstances, it is difficult to determine whether a date recorded reflected date of first clinical signs of disease, date of farmer reporting, date of investigation, or date of laboratory confirmation. In addition, data were often in aggregated forms (at the commune level), limiting the ability to carry out more detailed epidemiological analysis of factors influencing disease spread over short distance scales. Lack of standardised

forms to report case event details also created difficulties. To ensure that high quality data are collected in future, it is important that steps are taken to standardise recording systems so that outbreak event details are recorded consistently and accurately throughout the country, as demonstrated in Chapter 7.

Routine analysis of surveillance data and details from identified outbreaks should be able to demonstrate important changes in the HPAI H5N1 situation. If this information is promptly fed back to field personnel it should inform and then enhance disease control strategies. The reason for analysing outbreak data retrospectively is to explain the temporal and spatial patterns of disease. While descriptive spatial analyses are relatively straightforward to carry out, complex multivariate analyses (which have the potential to provide a more in-depth understanding of factors influencing disease spread) can be problematic because of inconsistencies in both the accuracy and precision of explanatory and outcome variables. Errors affecting the accuracy of accumulated data, particularly errors that vary on a geographical basis, can produce biased results and the magnitude and direction of this bias from the true situation can be difficult to predict. In the absence of perfect data, routine surveillance and outbreak event data in conjunction with details derived from other surveillance activities necessarily has to be used to assist decision making.

By analysing data gathered from national sources and field studies, the findings presented in this thesis can be used to support the optimisation of the current surveillance system in Vietnam and to enhance the robustness of the evidence on which control decision can be made. These analyses can be applied to other diseases such as foot-and-mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS), and classical swine fever (CSF) that have occurred in Vietnam in recent years.

## 8.2 Epidemiology of HPAI

Our studies have shown that the epidemiology of HPAI H5N1 in Vietnam is undergoing change. Ducks have played a large role in recent outbreaks of HPAI, indicating that ducks play an important role in the transmission and maintenance of H5N1 in this country. The concern is greatest in rural areas like the Mekong River Delta where traditional free-ranging ducks, chickens and wildlife freely mingle, frequently sharing the same source of water. It was of public health concern that ducks might be infected and shed virus for

long periods (Chen et al. 2006). Therefore the role of domestic ducks in the transmission cycle of H5N1 needs to be addressed as it might complicate efforts to control the disease in poultry and prevent human cases. While there was a strong, consistent seasonality of HPAI H5N1 outbreaks in the Mekong River Delta between December and March, the temporal pattern has changed over time in the Red River Delta with outbreaks occurring during both winter and summer months. Although it is uncertain to what extent factors such as temperature, humidity, and poultry density may influence the epidemiology of HPAI H5N1 in Vietnam, the seasonal appearance of HPAI H5N1 outbreaks in poultry needs to be investigated further since this may help to explain the mechanism for disease emergence (Park & Glass 2007). It is likely that outbreak patterns are influenced by intervention activities which were successful in reducing local spread of HPAI H5N1 infection in both deltas of Vietnam during 2005 – 2007 epidemics.

### **8.3 Surveillance strategies**

A surveillance system will be effective only if the quality of data is high and information is processed and analysed rapidly (Frerichs 1991). Effective surveillance systems permit a prompt diagnosis and response to HPAI outbreaks. Data collected from surveillance activities can be used to improve and modify disease control programmes. Over the many years following the first HPAI H5N1 epidemic in Vietnam in late 2003, surveillance programmes have mainly focused on disease reporting rather than targeted surveillance. Targeted surveillance is difficult to achieve because poultry production in Vietnam is characterised by backyard systems, which results in many steps from disease occurrence in a village to laboratory confirmation and disease notification. Following disease confirmation, disease control managers at the provincial, regional and national levels are notified. When outbreaks affect village poultry, reporting may be substantially delayed and the disease may have already spread widely before effective control measures can be implemented. The success of surveillance and reporting of HPAI depends on stakeholders' involvement and cooperation, and efficient networks being established within communities.

The analyses presented in this thesis indicate that it is essential to move away from simple case-reporting by poultry owners to case-finding, moving towards a structured surveil-



lance system which focuses on quantifying transmission pathways and risk factors, and implementing control programmes flexible for specific conditions in different areas and time periods. The findings from Chapter 3 show that humans were a sentinel for the disease caused by HPAI H5N1 during 2003 – 2007, implying that surveillance should be improved to early detect HPAI infections in poultry. It has been demonstrated in the case-control study (Chapter 6) that where case-finding surveillance is implemented, it yields substantially higher numbers of infections than those detected by routine surveillance. This indicates that levels of detection under the current surveillance system substantially underestimate the true level of HPAI H5N1 infection in the Mekong River Delta. The best prospect for controlling current HPAI H5N1 outbreaks in Vietnam should be based on a risk-based surveillance approach to identify areas at higher risk and how transmission is occurring. Control measures then should be adopted to reduce risky processes and break transmission pathways. If the intensity of case-finding surveillance is less than optimal, data analyses can still be processed. However, the ‘fine detail’ of outbreaks may be missed out, and this will slow the progress towards identifying universally effective preventive strategies.

In order to improve the routine surveillance of HPAI in Vietnam, it is important to have a clear case definition to facilitate recognition of HPAI suspected cases. Local staff should be aware of how surveillance data are used and provided with feedback. Aggregated data need to be analysed, interpreted and reported frequently. Outbreak investigations should be conducted to gather detailed outbreak information and to find secondary cases once an index case has been identified. Outbreak data should be detailed at the flock level allowing more in-depth analyses to be conducted, as demonstrated in Chapter 7. It is recommended that collection and presentation of routine surveillance data should be harmonised throughout the country.

## 8.4 Disease control and prevention

HPAI control strategies in Vietnam have shown that successful control requires an integrated approach and requires regular adjustment of prescribed interventions (FAO 2007*b*). During the early epidemic of HPAI H5N1 in 2004, massive culling was effective in reducing the size of the epidemic but could not eradicate disease. Once the virus was persistent

throughout the country, culling alone was unlikely to succeed, and new integrated approaches had to be adopted. Vaccination has appeared to be beneficial in reducing the virus load in the environment, shortening outbreaks and preventing new outbreaks. The effect of biosecurity and other control measures such as restrictions on animal movements and live bird markets, and the ban on the hatching of waterfowl are difficult to access separately. In areas like the Mekong River Delta where poultry productions are characterised by backyard and free-range systems with low levels of biosecurity, a combination of control measures should be applied to minimise the dissemination of HPAI H5N1 infection among neighbouring communes. This includes vaccination, enhanced biosecurity measures, movement controls, rapid destruction of affected flocks, and closure of live bird markets (FAO 2004a, Morris & Jackson 2005). Risk-based surveillance systems should be applied to identify important transmission pathways for HPAI H5N1, leading to integrated programmes for disease control and prevention in this area. In addition, changes in some traditional farming practices (e.g. field running ducks) are needed to decrease the incidence of disease, especially in rural areas. Such changes require careful evaluation with respect to social, economic, and policy aspects.

