The Epidemiology of Avian Influenza in the Mekong River Delta of Viet Nam

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

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— Abstract —

Between December 2003 and December 2012 a total of 4,349 commune-level outbreaks of highly pathogenic avian influenza (HPAI) H5N1 were recorded in domestic poultry flocks throughout Viet Nam. Throughout the same period there were 123 cases of HPAI H5N1 virus infection in humans, 61 of which were fatal.

The studies presented in this thesis are largely based on data collected from a prospective cohort study of domestic poultry in 157 flocks in the Mekong River Delta of Viet Nam between December 2008 and April 2010. The first research chapter (Chapter 3) provides a description of the components and design features of an animal health decision support system for use in Viet Nam. While not explicitly used for the prospective cohort study, the motivation for development of this system was to provide a means for recording and storing animal health data so as to minimise duplication of data collection efforts. A feature of the system is the inclusion of a flexible reporting tool that provides system users with the capability of developing reports to deal with virtually any animal health issue, not just avian influenza. The intent of this system is that it will allow the Vietnamese Department of Animal Health to identify and respond to existing and emerging threats to animal health in a timely and cost-effective manner.

Our descriptive analyses (Chapter 4) show that the overall incidence rate of influenza Type A and H5 virus infection in village poultry was relatively high throughout the 17-month follow up period of the prospective cohort study. This implies that interventions such as vaccination, movement controls and biosecurity measures need to be carried out continuously throughout the year rather than focusing only on the established high risk periods. Broiler ducks had an incidence rate of influenza H5 virus infection that was approximately four times greater than that of layer ducks and in-contact species. This indicates that broiler ducks should be the focus of disease surveillance and control strategies.

Survival analyses, accounting for the intermittent sampling of birds throughout the follow-up period of the prospective cohort study (by interval censoring) and for the hierarchical structure of the data set were used to determine the duration of immunity to H5N1 following vaccination (Chapter 5). After adjusting for the effect of known confounders and unmeasured variation at the flock level the duration of immunity to H5N1 following vaccination was estimated to be in the order of 56 (95% CI 51 – 61) days, considerably shorter than the duration of immunity previously reported in laboratory-based studies. A multilevel logistic regression analysis carried out to identify risk factors for influenza Type A virus infection in the prospective cohort study poultry population found that the relative contribution of unmeasured flock- and bird-level factors on influenza Type A virus infection risk were approximately equal (Chapter 6). Most of the significant fixed-effects were flock-level exposures indicating that interventions to reduce the maintenance and transmission of influenza Type A virus in domestic poultry in this area of Viet Nam should be applied at the individual bird and individual flock level.

Chapter 7 presents the results of a study of poultry movement events that occurred in the south of Viet Nam between September 2009 and June 2010. Poultry were more likely to be moved between communes with provincial roads and between communes with more than 1,000 poultry-owning households. Assuming a causal relationship exists between a commune-to-commune poultry movement activity and HPAI H5N1 risk, a conclusion from this study was that communes more likely to be connected to others as a result of movement should be targeted for disease control and surveillance.

The findings presented in each of these chapters of this thesis have broadened our knowledge of the epidemiology of not only the HPAI H5N1 subtype, but influenza Type A viruses in poultry in general. It should be stressed that the methodological techniques that have been used in this thesis can be applied to a wide range of animal health issues, not just HPAI H5N1.

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Nomenclature

AAHL	Australian Animal Health Laboratory
AFT	accelerated failure time
AI	avian influenza
ARAHIS	ASEAN Region Animal Health Information System
BSE	bovine spongiform encephalopathy
CI	confidence interval
Ct	(RRT-PCR) cycle threshold
CSF	classical swine fever
CSS	cascading style sheets
DAH	Department of Animal Health, Viet Nam
DEFRA	Department for Environment, Food and Rural Affairs, UK
DMS	database management system
DSS	decision support system
DVS	district veterinary station
EDR	estimated dissemination ratio
FAO	Food and Agriculture Organization of the United Nations
FMD	foot-and-mouth disease
FRD	field running duck
GIS	geographic information system
GUI	graphic user interface

HA	haemagglutinin
HPAI	highly pathogenic avian influenza
HI	haemagglutination inhibition
HTML	hyperText markup language
KML	keyhole markup language
LPAI	low pathogenic avian influenza
MARD	Ministry of Agriculture and Rural Development, Viet Nam
MRD	Mekong River Delta, Viet Nam
NA	neuraminidase
NLIS	National Livestock Identification System, Australia
NVDC	National Veterinary Diagnostic Centre, Viet Nam
NVSL	National Veterinary Services Laboratories
OIE	Office International des Epizooties
OR	odds ratio
RADAR	Rapid Analysis and Detection of Animal-Related Risks, UK
RAHO	Regional Animal Health Office, Viet Nam
ROC	receiver operating characteristic (curve)
RRD	Red River Delta, Viet Nam
PCR	polymerase chain reaction
RRT-PCR	real time reverse transcriptase polymerase chain reaction
SARS	severe acute respiratory syndrome
SDAH	Sub-department of Animal Health
SE	standard error
SISBOV	Serviço de Rastreabilidade da Cadeia Produtiva de Bovinos e Bubalinos, Brazil

SNA	social network analysis
TADinfo	Transboundary Animal Disease Information System
USDA	United States Department of Agriculture
WAHID	World Animal Health Information Database
WHO	World Health Organization

List of Publications

Long, N.V., Stevenson, M. and O'Leary, B. (2011) Decision Support Systems in Animal Health. Efficient Decision Support Systems — Practice and Challenges in Biomedical Related Domain. Edited by C.S. Jao. InTech, Rijeka, Croatia. ISBN: 978-953-307-258-6.

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CHAPTER 1

Introduction

Viet Nam is a developing country located in the Indochina Peninsula in Southeast Asia. Approximately 70% of the Vietnamese population live in rural areas and of that group approximately 50% are involved in agriculture. This means that agriculture plays an important role in the national economy (GSO 2012). In particular, in 2011 agriculture accounted for 22% of the country's Gross Domestic Product (GSO 2012). Livestock production constitutes approximately 30% of agriculture output. The scale and modes of animal production throughout Viet Nam are diverse. Estimates from the 2011 national animal census (GSO 2012) showed that approximately 90% of households kept poultry flocks comprised of less than 50 birds whereas only 3% of households kept flocks comprised of more than 100 birds. The Mekong River Delta (MRD) and the Red River Delta (RRD) are the two main areas for agriculture and animal production.

The MRD has a high density of poultry with a large number of waterfowl (approximately 30 million, (GSO 2012)). Poultry populations are highly dynamic because the majority of flocks are reared in a backyard system of production. In this region, duck raising is highly prevalent as ducks are used to scavenge rice fields to glean grains that fall to the ground during harvesting periods (as 'field running ducks'). Ducks may be moved over long distances since the time of harvest differs according to region (Desvaux et al. 2008, Men 2010). In addition highly pathogenic avian influenza (HPAI) H5N1 outbreaks have occurred frequently in the MRD since 2003 (Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009). Routine active surveillance has shown that the prevalence of HPAI H5N1 virus in this area of Viet Nam has been relatively high (Long 2008, 2009, 2010).

In Viet Nam, livestock production is adversely influenced by many infectious diseases

including HPAI H5N1, foot-and-mouth disease (FMD), porcine reproduction and respiratory syndrome (PRRS) and classical swine fever (CSF). Between December 2003 and December 2012 a total of 4,349 commune-level outbreaks of HPAI H5N1 were recorded throughout the country (Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, DAH 2012). Throughout that time HPAI H5N1 infected a total of 123 people resulting in 61 deaths (WHO 2012). HPAI H5N1 outbreaks have mainly been confined to the areas of intensive poultry production in the MRD and RRD (Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009).

Since December 2003 the Vietnamese government has implemented a number of measures to control HPAI H5N1, including culling of infected flocks, vaccination, improved management of the way livestock are moved throughout the country, and education campaigns to raise flock owner awareness about the disease. As a result, HPAI H5N1 has largely been brought under control with a small number of sporadic outbreaks (n = 44) in backyard flocks during the period March 2009 to December 2011 (DAH 2012). A number of observational studies have been carried to better understand the epidemiology of HPAI H5N1 in Viet Nam (Henning et al. 2009, Minh, Schauer, Stevenson, Jones, Morris & Noble 2009, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, Desvaux et al. 2011). In addition, active surveillance has been undertaken since 2005 to evaluate the efficacy of the HPAI H5N1 vaccination strategy and to monitor the evolution of the HPAI H5N1 virus (Wan et al. 2008, Nguyen et al. 2009, Long 2010, Diep 2011).

Records of surveillance activities and outbreak event details represent a diverse set of data that require considerable investment in terms of time and effort to be managed, analysed and interpreted appropriately. An on-going problem is that there are numerous projects developed to collect data about livestock populations in Viet Nam and these are invariably carried out independently. This means that over time a large number of data sets are accumulated (typically in different formats) with the potential for duplication (Taylor & Dung 2007, Long 2009, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, Diep 2011). To date, an effective decision support system to manage animal health data has been not available in Viet Nam. This results in difficulties when there is a requirement to use details of the animal population at risk and outbreak events as a basis to support decision making. This issue exists not only with HPAI H5N1 but also FMD and PRRS where there have been approximately 5,372 and 2,616 commune-level outbreaks,

respectively, over the period 2006 to 2012 (DAH 2012).

This thesis is comprised of a series of six studies presented in the format of either published papers or as manuscripts ready for publication in peer-reviewed scientific journals. The literature review (Chapter 2) provides a description of animal health decision support systems in use in various countries throughout the world with specific reference to how these systems might be implemented in developing countries. The development of an animal health decision support system for use in Viet Nam is described in Chapter 3. The system is comprised of a set of tools to facilitate the collection, storage, management and analysis of animal health data at any level (conceivably from the individual animal level to the national level). The ideas presented in Chapter 3 are based on many of the conclusions drawn from the research chapters.

Chapter 4 provides a descriptive analysis of the prospective cohort study of low pathogenic and highly pathogenic avian influenza that comprised the bulk of the field work collected as part of this research project. Factors influencing the duration of immunity in individual birds following HPAI H5N1 vaccination are presented in Chapter 5 and factors associated with influenza Type A infection are presented in Chapter 6.

An analysis of poultry movement data is presented in Chapter 7 using social network analyses (Wasserman & Faust 1994). In this study details of commune geography and human and poultry population counts are used as explanatory variables in an exponential-family random graph model (Hunter et al. 2008). The purpose of Chapter 7 is to provide a means for ranking communes within the MRD in terms of their likelihood of being involved in a poultry movement network. In turn, this should assist decision makers within the Vietnamese Department of Animal Health to focus their surveillance resources more effectively.

The thesis concludes with a general discussion presented in Chapter 8. Here, reflection is made on the investigatory approaches used throughout the thesis, the major findings and suggestions are provided to allow infectious diseases of livestock (not only poultry) to be better managed in Viet Nam.

CHAPTER 2

Literature review[1]

2.1 Introduction

The safety of food derived from animals has received significant public media attention in recent times and it is likely that this trend will continue in the short to medium term future. Examples of diseases in humans arising from the consumption of animals or animal products at the centre of recent food safety scares include variant Creutzfeld-Jakob disease, *Salmonella Typhimurium* DT104, *Salmonella Enteritidis* and *Escherichia coli* O157. Of additional concerns to the general public are infectious diseases of livestock, particularly those that have required large numbers of animals to be pre-emptively slaughtered or culled as part of control and eradication measures. Since 2000, there have been a number of large outbreaks of infectious disease in farmed animal populations that have been managed using pre-emptive slaughter or cull. This approach to disease management has raised questions about the legitimacy, animal ethics and long term future of intensive farming practices. Examples include the outbreak of classical swine fever (CSF) in The Netherlands in 2000 and foot-and-mouth disease (FMD) in the United Kingdom in 2001. In recent times infectious disease has impacted heavily on the health and productivity of livestock populations in Southeast Asian countries. Highly pathogenic avian influenza (HPAI H5N1) has emerged as a disease of international concern not only because of its ability to cause illness and death in poultry and humans, but also by its capacity to disrupt poultry trade and to threaten food security in resource-poor countries.

[1]Published as: Long, N.V., Stevenson, M.A. and OLeary, B. (2011) Decision Support Systems in Animal Health. Efficient Decision Support Systems — Practice and Challenges in Biomedical Related Domain. Edited by C.S. Jao. InTech Publishers, Rijeka, Croatia. ISBN: 978-953-307-258-6.

To deal with infectious disease outbreaks in domestic livestock populations it is essential that animal health authorities have access to appropriate information to guide decision making. Animal health information includes (but should not be restricted to) details of the population at risk and details of incident cases of disease conditions of interest. These core details allow the distribution of disease to be described in terms of the established epidemiological triad of individual, place and time. Individual-level analyses include estimates of the number of cases per head of population and for various subsections of the population (for example animals of a given age, sex or breed and type). Spatial analyses provide insight into geographical factors influencing the distribution of disease (e.g. proximity to pollutants, farming practices characteristic of a given area). Temporal analyses provide insight into short and long term variations in disease frequency. All three categories of analysis are useful in that firstly they provide an objectively measured point of comparison once control measures have been implemented and secondly, they provide information that can be used for hypothesis generation about factors associated with, or causing disease. Collection of additional information about the environment in which animals are located and events they are exposed to over their lifetime allows these hypotheses to be tested which in turn allow authorities to identify risk factors for disease. Concentrating surveillance and control activities on animals or farm premises with identified risk factors then allows (often scarce) resources to be more effectively targeted at the sector of the animal population in greatest need of attention.

In this chapter we provide an overview of the infrastructure necessary to carry out the analytical procedures outlined above using a national animal health decision support system (DSS). The chapter is divided into three main sections. In the first section, we provide a description of an animal health DSS and review DSSs currently in operation in various countries throughout the world. In the second section, we provide a description of how these systems are implemented in developing countries. The third and final sections look to the future, briefly outlining the planning that must be done by developing countries to ensure that current animal health DSSs continue to meet their needs well into the future. We conclude by proposing that a standardised format for recording and storing animal demographic, productivity and health data needs to be agreed on. Widespread adoption of this format will help to make animal health DSS components more readily transferable from one jurisdiction to another, ultimately reducing their cost and in doing so helping to

alleviate one of the important obstacles to the more widespread uptake of this technology.

2.2 Animal health decision support systems currently in use

An animal health DSS is an interactive, flexible and adaptable system (including, but not limited to, computer-based systems) comprised of relevant databases, technologies and appropriate analytical techniques to identify problems, predict consequences and provide optional solutions so that they can complete their decision processes with more cost-effective outcomes (Turban 1995). A DSS should have two main goals. The first goal is to provide animal health authorities with the ability to trace animals from 'farm to fork', an essential requirement for food safety and documenting health status for domestic and international trading partners. The second goal is to provide a means for facilitating the detection of re-emergence and new emergence of diseases, allowing appropriate deployment of field operations and resources to deal with identified problems if and when they occur. Such a system promotes transparency in the state of animal health, allowing animal health policies to be based on the best available evidence.

A key component of an animal health DSS is a so-called 'data warehouse' which provides the facilities to capture and store and link all relevant information about an animal population of interest (Figure 2.1). Relevant information in this context includes details of disease events, the population at risk, and the location of farm premises, animal movements and results of laboratory and residue analyses. We acknowledge that most (if not all) countries producing food from animals already have established facilities to record some, or even all, of this information. The key feature that distinguishes a DSS is that the various tables listed are linked using unique individual animal and/or farm identifiers to form a coherent relational database design. This allows analysts to easily extract details of (for example) diseased animals, their location of origin and the identities of farm locations they might have visited throughout their lifetime. At the population level, the burden of disease in an animal population can be quantified in terms of incidence and prevalence. Maps of the distribution of diseased animals or animal movements can be produced and interpreted in context of the distribution of the population at risk. An addi-

tional key component of a DSS is that it needs to be both flexible and user friendly for both those end-users interrogate the system and those people interpret information produced by the system.

EpiMAN (Sanson 1993) is an early example of an animal health DSS with a data architecture similar to that shown in Figure 2.1. The system combines a database management system, a geographic information system, a graphic user interface to allow the user to conduct descriptive analyses of infectious disease outbreak data, manage resources (e.g. scheduling of farm visits for patrol visits) and run a simulation model to evaluate the effect of alternative control measures. Initially, EpiMAN was developed to manage the data generated during the course of a large outbreak of FMD. The motivation for doing this was that timely analysis of outbreak event details would allow control and eradication activities to be fine-tuned as the epidemic progressed, as individual circumstances dictate (Sanson et al. 1999).

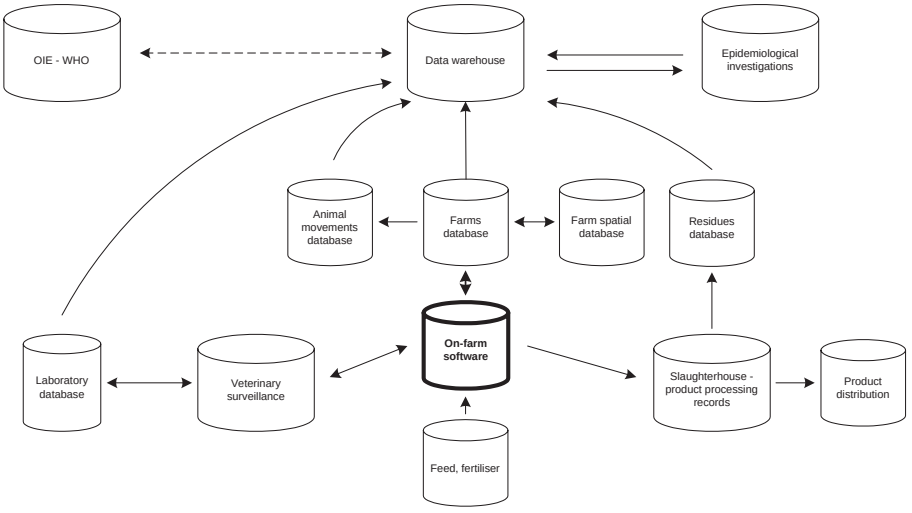


Figure 2.1: Schematic diagram showing how on-farm data, veterinary practice records, diagnostic laboratory data, slaughterhouse processing records, details of residue assessments and animal movements might be integrated within an active surveillance data network (Morris 1997, Stevenson et al. 2007)). OIE = Office International des Epizooties; WHO = World Health Organization. Reproduced with permission from the New Zealand Veterinary Journal.

Another recent animal health DSS is the Rapid Analysis and Detection of Animal-Related Risks (RADAR)[2] system which has been deployed in the United Kingdom (UK) since 2003 (Scudamore 2003, Smith et al. 2006, Paiba et al. 2007). RADAR brings animal health and demographic data together in a standardised format to support research and

[2]URL: `http://www.defra.gov.uk/foodfarm/farmanimal/diseases/vetsurveillance/radar/index.htm`

reports required by a wider group of stakeholders. RADAR integrates data sets from existing systems and transforms them into a common coding system called the extraction, transformation and loading (ETL) process. The system derives additional information from the raw source data automatically using a variety of calculations and algorithms with metadata fully adherent to the UK government's e-Government Metadata Standards (Roberts 2004).

Before RADAR animal health information in the UK was collected and stored by different authorities using different nomenclatures, collection standards and coding systems. This meant that collation of information was extremely slow and integration of different data sources difficult (Morris et al. 2002). The RADAR project has been conducted in three phases. The first phase (2003-2005) focused on the establishment of the data warehouse managing details of the cattle population, details of salmonella cases and exotic disease cases and analytical tools. The second phase (2005-2006) enhanced data cataloguing and reporting tools and extended the scope of data collection to other species (sheep, pigs, goats, and deer) and other diseases. This information has been used for the investigation and control of a range of animal health issues such as surveiallance for salmonella in dairy and poultry (Evans & Jordan 2003), movement of cattle herds (Vernon & Keeling 2009), transmission of bovine tuberculosis (Green et al. 2008), bluetongue vaccination (Wood et al. 2008), avian influenza (Knight-Jones et al. 2010, Lysons et al. 2007) and FMD (2007) (Paiba et al. 2007, Department for Environment, Food and Rural Affairs 2011). An example report from RADAR is shown in Figure 2.2. Figure 2.2 shows the density of poultry premises (expressed as the number of premises per 100 km^2) throughout Great Britain. By simply identifying the location of the poultry population at risk animal health authorities are better placed to carry out a range of activities to maintain poultry health: identify high risk areas for disease transmission, target surveillance activities and monitor poultry movement. The third and final phase of the RADAR project (2006-2013) is to expand the species coverage to include horses, companion animals and wildlife (Smith et al. 2006).

In 2002 the Swiss government funded development of a system titled KODAVET (*Koordiniertes Datenverwaltungs und Analysesystem des Veterinärdienstes Schweiz*). The purpose of the system is to manage any type of information related to both food producing and companion animals (Stärk et al. 2006, Schaller 2006). Unique, individual animal

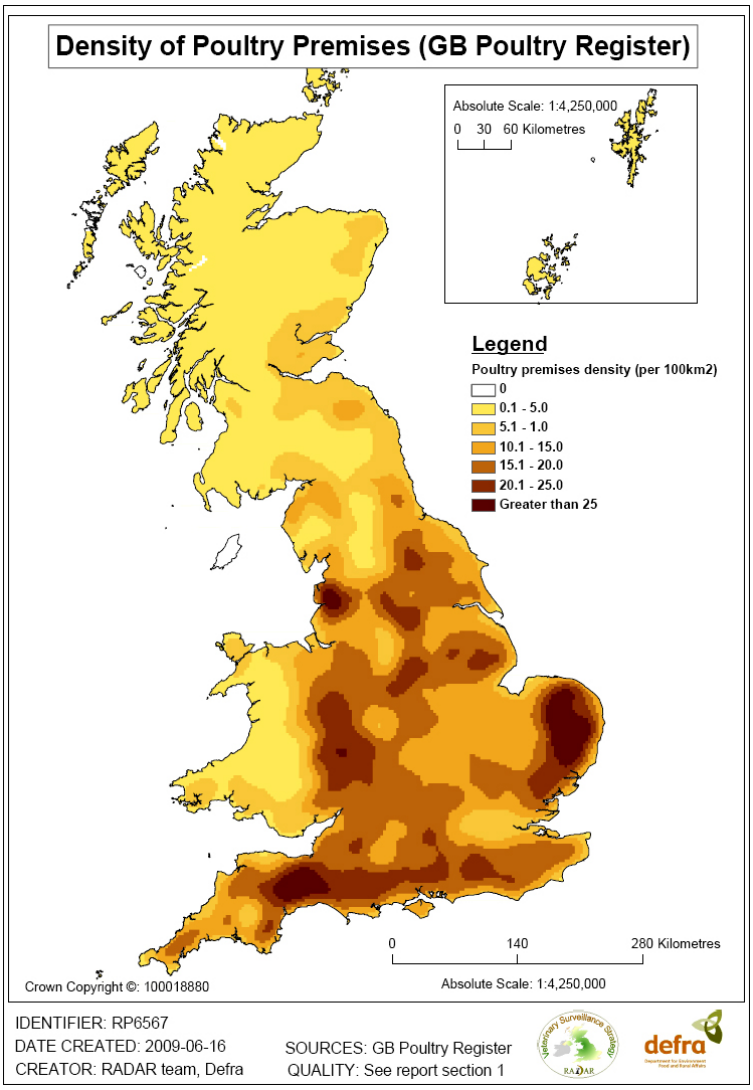


Figure 2.2: Map of Great Britain showing the density of poultry premises (expressed as numbers per 100 km^2).

identifiers and mandatory reporting of livestock movement events mean that animals can be tracked through the system throughout their lifetime. A feature of KODAVET is that it provides a standardised facility for generating and storing health certificates for producers as well as scheduling routine inspection visits by Swiss Federal Veterinary Office staff. The system links a number of databases, including data from local veterinary offices in the cantons, the National Animal Movement Database and the National Database of the Federal Office of Agriculture. This project is on-going and has provided information useful for decision making and efficient use of resources (Swiss Federal Veterinary Office 2009).

Partial animal health DSS systems exist in a number of countries such as Australia (Na-

tional Livestock Identification System, NLIS) [3] (Australian Department of Agriculture, Fisheries and Forestry 2006), Argentina (Sanitary Management System database, maintained by the National Service for Agrifood Health and Quality, SENASA) (Aznar et al. 2011), United States (National Animal Health Monitoring System, NAHMS) [4] (Wineland & Dargatz 1998), New Zealand (AgriBase) [5] (Sanson & Pearson 1997). In Brazil, for example, the *Serviço de Rastreabilidade da Cadeia Produtiva de Bovinos e Bubalinos* (SISBOV)[6] provides the facility to register all cattle and buffaloes born in Brazil or imported into the country since July 2002 (Cardoso & Cardellino 2004*a*, Bowling et al. 2008). The primary purpose of the system is to allow tracing of registered individuals to meet international market requirements. The system provides the government with information to enhance the control of cattle herds, particularly management of FMD free zones and high risk areas.

The examples cited here are by no means exhaustive and the reader is referred to Stevenson et al. (2007) for a more detailed review and critique of systems in place within individual countries. A critical point is that while most countries producing food derived from animals for export have components of a DSS in place few, if any, are capturing and using all of the data elements shown in Figure 2.1. The reason for this is that individual system components are expensive and time consuming to implement, often requiring substantial change in the behaviour of the users of the system. For example, implementation of an animal movement database means that livestock producers are no longer able to freely move animals from one location to another. A new layer of bureaucracy needs to be established to issue movement permits as well as a mechanism to police the system to ensure that all movement events are recorded and appropriate penalties are applied to those that ignore system directives. Substantial information technology infrastructure needs to be built to record and store collected information and finally, facilities need to be put in place to retrieve and analyse data that is actually recorded. Given the time and expense required to set up workable system components we predict that it will take a number of years before 'full' DSSs become commonplace. A powerful facilitator of progress has,

[3]URL: `https://www.nlis.mla.com.au/`

[4]URL: `http://www.aphis.usda.gov/animal_health/nahms/index.shtml`

[5]URL: `http://www.asurequality.com/geospatial-services/agribase.cfm`

[6]URL: `http://extranet.agricultura.gov.br/primeira_pagina/extranet/SISBOV.htm`

in the recent past, been the emergence of novel disease conditions such as bovine spongiform encephalopathy (BSE), severe acute respiratory syndrome (SARS), and HPAI. It is likely that progress in this area will be driven by a need to deal with hazards of this type in the immediate to short term future.

2.3 Animal health decision support systems in developing countries

The key difference between developed and developing countries in terms of recording of animal health information is that developing countries often lack a system for identifying and recording the location of individual farm enterprises. A second different point is that recording of disease event information in developing countries is generally restricted to diseases formerly classified as List A by the World Organisation for Animal Health (OIE).

Because data frames listing the identity of individual farm enterprises are not fully available in developing countries, it is common for animal health details to be aggregated to the tertiary administrative unit level. In the case of Viet Nam, this is the commune.[7] In the outbreaks of highly pathogenic avian influenza (HPAI) H5N1 that have occurred in Viet Nam since December 2003 the commune (tertiary administrative unit, the lowest level of the admistrative system in Viet Nam) has been official unit of interest to define outbreak locations (MARD 2005*b*, Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009).

Similar area-based systems are present in other Asian countries. For example, in the recent outbreaks of HPAI H5N1 in Thailand the location of affected flocks was assigned at the village level using codes managed by the Thai Department of Livestock Development. These details were then aggregated to provide summaries at the sub-district (tertiary administrative unit) level, as shown in Figure 2.3 (see Tiensin et al. 2007, Tiensin et al. 2009 and Souris et al. 2010 for examples). In the early days of the epidemic of HPAI H5N1 in Indonesia outbreak details were recorded down to the district (secondary administrative unit) level (Pfeiffer 2006, Gilbert et al. 2008). Recent improvements to the Participatory

[7] In Viet Nam in 2012 there were three geographic levels of administration: the province ($n = 63$), district ($n = 718$) and commune ($n = 11{,}129$).

Disease Surveillance and Response (PDSR) database now allow disease event information to be recorded at the *desa* (village) level (Perry et al. 2009).

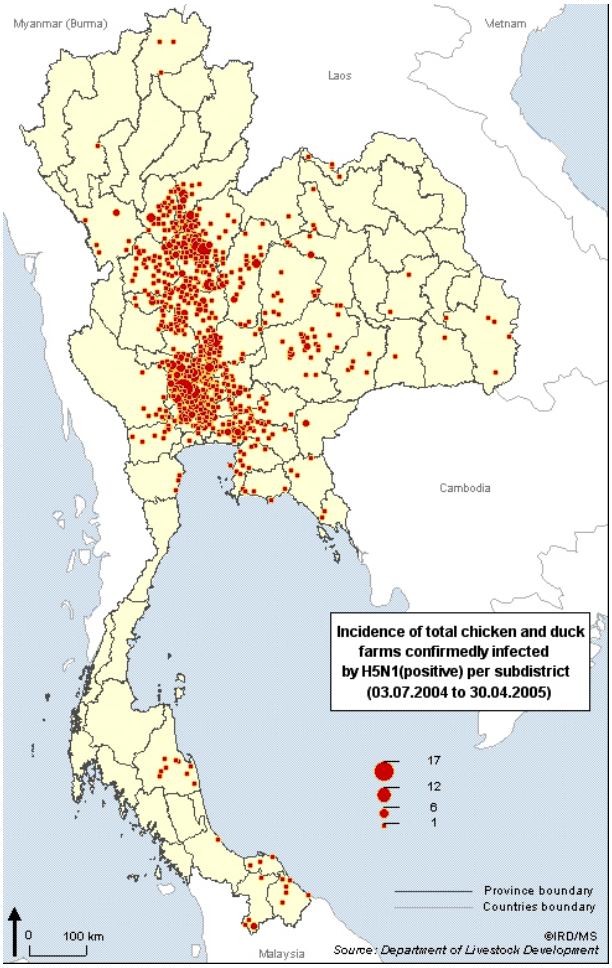


Figure 2.3: Map of Thailand showing the incidence of chicken and duck farms confirmed with HPAI H5N1, by sub-district (Souris et al. 2010). Reproduced with permission from the International Journal of Health Geographics.

A number of factors work against animal health authorities in developing countries in terms of the amount and level of detail of animal health information able to be routinely recorded. The first is that majority of production units are small-scale and run at the individual household level. For example, backyard poultry are ubiquitous in many Southeast Asian countries such as Cambodia, Lao PDR, Indonesia, Thailand and Viet Nam. In addition, the distribution of backyard herds and poultry flocks (in particular) are under a constant state of change as stock are frequently moved and sold (Minh et al. 2010). Rural areas, where animal disease problems tend to be greater, are characterised by poor communication and transport infrastructure (Baldock et al. 1999). Livestock owners are often unable to contact veterinary staff and it is difficult for veterinary staff to access livestock,

which means that provision of services and collection of the necessary information related to outbreak events is problematic. In addition, collection of detailed information at the individual herd or flock level depends heavily on political and financial incentives, which are not likely to be present in all developing countries.

As a case study, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson (2009) conducted a study of the spatial-temporal epidemiology of HPAI H5N1 in the MRD, Viet Nam. Using commune-level data these authors concluded that HPAI outbreaks that occurred between 2003-2007 were clustered in both space and time, and that disease transmission occurs by a combination of local and long-distance spread. A similar study was conducted in the same region in 2010. On this occassion the exact details of affected households were recorded using a global positioning device (Minh et al. 2011). In this study it was found that HPAI-positive households posed a risk to other households where poultry were present over a distance of up to 10 km and for a period of up to 12 days following detection. The second study, by capturing and analysing data at a much higher level of detail, confirmed the findings of the earlier study and was able to provide information that was more useful for field staff for disease control.

The Transboundary Animal Disease Information System (TADinfo),[8] developed by the Food and Agriculture Organization of the United Nations, provides an off-the-shelf and flexible solution for recording many of the items listed in Figure 2.1 (Kamata et al. 2009). The system is adaptable and fully customisable, combining a relational database with a mapping system allowing users to record details of disease observations, abattoir surveillance, livestock census details and vaccination records. The smallest unit of interest within the system is user defined with typical choices being either the individual farm or small area (such as the town or commune, in the case of Viet Nam).

Choice of an appropriate unit of interest at the time the system is implemented is important. If, for example, a country initially uses the small area as the unit of interest and then, at some later date decides to change over to a system based on individual farms data incompatability issues will arise. On the other hand, switching from an individual farm based system to one based on small areas presents little problems since the data are easily aggregated. These issues need to be thought thoroughly by animal health authorities at

[8]URL: `http://www.fao.org/ag/againfo/programmes/en/empres/tadinfo/about.html`

the time systems are implemented. In particular, thought needs to be given to the likely animal health information needs of the country 10 – 15 years into the future as well as immediate requirements.

2.4 The future

In developed countries, a major focus of animal health is to maintain food safety and provide transparent evidence of a country's disease status for the purpose of international trade. In developing countries, on the other hand, animal health activities tend to mainly concentrate on the control and management of infectious diseases such as FMD, CSF and HPAI H5N1. Some countries, such as Thailand, are at a cross road between the two, exporting food of animal origin (chicken meat) to other countries (Bowles et al. 2005) while at the same time having to deal with infectious disease outbreaks affecting predominantly household poultry populations. It is likely that many developing Asian countries will follow in the footsteps of Thailand, moving away from predominantly subsistence farming to being exporters of animal products. For this development to progress smoothly it is essential that a credible track record in food safety is developed and maintained so that these issues do not become a future barrier to trade. On-farm pesticide, anthelmintic, antibiotic and fertilizer use is an additional data component that could be recorded within a DSS providing an additional safeguard against chemical residues being held up as a trade barrier. This said, developing countries therefore need to develop a planned approach to data management, recognising that over time there will be a need to move away from using animal health data to control infectious disease outbreaks towards documentation of disease status to trading partners and consumers in relation to issues around food safety.

Given the expense of designing and implementing DSS components we propose that a standardised format for recording and storing animal demographic, productivity and health data needs to be agreed on. Widespread adoption of this format will help to make animal health DSS components more readily transferable from one jurisdiction to another, ultimately reducing their cost, other resources and in doing so helping to alleviate one of the important obstacles to more widespread uptake of this technology.

Just as, if not more important, than the development of the technology and infrastructure for data collection, it is vital to provide veterinary staff (particularly those in developing

countries) with the appropriate analytical skills so that maximum value is derived from the data that is actually collected. As an example, extremely large numbers of records are collected by animal movement databases, even in countries of moderate size. One of the skills required when analysing this data is the ability to summarise movements at the national level and then to 'drill down' on specific areas of interest identified from summary analyses (Martínez-López et al. 2009, Aznar et al. 2011). Extracting the maximum value out of animal movement data sets requires a broad range of analytical skills including epidemiology, social network analysis, and spatial statistics. Other analytical procedures, for example the design of surveillance strategies will rely on a different skill set including knowledge of sampling theory and economics.

Issues related to system sensitivity and specificity arise when DSSs are used to detect emerging diseases in an animal population. If the analyst is too sensitive in terms of declaring a pattern of disease events as indicative of an emerging syndrome investigative resources will be wasted. If, on the other hand, the analyst is too specific then it is possible that emerging syndromes that should have been identified and acted upon will be missed. To 'get the balance right' it is essential that those involved in the analysis of DSS data are given the opportunity to become familiar with its features, particularly temporal (e.g. seasonal) and spatial trends. Our only suggestion here is that these skills take many years to develop and part of the costing of implementing a DSS should include a component to select, train and appropriately renumerate skilled system analysts. Also, it is important for funding agencies and decision makers to realise that a DSS is not a replacement for clinical experience and a detailed local knowledge.

2.5 Conclusions

A decision support system of the type described in this chapter provides authorities with a valuable resource for recording, validating, storing and analysing animal health data. Outputs from such systems can be used to manage and control outbreaks of infectious disease in animals, identify factors associated with the presence of disease, provide an objectively measured point of comparison once control measures have been implemented and finally, provide an additional means for detection of emerging disease syndromes.

As we have shown a DSS is comprised of a number of components, some of which are

expensive and time consuming to implement. For this reason, the current situation is that many food producing countries throughout the world have individual components of a full system in place and fully operational systems are the exception rather than the rule.

Unlike the situation in developed countries, developing countries tend to use DSSs for the management and control of infectious disease outbreaks with data typically aggregated at the small area level. Animal health managers in developing countries should be aware that, over time, focus will shift from management and control of infectious diseases to food safety and transparent documentation of animal disease status. A planned approach to animal health data management is therefore required, inevitably meaning that the unit of interest of DSSs need to shift away from the small area level to that of the individual producer.

Given the expense of designing and implementing DSS components we propose that a standardised format for recording and storing animal demographic, productivity and health data needs to be agreed on. Widespread adoption of this format will help to make animal health DSS components more readily transferable from one jurisdiction to another, ultimately reducing their cost. Just as important is the need to provide veterinary staff with the appropriate analytical skills so that maximum value is derived from the data actually collected.