## 8.5 Conclusion

This thesis has presented a range of analytical approaches for analysing routine surveillance and outbreak data, providing insight into the epidemiology of HPAI H5N1 in Vietnam. In summary, our recommendations for improving surveillance and outbreak response can be listed as follows:

- Recording and reporting protocols need to be standardised within and between regional animal health offices throughout the country (Chapter 2).
- A case-finding, rather than a case-reporting approach for HPAI H5N1 needs to be adopted. Specifically this means that once a primary case household has been detected in an area, poultry owning households in the immediate vicinity need to be pro-actively visited to detect disease promptly (Chapters 6 and 7).
- Every effort should be made to collect and maintain detailed information on the

domestic animal population at risk. Typically this would require a detailed census of livestock to be conducted at least every five years.

- Variables known to influence the occurrence of disease (i.e. risk factors such as climatic zone, land use, and animal movement patterns) need to be recorded with uniform precision across the country. This will allow multivariate models to provide more useful information for decision makers since estimation of disease risk will be more consistently estimated across regions. Ultimately this will enhance resource allocation since high and low risk areas will be identified with greater certainty.

This thesis has demonstrated that the application of spatial, temporal, and spatio-temporal analyses for routine surveillance data can improve the understanding of the epidemiology of HPAI H5N1 in Vietnam. Detailed investigations need to be routinely conducted following future outbreaks so that detailed information is available for further analytical studies. This will mean that the techniques presented here can be more readily applied to allow timely and effective decision making during infectious disease outbreaks.



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**APPENDIX A**

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**Appendix 1**

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# Questionnaire on poultry management in the Mekong River Delta

(For village headmen)

Date of interview \_\_\_\_\_

Interviewer ID \_\_\_\_\_

Village ID \_\_\_\_\_

Good morning/afternoon/evening. I would like to ask you some questions about your village. I am ....., a veterinary staff member of district..... I am here to ask you about poultry production in your village, especially about field running ducks. The purpose of this interview is to study poultry practice and avian influenza control and prevention. We would appreciate if you could spend few minutes to take part in the interview. If at any time during the interview you are not clear about the question, be sure to ask me.

## I. General information

1. How old are you? (*Tick one*)

< 20 years    20 to 29 years    30 to 39 years    40 to 49 years     $\geq$  50 years

2. Are you raising poultry yourself?

Yes    No

3. What is the total size of the area your village covers (including residential areas, rice fields, etc.)?

\_\_\_\_\_ m<sup>2</sup> or \_\_\_\_\_ ha

Not sure (*Estimated by interviewer: \_\_\_\_\_ m<sup>2</sup> or \_\_\_\_\_ ha*)

4. What is the percentage of the total village area used for rice production?

\_\_\_\_\_ %    None → Skip to question 7    Not sure

5. Please list the periods when people in your village grow rice (months from seed to harvest in lunar calendar):

Cycle 1: from \_\_\_\_\_ to \_\_\_\_\_    Not sure

Cycle 2: from \_\_\_\_\_ to \_\_\_\_\_    Not sure

Cycle 3: from \_\_\_\_\_ to \_\_\_\_\_    Not sure

6. Do people in your village use rice after growth for ducks:

Yes    No

If yes, please indicate period people use rice after growth for ducks

After cycle 1    After cycle 2    After cycle 3    Other (specify) \_\_\_\_\_    Not sure

7. Please estimate the percentage of the total village area that is covered by water during rainy season, please indicate rainy season (as months lunar calendar)

\_\_\_\_\_ %      From (month) \_\_\_\_\_ to (month) \_\_\_\_\_       Not sure

8. How many households are in your village?

\_\_\_\_\_       Not sure

9. Which ethnic groups are there in your village? Please estimate the percentage of these groups.

Kinh: \_\_\_\_\_%    Khmer: \_\_\_\_\_%    Other (specify): \_\_\_\_\_

10. What is the distance from your village's centre to the nearest market? (*Tick one*)

in the village      \_\_\_\_\_ km       Not sure

11. What is the distance to the nearest inter-commune road? (*Tick one*)

in the village      \_\_\_\_\_ km       Not sure

**II. Poultry production**

12. Please estimate the number of householders keeping poultry in your village?

\_\_\_\_\_ or \_\_\_\_\_%       Not sure

13. Please estimate the total number of chickens, ducks and muscovy in your village?

Chicken: \_\_\_\_\_       Not sure

Duck: \_\_\_\_\_       Not sure

Muscovy: \_\_\_\_\_       Not sure

14. Please indicate which of the following poultry species raised in your village? (*Tick one or more*)

Geese     Fighting cock     Quail     Pigeon     Pet bird

15. Where do people in your village often purchase breeding poultry? Select one or more categories per column and rank them according to 1 = most frequent, 2 = second most frequent, etc.

Selection	Rank
<input type="radio"/> The same village	_____
<input type="radio"/> Other villages in your province	_____
<input type="radio"/> Other provinces	_____
<input type="radio"/> Middlemen	_____
<input type="radio"/> Other (specify) _____	_____
<input type="radio"/> Not sure	_____



16. Where do people in your village often sell live poultry? Select one or more categories per column and rank them according to 1 = most frequent, 2 = second most frequent, etc.

Selection	Rank
<input type="radio"/> The same village	_____
<input type="radio"/> Other villages in your province	_____
<input type="radio"/> Other provinces	_____
<input type="radio"/> Middlemen	_____
<input type="radio"/> Other (specify) _____	_____
<input type="radio"/> Not sure	_____

17. What kind of transport do people in your village use to move poultry? Select one or more of the following categories. Select one or more categories per column and rank them according to 1 = most frequent, 2 = second most frequent, etc.

Selection	Rank
<input type="radio"/> truck	_____
<input type="radio"/> Ship/boat	_____
<input type="radio"/> Motorbike/bicycle	_____
<input type="radio"/> Foot	_____
<input type="radio"/> walking herded	_____
<input type="radio"/> other (specify) _____	_____
<input type="radio"/> Not sure	_____

**III. Field running ducks** (Please notice that we only consider field running ducks in the following section. Field running ducks are ducks scavenging in rice fields, which are owned by people in your village)

18. We want to know for what purposes people in your village keep field running ducks? Please indicate the importance of each of the following production purposes for people in your village (*Tick one box per row*)

Purpose	Very important	Important	Not very important	Not applicable
Sale of ducks (live or culled)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of eggs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of hatched ducklings	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Home consumption of meat	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Home consumption of eggs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of feathers	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of manure	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other (specify)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

19. Please estimate the number of field running duck owners in your village.

Number of owners: \_\_\_\_\_, of which

Number of owners keeping broiler flocks only: \_\_\_\_\_  Not sure

Number of owners keeping layer flocks only: \_\_\_\_\_  Not sure

Number of owners keeping both broiler and layer flocks: \_\_\_\_\_  Not sure

Not sure

20. Do people in your village move field running ducks out of the village for scavenging rice?

Yes (Go to question 21)

No (Skip to question 23)

Not sure (Skip to question 23)

21. Please estimate the number of owners who run their ducks out of your village during September and February (lunar calendar).