CHAPTER 3

The design of a decision support system for animal health management in Viet Nam

Abstract – Infectious animal diseases including highly pathogenic avian influenza caused by H5N1 virus, foot-and-mouth disease, porcine reproductive and respiratory syndrome and classical swine fever regularly occur in Viet Nam. A goal of the Vietnamese Department of Animal Health is to control these diseases and, ultimately, to eradicate them. To this it is essential that animal health authorities have up to date information on animal populations at risk and incident cases of disease. This paper describes the components and design features of an animal health decision support system for use in Viet Nam. The objectives of the system are to: (1) enhance data management by improving mechanisms for data collection, validation and storage; and (2) provide tools for epidemiological analyses and surveillance design. A feature of our design is that it is web-enabled allowing authorised users, no matter where they are located throughout the country, to query and report on data entered into the system. A second feature is the inclusion of a powerful and flexible reporting tool that will provide users with the capability of developing reports to deal with virtually any animal health issue. Continued use of this system will allow the Vietnamese Department of Animal Health to identify and respond to existing and emerging threats to animal health in a timely and cost-effective manner.

Long, N.V., Stevenson, M., OLeary, B., Sujau, M. and Morris, R. (2013) The design of a decision support system for animal health management in Viet Nam.

3.1 Introduction

A decision support system (DSS) can be defined as an interactive, flexible and adaptable system (including, but not limited to, computer-based systems) comprised of relevant databases, technologies and appropriate analytical techniques to identify problems, predict consequences and provide solutions for improved decision making (Turban 1995). In the context of animal health a DSS can be used to collect, store, and manage data that can be used for a number of purposes including descriptive and analytical epidemiological analyses, the design of disease surveillance strategies and risk analyses. In a broader context, and extending the concept of 'One Health' (King et al. 2008) to that of 'Global Health' an animal health decision support should be thought of as a single component within a suite of systems to record, monitor and report on factors influencing the health of human and wildlife populations as well as the environment.

EpiMAN was one of the first decision support systems used in animal health (Sanson 1993). It was comprised of a database management system (DMS), a geographic information system (GIS) and a graphic user interface (GUI). Primarily intended to support decision making during infectious disease outbreaks in farmed livestock populations, EpiMAN was designed to allow users to conduct descriptive analyses of infectious disease outbreak data, manage human resources during an infectious disease outbreak (e.g. scheduling of patrol visits) and to carry out disease simulation modelling. Recent examples of animal health DSSs include the RADAR (Rapid Analysis and Detection of Animal-Related Risks) system in the United Kingdom (Lysons et al. 2007, Paiba et al. 2007, Department for Environment, Food and Rural Affairs 2011) and KODAVET (Koordiniertes Datenverwaltungs und Analysesystem des Veterinärdienstes Schweiz), a system developed to manage information about food producing and companion animals in Switzerland (Presi & Heim 2010).

Partial animal health DSSs exist in a number of countries such as the Australian National Livestock Identification System (Australian Department of Agriculture, Fisheries and Forestry 2006), the National Animal Identification Tracing System in New Zealand (NAIT 2011), the Sanitary Management System database, maintained by the National Service for Agrifood Health and Quality in Argentina (Bowling et al. 2008) and the Serviço de Rastreabilidade da Cadeia Produtiva de Bovinos e Bubalinos (SISBOV) in Brazil (Car-

doso & Cardellino 2004*b*). A general comment is that these systems are highly specific in that they have been set up to manage the identification of animals within a given jurisdiction and to keep records of the movement of animals from one location to another.

This paper describes the components and design features of an animal health DSS for use in Viet Nam. Although the focus of the system in this paper is on how it might be used in a Vietnamese context it should be noted that the system has been designed to be generic; that is, it can be readily adapted for use in other countries. Our description of the system is divided into three main sections. In the first we describe the overall system design. The second section provides a detailed description of the first released system currently operating at the Department of Animal Health in Viet Nam. The third and final section provides a brief discussion of the development and use of animal health DSSs in developing countries.

3.2 System design

The Vietnamese animal health DSS[1] is a secured web-based system comprised of four main components (Figure 3.1): (1) a data warehouse, (2) a graphic user interface, (3) a set of reporting tools, and (4) a spatial database and geographic information system to support spatial queries of the data and mapping.

3.2.1 Database structure

Details of domestic animal populations at risk in Viet Nam (e.g. cattle, buffaloes, chickens, and ducks) and details of health events affecting these populations are stored in a series of tables in the DSS collectively referred to as the 'data warehouse'. The data warehouse (Figure 3.1) is a relational database that allows data to be stored, managed and queried. The Vietnamese DSS data warehouse was designed to follow a three-level data architecture principle (Connolly & Begg 2001): external, conceptual and internal. A primary objective of this three-level architecture is to have data independence, which means that changes in each of the three levels such as adding, removing and modifying entities, data attributes, relationships and reports do not result in changes in the other two levels.

[1]URL: `http://www.epimanager.com/dss`. Username: `dss`. Password: `pass`.

The external level is a specific view of a database and is comprised of entities, data attributes, data relationships and reports available to a given user of the system. An important characteristic of the external level is that any change to a user view is unique to that user — the view seen by other users of the system is unaffected.

The conceptual level defines what data are actually stored in the system and the relationships that exist between data components (Figure 3.2). This is one of the important components of the system because it presents all entities, data attributes and relationships, the constraints on the data, the semantic information about data and system security and integrity information. The internal level is the physical representation of the database and describes how data are stored in the system. A typical data warehouse has at least ten services (Connolly & Begg 2001), as summarised in Table 3.1.

A data warehouse can be developed using two main approaches: either a static object model or a dynamic object model. Most small-scaled database systems are based on a static object model which means that details of the database design are fixed at the time of system design. On the other hand, complex database systems are based on a dynamic object model (Riehle et al. 2000) which means that additional tables and relationships can be added to the system (as individual needs arise) and the system interprets these changes accordingly. The Vietnamese animal health DSS has been developed using a dynamic object model. This approach allows users to add new objects to the system (e.g. additional variables in existing data tables) or to modify existing objects at runtime without having to re-program the system. For example, descriptive analyses of post vaccination serosurveillance data in poultry might lead investigators to develop the hypothesis that vaccinator technique might be an important determinant of vaccination success. To test this hypothesis it would be necessary to include the name and identity details of individuals carrying out vaccination events in poultry flocks. If a field did not already exist in the vaccinations table to identify the individual carrying out a vaccination event the dynamic object model approach would allow the vaccination table to be modified to include this additional variable.

Data within the dynamic object model-based system can be classified into two main groups: ‘objects’ and ‘events’. An object is defined as a physical entity within the system such as a commune, a farm, slaughterhouse or an individual animal. Within the system details objects can have attribute information for example the date the object entered the

system (i.e. birth date in the case of an individual animal) and the date the object left the system (the date of death or slaughter). An event, on the other hand, is something that can happen to an object during the time it is present within the system. An object (for example, a commune) may experience several animal disease outbreaks that can be recorded as events over a given observation period. Object and event data are linked together through the 'entity' relationship using an internal key system derived from the schema. The entity relationship can be one-to-one, one-to-many or many-to-many. An example of one-to-one relationship in the system is that each commune has only one veterinary chief at any given time. On the other hand, single communes can have several veterinarians which would be an example of a one-to-many relationship. Although records for animal disease events are, at the time of writing, typically recorded at the commune level (the lowest administrative unit in Viet Nam), a time will come when data will need to be recorded at either the individual village, individual household or individual animal level. The data warehouse of the Vietnamese animal health DSS therefore needs to be easily extendable to accomodate these changes. The data warehouse of the Vietnamese DSS was developed using the Microsoft .NET framework 4.0 and Microsoft SQL Server 2008 R2 (Microsoft Corporation 2010).

3.2.2 Graphic user interface

The graphic user interface (GUI) is the representation of the external level of the three-level architecture described above. A typical GUI has two components: input and output objects that are typically designed with common icons such as buttons and pull-down menus to allow end-users to communicate efficiently with the system. The GUI of the Vietnamese animal health DSS is based on a web based design using hypertext markup language (HTML), cascading style sheets (CSS) and JavaScript. The system is designed to operate using all of the major web browsers currently in use including Firefox,[2] Google Chrome,[3] and Microsoft Internet Explorer.[4] The design of the GUI follows a typical Microsoft Windows architecture-behaviour. Using the GUI system users can import and query data within the system using filters. The GUI provides system users with the ability

[2]URL:`http://www.mozilla.org`
[3]URL:`http://www.google.com/chrome`
[4]URL:`http://www.microsoft.com`

to present the results of analyses in the form of summary tables, graphs and maps. In addition, it allows system administrators to manage system security using a Role-Based Access Control (RBAC) pattern.

The GUI of the Vietnamese animal health DSS is comprised of three main windows: a dashboard window, a projects window and a setup window (Figure 3.3).

The dashboard window is the default window for the GUI and is comprised of three sub-windows: (1) a window listing the active projects the log-in user is assigned to work on, (2) a window of reports showing the latest set of results, and (3) a window listing reports relevant to the log-in user. These three sub-windows can be minimised individually to save screen space. A refresh button allows all reports to be updated as new information is entered into the system. This facility is of most use during (for example) a foot-and-mouth disease outbreak when the number of infected places is changing rapidly.

The project window lists the name and description of the projects assigned to the log-in user. Each of the projects listed in this window provides: a tab providing summary information about the project, a properties tab which allows users to configure their project, a tab for importing data from external sources, and a tab for searching data within the system. In addition, the projects window provides the user with customisable labels to group data and reports for quick access.

The setup window is one of the most important interface components of the Vietnamese animal health DSS. Four main activities can be configured using this window: (1) the project manager allows users to create, modify or delete any projects (if they have appropriate permissions to do so); (2) the organisation manager allows the system manager to add, modify or remove organisations currently using the system; (3) the user manager allows the system manager to create new users and then specify their roles within the system; and (4) the translation manager allows the system manager to configure the system in using their choice of language. The current system is configured in both English and Vietnamese.

A strong point of the DSS is that users are not required to enter data into the system while they are online. Data can be entered directly into a spread sheet and then uploaded into the system when an Internet connection becomes available. In addition, data tables common to individual users can be shared using a link function. This facility minimises the

likelihood of recording duplicate data such as geographic details and animal demographic data (e.g. the number of individual livestock species per commune).

3.2.3 Reporting tools

Several steps need to be carried out before a regular user of the system can view reports generated by the system. Firstly, data need to be prepared for analysis using the filtering tools. The filter allows a user to query the data using specific criteria (e.g. details of events on the basis of location and/or time). Under the setup window of a given project, a filter can be created to query data from a single or multiple tables. From a single table, the user can select any number of variables and add criteria if necessary. Data from multiple tables can be queried using a join function whereby tables are linked ('joined') using the primary keys present in each table. Once created filtered tables can be used for reporting.

Table 3.2, Figure 3.4 and Figure 3.5 provide an example of a typical set of reports produced by the Vietnamese animal health DSS system. The reporting engine is powered by the open source statistical package, R (R Development Core Team 2012). This provides users and/or system administrators with complete freedom to develop their own reports (tables, graphs, maps), taking advantage of the vast analytical capabilities within the R system itself and packages contributed to R by its large group of users. There are two main options for generating reports within the analysis window. Option one allows users to run pre-defined reports that come pre-loaded with the project. In practice these reports would be designed by a senior veterinary epidemiologist managing a given project. Typical examples of pre-defined reports for infectious disease outbreak data would include epidemic curves and EDR (estimated dissemination ratio) plots. Option two allows users to generate their own reports. This can be done by developing their own R code from scratch or by copying the code required to run an existing report and then modifying it.

Geographic information system

A Geographic Information System (GIS) is defined as a computer system for capturing, storing, checking, integrating, manipulating, analysing and displaying data related to positions on the Earth's surface. The Vietnamese animal health DSS has a built-in GIS which allows users to specify the location of objects within the system (e.g. communes,

Table 3.1: Ten services of the Vietnamese animal health DSS data warehouse.

Service	Functions within the Vietnamese animal health DSS
Store, update and retrieve data	Allow users to enter or import, update and query data.
User-accessible catalogue	Provide a means for describing data items stored and accessible to users.
Support data transactions	Regulate all updates corresponding to a given data transaction.
Concurrency control services	Regulate all data to be updated correctly although these updates can be carried out by multiple users.
Data recovery	Automatically back up the database at regular intervals.
Service authorisation	Ensure that only authorised users have access to components of the system.
Data communication support	Allow integration with other database systems.
Integrity services	Manage all data within the system and ensure that changes follow defined business rules.
Services to promote data independence	Support independence of programs from the actual structure of the database.
Utilities	Provide system utilities for database management.

herds-flocks of animals). At the level of the data table within the system a variable type of 'spatial' can be defined which allows the user to specify the character of the spatial variable of interest as either a polygon, line or point. In Viet Nam it is frequent that the boundaries of administrative areas change as a result of rearrangement or division, and for this reason it is important to that the system is able to store historical spatial data sets by including a variable to record the date on which a spatial variable was updated.

Spatial data used in the Vietnamese animal health DSS are derived from three main sources: (1) Open Street Map (Haklay et al. 2008), (2) Google Maps (Google Corporation 2013), and (3) the government of Viet Nam (NARENCA 2012). The NARENCA data are typically imported into a dedicated server computer and updated as the need arises. Online GIS data sources are rely on an Internet connection and persistent provision of data from the relevant data providers. Similar to the approach described for data recorded in text format, spatial data can be queried and the results of spatial queries used for reporting.

Administration

The Vietnamese animal health DSS is a web-secured system designed to run on centrally managed servers, with broadband Internet access required to allow users in remote locations (e.g. regional offices) access the system. Where broadband Internet access is not available it is anticipated that data will be recorded in hard copy format and then transferred into the system by download at a later date. All users of the system will have to be authorised by a system administrator, with login credentials and security permissions.

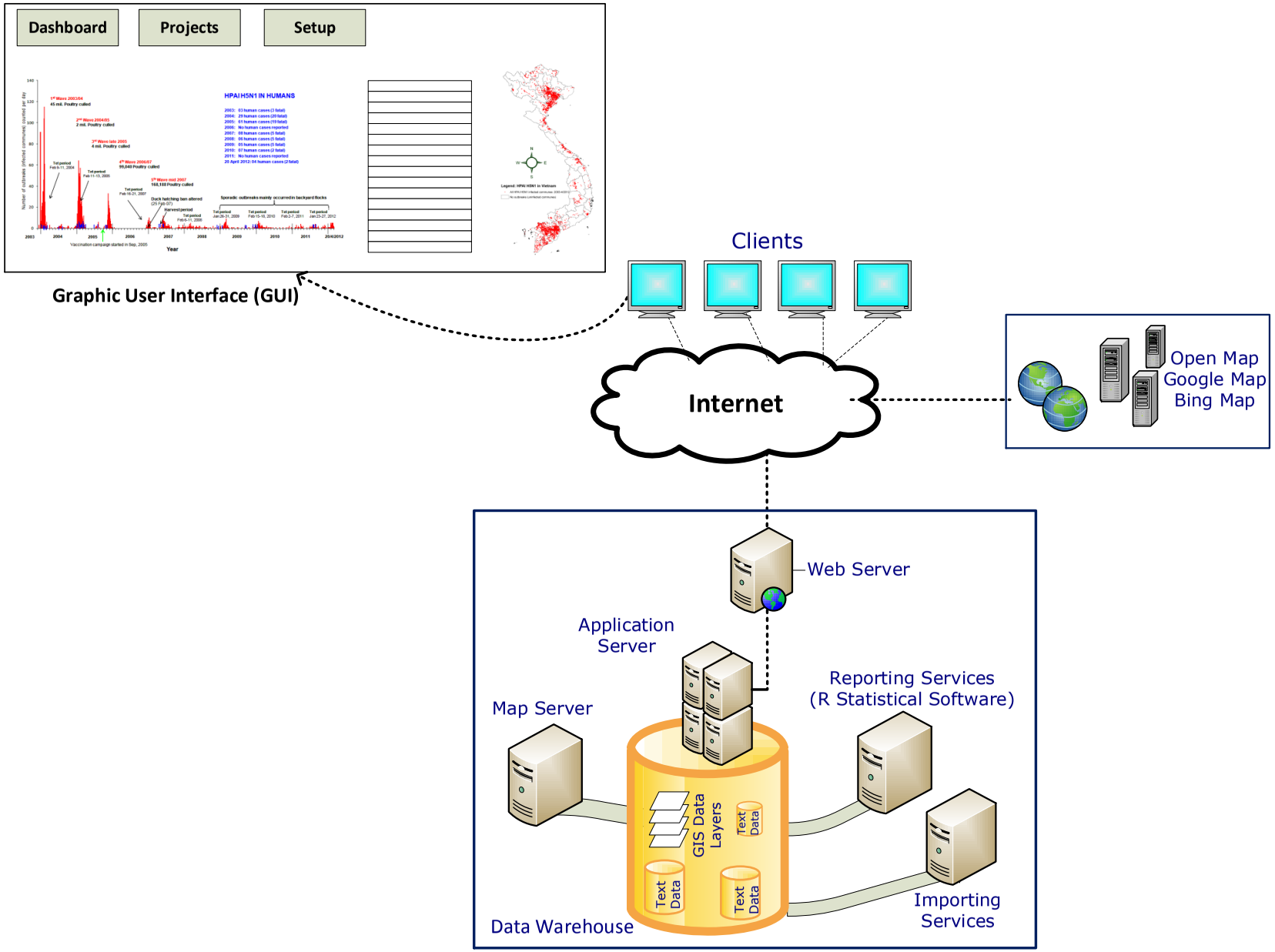


Figure 3.1: Diagram showing the structure of the Vietnamese animal health DSS. The system is comprised of three components: A data warehouse, reporting tools and a graphic user interface.

Table 3.2: Example tabular report produced by the Vietnamese animal health DSS. Table presenting the incidence risk of HPAI H5N1 infected communes for the period 1 January to 31 December 2004.