\_\_\_\_\_ owners, or \_\_\_\_\_%  Not sure

22. Where do people move their ducks to between September and February (lunar calendar)? Select one or more categories and rank them according to (1) = frequently, (2) = occasionally.

Place	Broiler flocks	Layer flocks
the same commune	<input type="radio"/> ( )	<input type="radio"/> ( )
other communes of the same district	<input type="radio"/> ( )	<input type="radio"/> ( )
other districts of the same province	<input type="radio"/> ( )	<input type="radio"/> ( )
other provinces	<input type="radio"/> ( )	<input type="radio"/> ( )
Not sure	<input type="radio"/>	<input type="radio"/>

23. Do field running ducks from outside come to your village?

Yes

No → (Skip to question 27)

24. How many different duck herders do you expect in your village after each rice harvest?

After cycle 1: \_\_\_\_\_  Not sure

After cycle 2: \_\_\_\_\_  Not sure

After cycle 3: \_\_\_\_\_  Not sure

25. Where do field running duck herders from outside your village usually come from? Please select one or more categories. If you select more than one category per column, please rank them according to 1 = frequently, 2 = occasionally,

the same commune \_\_\_\_\_

other communes of the same district \_\_\_\_\_

other districts of the same province \_\_\_\_\_

other provinces \_\_\_\_\_

Not sure

26. Do you generally see the same field running duck herders come to your village every year?

- Yes             No             Not sure

#### IV. Biosecurity

27. Have you ever seen wild waterfowl in your village area (including rice fields) during the last 12 months?

- Yes             No → (Skip to question 30)

28. Please list the names of waterfowl species you have seen in your village during the last 12 months)

Species:.....

- Do not know

29. Please list the periods when you often see wild waterfowl during the year (lunar calendar)

From (month) -- To (month)

30. What do people in your village generally do with dead birds? (*Tick one or more*)

- |  |                                      |
|--|--------------------------------------|
| <input type="radio"/> Separate them from other birds   | <input type="radio"/> Consume them   |
| <input type="radio"/> Call vet/head of village         | <input type="radio"/> Sell them      |
| <input type="radio"/> Throw them in pond/field/river   | <input type="radio"/> Burn/bury them |
| <input type="radio"/> Use antibiotics/other treatments | <input type="radio"/> Nothing        |
|  | <input type="radio"/> Not sure       |

31. What do people in your village generally do when they observe unusual high mortality amongst their birds? (*Tick one or more*)


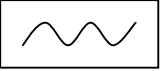
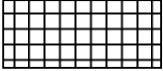
- |  |  |
|--|--|
| <input type="radio"/> Consult other farmers      | <input type="radio"/> Burn/bury            |
| <input type="radio"/> Notify the village headman | <input type="radio"/> Other (specify)_____ |
| <input type="radio"/> Notify the vet             | <input type="radio"/> Not sure             |

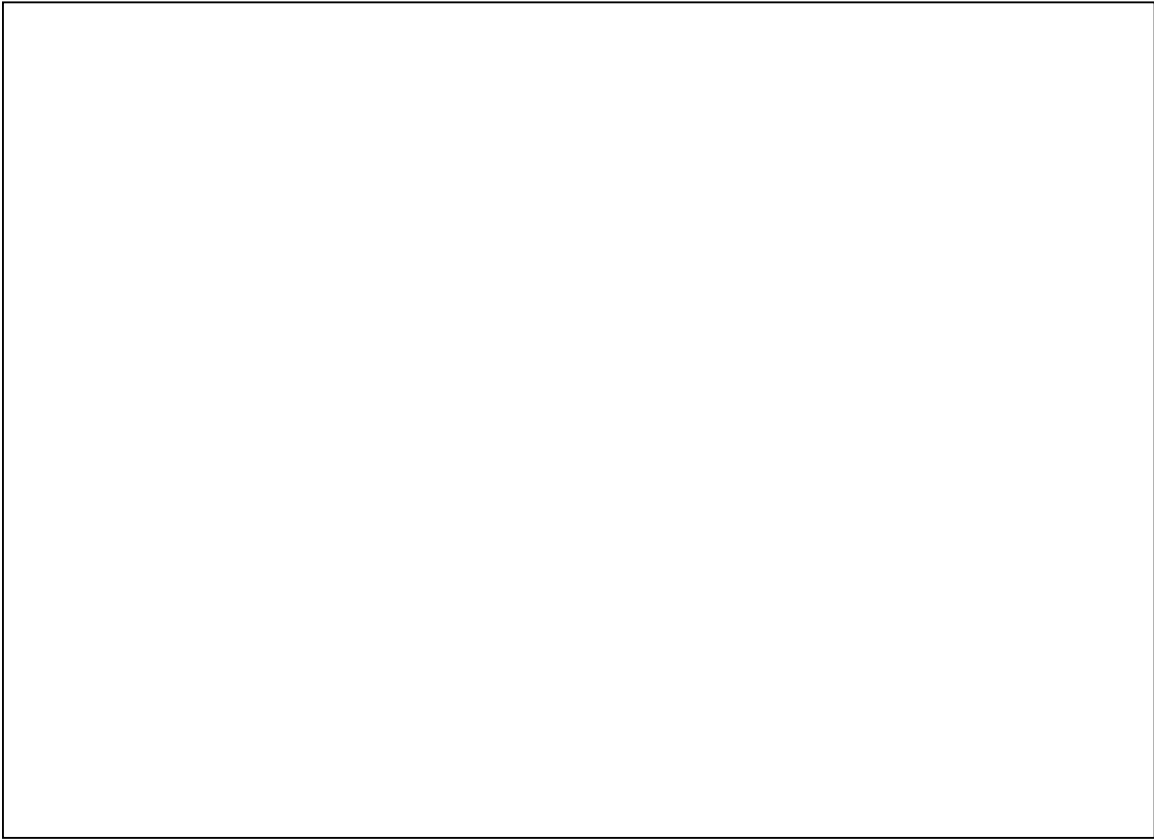
32. Did you observe unusual high mortality in your village over the last five years? (if possible, specify times (e.g. May 2005 and Jan 2007) and animal species) (*Tick one or more*)

- In village poultry; .....
- In wild birds; .....
- No
- Not sure

## V. Avian influenza

33. Have you heard of HPAI outbreaks in your (*Tick one or more*)
- Your village
  - Other villages in your commune
  - Other communes in your district
  - Other districts in your province
  - Other provinces
  - No → (Skip to 35)
34. What did people in your village do with their poultry after they heard about HPAI outbreaks (in question 33)? (*Tick one or more*)
- Vaccination of poultry
  - Confinement of poultry
  - Clean/disinfect their premises
  - Restricted sources of hatchlings
  - No mixing of poultry species
  - Other (please specify): \_\_\_\_\_
  - Nothing specific
  - Not sure
35. What is the percentage of poultry in your village being vaccinated against avian influenza during the last 6 months? (*Tick one for each category*)
- For chicken:
- None     < 20%     21 to 50%     51 to 80%     > 80%     Not sure
- For ducks:
- None     < 20%     21 to 50%     51 to 80%     > 80%     Not sure
36. How do you rank the importance of avian influenza? (*Tick one*)
- Very important     Important     Moderate concern     Not important     Not sure

Please draw a crude map of your village. The drawing should emphasize rice fields, water sources (such as rivers, ponds), and residential areas. Please use the following patterns for rice fields , water sources , and residential areas .



**Do you have any comments on whether this village is typical of its conditions experienced in this province, or if there is something unusual or atypical worth noting?**

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Thank you very much for your cooperation. Could you please give me a list of households raising field running ducks so that I can select five households for further interviews?

**Questionnaire on poultry management in the Mekong River Delta**  
**(For individual households)**

Date of interview \_\_\_\_\_

Interviewer ID \_\_\_\_\_

Village ID \_\_\_\_\_

Respondent ID \_\_\_\_\_

Position on farm of interviewee \_\_\_\_\_

Good morning/afternoon/evening. I would like to ask you some questions about your village. I am ....., a veterinary staff member of district..... I am here to ask you about your poultry production, especially about field running ducks. The purpose of this interview is to study poultry practice and avian influenza control and prevention. We would appreciate if you could spend few minutes to take part in the interview. If at any time during the interview you are not clear about the question, be sure to ask me.

**I. General information**

1. How old are you? (*Tick one*)

- < 20 years     20 to 29 years     30 to 39 years     40 to 49 years     ≥ 50 years

2. Do you grow rice?

- Yes                       No → (Skip to question 9)

3. Please list the periods when you grow rice (months from seed to harvest, use lunar calendar):

Cycle 1: from \_\_\_\_\_ to \_\_\_\_\_     Not sure

Cycle 2: from \_\_\_\_\_ to \_\_\_\_\_     Not sure

Cycle 3: from \_\_\_\_\_ to \_\_\_\_\_     Not sure

4. Do you raise your own ducks during the growing periods of rice?

- Yes                       No

5. Do you use rice after growth for your own ducks?

- Yes                       No

If yes, please indicate during which periods you often use rice after growth for your ducks?

- After cycle 1     After cycle 2     After cycle 3     Other (specify)\_\_\_\_     Not sure

6. What do you use to fertilize your rice field? (Tick one or more)

- Poultry manure       Other (specify) \_\_\_\_\_
- Other manure       None
- Artificial fertilizer

7. Do field running duck herders from outside your village come to your rice fields?

- Yes       No → (Skip to question 10)

8. Please indicate periods when field running ducks from outside your village are often moved to your rice fields?

- After cycle 1    After cycle 2    After cycle 3    Other (specify) \_\_\_\_    Not sure

**II. Poultry production**

9. How many of the following poultry species do you have?

Species	Number of poultry	Species	Number of poultry
Chicken		Fighting cock	
Duck		Quail	
Muscovy		Pigeon	
Goose		Pet bird	

10. Do you run your different poultry species together?

- Yes       No

11. Which water sources do you use for your poultry? If you select more than one water source, please rank them according to 1 = frequently, 2 = occasionally.

- | Selection                                 | Rank  |
|---|-------|
| <input type="radio"/> Municipality supply | _____ |
| <input type="radio"/> Pond                | _____ |
| <input type="radio"/> Well/bore           | _____ |
| <input type="radio"/> Rain collected      | _____ |
| <input type="radio"/> River/canal         | _____ |
| <input type="radio"/> Other:.....         | _____ |

12. Please indicate purposes of your poultry production? If you select more than one, please rank them according to 1 = most important, 2 = second most important.

- |  | Rank  |
|--|-------|
| <input type="radio"/> Source of food for your family               | _____ |
| <input type="radio"/> Source of income                             | _____ |
| <input type="radio"/> Social function (ceremonies, gifts, rituals) | _____ |
| <input type="radio"/> Other (specify) _____                        | _____ |

13. What are the main methods for exchanging your poultry products? (*Tick one or more*)  
 Sell/buy     Gift     Barter     Other (specify)\_\_\_\_\_
14. Where do you purchase breeding poultry? If you select more than one, please rank them according to 1 = frequently, 2 = occasionally.
- | Selection   | Rank  |
|---|-------|
| <input type="radio"/> Within your village             | _____ |
| <input type="radio"/> Other villages in your province | _____ |
| <input type="radio"/> Other provinces                 | _____ |
| <input type="radio"/> Middlemen                       | _____ |
| <input type="radio"/> Other (specify)                 | _____ |
| <input type="radio"/> Not sure                        |       |
15. Where do you sell your live poultry? If you select more than one, please rank them according to 1 = frequently, 2 = occasionally.
- | Selection   | Rank  |
|---|-------|
| <input type="radio"/> Within your village             | _____ |
| <input type="radio"/> Other villages in your province | _____ |
| <input type="radio"/> Other provinces                 | _____ |
| <input type="radio"/> Middlemen                       | _____ |
| <input type="radio"/> Other (specify)                 | _____ |
| <input type="radio"/> Not sure                        |       |

**III. Field running ducks** (Please notice that we only consider field running ducks in the following section. Field running ducks are ducks scavenging in rice fields)

16. For what purposes do you keep field running ducks? (*Tick one box per row*)

	Very important	Important	Not very important	Not applicable
Sale of ducks (live or culled)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of eggs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of hatched ducklings	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Home consumption of meat	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Home consumption of eggs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of feathers	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sale of manure	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other (specify)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>



17. Please indicate the number of broiler and layer field running ducks you have in each of the following age groups.

Broiler		Layer	
Age group	Number of ducks	Age group	Number of ducks
< 1 month	_____	< 1 month	_____
1 -- 2 months	_____	1 -- 2 months	_____
2 -- 3 months	_____	2 -- 3 months	_____
> 3 months	_____	3 -- 6 months	_____
		6 -- 12 months	_____
		1 -- 1.5 years	_____
		> 1.5 years	_____

18. Where do you often run ducks? Please indicate for each selected category how frequently ducks come back to your house.