ID	Province name	Infected districts	Infected communes	Poultry households at risk	Infected households	Poultry at risk	Infected poultry	Household incidence risk	Poultry incidence risk
		n	n	n	n	n	n	(95% CI)	(95% CI)
1	Ba Ria Vung Tau	1	2	116	2	4,752	1,922	1.72 (0.21 –6.09)	40.45 (39.05 – 41.86)
2	Bac Lieu	4	10	14,552	10	551,621	16,821	0.07 (0.03 – 0.13)	3.05 (3.00 – 3.09)
3	Bac Ninh	1	1	1,510	1	41,399	49	0.07 (0.00 – 0.37	0.12 (0.09 – 0.16)
4	Ben Tre	4	11	13,496	11	224,713	38,703	0.08 (0.04 – 0.15)	17.22 (17.07 – 17.38)
5	Binh Phuoc	1	1	816	1	15,993	28	0.12 (0.00 – 0.68)	0.18 (0.12 – 0.25)
6	Ca Mau	1	2	2,320	2	104,860	2,183	0.09 (0.01 – 0.31)	2.08 (2.00 – 2.17)
7	Can Tho	7	13	12,037	16	139,048	21,699	0.13 (0.08 – 0.22)	15.61 (15.42 – 15.80)
8	Dong Thap	6	15	19,402	17	303,666	22,914	0.09 (0.05 – 0.14)	7.55 (7.46 – 7.64)
9	Ha Noi	3	4	2,320	4	492,383	2,622	0.17 (0.05 – 0.44)	0.53 (0.51 – 0.55)
10	Hai Duong	6	8	11,091	8	336,464	21,1463	0.07 (0.03 – 0.14)	62.85 (62.72 – 62.98)
11	Hai Phong	5	8	9,606	8	352,012	26,201	0.08 (0.04 – 0.16)	7.44 (7.36 – 7.53)
12	Hau Giang	3	5	11,088	6	300,514	9,147	0.05 (0.02 – 0.12)	3.04 (2.98 – 3.10)
13	Kien Giang	1	1	1,156	1	35,590	5,500	0.09 (0.00 – 0.48)	15.45 (15.08 – 15.83)
14	Long An	4	5	4,444	6	1,326,547	30,840	0.14 (0.05 – 0.29)	2.32 (2.30 – 2.35)
15	Ninh Thuan	1	2	247	2	16,682	1,490	0.81 (0.10 – 2.89)	8.93 (8.50 – 9.37)
16	Quang Nam	1	1	1,652	1	22,092	225	0.06 (0.00 – 0.34)	1.02 (0.89 – 1.16)
17	Quang Ninh	2	2	2,220	2	67,897	1,793	0.09 (0.01 – 0.33)	2.64 (2.52 – 2.76)
18	Quang Tri	1	1	1,183	1	26,700	100	0.08 (0.00 – 0.47)	0.37 (0.30 – 0.46)
19	Soc Trang	1	1	-	-	-	880	-	-
20	Son La	2	2	1,876	2	6,057	53	0.11 (0.01 – 0.38)	0.88 (0.66 – 1.14)
21	Tay Ninh	1	1	1,317	1	27,960	11,287	0.08 (0.00 – 0.42)	40.37 (39.79 – 40.95)
22	Thai Nguyen	1	1	2,018	1	42,540	30	0.05 (0.00 – 0.28)	0.07 (0.05 – 0.1)
23	Thanh Hoa	5	7	10,934	7	114,212	3,887	0.06 (0.03 – 0.13)	3.40 (3.30 – 3.51)
24	Tien Giang	5	10	13,317	10	533,275	15,237	0.08 (0.04 – 0.14)	2.86 (2.81 – 2.90)
25	Tra Vinh	2	2	2,457	2	43,068	10,100	0.08 (0.01 – 0.29)	23.45 (23.05 – 23.85)
26	Vinh Long	1	2	2,776	2	39,921	4,000	0.07 (0.01 – 0.26)	10.02 (9.73 – 10.32)
26	Vinh Phuc	3	3	2,381	3	113,362	1,108	0.13 (0.03 – 0.37)	0.98 (0.92 – 1.04)

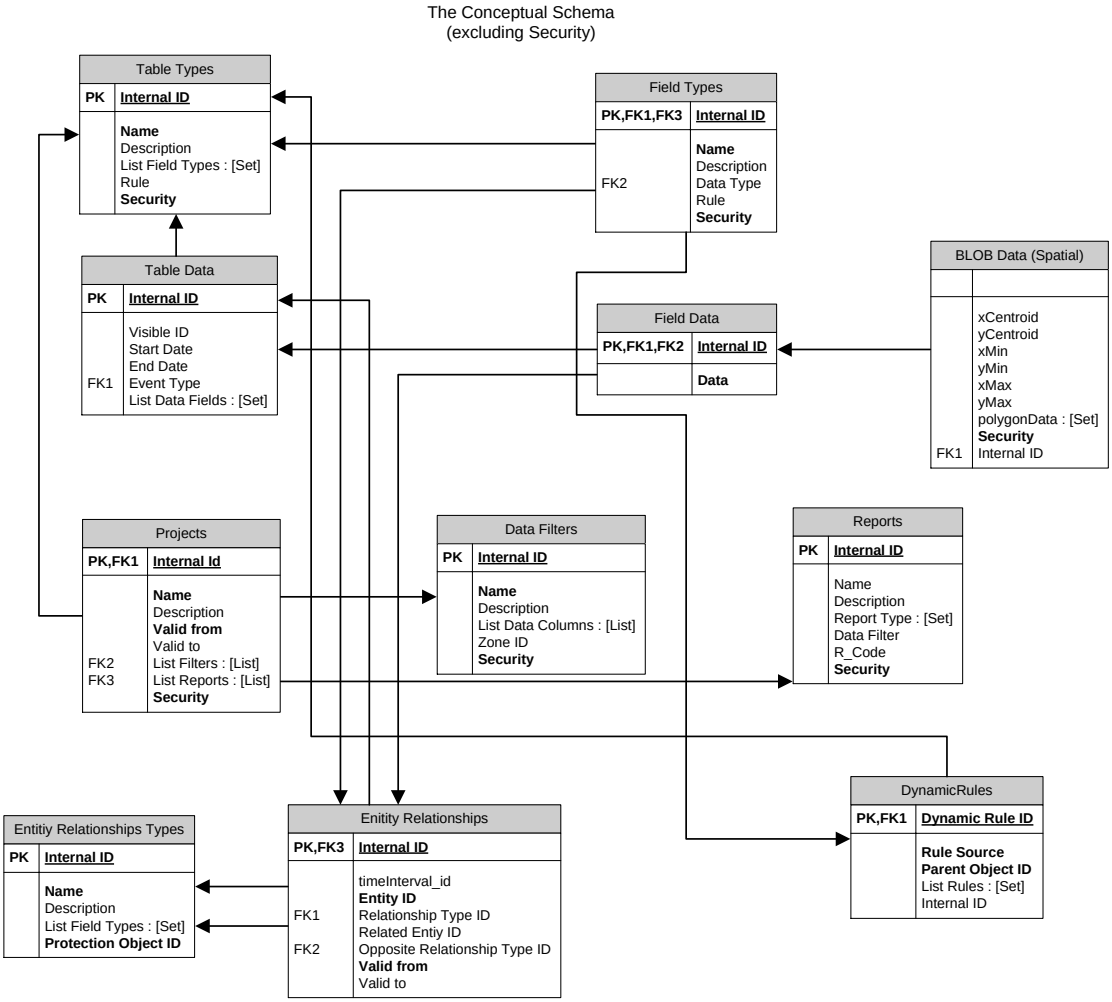


Figure 3.2: System diagram representing the conceptual design of the data warehouse.

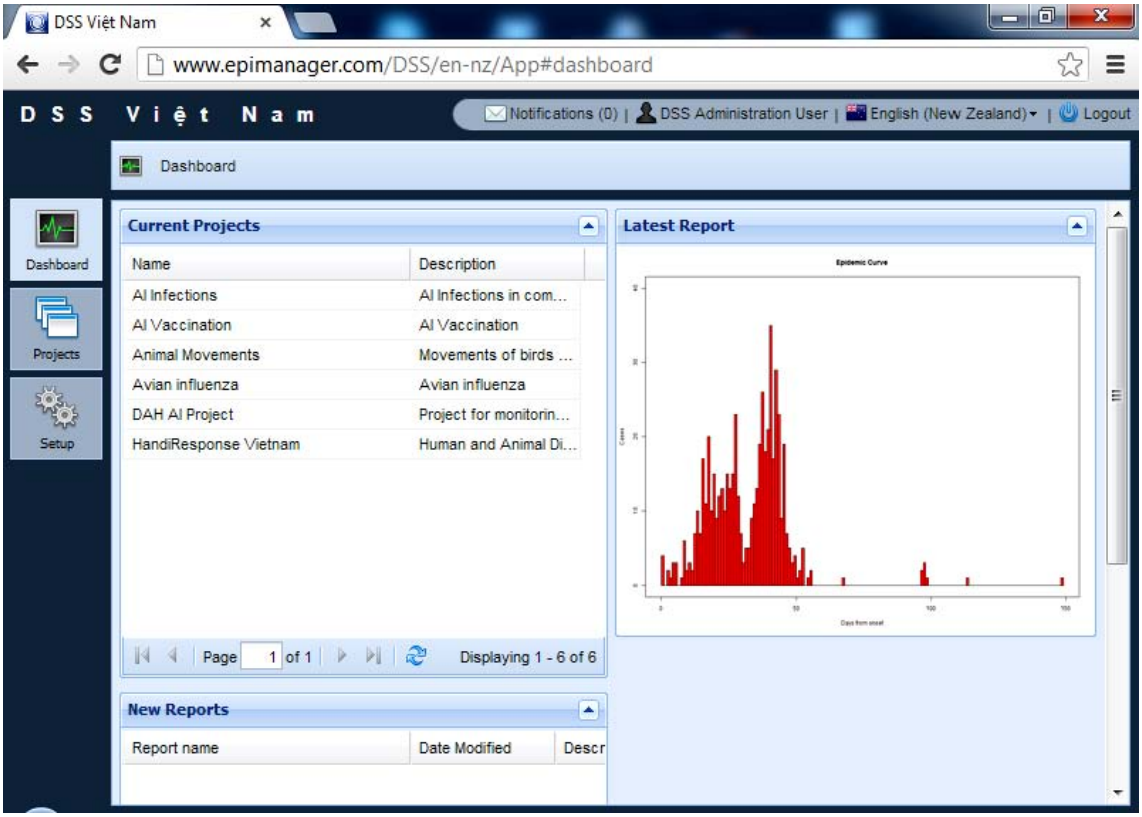


Figure 3.3: The graphic user interface of the Vietnamese animal health DSS.

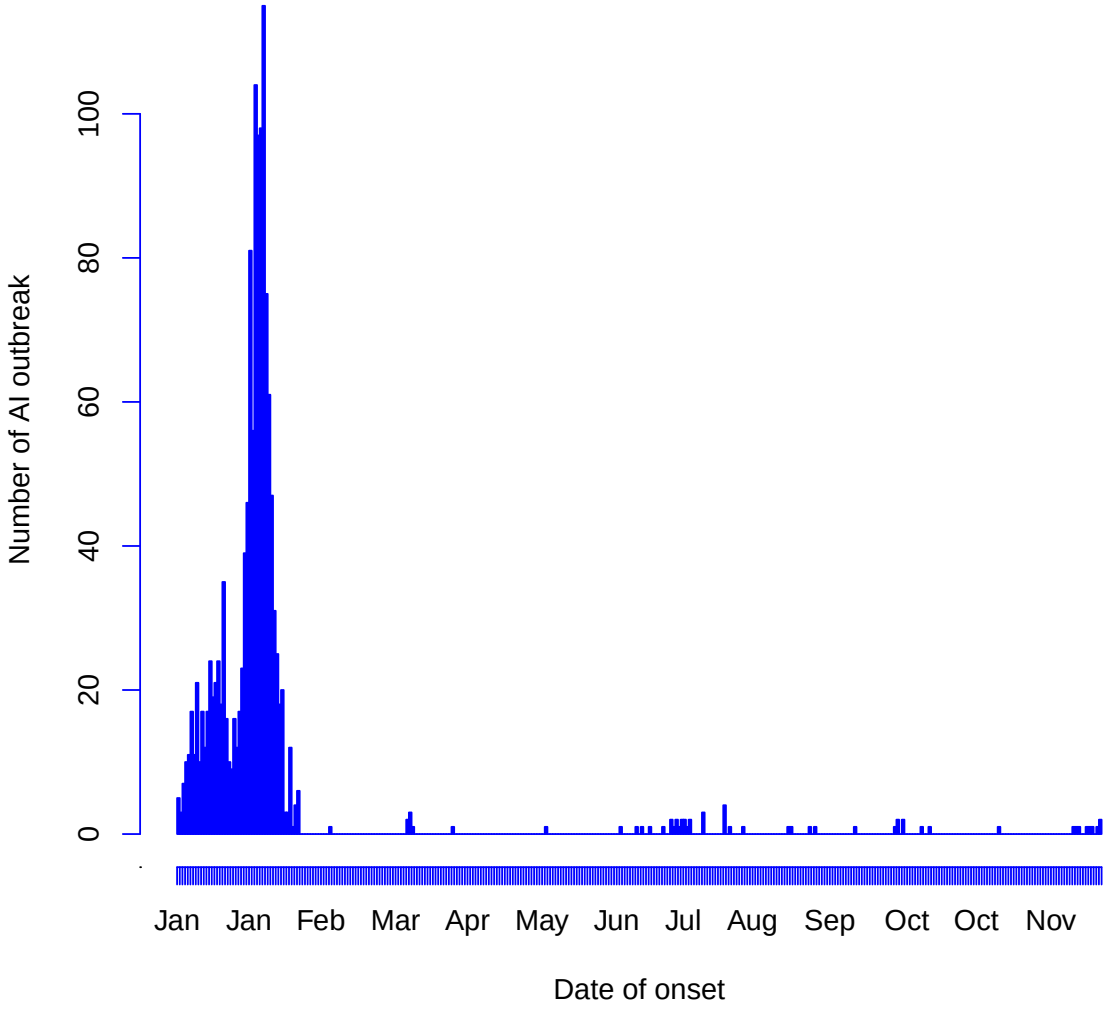


Figure 3.4: Example graphical report produced by the Vietnamese animal health DSS. Epidemic curve of HPAI H5N1 outbreaks (the number of newly infected communes per day) for the period 1 January to 31 December 2004.

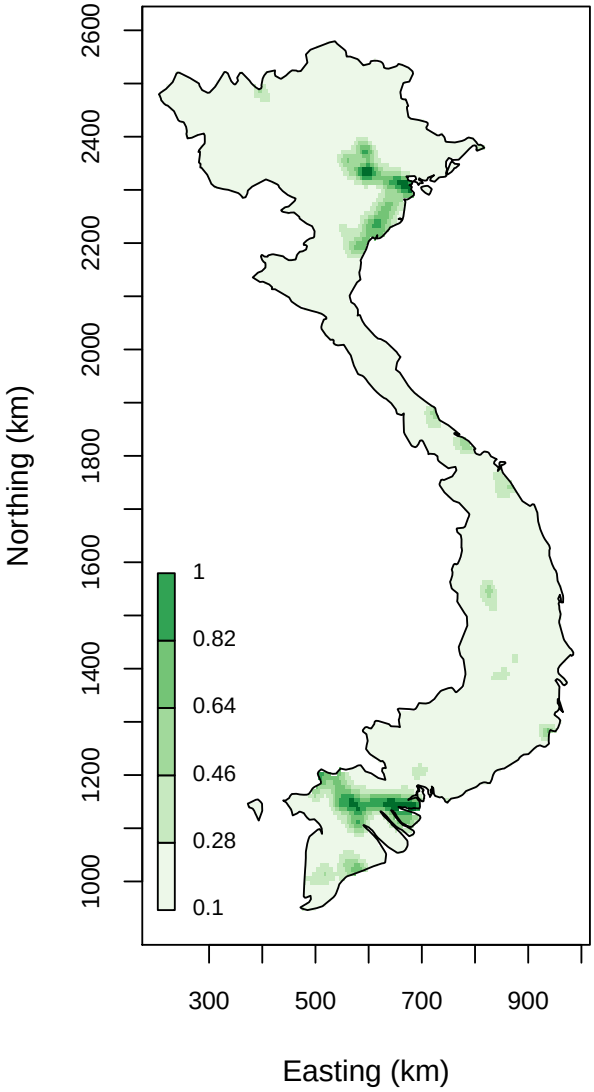


Figure 3.5: Example map report produced by the Vietnamese animal health DSS. Kernel smoothed raster surface showing the proportion of HPAI H5N1 infected communes per square kilometre for the period 1 January to 31 December 2004.

3.3 Discussion

A key objective of the Vietnamese animal health DSS is to provide a means to reduce the number of errors in animal health data and to develop a system whereby animal health data can be analysed quickly and easily to support decision making. As well as providing tools to assist in the management of endemic infectious diseases of livestock we believe that ultimately the system will allow emerging and re-emerging diseases to be detected, ensuring that appropriate field operations and resources are deployed to deal with identified problems if and when they occur. Such a system will provide greater transparency in Viet Nam's animal health status, improving trading partner confidence and allowing animal health policies to be based on the best available evidence.

An effective DSS should not only be well designed but also be supported by an appropriate level of hardware infrastructure. To achieve this objective high capacity computer servers and broadband Internet access are essential to allow the system to operate properly for all users, particularly those located in remote areas of the country. It is important to remember that investments in computer hardware are not one-off costs and it is important that funds are made available to maintain and upgrade system hardware when the need arises. This issue is particularly important when the development of the system gets to the point where it is actively used by staff in all regional DAH offices. In addition, it is important to provide users of the system with on-going training to allow them to derive maximum benefit from the system.

In developed countries either the herd (flock) or the individual animal are typically the units of interest when analysing animal health data. In developing countries, because of the lack of infrastructure to uniquely identify groups of animals (herds or flocks) and/or individual animals, the units of interest are typically at a higher level, often the village or, in the case of Viet Nam, the commune. As systems to uniquely identify groups of animals become established in Viet Nam it is important that the tabular architecture within the DSS is able to be modified accordingly. The dynamic object model which forms the basis of the design of the data warehouse ensures that this transition will take place without the need for a major re-design of the system architecture.

Simulation models are powerful tools that can be used to support decision making around the most appropriate way to handle infectious disease outbreaks in animal populations

(deJong 1994, Saatkamp et al. 1996, Morris et al. 2001, Sorensen et al. 2001, Keeling et al. 2003, Yoon et al. 2006, Stevenson et al. 2013). Although they have the potential to be an extremely useful when managing infectious disease outbreaks, disease simulation models need to be populated with a considerable amount of detailed data such as the number of susceptible animal species present at each farm (or commune) location as well as details of the frequency of animal movement events and the distances over which animals travel when movement events take place (Stevenson et al. 2013). An additional important function within an animal health DSS therefore, is to provide the facilities that will allow data to be recorded and then reported in a format that makes it easy for end users to develop simulation models of animal disease. As noted earlier the dynamic object model design on which the Vietnamese animal health DSS is based makes this objective achievable, particularly if animal movement events are routinely entered into the system.

3.4 Conclusions

This paper describes the components and design features of an animal health DSS for use in Viet Nam. Although the focus of the system in this paper is on how it might be used in a Vietnamese context it should be noted that the system has been designed to be generic and therefore readily adaptable for use in other countries. We conclude that the system provides a means for recording and storing animal health data that reduces the likelihood of data duplication. The powerful reporting tool built into the system provides users with a reporting capability flexible enough to deal with virtually any animal health issue. It is important that the system is supported by computer servers with appropriate capacity to allow the system to operate properly for all users, particularly those located in remote areas of the country.