- | Place of running   | Frequency of coming back ( <i>Tick one per row</i> )   |
|--|--|
| <input type="radio"/> Within your village                | <input type="radio"/> Daily <input type="radio"/> Weekly <input type="radio"/> Monthly <input type="radio"/> Never <input type="radio"/> Other:..... |
| <input type="radio"/> Other villages in the same commune | <input type="radio"/> Daily <input type="radio"/> Weekly <input type="radio"/> Monthly <input type="radio"/> Never <input type="radio"/> Other:..... |
| <input type="radio"/> Other communes of your district    | <input type="radio"/> Daily <input type="radio"/> Weekly <input type="radio"/> Monthly <input type="radio"/> Never <input type="radio"/> Other:..... |
| <input type="radio"/> Other districts of your province   | <input type="radio"/> Daily <input type="radio"/> Weekly <input type="radio"/> Monthly <input type="radio"/> Never <input type="radio"/> Other:..... |
| <input type="radio"/> Other provinces                    | <input type="radio"/> Daily <input type="radio"/> Weekly <input type="radio"/> Monthly <input type="radio"/> Never <input type="radio"/> Other:..... |

19. Please list the periods during the year when you often move your ducks to rice fields outside the village (lunar calendar)

From (month) -- To (month)

No

20. Have you seen wild waterfowl in the same field(s) with your field running ducks during last 12 months?

Yes  No → (Skip to question 23)

21. Please list the names of wild waterfowl species that have appeared in the same rice field(s) with your field running ducks during the last 12 months

Species:.....

Do not know

22. Please list the periods when you have seen wild waterfowl appearing in the same field(s) with your field running ducks during the last 12 months (lunar calendar).

From (month) -- To (month)

23. Who is directly involved with raising ducks? (*Tick one or more*)

Family members

Hired labour from  the same village  other villages

Other (specify) \_\_\_\_\_

24. Do you breed ducks from your own flocks?

Always → (Skip to question 27)  Sometimes  Never

25. Where do you purchase your breeding ducks for field running, please indicate types of ducks?

Source	Are they sourced as
<input type="radio"/> Your commune	<input type="radio"/> ready to hatch eggs <input type="radio"/> ducklings <input type="radio"/> Adults
<input type="radio"/> Other communes in your district	<input type="radio"/> ready to hatch eggs <input type="radio"/> ducklings <input type="radio"/> Adults
<input type="radio"/> Other districts in your province	<input type="radio"/> ready to hatch eggs <input type="radio"/> ducklings <input type="radio"/> Adults
<input type="radio"/> Other provinces	<input type="radio"/> ready to hatch eggs <input type="radio"/> ducklings <input type="radio"/> Adults
<input type="radio"/> other sources (specify):	<input type="radio"/> ready to hatch eggs <input type="radio"/> ducklings <input type="radio"/> Adults

26. By what method are ducks transported to your farm? (*Tick one or more*)

Truck  Foot  Walking herded  other (specify) \_\_\_\_\_

Ship/boat  Motorbike/bicycle  Pushbike

27. Where do you often sell your field running ducks? (*Tick one or more*)

Farm-gate to neighbours

Farm-gate to traders

Local market

Distance market

- Along the way of movement
  - Other (specify)\_\_\_\_\_
28. By what method are your ducks transported to the place of sale? (*Tick one or more*)
- Truck       Pushbike       Walking herded       Other (specify)\_\_\_\_\_
  - Ship/boat       Motorbike/bicycle       Foot
29. Which of the following documents do you have for your field running duck flocks (*Tick one or more*)
- Certificate of HPAI vaccination
  - Register book for your field running ducks
  - Quarantine certificate when you move your ducks to other provinces
  - Other documents (specify)\_\_\_\_\_
  - No document
  - Not sure

#### **IV. Biosecurity**

30. Do you sell your ducks in markets?
- Yes       No → (Skip to question 33)
31. What do you do with poultry you couldn't sell in markets (*Tick one*)?
- Bring them back to your farm, and
    - Re-mix them with other birds
    - Keep them separate from other birds
  - Keep them in markets
  - Other (please specify)\_\_\_\_\_
32. How often do you clean your premises (*Tick one*)
- daily     2 -- 5 times/week     weekly     monthly    Other:\_\_\_\_\_     Never
33. How often do you disinfect your premises (*Tick one*)?
- daily     2 -- 5 times/week     weekly     monthly    Other:\_\_\_\_\_     Never
34. How often does a veterinarian visit your farm? (*Tick one*)
- weekly     monthly     quarterly    Other:\_\_\_\_\_     Never
35. Do you control entries of wild birds for your backyard poultry?
- Yes, please specify how:.....
  - No
36. Do traders/middlemen enter your farm yard?
- Yes       No

37. Do you wash your hands with clean water and soap before and after handling poultry? *(Tick one)*
- Frequently       Occasionally       Never
38. Do you wear protective shoes and change clothes while you are in contact with your poultry? *(Tick one)*
- Frequently       Occasionally       Never
39. Do you wear protective gears while you slaughter your poultry? *(Tick one)*
- Frequently       Occasionally       Never
40. What do you do with sick/dead birds? *(Tick one or more)*
- Separate from other birds       Consume them
- Call vet/head of village       Give them to others
- Throw them in pond/field/river       Sell them
- Use antibiotics/other treatments       Burn/bury them
- Nothing
- Not sure
41. What do you do when you observe unusual high mortality amongst your birds? *(Tick one or more)*
- Consult other farmers       Notify the vet
- Notify the village headman       Burn/burly
- Nothing
42. Did you observe unusual high mortality over the last five years? (if possible, specify times (e.g. May 2005 and Jan 2007) and animal species) *(Tick one or more)*
- Your poultry; .....
- In village poultry; .....
- In wild birds; .....
- No
- Not sure

## **V. Avian influenza**

43. Have you heard of HPAI outbreaks in your *(Tick one or more)*
- Your village
- Other villages in your commune
- Other communes in your district
- Other districts in your province
- Other provinces
- No → (Skip to question 45)

44. How much did the information about HPAI outbreaks (in question 43) affect your poultry management? (*Tick one or more*)
- Vaccination of your poultry
  - Confinement of your poultry during  night  both day and night
  - Clean/disinfect your premises
  - Restricted sources of hatchlings
  - No mixing of type of birds
  - Other (please specify): \_\_\_\_\_
  - Nothing at all
  - Not sure
45. Have you ever had avian influenza in your farm?
- Yes → Dates of the last time: \_\_\_\_\_  No
46. Have your poultry been vaccinated against HPAI during the last 6 months?
- Yes  No  Not sure
47. How high is the percentage of your poultry being vaccinated against avian influenza during the last 6 months? (*Tick one for each category*)
- For chickens:
- None  < 20%  21 to 50%  51 to 80%  > 80%  Not sure
- For ducks:
- None  < 20%  21 to 50%  51 to 80%  > 80%  Not sure
48. If some of your poultry were not vaccinated, for what reasons were these poultry not vaccinated? If all your poultry are vaccinated, skip to question 49. (*Tick one or more*)
- Vaccine unavailable
  - No one available to administer vaccine
  - Unable to catch the ducks
  - Other (specify) \_\_\_\_\_
  - Do not know
49. What are the reasons for you to vaccinate your poultry? (*Tick one or more*)
- You obtain new birds
  - Vets (or other person) arrive in the village to vaccinate
  - Occurrence of HPAI outbreaks in the area
  - Other (specify) \_\_\_\_\_
  - Not sure
50. How do you rank the importance of avian influenza? (*Tick one*)
- Very important  Important  Moderate concern  Not important  Not sure

**Any comment on whether your poultry production system is typical of conditions experienced in this village, or if there is something unusual or atypical worth noting**

A large, empty rectangular box with a thin black border, intended for the respondent to provide their comments on their poultry production system.