CHAPTER 4

Descriptive results of a prospective cohort study of avian influenza in the Mekong River Delta of Viet Nam, 2008 – 2010

Abstract – A prospective cohort study of avian influenza infection in poultry flocks was carried out in the Mekong River Delta of Viet Nam between December 2008 and April 2010. Our objectives were to: (1) estimate the prevalence and incidence of avian influenza virus infection, and (2) assess the efficacy of H5N1 vaccination programs as indicated by the presence of H5 antibody in vaccinated and unvaccinated poultry.

Real-time PCR and H5 Multiplex assays were used to detect the antigen of avian influenza viruses from swab samples. The haemagglutination inhibition test was used to detect H5 antibody. A total of 17,968 swab and 14,878 blood samples were collected from 5,476 birds over the study period. The overall incidence rate of influenza Type A virus infection was 5 (95% CI 4 – 7) positive birds per 100 bird-months at risk. The overall incidence rate of H5 virus infection was 0.2 (95% CI 0.1 – 0.5) positive birds per 100 bird-months at risk. Fifty (95% CI 48 – 52) birds per 100 tested birds were H5 HI positive in the unvaccinated group compared with 71 (95% CI 69 – 73) birds per 100 in the vaccinated group.

Influenza Type A and H5 viruses were circulating in village poultry throughout the study period with no recorded signs of clinical disease. This implies that interventions need to be carried out continuously throughout the year rather than only focusing on the established high risk periods. Broiler ducks had an incidence rate of influenza H5 virus infection approximately four times greater than that of layer ducks and in-contact species. We conclude that broiler ducks are likely to be the main entry route for H5 virus into poultry flocks in the MRD. Control efforts would benefit from understanding why there is a difference between villages in H5 incidence, and developing strategies to provide greater protection to broiler ducks.

Long, N.V., Stevenson, M., Schauer, B., Diep, N.T., Quy, T.D., Tien, T.N., Phuong, T.T.T., Prattley, D. and Morris, R. (2013) Descriptive results of a prospective cohort study of avian influenza in the Mekong River Delta of Viet Nam. *Transboundary and Emerging Diseases*. doi: 10.1111/tbed.12055.

4.1 Introduction

Avian influenza (AI) caused by the highly pathogenic avian influenza (HPAI) H5N1 virus has the potential to produce severe disease not only in animals but also in human populations. Since 2003, HPAI H5N1 outbreaks have been reported in domestic and wild avian populations throughout the Asia-Pacific and Middle Eastern regions, Europe and Africa (OIE 2012). HPAI H5N1 outbreaks have resulted in the loss of millions of domestic poultry, principally as a result of culling. This has had a substantial negative impact on rural economies, particularly those in developing countries such as Viet Nam. Up to 10 August 2012 a total of 608 cases of HPAI H5N1 in humans, 359 of them fatal, have been reported to the World Health Organization (WHO 2012). After Indonesia (n = 191) and Egypt (n = 168), Viet Nam has reported the third highest number of human cases (n = 123) with 61 deaths (WHO 2012).

From late 2003 to December 2011 there were over 4,000 commune-level outbreaks of HPAI H5N1 in Viet Nam (DAH 2012) where the diagnosis of disease was based on either the presence of characteristic clinical signs and/or real-time reverse transcription polymerase chain reaction (RRT-PCR) confirmation. The disease was mainly confined to areas of intensive poultry production in the Mekong and Red River Deltas (MARD 2009). Since 2005, the Vietnamese government has implemented a number of measures to control HPAI H5N1 in poultry including vaccination, steps to reduce the overall size of the duck population and education campaigns to raise flock owner awareness about the disease. As a result, HPAI H5N1 has largely been brought under control, such that in the period March 2009 to December 2011 there was only a small number of sporadic outbreaks (n = 44) in backyard flocks (DAH 2012). In addition, active surveillance has been undertaken since 2005 to evaluate the efficacy of the HPAI H5N1 vaccination strategy and to monitor the evolution of the HPAI H5N1 virus (Wan et al. 2008, Nguyen et al. 2009, Long 2010, Diep 2011).

Several studies have been carried out to better understand the epidemiology of HPAI H5N1 in Viet Nam. A case-control study was conducted in the Mekong River Delta (MRD) to investigate risk factors for the 2006 – 2007 HPAI H5N1 outbreaks (Henning et al. 2009). This study found that there was an increase in the risk of HPAI H5N1 outbreaks in poultry flocks that shared scavenging areas. A longitudinal study in the

same region was carried out between May 2007 and May 2008 (Henning et al. 2011). The aim of this study was to estimate the prevalence of H5 viruses in unvaccinated and vaccinated poultry flocks using serology (haemagglutination inhibition, HI) and antigen detection (RRT-PCR) tests. (Henning et al. 2011) found that 17% (95% CI 14 – 21%) of unvaccinated ducks and 11% (95% CI 7 – 14%) of unvaccinated, in-contact species were seropositive with an overall flock-level virus prevalence, as estimated by RRT-PCR, of 0.7% (95% CI 0 – 2%). In the Red River Delta, a case-control study was carried out to identify risk factors for HPAI H5N1 at the village and poultry-owning household levels (Desvaux et al. 2011). Desvaux and colleagues showed that villages with traders and villages with high proportions of broiler duck flocks had an increased risk of the disease. At the individual household level the risk of HPAI H5N1 outbreaks was increased when multiple animal species were present, where there were large numbers of birds and where there was ready access to pond water.

Besides HPAI H5N1 virus, low pathogenic avian influenza (LPAI) viruses including the H3, H4, H9 and H11 subtypes are known to circulate in Viet Nam (Nguyen et al. 2005, Nomura et al. 2011). The concurrent circulation of these viruses is thought to influence the epidemiology of HPAI H5N1 due to interaction and the potential for viral reassortment. Knowledge of LPAI viruses that circulate within a poultry population provides useful information for understanding factors influencing the transmission of HPAI viruses. This is because LPAI viruses generally circulate at a higher prevalence within a population which means that factors influencing transmission dynamics can be quantified without the need to enrol large numbers of study subjects.

A number of questions still need to be answered about the epidemiology of AI in Viet Nam. Firstly, it is not known where the virus resides between epidemics. Survival of the virus in the environment needs to be better understood, as does whether or not the virus is changing in ways that affect its circulation. Secondly, the roles of itinerant grazing (field running) ducks, wild birds, smuggled birds, poultry products and other species (such as fighting cocks and Muscovy ducks) in the epidemiology of the disease need to be better understood. In addition, there is a lack of detailed knowledge about the role that movement of poultry plays in spreading disease.

To address some of these questions, a prospective cohort study of AI infection in field running ducks (broilers and layers) and in-contact species was carried out in the MRD of Viet

Nam between December 2008 and April 2010. The overall objectives of the study were to: (1) identify transmission and maintenance mechanisms for LPAI and HPAI viruses in field running duck and other in-contact species flocks; (2) identify risk factors for AI virus transmission associated with field running duck farming; and (3) monitor the course of antibody response following vaccination and/or natural infection and its effect on virus shedding and transmission. This paper addresses part of the overall study objectives by providing: (1) summary estimates of the period prevalence and incidence rate of influenza Type A and H5 viruses in the study population through viral gene detection; and (2) an assessment of the efficacy of H5N1 vaccination programmes as indicated by the presence of H5 antibody in vaccinated poultry.

4.2 Materials and methods

4.2.1 Study areas and study population

This was a prospective cohort study carried out between 1 December 2008 and 3 April 2010 using a stratified, three-stage cluster sampling design. Four districts from the 129 districts in the MRD (Figure 4.1) were purposively selected for study and were treated as strata. Villages within districts comprised the first stage, poultry flocks within villages comprised the second stage and finally, individual birds within flocks comprised the third stage of sampling.

To be eligible for the study, villages were required to have at least three layer duck flocks and two of these flocks were required to be moved out of their home village for field running throughout the study period. The second criterion was that each village had at least one broiler duck flock. Details of villages that met the eligibility criteria were provided by a cross-sectional survey conducted in the study districts in March 2008 (Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009). In total 27 of 60 villages met the eligibility criteria. Each eligible village was numbered from 1 to 27 and a sample of eight numbers corresponding to the village identifiers selected without replacement using the random number function within a spreadsheet.

Twenty birds from each of the enrolled flocks were sampled. This number was largely based on practical grounds (Bennett et al. 1991) because it was easy for field staff to

collect and then manage 20 samples given the amount of time available for each flock. In addition, a sample size of 20 was considered appropriate based on the assumption that if virus was present within a flock, the prevalence of infection was likely to be greater than 80%.

The number of flocks to be sampled was calculated assuming that at the population level, the individual bird-level prevalence of infection was approximately 15% (Nguyen et al. 2005, Long 2008, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, Minh et al. 2010). A total of 157 flocks needed to be sampled to be 95% confident that the estimated individual bird-level prevalence was between 5% and 25% (i.e. an absolute error of 10%) when 20 birds were sampled per flock. For these calculations we assumed a design effect of 2 since no information on clustering of AI virus infection at the flock level was available. A design effect of 2 is similar to that determined previously for Newcastle disease (Otto & Kristensen 2004).

A sampling frame of all poultry flocks within the eight selected villages was compiled by staff of the Sub-Department of Animal Health in cooperation with staff from the District Veterinary Station and commune veterinarians. In total, 20 poultry flocks per village were selected at random using the procedure described above. The owners of the 160 (that is 8×20) selected poultry flocks were then contacted by their respective commune veterinarians to seek their consent to participate in the study; the owners (n = 97) of 157 flocks agreed to take part.

At the first sampling round individual birds were selected using a systematic simple random sampling procedure. Selected birds were then identified using two leg bands and permanent aerosol dye. Ten of the 20 birds were kept without vaccination as sentinels; the other 10 together with all other (non-study) birds in the study flock were vaccinated under the requirements of the national vaccination campaign (MARD 2009).

In this paper we use the term 'flock' to refer to the group of birds present at an initial selection visit and then repeatedly sampled throughout the study period. Using this definition, a flock is, in effect, a cohort of birds. Some households had many flocks throughout the study period (population turnover was rapid), whereas others had only a single flock that persisted for the entire period.

At the start of the study, a total of 76 flocks, including 19 broiler duck, 29 layer duck and 28 in-contact species (chickens, Muscovy ducks and geese) flocks were enrolled.

Any flock that dropped out of the study was replaced by a new flock that met the selection criteria described earlier. The first 19 broiler duck flocks were enrolled between December 2008 and March 2009 and an additional 19 broiler duck flocks were enrolled between December 2009 and April 2010. No broiler flocks were enrolled between March and November 2009 since this was considered to be the low-risk period for HPAI H5N1 in the MRD, and broiler duck production is seasonal. After enrolment broiler duck flocks were monitored for three sampling rounds. In contrast, layer duck and in-contact species flocks were enrolled at the start of the study and monitored over the entire study period. Owners of in-contact flocks were permitted to sell their birds after a minimum of three sampling rounds.

A total of 5,476 birds from 157 flocks were enrolled from 97 contracted households in the eight study villages. No broiler duck flocks needed to be replaced throughout the study period. In contrast, 59% (17 of 29) of layer duck flocks dropped out and were replaced by new flocks one time over the study period. Four (14%) layer duck flocks dropped out and replaced by new layer flocks twice over the study period. One of the main reasons for loss of layer duck flocks was that during the 17-month study period the price of eggs decreased sharply making it necessary for many flock owners to sell their birds. All (28 of 28) in-contact species flocks were replaced because their owners were permitted to sell them after every three sampling rounds. Eleven (38%) in-contact species flocks were replaced twice and two (7%) in-contact species flocks were replaced three times over the study period.

Contracted flock owners were asked to stop vaccinating their 10 sentinel birds after the date of flock enrolment. Replacement birds were enrolled when any of the study birds were removed for reasons such as death, loss, consumption or sale. Five additional, unvaccinated birds were kept to allow replacement of lost sentinel birds.

4.2.2 Study period

The study was conducted over a period of 17 months, from 1 December 2008 to 3 April 2010 (inclusive). Flocks were visited on 14 occasions termed ‘sampling rounds’ in the remainder of this paper. Sampling rounds were conducted during the two high risk periods (rounds 1 to 5 between December 2008 and March 2009 and rounds 10 to 14 between De-

cember 2009 and April 2010) and one low risk period (rounds 6 to 9 between March 2009 and November 2009). The distinction between low and high risk periods was based on a frequency analysis of previous AI outbreaks (Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009) and the prevalence of HPAI H5N1 circulation under the national surveillance programmes (Taylor & Dung 2007, Long 2008). Sampling was carried out at 28-day intervals during the two high risk periods and at 70-day intervals during the low risk period.

4.2.3 Collection of samples

Oropharyngeal and cloacal swab samples were collected from all study birds during each sampling round. The two swabs from each bird were mixed together in a single 15 mL tube containing 2.5 mL viral transport media. Viral transport media was validated by a sub-experiment to evaluate factors influencing the survival of AI viruses. Samples were kept in cool boxes at 10–12 °C. These were either transported to provincial offices where they were refrigerated at 4 °C for a maximum of two days and then transported to the laboratory, or were transported directly to the regional animal health laboratory (RAHO7) on the day of sampling. Blood samples were collected from birds that were greater than four weeks of age at the time of sampling.

4.2.4 Laboratory procedures

Individual swab samples were mixed by vortexing. The mixed material was then centrifuged at 3,500 rpm for 5 minutes. The supernatant solution was transferred into two tubes of 1.5 mL (one aliquot was used for virus isolation and the other used for RRT-PCR M gene detection). The aliquot used for virus isolation was stored at -80 °C, while the aliquot used for RRT-PCR was stored at -20 °C. Five of the individual RRT-PCR aliquots were then pooled and tested using M gene RRT-PCR. Four pools per flock were constructed at each sampling round (Figure 4.2).

Serum was treated at 56 °C for 30 minutes to inactivate non-specific inhibitors of haemagglutination. Treated serum samples were stored at 4 °C prior to further testing or at -20 °C for long-term storage.

The USDA-validated M gene RRT-PCR (Spackman et al. 2002, NVSL 2008), customised by the Vietnamese Department of Animal Health (MARD 2005*a*) was used to detect the M gene of AI viruses. The RRT-PCR was firstly used as a screening test for pooled samples. Following screening all individual samples from RRT-PCR positive and suspect pools were further tested for M gene using the same RRT-PCR technique.

RNA was extracted using the MagMAX-96 AI/ND RNA Isolation Kit (Ambion). The Quiagen one-step RT-PCR kit (QIAGEN 2008) was used to prepare the master mix, and amplification and fluorescence detection were carried out using a BioRad real-time PCR cycler. After an initial reverse transcription step at 50 °C for 30 minutes and an initial denaturation step at 95 °C for 15 minutes, 45 cycles (94 °C for 15 seconds followed by 60 °C for one minute) were performed with fluorescence detection at the end of the annealing-extension step. Samples were declared positive when the cycle threshold (Ct) for the sample was less than 40.

The H5 Multiplex assay used in this study was developed and validated by the Australian Animal Health Laboratory (Australian Animal Health Laboratory 2010). The Multiplex TaqMan assay consists of two sets of primers and probes targeting two different regions (C-terminus and N-terminus) of the HA gene of H5 viruses.

The Quiagen one-step RT-PCR kit was used to prepare the master mix and amplification and fluorescence detection were carried out on a BioRad real-time PCR cycler. After an initial reverse transcription step at 45 °C for 10 minutes and an initial denaturation step at 95 °C for 10 minutes, 45 cycles (95 °C for 15 seconds followed by 60 °C for 45 seconds) were performed with fluorescence detection at the end of the annealing-extension step. Positive controls consisted of the H5 antigens that had been designed from Vietnamese H5 viruses. Negative controls were RNase-free water. Samples were declared positive when the cycle threshold for the sample was less than 40.

The HI test was used to detect the amount of antibody against H5 viruses. The protocol for this test is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2009) and has previously been customised for use under Vietnamese laboratory conditions (MARD 2005*a*). Ten dilutions were performed to titrate the quantity of the specific antiserum in each serum sample. Both positive and negative control antigens and antisera were used in each HI testing round. HI titres were measured based on the inhibition serum dilutions, which were positive if the dilutions were at least 1/16.

4.2.5 Data management

Test results were recorded for each study bird following each sampling round. All test results were entered into a commercial spreadsheet package by laboratory staff. These data were then imported into a relational database (subsequently referred to as the laboratory database), which also incorporated individual bird-level details recorded at the time of sampling.

4.2.6 Statistical analyses

The period prevalence and incidence rate of influenza Type A and H5 virus infection were estimated at both the bird and flock level. Period prevalence for a given time interval (e.g. the low and high risk periods or the entire study period) was defined as the number of birds (or flocks) positive at the start of the interval plus the number of birds (or flocks) identified as positive throughout the interval divided by the total number of birds (or flocks) that were tested during the interval. A flock was declared positive if it had at least one bird that was positive to the M gene RRT-PCR or H5 Multiplex assay. Incidence rate was defined as the total number of newly infected birds (or flocks) divided by the total number of bird (or flock)-months at risk for that interval.

Standard errors for the period prevalence and incidence rate estimates respecting the multistage sampling design were calculated using the survey package (Lumley 2004) implemented within R (R Development Core Team 2012). Sampling weights (Lohr 2009) were calculated as the inverse of the probability of selection. At the flock level the probability of selection was equal to (the number of selected villages divided by the total number of villages within the selected districts) $\times$ (the number of selected flocks divided by the total number of flocks within the selected villages). At the individual bird level the probability of selection was equal to the flock level probability of selection multiplied by (the total number of study birds divided by an estimate of the total number of birds within the study flocks). Our estimates of the standard errors of period prevalence and incidence rate did not account for the repeated sampling of individual birds over time. Our reasons for this were firstly because of the complexity of having to deal with birds joining and leaving the study population throughout the study period and secondly because accounting for levels beyond the first stage strata (i.e. district) and first stage cluster (i.e. village) has minimal

effect on the sample variance estimates (Wolter 2007). Using this approach sampling was assumed to have occurred with replacement at the final sampling stage (i.e. flock, for the flock-level analyses and bird for the individual bird-level analyses).

A scatter plot was constructed to represent changes in Ct value estimates as a function of calendar date, stratified by village. Superimposed on each scatterplot was a line of best fit and its confidence interval calculated using a local polynomial regression method.

4.3 Results

4.3.1 Detection of influenza Type A viruses

In total 17,968 mixed swabs (oropharyngeal and cloacal) were collected. These were then used to construct 3,601 pools which were tested using the M gene RRT-PCR (Figure 4.2). A total of 358 pools were positive and 16 were suspect. Ninety four per cent (1,767 of 1,871) of the individual samples which comprised these positive and suspect pools were then tested using the same M gene RRT-PCR. Six per cent (n = 104) of the remaining individual samples were not tested since they were either used for another study (n = 55 samples), sent to other laboratories for duplicate virus isolation (n = 30) or were not found after the prolonged period of storage (n = 18).

The measured Ct values ranged from 18.70 to 44.91 (mean 34.84) at the individual bird level. Individual bird Ct values varied between study villages and sampling rounds (Figure 4.3). It also shows a relatively high prevalence of influenza Type A viruses throughout the year, with little clear differentiation between the high and low risk periods.

Table 4.1 shows the results of the M gene RRT-PCR analyses at the individual bird level. The incidence rates were approximately 2 – 2.5 times higher for the first and second high risk period compared with that found in the low risk period. Evidence of influenza Type A virus infection was found in all study villages, but the period prevalence and the incidence rates varied by village. Broiler ducks had an incidence rate of influenza Type A virus infection that was substantially greater than that recorded for layer ducks and in-contact species. The incidence rates of influenza Type A virus infection for vaccinated and unvaccinated birds were similar. Birds that were less than 90 days of age at the time of sampling had the highest incidence rate of positivity with 9 (95% CI 6 – 13) positives per

100 bird-months at risk, approximately four times higher than that of birds aged greater than 365 days.

At the flock level, the overall incidence rate was relatively high with 19 (95% CI 17 – 22) positive flocks per 100 flock-months at risk (Table 4.1). The period prevalence did not follow the high-low-high pattern that was expected for the high, low and high risk periods. Similar to the individual bird level analyses, the incidence rate of influenza Type A viruses for broiler ducks was highest, followed by layer ducks and in-contact species.

4.3.2 Detection of H5 viruses

A total of 711 individual M gene positive (n = 657) or suspect (n = 54) M gene RRT-PCR samples from 86 study flocks were further tested to estimate the period prevalence and incidence rate of H5 virus infection using the H5 Multiplex assay.

Table 4.2 shows the period prevalence and incidence rate of H5 virus infection at the individual bird level. The overall incidence rate was 0.2 (95% CI 0.1 – 0.5) positive birds per 100 bird-months at risk. In contrast to the M gene RRT-PCR results at the individual bird level (Table 4.1) the incidence rate of H5 virus detection was greater during the low risk period compared with the two high risk periods. All study villages showed evidence of H5 virus circulation during the study, except for Dong Thanh. Village Ap 4 had the highest incidence rate of H5 virus detection with 0.9 (95% CI 0.7 – 1.1) H5 positive birds per 100 bird-months at risk. Broiler ducks had an incidence rate of influenza H5 virus infection that was approximately four times greater than that recorded for layer ducks and in-contact species. For vaccinated poultry, H5 virus PCR positives were detected in 0.6 (95% CI 0.4 – 1.0) per 100 tested birds. This was numerically lower than the 1.0 (95% CI 0.7 – 1.4) positives per 100 in unvaccinated birds. Of all age groups, birds less than 90 days of age at the time of testing had the highest H5 incidence rate with 0.4 (95% CI 0.2 – 0.7) positives per 100 bird-months at risk (Table 4.2).

At the flock level, the overall period prevalence of H5 virus detection was 16 (95% CI 11 – 23) positive flocks per 100 tested flocks (Table 4.2). Similar to the individual bird level analyses, the period prevalence and the incidence rate of H5 virus detection varied by village.

4.3.3 Evaluation of immune response of poultry against H5 viruses

A total of 14,878 blood samples were collected throughout the study period. This represented 83% of the total number of bird-rounds that were eligible for sampling. Blood was not collected from the remaining 17% (n = 3,090) of bird-rounds primarily because birds were less than 4 weeks of age at the time of sampling. A small number of serum samples (0.7%) were ineligible for testing because of faulty storage or handling (e.g. haemolysis). Eighty four per cent (n = 12,536) of collected serum samples were tested using the H5 HI test.

Figure 4.4 shows the distribution of H5 HI antibody titres of 12,536 serum samples collected from 5,014 individual birds (2,751 unvaccinated and 2,533 vaccinated) throughout the study period. A total of 2,893 (57%) individual birds (1,638 vaccinated and 1,255 unvaccinated) had positive titres (HI titre $\geq$ 1/16).

Table 4.3 shows the period prevalence and incidence rate of H5 seropositivity (HI titre $\geq$ 4log2) for vaccinated and unvaccinated birds. The overall incidence rate of H5 seropositivity was much higher than that of H5 antigen detection. The period prevalence of H5 HI positivity varied by village. The incidence rate of H5 HI positivity in vaccinated birds was greater than that recorded for unvaccinated birds.

For unvaccinated birds the period prevalence of H5 seropositivity for layer ducks was approximately three times greater than that recorded for broiler ducks and in-contact species.

4.3.4 Expression of clinical signs consistent with H5N1

No clinical signs consistent with avian influenza H5N1 were reported in either poultry or people in the sampled villages throughout the study period.

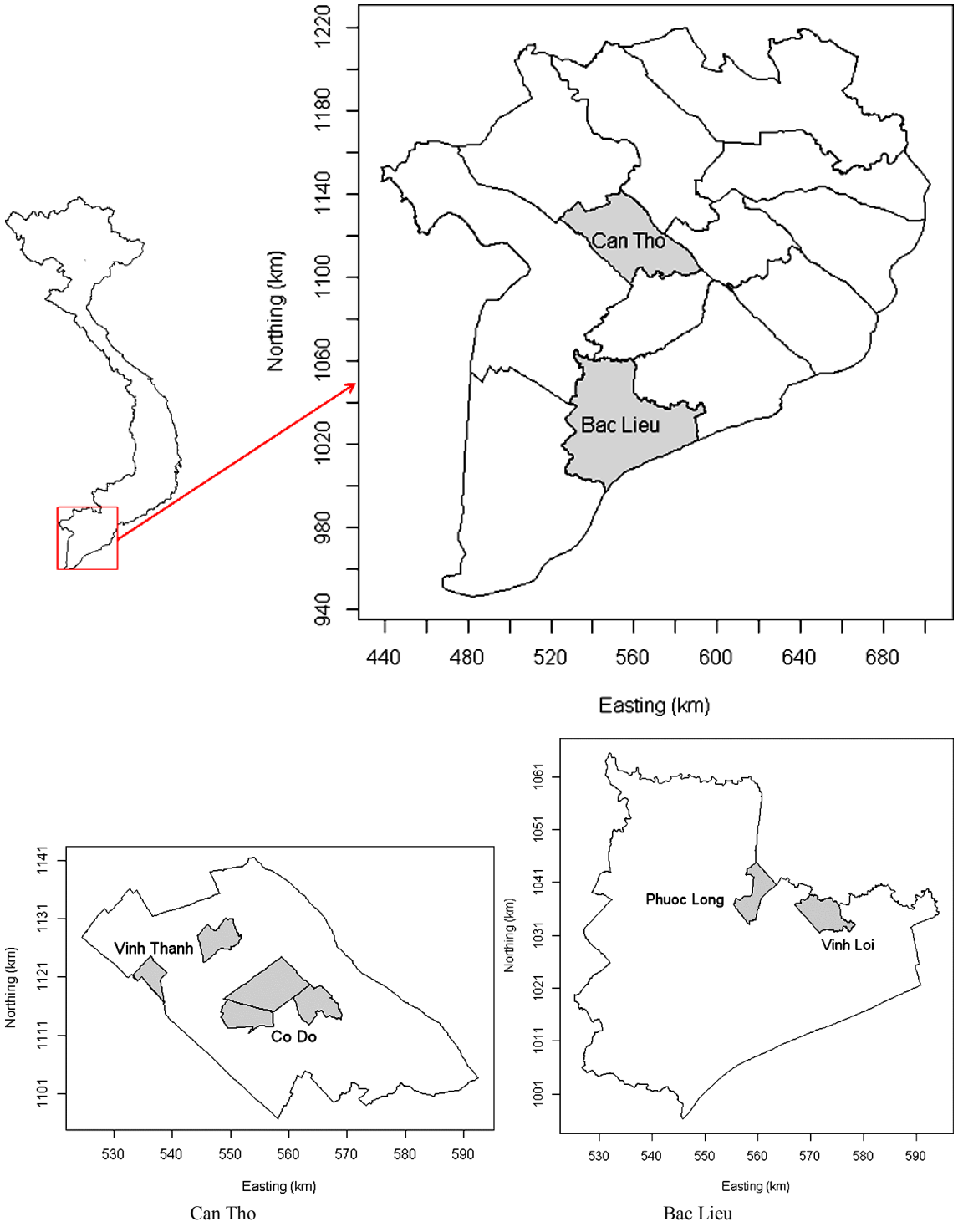


Figure 4.1: Map of Viet Nam showing the study areas. The upper figure shows the Mekong River Delta and the two study provinces (Can Tho and Bac Lieu). The lower figure shows the two study provinces with the shade showing study communes of selected districts (Vinh Thanh and Co Do of Can Tho, Phuoc Long and Vinh Loi of Bac Lieu).

Table 4.1: M gene RRT-PCR results showing the period prevalence corrected according to sampling design and incidence rate of influenza Type A infection in the study birds and flocks in the Mekong River Delta, Viet Nam.

Variable	Bird level						Flock level					
	n bird positive	*n* bird tested	*n* bird-months positive	*n* bird-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)	*n* flock positive	*n* flock tested	*n* flock-months positive	*n* flock-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)
Period:												
High risk period [a]	343	2,493	394	8,321	19 (17 – 20)	5 (3 – 9)	58	104	114	713	56 (47 – 65)	17 (14 – 19)
Low risk period [b]	164	1,658	166	4,662	8 (7 – 10)	3 (1 – 5)	19	28	30	121	69 (51 – 83)	23 (17 – 30)
High risk period [c]	92	1,325	97	2,325	13 (11 – 15)	7 (4 – 10)	9	25	17	52	50 (32 – 68)	49 (32 – 75)
Village:												
Ap 4	52	551	58	1,951	10 (7 – 12)	3 (2 – 3)	7	19	14	112	37 (19 – 59)	13 (12 – 13)
Thoi Phuoc 2	84	557	91	1,580	17 (14 – 21)	5 (5 – 6)	8	13	18	97	62 (36 – 82)	19 (19 – 19)
Dong Thanh	24	610	24	1,662	3 (2 – 5)	1 (1 – 1)	5	18	5	90	28 (12 – 51)	6 (6 – 6)
Tan Lap	69	780	72	2,351	13 (11 – 16)	4 (3 – 4)	15	21	32	139	71 (50 – 86)	23 (23 – 23)
Phung Quoi A	86	632	104	2,188	14 (11 – 16)	4 (3 – 4)	12	20	26	126	60 (39 – 78)	21 (21 – 21)
Tra Hat	111	764	130	1,873	25 (22 – 28)	11 (10 – 11)	12	22	27	111	55 (35 – 73)	24 (24 – 24)
My Trinh	62	819	63	2,090	9 (7 – 11)	3 (3 – 3)	14	24	20	119	58 (39 – 76)	17 (17 – 17)
My Tuong II	111	763	115	1,613	18 (15 – 21)	10 (9 – 11)	13	20	19	92	65 (43 – 82)	21 (21 – 21)
Type:												
Broiler duck	246	800	271	1,438	35 (32 – 39)	22 (14 – 33)	24	42	42	71	69 (53 – 81)	66 (54 – 81)
Layer duck	301	1,752	333	7,786	17 (16 – 19)	4 (3 – 6)	14	96	96	419	89 (77 – 95)	24 (21 – 28)
In-contact species	52	2,924	53	6,084	1 (1 – 2)	1 (0 – 1)	18	23	23	396	29 (20 – 41)	6 (5 – 9)
Vaccination status:												
Unvaccinated	289	2,766	316	7,746	14 (13 – 15)	5 (3 – 6)	-	-	-	-	-	-
Vaccinated	310	2,710	341	7,562	16 (14 – 17)	5 (4 – 7)	-	-	-	-	-	-
Age at time of sampling (days):												
≤90	363	3,193	402	6,479	17 (16 – 18)	9 (6 – 13)	-	-	-	-	-	-
91−365	140	1,204	154	4,519	16 (14 – 18)	5 (3 – 6)	-	-	-	-	-	-
>365	96	1,075	101	4,300	10 (8 – 12)	2 (1 – 3)	-	-	-	-	-	-
Missing	0	4	0	10	0 (0 – 49)	0 (0 – 0)	-	-	-	-	-	-
Total:	599	5,476	657	15,308	15 (12 – 18)	5 (4 – 7)	86	157	161	886	58 (50 – 65)	19 (17 – 22)

n number of birds or flocks; CI: confidence interval.
[a] December 2008 to March 2009. [b] April to November 2009. [c] December 2009 to March 2010.
[d] Period prevalence expressed as the number of positive birds or flocks per 100 birds tested, corrected according to sampling design.
[e] Number of positive birds or flocks per 100 bird-months or flock-months at risk.

Table 4.2: H5 Multiplex assay results showing the period prevalence corrected according to sampling design and incidence rate of influenza H5 virus infection in the study birds and flocks in the Mekong River Delta, Viet Nam.

Variable	Bird level						Flock level					
	n bird positive	*n* bird tested	*n* bird-months positive	*n* bird-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)	*n* flock positive	*n* flock tested	*n* flock-months positive	*n* flock-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)
Period:												
High risk period [a]	39	2,493	39	8,321	0.9 (0.6 – 1.4)	0.2 (0.1 – 0.4)	17	104	19	713	18 (12 – 26)	2.9 (1.9 – 4.6)
Low risk period [b]	12	1,658	12	4,662	0.9 (0.6 – 1.5)	0.3 (0.05 – 1.5)	5	28	5	121	14 (6 – 31)	3.4 (1.8 – 6.7)
High risk period [c]	16	1,325	16	2,325	0.4 (0.2 – 0.9)	0.2 (0.1 – 0.3)	2	25	2	52	13 (5 – 31)	6.2 (3.0 – 13.0)
Village:												
Ap 4	20	551	20	1,951	3.5 (2.2 – 5.3)	0.9 (0.7 – 1.1)	4	19	4	112	21 (9 – 43)	3.6 (3.6 – 3.6)
Thoi Phuoc 2	7	557	7	1,580	0.9 (0.4 – 2.0)	0.2 (0.2 – 0.4)	1	13	2	97	8 (1 – 33)	2.1 (2.1 – 2.1)
Dong Thanh	0	610	0	1,662	0.0 (0.0 – 0.6)	0.0 (0.0 – 0.0)	0	18	0	90	0 (0 – 18)	0.0 (0.0 – 0.0)
Tan Lap	7	780	7	2,351	2.0 (1.2 – 3.2)	0.5 (0.4 – 0.6)	4	21	4	139	19 (8 – 40)	2.9 (2.9 – 2.9)
Phung Quoi A	7	632	7	2,188	0.5 (0.2 – 1.4)	0.1 (0.1 – 0.1)	4	20	4	126	20 (8 – 42)	3.2 (3.2 – 3.2)
Tra Hat	7	764	7	1,873	0.1 (0.0 – 0.7)	0.1 (0.0 – 0.1)	5	22	2	111	9 (3 – 28)	1.8 (1.8 – 1.8)
My Trinh	10	819	10	2,090	1.5 (0.9 – 2.6)	0.5 (0.4 – 0.6)	5	24	5	119	21 (9 – 40)	4.2 (4.2 – 4.2)
My Tuong II	9	763	9	1,613	0.4 (0.2 – 1.2)	0.2 (0.2 – 0.3)	4	20	5	92	20 (8 – 42)	5.4 (5.4 – 5.4)
Type:												
Broiler duck	28	800	28	1,438	1.3 (0.7 – 2.4)	0.8 (0.4 – 1.4)	5	38	6	71	16 (8 – 31)	9.8 (3.7 – 26.0)
Layer duck	19	1,752	19	7,786	1.0 (0.6 – 1.5)	0.2 (0.1 – 0.7)	10	50	10	419	14 (7 – 27)	1.7 (0.8 – 3.6)
In-contact species	20	2,924	20	6,084	0.3 (0.2 – 0.6)	0.1 (0.0 – 0.5)	9	69	10	396	18 (11 – 28)	3.5 (1.6 – 7.7)
Vaccination status:												
Unvaccinated	41	2,766	41	7,746	1.0 (0.7 – 1.4)	0.3 (0.1 – 0.6)	-	-	-	-	-	-
Vaccinated	26	2,710	26	7,562	0.6 (0.4 – 1.0)	0.2 (0.1 – 0.6)	-	-	-	-	-	-
Age at time of sampling (days):												
≤90	48	3,193	48	6,479	0.8 (0.5 – 1.1)	0.4 (0.2 – 0.7)	-	-	-	-	-	-
91−365	9	1,204	9	4,519	0.8 (0.4 – 1.4)	0.2 (0.1 – 0.5)	-	-	-	-	-	-
>365	10	1,075	10	4,300	0.8 (0.4 – 1.5)	0.2 (0.0 – 1.5)	-	-	-	-	-	-
Missing	0	4	0	10	0.0 (0.0 – 49.0)	0.0 (0.0 – 0.0)	-	-	-	-	-	-
Total:	67	5,476	67	15,308	0.8 (0.6 – 1.1)	0.2 (0.1 – 0.5)	24	157	26	886	16 (11 – 23)	3.2 (2.3 – 4.4)

n number of birds or flocks; CI: confidence interval.

[a] December 2008 to March 2009. [b] April to November 2009. [c] December 2009 to March 2010.

[d] Period prevalence expressed as the number of positive birds or flocks per 100 birds tested, corrected according to sampling design.

[e] Number of positive birds or flocks per 100 bird-months or flock-months at risk.

Table 4.3: H5 HI results showing the period prevalence corrected according to sampling design and incidence rate of H5 seroconversion in the study birds in the Mekong River Delta, Viet Nam.