*Thank you for your time!*

**APPENDIX B**

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**Appendix 2**

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## Questionnaire for case-control study For individual households

Date of investigation	
Code of investigator	
Name of investigator	

Good morning/afternoon/evening. I am ....., a veterinarian of..... I am here to ask a few questions about your poultry production. The purpose of this interview is to study poultry practice and measures of avian influenza control and prevention. Can I speak to the person who is mainly responsible for poultry raising in this household? I would appreciate if you could spend approximately 20 minutes to take part in the interview. If you are not clear about the question at any time during the interview, be sure to ask me.

### Address

	Province	
	District	
	Commune	
	Village	
	Village ID <sup>1</sup>	
	Household ID <sup>2</sup>	
	Name of poultry owner	
	Position of interviewee (owner, manager, worker, etc.)	
	Coordinates (investigators use a GPS device)	Longitude:..... Latitude:.....

<sup>1</sup> Village ID: for each case control investigation: x.1 & x.2 for 02 control villages; x.3 for case village >> x = 1 ÷ 40 (40 investigations). For example, for the first investigation, IDs for two control villages are 1.1 & 1.2, and ID for case village is 1.3

<sup>2</sup> Household ID: numbered from 1 to 5 for each case-control investigation: 1 & 2 for two control households in two neighbouring villages, 3 & 4 for two control households in the same village with case; 5 for the case household

## **General information**

*Firstly, I would like to get some general information about your household.*

1. How old are you? (*Tick one*)

< 20 years     20 to 29 years     30 to 39 years     40 to 49 years     ≥ 50 years

2. How many people are currently living in your household? (*Write down a number*)

\_\_\_\_\_ people

3. What is your highest education level (*Tick one*)?

None                                       High school  
 Primary school                       Other (specify) \_\_\_\_\_  
 Secondary school

4. Do you grow rice in your family?

Yes                       No → (Skip to question 6)

If yes, please list the periods when you grow rice (months from seed to harvest, use lunar calendar).

Cycle 1: from \_\_\_\_\_ to \_\_\_\_\_     Not sure of period

Cycle 2: from \_\_\_\_\_ to \_\_\_\_\_     Not sure of period                       No second cycle

Cycle 3: from \_\_\_\_\_ to \_\_\_\_\_     Not sure of period                       No third cycle

5. Have duck flocks from outside your village visited your rice field in the last month?

Yes                       No                       Not known

If yes, where did ducks from outside your village come from? (*Tick one or more; please, specify name(s) of location(s), eg. village, commune, district, and province*)

Other villages within the same commune (specify village name)

\_\_\_\_\_

Other communes within the same district (specify commune name)

\_\_\_\_\_

Other districts within the same province (specify commune and district name)

\_\_\_\_\_

Other provinces (specify district and province name)

\_\_\_\_\_

Don't know

## **Poultry production**

Next, I would like to ask you some information about your poultry production.

6. How many of each poultry species do you have? <sup>3</sup> Please specify ages of your poultry flocks. (Fill in all rows; write "0" if not applicable)

<b>Species</b>	<b>Number of poultry</b>	<b>Age in months <sup>4</sup></b>
Chickens		
Ducks		
Muscovy ducks		
Geese		
Fighting cocks		
Quail		
Pigeons		
Pet birds		
Other (specify)_____		

7. Do you mix your chickens with other poultry species? (Tick one)

Yes       No       Not applicable<sup>5</sup>

8. Do you keep any pigs?

Yes       No

9. Where do you seek information about poultry raising? (Tick one or more)

Other farmers       TV/radio  
 Vets       Social organizations (Woman, Youth, Veteran, etc.)  
 Village headman       Other (specify)\_\_\_\_\_

Booklets/manuals       Not seeking for information

10. Please indicate the proportion of your family's income which is derived from poultry production under normal circumstances. (Tick one)

0 %     Low (1 to 50%)     Medium (51 to 75%)     High (> 75%)     Don't know

<sup>3</sup> For cases: total population before the outbreak

<sup>4</sup> If there is more than one flock with different age groups for each species, please separate flocks by ";" (for example: 2 -- 3; 6; >18 means **three** flocks with ages of (1) 2 -- 3 months, (2) 6 months, and (3) more than 18 months), OR write "mixed"

<sup>5</sup> Do not keep chickens, or keep chickens only, but not other species.

11. Please indicate a level of each of the following purposes for raising poultry? Please rank according to the importance of each purpose for chicken and duck production separately. (Tick one per row)

**Chicken production:**

Purposes	Levels			
	Usually (> 90%)	Sometimes (20 -- 90%)	Rarely (< 20%)	Never
Sale of live chickens	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of chicken meat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of chicken eggs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of chicks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home consumption of chicken meat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home consumption of chicken eggs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other (specify)_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**Duck production:**

Purposes	Levels			
	Usually (> 90%)	Sometimes (20 -- 90%)	Rarely (< 20%)	Never
Sale of live ducks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of duck meat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of duck eggs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sale of ducklings	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home consumption of duck meat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home consumption of duck eggs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other (specify)_____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

12. Where do you keep your poultry during the day? (*Tick one category for each poultry species*).

Category	Chickens	Ducks	Muscovy ducks
Confined with roof	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Confined by fence (with no roof or partly roofed) with access to yard only	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Confined by fence (with no roof or partly roofed) with access to yard and waterways	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Free range on your own property	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Free range on your own and neighbour's yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Free range with access to waterways	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Not raising	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

13. Where do you keep your poultry during the night? (*Tick one category for each poultry species*).

Category	Chickens	Ducks	Muscovy ducks
Confined with roof	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Confined by fence (with no roof or partly roofed) with access to yard only	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Confined by fence (with no roof or partly roofed) with access to yard and waterways	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Keep freely on your own property	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Keep freely on your own and neighbour's yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Keep freely with access to waterways	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Not raising	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

14. Do you employ people to help you raise poultry?

Yes       No

15. Do you purchase feed for your poultry?

Yes       No

If yes, please estimate the percentage of total feed you buy: \_\_\_\_\_ %       Don't know

Other comments: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

16. What do you do with poultry manure? (*Tick one or more*)

- |   |  |
|---|--|
| <input type="checkbox"/> Sell it                      | <input type="checkbox"/> Rake it up in one heap and leave it |
| <input type="checkbox"/> Feed to your fishes/prawns   | <input type="checkbox"/> Other (specify).....                |
| <input type="checkbox"/> Fertilise your garden(s)     | <input type="checkbox"/> Nothing                             |
| <input type="checkbox"/> Fertilise your rice field(s) | <input type="checkbox"/> Don't know                          |

17. Did you sell live poultry/poultry products during the last month? (*Tick one*).

- Yes     No → (Skip to question 20)     Don't know → (Skip to question 20)

18. Where did you sell your live poultry/poultry products (meat, eggs, offal, feather, manure) during the last month? If you select more than one category, please rank them according to 1= most frequent, 2 = second most frequent.