Variable	Vaccinated birds						Unvaccinated birds					
	n bird positive	*n* bird tested	*n* bird-months positive	*n* bird-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)	*n* bird positive	*n* bird tested	*n* bird-months positive	*n* bird-months tested	Prevalence[d] (95% CI)	Incidence rate[e] (95% CI)
Period:												
High risk period [a]	693	1,163	1,822	4,012	64 (62 – 67)	47 (43 – 52)	551	1,230	1,537	4,309	47 (44 – 50)	36 (31 – 42)
Low risk period [b]	531	749	1,185	2,381	75 (71 – 78)	54 (49 – 60)	374	751	798	2,281	54 (50 – 57)	38 (26 – 54)
High risk period [c]	414	621	698	1,169	82 (79 – 85)	66 (59 – 73)	330	590	560	1,156	54 (50 – 58)	46 (36 – 58)
Village:												
Ap 4	169	245	474	967	85 (80 – 89)	55 (52 – 58)	130	226	408	984	79 (73 – 84)	50 (48 – 53)
Thoi Phuoc 2	156	262	401	784	76 (70 – 81)	56 (54 – 58)	149	262	373	796	73 (67 – 78)	51 (49 – 53)
Dong Thanh	206	291	462	829	79 (74 – 83)	58 (55 – 61)	166	294	395	833	70 (64 – 75)	50 (46 – 54)
Tan Lap	244	369	593	1,182	78 (74 – 82)	57 (55 – 59)	222	381	499	1,169	74 (70 – 78)	52 (50 – 54)
Phung Quoi A	201	314	529	1,091	75 (69 – 79)	51 (49 – 53)	124	316	320	1,097	46 (41 – 52)	34 (31 – 37)
Tra Hat	209	348	355	901	56 (50 – 61)	34 (33 – 36)	148	350	231	972	37 (32 – 42)	19 (18 – 20)
My Trinh	238	367	505	1,008	67 (62 – 72)	52 (51 – 54)	147	388	397	1,082	44 (39 – 49)	45 (42 – 48)
My Tuong II	215	337	386	800	63 (57 – 68)	54 (52 – 56)	169	354	272	813	40 (35 – 45)	35 (33 – 37)
Type:												
Broiler duck	205	395	296	719	61 (56 – 66)	53 (35 – 80)	84	389	111	719	27 (22 – 31)	20 (14 – 29)
Layer duck	771	878	2,389	3,854	91 (89 – 93)	58 (53 – 64)	715	845	2,142	3,932	86 (84 – 88)	50 (42 – 59)
In-contact species	662	1,260	1,020	2,989	50 (48 – 53)	33 (28 – 38)	456	1,337	642	3,095	22 (19 – 24)	12 (7.4 – 21)
Age at time of sampling (days):												
≤90	744	1,354	1,119	2,947	57 (54 – 59)	38 (32 – 45)	533	1,531	770	3,532	30 (28 – 33)	19 (17 – 21)
91−365	449	663	1,189	2,437	72 (69 – 76)	50 (47 – 53)	319	518	913	2,082	55 (51 – 60)	36 (26 – 49)
>365	443	1,391	1,391	2,169	92 (90 – 94)	61 (57 – 66)	402	521	1,211	2,131	83 (79 – 86)	53 (45 – 63)
Missing	2	3	6	9	67 (21 – 94)	67 (67 – 67)	1	1	1	1	100 (21 – 100)	100 (21 – 100)
Total:	1,638	3,705	3,705	7,562	71 (69 – 73)	51 (48 – 55)	1,255	2,571	2,895	7,746	50 (48 – 52)	38 (31 – 46)

n number of birds or flocks; CI: confidence interval.

[a] December 2008 to March 2009. [b] April to November 2009. [c] December 2009 to March 2010.

[d] Period prevalence expressed as the number of positive birds per 100 birds tested, corrected according to sampling design.

[e] Number of positive birds per 100 bird-months at risk.

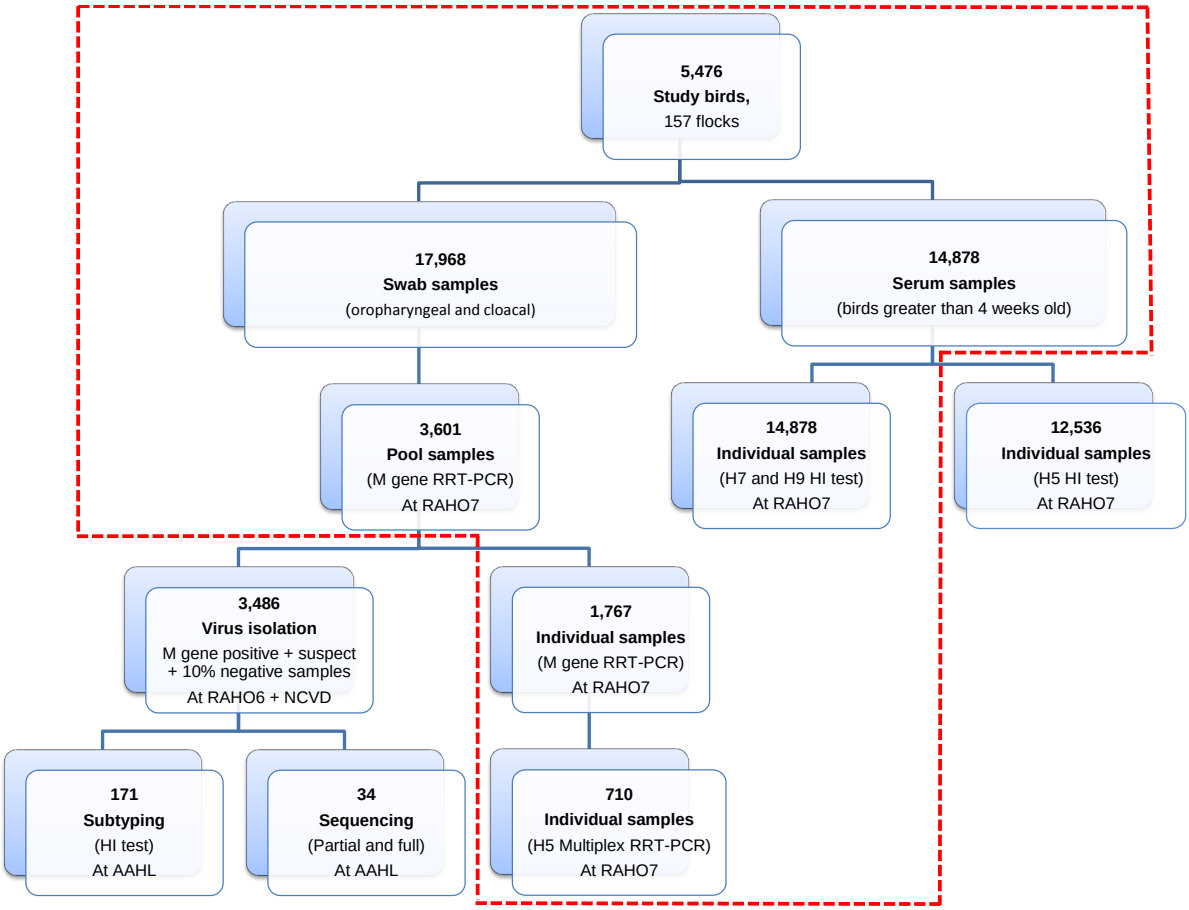


Figure 4.2: Flow chart of sample testing procedures. The numbers in each box indicating the total number of samples that were collected and tested at individual or pool level. The dashed red line indicates samples and results presented in this paper.

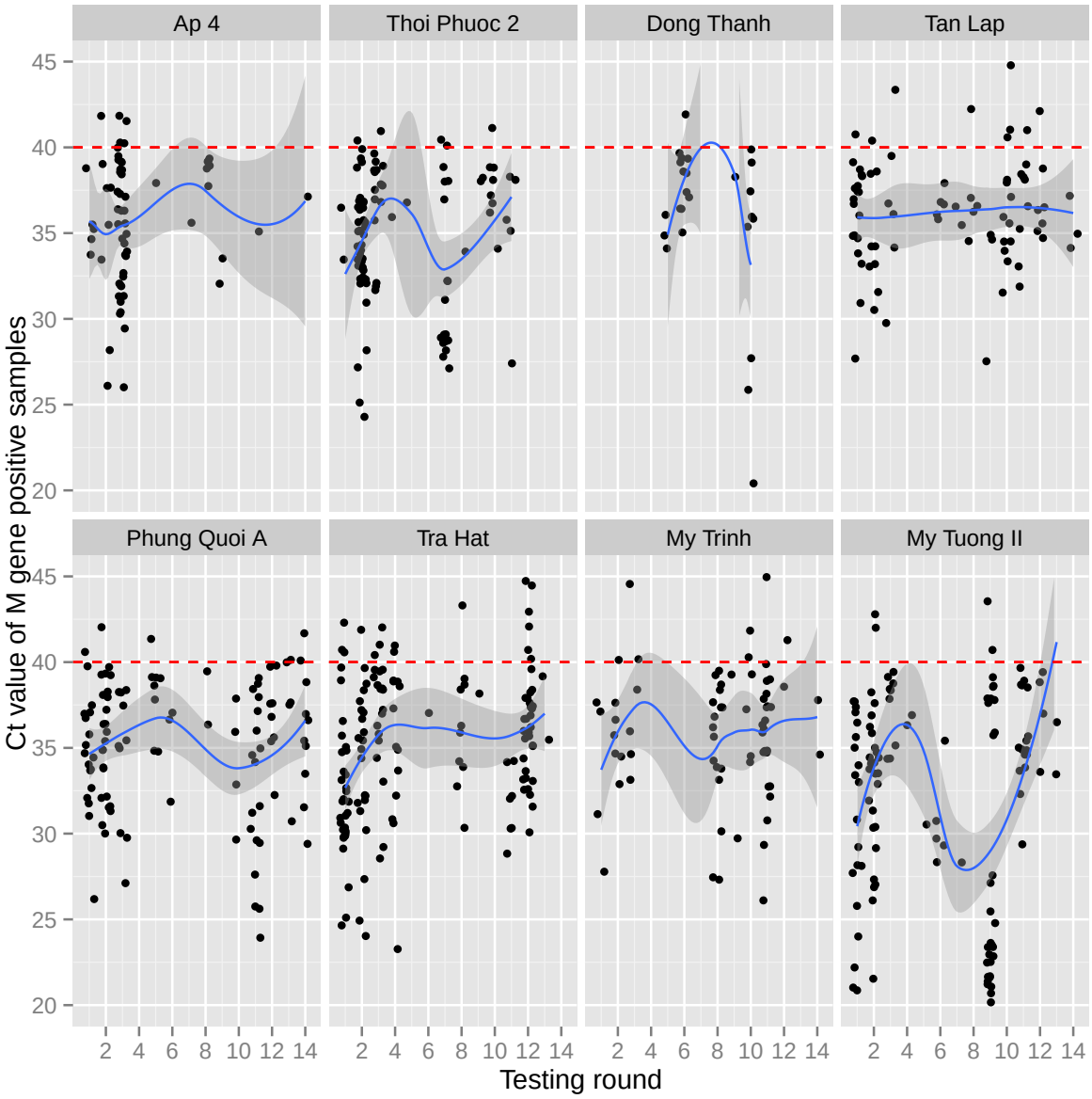


Figure 4.3: Scatter plots showing Ct value of individual samples as a function of testing round, stratified by village. The dashed line indicates the cut-off value for Ct values. Ct values of less than 40 were declared positive by the M gene RRT-PCR test. The line of best fit shows the average Ct value of tested samples. The shading shows the lower and upper limits of the confidence intervals.

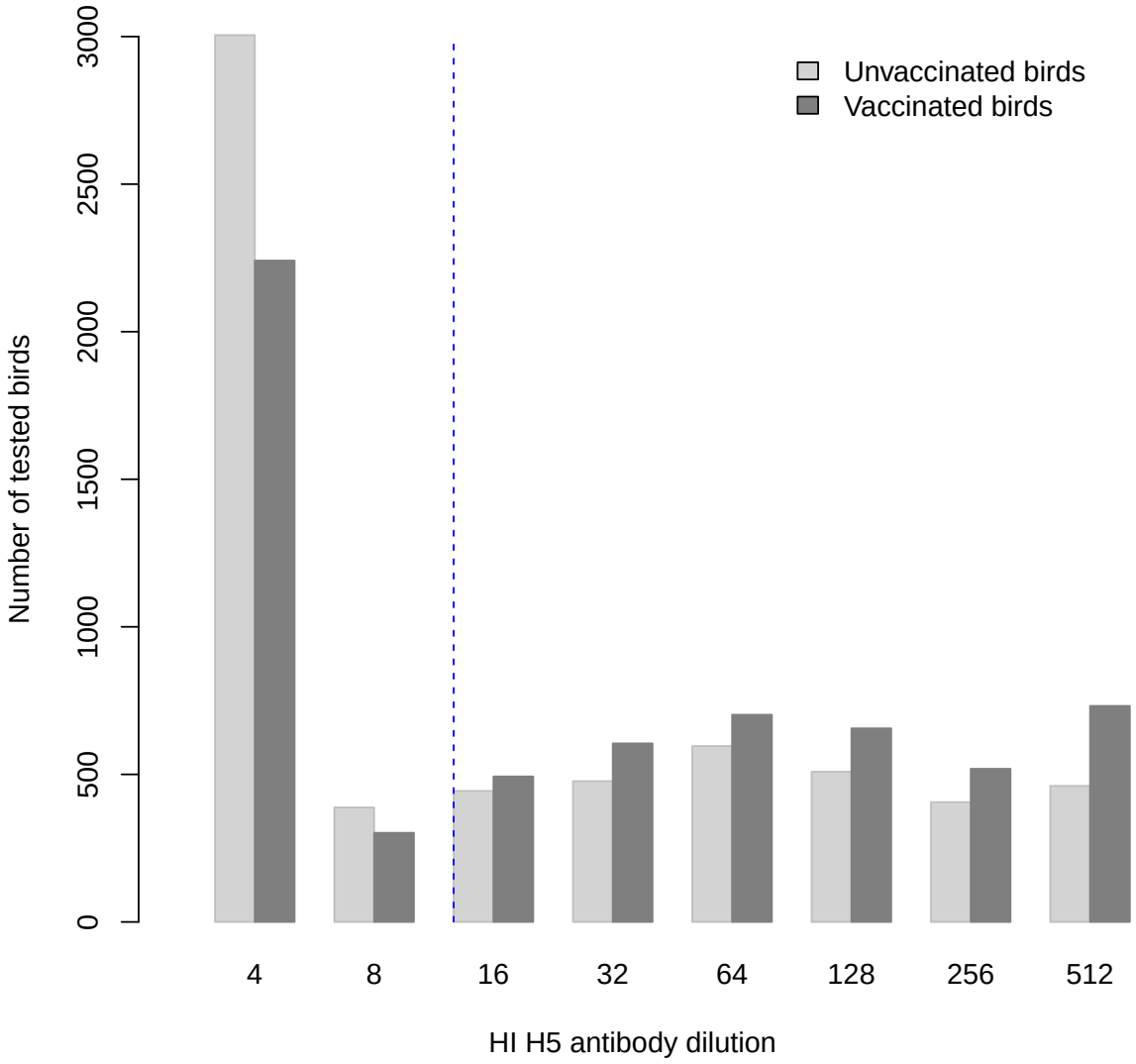


Figure 4.4: Distribution of H5 HI antibody titres of 12,536 serum samples collected from 5,014 birds (2,751 unvaccinated and 2,533 vaccinated) in the Mekong River Delta during the study period (December 2008 to April 2010). The dashed vertical line shows the cut-off value (HI titre $\geq$ 1/16 or 4 $\log_2$) for declaring samples positive.

4.4 Discussion

This study was designed to estimate the frequency of influenza Type A and H5 virus infection in 157 village poultry flocks, comprised of both vaccinated and unvaccinated birds, in the Mekong River Delta of Viet Nam. Occurrence of infection was quantified using period prevalence and incidence rates estimated at the individual bird and individual flock level. The study also investigated the relationship between vaccination history and infection. This is believed to be the first study which has jointly examined H5 infection and the wider population of influenza Type A viruses in village poultry using quantitative methods. While our estimates of prevalence and incidence rate and their standard errors at the individual bird level have been adjusted to account for the stratified, three-stage cluster sampling design used in this study we provide no adjustment for the flock effect, and our comparisons of infection frequency across subgroups (e.g. vaccinated versus unvaccinated birds) should be interpreted accordingly.

The incidence rate of influenza Type A virus infection in individual birds peaked during the high risk periods (December to March) in both years and was lower during the April to November low-risk period (Table 4.1). The reason for this seasonality of incidence remains unresolved. It is likely to be multifactorial and due to factors such as the occurrence of a peak in rice harvesting and consequent movement of itinerant grazing ducks used for post-harvest scavenging and expanded poultry production preceding the Têt New Year celebrations, when demand for poultry for consumption is high. This peak was particularly evident in broiler ducks and both the prevalence and incidence rate of Type A virus infection were considerably higher in broiler ducks than in the other populations that were sampled (Table 4.1). In addition, the frequency of H5 virus infection was much greater compared with another longitudinal study conducted in the MRD between May 2007 and May 2008, which found only a very small proportion (0.7%) of flocks H5 positive (Henning et al. 2011). The presence of a non-negligible incidence of H5 virus infection during the low risk period (Table 4.2) might account for the sporadic occurrence of AI outbreaks in this area of Viet Nam during recent years (DAH 2011).

The incidence rate of H5 virus infection was approximately 5% of total influenza Type A virus incidence rate, demonstrating that around 95% of influenza virus activity was associated with low pathogenic strains (Table 4.1 and Table 4.2). These findings provide

important information to inform control strategies for AI since the circulation of possibly multiple AI subtype viruses in a highly dynamic, vaccinated population provides conditions favourable for virus mutation and reassortment (Webster et al. 1992, Webby et al. 2002). The epidemiology of AI in the MRD region may be further complicated because influenza viruses have the capacity to circulate in other species (humans and pigs), further increasing the potential for reassortment. Evidence of the circulation of influenza H3N2 virus in pigs in the south of Viet Nam was identified recently (Ngo et al. 2011). A survey conducted at pig slaughterhouses between October 2009 and May 2010 in the Red River Delta showed that the seroprevalence of influenza pandemic H1N1 virus infection was 29 (95% CI 23 – 35) H1N1 positive farms per 100 farms at risk (Trevennec et al. 2011). Evidence of reassortment of influenza viruses originating from different species was identified during the 2009 H1N1 pandemic (Smith et al. 2009). This is a concern because in Viet Nam poultry flocks are often kept with other animals (such as pigs) with little or no biosecurity measures. A better understanding of the epidemiology of influenza Type A viruses may help to clarify many aspects of the epidemiology of H5N1, which has not yet been fully understood because H5N1 infected flocks have usually been culled immediately on detection following current disease control regulations (MARD 2005*b*).

Broiler ducks, which are only kept for about three months in the MRD, had the highest prevalence and incidence rate of both influenza Type A and H5 virus infection (Tables 4.1 and Table 4.2). Broilers were also the youngest birds, which explains the high incidence rate in birds that were less than or equal to 90 days of age. Although the birds in this study had their vaccination status determined by the study design, under normal husbandry conditions in this region broiler ducks may arrive, be reared, be moved around their region as field running ducks (Minh et al. 2010) and then be sold for consumption, all before the national vaccination program may visit their home village. These characteristics of broiler duck management makes them centrally important to the epidemiology of influenza Type A and H5 viruses. The vaccination strategy that has been used as part of disease control measures needs to take these particular features of poultry management into account.

Compared with broilers, layer ducks stay in production for much longer periods (Minh et al. 2010) and therefore have a greater probability of being vaccinated on either one or several occasions throughout their lifetime. Even so, layer ducks had a non-negligible incidence of H5 infection (Table 4.2). Based on these results it appears that vaccination

does not provide full protection against establishment of infection with H5 viruses. Layer ducks are also involved in the maintenance of infection with low pathogenic influenza Type A strains, with a period prevalence approximately 50% of that in broiler ducks (Table 4.1).