Sell live poultry	Rank	Sell poultry products (meat, eggs, offal, feathers, manure)	Rank
<input type="checkbox"/> Markets in your village	_____	<input type="checkbox"/> Markets in your village	_____
<input type="checkbox"/> Markets outside your village*	_____	<input type="checkbox"/> Markets outside your village*	_____
<input type="checkbox"/> Other farmers in your village	_____	<input type="checkbox"/> Other farmers in your village	_____
<input type="checkbox"/> Traders from the same village	_____	<input type="checkbox"/> Traders from the same village	_____
<input type="checkbox"/> Traders from outside your village	_____	<input type="checkbox"/> Traders from outside your village	_____
<input type="checkbox"/> Other (specify) _____	_____	<input type="checkbox"/> Other (specify) _____	_____
<input type="checkbox"/> No sale	_____	<input type="checkbox"/> No sale	_____
<input type="checkbox"/> Don't know	_____	<input type="checkbox"/> Don't know	_____

\* If you sold poultry/poultry products to markets outside your village, please specify name(s) and its location(s) (village, commune, district, province).

Sell live poultry	Sell poultry products

19. Did you bring any unsold live poultry back to your house during the last month?

- Yes     No     Don't know

20. Did you purchase live poultry/poultry products (meat, eggs, offal, feather, manure) during the last month (*Tick one*)?

- Yes       No → (Skip to question 23)       Don't know → (Skip to question 23)

21. Where did you purchase live poultry/poultry products during the last month? If you select more than one category, please rank them according to 1= most frequent, 2 = second most frequent.

Purchase live poultry	Rank	Purchase poultry products (eggs, meat, offal, and feather)	Rank
<input type="checkbox"/> Markets in your village	_____	<input type="checkbox"/> Markets in your village	_____
<input type="checkbox"/> Markets outside your village*	_____	<input type="checkbox"/> Markets outside your village*	_____
<input type="checkbox"/> Other farmers in your village	_____	<input type="checkbox"/> Other farmers in your village	_____
<input type="checkbox"/> Traders from the same village	_____	<input type="checkbox"/> Traders from the same village	_____
<input type="checkbox"/> Traders from outside your village	_____	<input type="checkbox"/> Traders from outside your village	_____
<input type="checkbox"/> Other (specify) _____	_____	<input type="checkbox"/> Other (specify) _____	_____
<input type="checkbox"/> No purchase	_____	<input type="checkbox"/> No purchase	_____
<input type="checkbox"/> Don't know	_____	<input type="checkbox"/> Don't know	_____

\* If you purchased poultry/poultry products from markets outside your village, please specify name(s) and its location(s) (village, commune, district, province).

Purchase live poultry	Purchase poultry products

22. Did you separate live poultry that you purchased during the last month from your old flocks for at least 3 days?

- Yes       No       No purchase of live poultry

If yes, please indicate where you separated the poultry

- In the same premises with current poultry  
 Outside of your premises

23. Please indicate if there have been any changes in market price of poultry/poultry products during the last month compared to previous months in your region? (*Tick one*)

- Higher       Lower       Stable       Don't know

24. Please indicate whether any of the following activities have happened during the last month (*Tick one per row*).

	Yes	No	Not sure
You purchased or received (gift, barter) new poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Your poultry had contact with other village poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Your poultry had contact with poultry from other villages	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Your poultry were in close contact with wild birds	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Paravets visited the place you are raising poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Poultry traders visited the place you are raising poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
People from the same village visited the place you are raising poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
People from outside the village visited the place you are raising poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Your family members or employees returned from a visit to other villages	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

25. Have you observed unusual high mortality in your poultry flock over the last year? (specify times, percentage of death, and species affected).

Yes, \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

No

Don't know



**Duck production** (if the household does not keep ducks, skip to question 31)

*Next, I would like to ask you a few questions about your duck production*

26. For what purposes do you keep ducks? (*Tick one or more*)

- Broiler       Layer       Other (specify).....       Don't know

27. You already indicated your total duck population. Now, please indicate in more details about age groups and purposes of duck raising?

Purpose (broiler/layer)	Age in months	Number of ducks

- Don't know

28. Do you have integrated fish-duck farming (share ponds)?

- Yes       No       Don't know

29. Do you run ducks on rice fields?

- Yes     No → (Skip to question 31)

30. Where have you run your ducks during last month? (*Tick one or more; specify name(s) of location(s), eg. village, commune, district, and province*)

- Within your village  
 Other villages within the same commune (specify village name)

- 
- Other communes within the same district (specify commune name)

- 
- Other districts within the same province (specify commune and district name)

- 
- Other provinces (specify district and province name)

- 
- Don't know

## **Biosecurity**

Now, I would like to ask you some information about poultry management and protection

31. How do you usually clean the places of keeping poultry? (*Tick one or more*)

- sweeping       disinfect       other: \_\_\_\_\_       Not done

32. How often do you clean (sweeping or remove manure) the places of keeping poultry? (*Tick one*)

- daily                       weekly                       monthly  
 after selling poultry       other: \_\_\_\_\_       never

33. How often do veterinarians visit your house? (*Tick one*)

- weekly                       monthly                       quarterly  
 during HPAI vaccination       other: \_\_\_\_\_       never

34. Do poultry traders enter the places of raising your poultry?

- Yes                       No

35. Do you wash your hands after handling poultry?

- Usually                       Occasionally                       Rarely                       Never

36. Do you wear protective gear while you are in contact with poultry? *Please indicate which types of protective gear (gum boots, gloves, etc.)*

- Usually: \_\_\_\_\_       Occasionally: \_\_\_\_\_       Never

37. What do you do with your sick birds? (*Tick one or more*)

- Separate from other birds                       Consume them  
 Inform vets                       Sell them  
 Inform the village headman                       Nothing  
 Use antibiotics/other treatments

38. What do you do with your dead birds? (*Tick one or more*)

- Inform vets                       Sell them  
 Inform the village headman                       Burn/bury them  
 Throw them in pond/field/river                       Nothing  
 Consume them

39. Have you vaccinated your current poultry with HPAI vaccine? <sup>6</sup>

Yes

No → (Skip to question 42)

Don't know → (Skip to question 42)

Not applicable<sup>7</sup> → (Skip to question 42)

▪ If yes, please indicate:

▪ The date of the most recent vaccination: \_\_\_\_\_  Don't know

▪ Number of poultry being vaccinated at the time

Chicken: \_\_\_\_\_ (%)  Don't know  No chicken

Duck: \_\_\_\_\_ (%)  Don't know  No duck

40. How many new poultry have entered your poultry flocks since the last vaccination? (*write '0' if no poultry entered*)

Chicken: \_\_\_\_\_  Don't know

Duck: \_\_\_\_\_  Don't know

41. Who vaccinated your poultry? (*Tick one or more*)

Your self

Vets/trained personnel from other villages

Your family member

Other (specify) \_\_\_\_\_

Your hired labour

Don't know

Vets/trained personnel from your village

42. Have you ever heard of any HPAI outbreaks in your village during the last month?

Yes  No

43. Have you ever thought your poultry flocks may be affected by HPAI?

Yes  No

If yes, please explain why?

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<sup>6</sup> For cases: vaccination of poultry before outbreaks

<sup>7</sup> No chickens/ducks

**Outbreak details** (used for cases only)

Finally, I would like to ask you some information about your infected flock(s)

44. When was the date of the first signs of disease?

Date: \_\_\_\_\_  Don't know

45. Which of the following categories did you observe from your HPAI infected poultry?  
(Tick one or more, and indicate numbers or percentage)

Sudden death is reported for more than one day on some birds

Clinical signs of HPAI on some birds

Egg production drop

Feeding intake decrease

Other (specify) \_\_\_\_\_

Don't know

46. Who is the person who first thought your poultry were HPAI infection? (Tick one)

Your self  Village headman

Your family member  Other (specify) \_\_\_\_\_

Your hire labour  Don't know

Commune/village vet

47. Please indicate the number of species infected in the flock

Species	Age in months	Number of sick	Number of deaths	Number in the flock

48. Please indicate possible sources which you think may have caused your poultry's infection. (Tick one per row)

Introducing new poultry into your flock  Yes  No  Not known

Movement of your poultry flocks  Yes  No  Not known

Your poultry contacted with wild birds  Yes  No  Not known

Fomites (vehicle of poultry traders, possibly contaminated material...)  Yes  No  Not known

People (visitors, farmers...)  Yes  No  Not known

Other (specify) \_\_\_\_\_

49. Has any sick or dead poultry with clinical signs of HPAI infection been observed in your neighbours during last month?

Yes       No       Do not know

If yes, please tell us date when this event was observed, and list all clinical signs observed.

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## Questionnaire for case-control study For village headmen<sup>8</sup>

Date of investigation	<input type="text"/>
Code of investigator	<input type="text"/>
Name of investigator	<input type="text"/>

Good morning/afternoon/evening. I am ....., a veterinarian of..... I am here to ask a few questions about poultry production in your village. The purpose of this interview is to study poultry practice and measures of avian influenza control and prevention. I would appreciate if you could spend approximately 10 minutes to take part in the interview. If at any time during the interview you are not clear about the question, be sure to ask me.

### Address

Province	<input type="text"/>
District	<input type="text"/>
Commune	<input type="text"/>
Village	<input type="text"/>
Village ID <sup>9</sup>	<input type="text"/>
Name of village headman	<input type="text"/>

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<sup>8</sup> Ask **three** village headmen for each case-control: **01** headman of **infected village** (where we recruit 01 case and 02 controls-> the investigator interviews the village headman and may fill in the questionnaire for village headman attached for the case; do not need to fill in this part for 02 controls in the same village), and **02** headmen of **non-infected villages** (where we recruit 02 other controls).

<sup>9</sup> Village ID for each case control investigation: x.1 & x.2 for 02 control villages; x.3 for case village>> x = 1 ÷ 40 (40 investigations). This village ID is the same with one in the front page of this questionnaire

1. What is the total area of your village (including residence and rice field)?  
 \_\_\_\_\_ km<sup>2</sup> or \_\_\_\_\_ ha  Don't know
2. What is the percentage (or areas) of the above area used for rice production?  
 \_\_\_\_\_ % or \_\_\_\_\_ km<sup>2</sup> or \_\_\_\_\_ ha  Don't know
3. How many rice cycles do most farmers in your village have per year?  
 \_\_\_\_\_ Cycles  Don't know
4. How many households are in your village?  
 .....households  Don't know
5. What is the human population of your village?  
 .....people  Don't know
6. Please estimate the number (or percentage) of households in your village that are raising poultry?  
 ..... households or .....%  Don't know
7. Is there any live bird market in your village?  
 Yes  No  Don't know

8. How many of each following poultry species are in your village?

Species	Number of poultry
Chickens	<input type="checkbox"/> Don't know
Ducks	<input type="checkbox"/> Don't know
Muscovy ducks	<input type="checkbox"/> Don't know

9. Please indicate which of the following poultry species raised in your village? (*Tick one for each species*)

Species	Yes	No	Don't know
Geese	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fighting cocks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Quail	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pigeons	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pet birds	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

10. Please estimate the number of field running duck owners in your village.

Number of owners: \_\_\_\_\_, of which

Number of owners keeping broiler flocks only: \_\_\_\_\_  Don't know

Number of owners keeping layer flocks only: \_\_\_\_\_  Don't know

Number of owners keeping both broiler and layer flocks: \_\_\_\_\_  Don't know

Don't know

11. Have field running duck owners in your village moved ducks to rice fields outside your village during the last month?

Yes  No  Don't know

12. Have duck flocks from other villages come your village during the last month?

Yes  No → (Skip to question 14)  Don't know → (Skip to question 14)

13. Where did ducks from outside your village come from? (*Tick one or more; specify name(s) of location(s), eg. commune, district, and province*)

Other villages within the same commune (specify village name)

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Other communes within the same district (specify commune name)

---

Other districts within the same province (specify commune and district name)

---

Other provinces (specify district and province name)

---

Don't know

14. Have you seen more than usual numbers of wild waterfowl in the fields in your village during the last month?

Yes  No  Don't know

15. Please estimate the percentage of current chickens and ducks in the village being vaccinated against avian influenza during the last 6 months.

Chickens:

None  < 20%  21 to 50%  51 to 80%  > 80%  Don't know

Ducks:

None  < 20%  21 to 50%  51 to 80%  > 80%  Don't know



16. Has your village ever experienced HPAI outbreaks during the last two years? *(If yes, please specify the date of most recent outbreak and affected species)*

Yes, \_\_\_\_\_


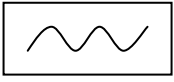
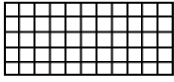
\_\_\_\_\_


No

Don't know

**FOR INVESTIGATOR**

Please draw a crude map of the village (or obtain a copy of village/commune’s map if it is available). The drawing should emphasize rice fields, water sources (such as rivers, ponds), and residential areas. Please use the following patterns for

rice fields , water sources , residential areas , a case (infected household) ●, and controls ○



**Comments**

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Signature of the investigator 

**A copy of this form must be sent to the project management team and another must be kept at RAHO**