Table 4.2 shows that the incidence rate of H5 virus infection was lower in vaccinated sentinel birds (0.2 positive birds per 100 bird-months at risk) than in unvaccinated sentinels (0.3 positive birds per 100 bird-months at risk). Vaccinated birds had a higher incidence rate of influenza Type A virus infection compared with unvaccinated birds, but a lower incidence rate of H5 virus infection (Tables 4.1 and Table 4.2). These results indicate that interventions such as mass HPAI H5N1 vaccination, using the protocol currently practiced in Viet Nam, does not fully prevent birds from developing H5 virus infection. Assuming there are no issues with the implementation of the vaccination program (e.g. maintenance of cold chain, application of correct vaccination technique) we hypothesise that new or sub-clades of HPAI H5N1 or LPAI H5 viruses are either regularly or intermittently introduced into these poultry populations. This hypothesis is supported by experimental studies (Tung & Inui 2011) which found that new sub-clades of H5N1 viruses caused vaccination failures in the north of Viet Nam. In addition, a repeated cross-sectional study conducted in the MRD between April 2009 and March 2012 identified the presence of multiple AI subtypes and H5N1 viruses in poultry (Nomura et al. 2011). The HPAI H5N1 viruses detected from healthy ducks in this region were genetically confirmed as belonging to clade 1, but they were divided into three subgroups (Sakurai et al. 2012). In our study, genetic analysis of a limited number of H5 virus isolates (Diep 2011) showed that HPAI H5N1 viruses circulate in apparently healthy ducks.

Serological results (Table 4.3) show that 91% of vaccinated layer ducks had positive H5 antibody titres at 1:16 or higher, compared with 86% recorded for unvaccinated layer ducks. This similarity is most likely to have occurred because it is possible that some of the unvaccinated layers had received one or more vaccinations prior to the study start. Sixty one *percent* of vaccinated broiler ducks had positive titres compared with 27% of unvaccinated sentinel broilers. Similar findings were evident for in-contact species. These results show that virus can circulate in these populations in the presence of vaccination. As birds age there is a greater likelihood of them having positive H5 antibody titres because they are available for more vaccination cycles. The period prevalence of positive HI titres

to H5 in unvaccinated sentinels (50%) was remarkably high suggesting that this is due to a combination of vaccination of some birds prior to the project, plus natural exposure to virus.

Of the eight villages that took part in the study, Dong Thanh is notable because no H5 virus positive samples were obtained from this village even though virus was circulating in the other seven villages (Table 4.2). Dong Thanh also had the lowest period prevalence of low pathogenic AI viruses (Table 4.1). It had one of the highest levels of HI positives in vaccinated birds but not in unvaccinated birds (Table 4.3). Differences in immunity were too small to completely explain the absence of H5 infection in Dong Thanh. It seems likely that flock owners in Dong Thanh had good biosecurity practices[1], and this finding deserves further investigation. In contrast, village Ap 4 had a relatively high incidence rate of H5 infection (Table 4.2) but its immunity level, as measured by HI, was similar to Dong Thanh (Table 4.3). The period prevalence of positive HI titres in unvaccinated birds varied between 37% and 79% across the eight villages, and between 34% and 58% in vaccinated birds (Table 4.3) but this did not explain the differences in H5 incidence across villages. Clearly, there are some management or behavioural factors which determine whether H5 virus circulates at high, low or zero levels in a particular village. At the flock level, by far the highest incidence rates of both H5 and LPAI virus infection occurred in broiler ducks (Tables 4.1 and Table 4.2). We conclude that broiler ducks are likely to be the principal entry route of influenza viruses into poultry flocks in the MRD.

Although H5 virus was circulating throughout the study period, no clinical disease was recorded in any of the study flocks, or from other flocks in the study villages. There were no human H5N1 cases reported in any of the study villages despite the continuous circulation of H5N1 virus in poultry populations. Although the vaccination program did not prevent transmission of H5 viruses in seven of the eight villages, it appears that it did contribute to preventing clinical disease.

[1]'Good' biosecurity practices in this context could include activities such as the daily cleaning and disinfection of premises, efforts by local veterinarians to ensure that flocks are vaccinated in the correct manner at an appropriate time, and/or high levels of awareness about disease meaning that poultry are not moved to other places where there has been a history of HPAI H5N1.

4.5 Conclusions

We provide evidence that both influenza Type A and H5 viruses were circulating in the population of village poultry throughout the study period with no obvious signs of clinical disease. This implies that interventions such as vaccination, movement controls and biosecurity measures need to be carried out continuously throughout the year rather than only focusing on the established high risk periods. Broiler ducks had an incidence rate of influenza H5 virus infection that was approximately four times greater than that recorded for layer ducks and in-contact species. We conclude that broiler ducks are likely to be the main entry route for H5 virus into poultry flocks in the MRD. Control efforts would benefit from understanding why there is a difference between villages in H5 incidence, and developing strategies to provide greater protection to broiler ducks.

CHAPTER 5

Factors influencing the duration of immunity in poultry vaccinated against highly pathogenic avian influenza H5N1 in the Mekong River Delta of Viet Nam, 2008 – 2010

Abstract – A prospective cohort study was carried out to document factors influencing the duration of immunity in poultry vaccinated against highly pathogenic avian influenza H5N1 in the Mekong River Delta of Viet Nam between December 2008 and April 2010. A total of 12,536 serum samples from 5,104 birds in 157 poultry flocks were tested using the haemagluttination inhibition test.

Survival analyses were used to quantify the timing of seronegative events relative to the date of the last H5N1 vaccination. Because birds were tested at set time intervals thoughout the follow-up period the data were treated as interval censored. The overall time taken for H5 seropositive birds to become seronegative to HPAI H5N1 following a vaccination event was 56 (95% CI 51 – 61) days. The time taken for H5 seropositive birds aged less than 90 days, aged between 91 and 356 days and aged more than 365 days to become seronegative was 46 (95% CI 39 – 54), 69 (95% CI 49 – 97) and 96 (95% CI 72 – 132) days, respectively. The time taken for H5 seropositive birds vaccinated during the two dry seasons (from December to March 2009 and from December to March 2010) was 40 (95% CI 29 – 55) and 39 (95% CI 28 – 54) days, respectively. These are shorter than the 46 (95% CI 39 – 54) days recorded for birds vaccinated during the rainy season (from April to November 2009). Under field conditions the overall duration of immunity in vaccinated poultries was considerably shorter than documented in laboratory-based studies. Age of poultry at the time of vaccination and time of the year when vaccination was carried out significantly influenced duration of immunity in vaccinated birds. These two factors should be taken into account if the current vaccination strategy is to be continuously practiced in Viet Nam.

Long, N.V., Stevenson, M., Schauer, B. and Thuy, N.T. (2013) Factors influencing the duration of immunity in poultry vaccinated against highly pathogenic avian influenza H5N1 in the Mekong River Delta of Viet Nam, 2008 – 2010. Presented as 'Factors influencing the immune response of vaccinated poultry in the Mekong region of Viet Nam' at the 13th International Symposium on Veterinary Epidemiology and Economics, 20 – 24 August 2012, Maastricht, The Netherlands.

5.1 Introduction

Highly pathogenic avian influenza (HPAI) caused by H5N1 virus has resulted in a large number of outbreaks in poultry in Viet Nam since late 2003. The country has applied a number of control measures, including vaccination since late 2005, to control HPAI H5N1. Two vaccination rounds (in April and November) carried out each year have involved the administration of approximately 500 million doses of vaccine. At approximately USD 0.02 per dose the cost of vaccine alone for each vaccination round has been estimated to be approximately USD 5 million (DAH 2009, MARD 2009). Using vaccination and other measures such as movement restrictions and culling of infected poultry flocks, HPAI H5N1 outbreaks have largely been brought under control such that in the period March 2009 to December 2011 throughout Viet Nam there was only a small number of sporadic outbreaks (n = 44) in backyard poultry flocks (DAH 2012).

In Viet Nam, the efficacy of the candidate HPAI H5N1 vaccine was assessed firstly in the laboratory, which indicated that following vaccination poultry would be protected against H5N1 viruses for a period of up to 10 months (Tian et al. 2005). In August 2005 the candidate vaccine was trialled in two provinces (Nam Dinh in the north and Tien Giang in the south) of Viet Nam with follow-up serological testing indicating that the vaccine provided good protection under field conditions (MARD 2007). The evidence provided by these two studies was the main justification for the two massive vaccination campaigns held twice per year in Viet Nam. In addition, laboratory challenge experiments have been conducted every year to ensure that the vaccine used in the campaigns was actually protective (Tian et al. 2005, Thanh 2007, NCVD 2009, Tian et al. 2010, Tung & Inui 2011). Since 2005 field assessment of the vaccination program has been assessed using biannual cross-sectional studies (Taylor & Dung 2007, Long 2008, Diep 2011). It should be noted however, that these studies only provide an estimate of H5 seroconversion in the standing poultry population at single time points through the year. Vaccination efficacy is influenced by a number of factors such as the introductions of different clades of HPAI H5N1 viruses, which have been found in apparently healthy geese and domestic ducks in Viet Nam (Nguyen et al. 2005, 2009, 2012). To date, none of the observational epidemiological studies carried out in Viet Nam have documented the length of immune response of vaccinated poultry under field conditions. In addition, routine surveillance

such as the biannual cross-sectional studies described above, do not allow risk factors for vaccination failure under field conditions to be determined with certainty.

To address these knowledge gaps, our objectives in this study were to: (1) determine the median time taken for a H5N1 vaccinated, seropositive birds to become seronegative (that is, quantify the duration of immunity in vaccinated poultry), and (2) quantify the effect of flock-level factors influencing the duration of immunity. This information is necessary if Viet Nam is to take steps to further improve the overall effectiveness of vaccination as a control measure for HPAI H5N1.

5.2 Materials and methods

5.2.1 Study area and study population

A prospective cohort study of avian influenza (AI) infection and HPAI H5N1 vaccination of duck and in-contact species (chickens, Muscovy ducks and geese) flocks was carried out in the Mekong River Delta (MRD) of Viet Nam between 1 December 2008 and 3 April 2010. The study population comprised selected poultry flocks in eight villages from four districts in Can Tho and Bac Lieu province in the MRD (Figure 5.1). Villages and poultry households were selected based on the criteria described previously (Long, Minh, Schauer, Diep, Phuong & Thuy 2012, Long et al. 2013). A total of 157 poultry flocks from 97 contracted households were enrolled into the study. Within each study flock 10 birds that were vaccinated under the requirements of the national vaccination campaign (MARD 2009) were selected using a systematic simple random sampling procedure, and assigned to a vaccinated group. Ten additional unvaccinated birds were assigned to an unvaccinated, sentinel group.

Throughout the 17-month study period (comprised of 14 sampling rounds) a total of 15,478 blood samples were collected from 5,476 study birds (equivalent to 17,968 bird-rounds). This represented 85% of the total number of bird-rounds eligible for sampling. Blood samples were not collected from the remaining 15% (n = 2,720) of eligible bird-rounds because poultry were less than 4 weeks of age at the time of the sampling visit. A small number of serum samples (n = 113 or 0.7%) were ineligible for testing because of conditions related to faulty storage or handling (e.g. haemolysis). Eighty one per cent

(n = 12,536) of the total number of collected serum samples of 5,104 birds were tested (Figure 5.2).

5.2.2 Data management

The haemagglutination inhibition (HI) test was used to detect the presence and amount of antibody against H5 influenza viruses. The HI protocol is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2009) and was customised for use under Vietnamese laboratory conditions (MARD 2005*a*). Positive and negative control antigens and antisera were used in each HI testing plate. H5 HI titres were measured based on the inhibition serum dilutions for each sample. A sample was declared positive if the H5 HI titre was greater than 1/16.

Details of flock management were collected from flock owners at each sampling round using two questionnaires (written in Vietnamese). A baseline questionnaire form (Appendix A) was designed to collect information at the first sampling of the enrolled households. This questionnaire was comprised of 51 questions divided into five parts seeking information about the enrolled households, poultry and animal production, biosecurity, AI control measures and household contact details.

Follow-up questionnaires (Appendix B) were administered at each of the subsequent sampling rounds. These questionnaires were identical, comprised of 32 questions divided into three main parts. The intention was to solicit information on risk factors that may have changed over the course of the study period. In particular, part one was to update contact details of the enrolled flock owners. Part two was to update information about poultry and animal numbers (e.g. number of poultry, numbers of other domestic species kept in the household), poultry movements, the origin of newly introduced poultry and the quarantine procedures applied to them, the presence or absence of contact between the enrolled poultry flocks and outside flocks, and feeding and rice cultivation activities. Part three collected information about AI prevention and control measures such as biosecurity, vaccinations, the presence or absence of HPAI in the home village, treatment and management of sick and dead birds and plans to buy and sell new poultry (flocks).

Baseline and follow-up questionnaires were completed by trained field staff (provincial SDAH and/or district veterinarians) with assistance from commune veterinarians and the

village headman. Field staff were supervised by project staff at every sampling round. At each visit, field staff filled in two separate recording forms: the sampling form used (Appendix C) to collect information on samples and a questionnaire form.

Laboratory data were recorded for each study bird at each round of sampling. All records were entered into a purposely designed spreadsheet by laboratory staff. These data were then imported into a relational database (called the laboratory database), which incorporated information from the sampling form. HI results were coded as 0 for negative serum samples (H5 HI titre $<$ 1/16) or 1 for positive serum samples (H5 HI titre $\geq$ 1/16).

Baseline and follow-up questionnaires were coded with the unique questionnaire identification numbers representing the household and corresponding sampling round. Questionnaire responses were entered into a second relational database (the questionnaire database) by project staff. The laboratory and questionnaire databases were linked together using the respective household and sampling round identifiers.

5.2.3 Statistical analyses

The outcome of interest for this study was an estimate of the duration of vaccine acquired immunity, expressed in days. Duration of immunity was defined as the date on which a bird was first identified as seronegative to the H5 HI test minus the date of the last H5N1 vaccination event. A key assumption here was that H5 HI seropositivity occurred following a vaccination event. Birds were sampled at each of the sampling rounds which equated to sampling intervals of approximately one month during the two high risk periods and two and half months during the single low risk period. The two high risk periods (rounds 1 to 5 between December 2008 and March 2009 and rounds 10 to 14 between December 2009 and March 2010) and one low risk period (rounds 6 to 9 between April 2009 and November 2009) were defined on the basis of a frequency analysis of previous HPAI H5N1 outbreaks in Viet Nam (Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009).

Survival analyses were used to quantify the timing of seronegative events relative to the date of the last H5N1 vaccination. Because assessment of immune response occurred intermittently throughout the study period (i.e. at each sampling date) the exact date on which a seropositive bird became seronegative was not known. For this reason sur-

vival analyses were carried out using an interval censoring approach (Kleinbaum & Klein 2012).

Initially, 20 poultry were selected from each study flock and then assigned to a vaccinated (n = 10) and an unvaccinated, sentinel group (n = 10) group, as described above. As the aim of this study was to estimate the duration of immunity developed from vaccination, only data from vaccinated birds (n = 2,233 birds, equivalent to 5,387 serum samples tested) were used for the interval censored survival analyses (Figure 5.2).

A survival analysis respecting the presence of interval censoring requires a data set that has the dates on which individual birds entered the study, the dates on which birds were last identified as H5 HI positive, and the dates of each of sampling round on which birds were identified as H5 HI negative. It should be noted that many birds had several vaccination events throughout the study period which meant that the total number of observed bird-vaccination intervals (n = 3,797) was greater than the total number of vaccinated birds (n = 2,233, Figure 5.2).

Details of 17 factors (explanatory variables) thought to influence the duration of immunity were collected throughout the study period. These explanatory variables were broadly grouped into those related to geographical location (e.g. the village in which a flock was located), time of vaccination (the two high and one low risk periods) and bird (production type, flock size, age at the time of vaccination). Other, time varying covariates, included whether or not birds were moved out of their home district, the origin of birds introduced into a flock, changes in flock size, the presence of sick birds in the household between sampling rounds, the presence of sick birds in the home village between sampling rounds, the presence of multiple animal species in the household, the presence of pigs in the household, whether or not the household was visited by poultry traders between two consecutive sampling rounds, whether or not the household flock was visited by other poultry flocks (shared rice fields) between two consecutive sampling rounds, whether the flock owner purchased feed for poultry between consecutive sampling rounds, and the presence or absence of cleaning activities between two consecutive sampling rounds. Continuously distributed variables, for example age at the time of vaccination (expressed in days) and flock size were re-coded as categorical variables. The turnover of birds in the population, stratified by production type, was described using a Lexis diagram (Keiding 1990).

The effect of each candidate explanatory variable on the duration of immunity was as-

sessed using the Kaplan-Meier technique (Kaplan & Meier 1958). The homogeneity of time to event was tested using the log-rank statistic. Explanatory variables associated with time to event at P $<$ 0.20 were selected for inclusion in the multivariate analyses. The logarithm of the negative log of survival probability was plotted as a function of the logarithm of follow-up time, and was approximately linear. On this basis we assumed that the time taken for seropositive birds to become seronegative was consistent with the Weibull distribution.

The effect of the identified putative explanatory variables on time to event was quantified using an acceleration failure time (AFT) model (Kalbfleisch & Prentice 1980):

$$\mathcal{S}(t) = exp(-\lambda t^p) = exp\left[-(\lambda^{\frac{1}{p}}t)^p\right] \tag{5.1}$$

where

$$\lambda^{\frac{1}{p}} = exp\left[-(\beta_0 + \beta_1 x_{1ij} + ... + \beta_m x_{mij} + F_i + B_j)\right] \tag{5.2}$$

In Equation 5.1 $S(t)$ represents the probability that a bird remains seropositive up to time t. In Equation 5.2 the terms λ and p represent (respectively) the scale and shape parameter for the Weibull distribution. The term β_0 is the intercept term and $\beta_{1,...m}$ represent the regression coefficients for each of the m explanatory variables included in the model. The terms B_j and F_i represent (respectively) frailty terms for each of the j birds within each of the i flocks.

With this formulation the median duration of immunity (expressed in days) as a function of the parameterised explanatory variables is given by:

$$t = [-lnS(t)]^{\frac{1}{p}} \times \left[\lambda^{\frac{1}{p}}\right]^{-1} \tag{5.3}$$

Equation 5.3 The results of an AFT model can be expressed in terms of a time ratio, representing the increase (or decrease) in time to event as a function of the parameterised explanatory variables, compared with a specified reference category.

A backward-stepwise procedure was used to build the Weibull AFT model, which started with inclusion of all putative explanatory variables that were associated with time to event with a P-value of less than 0.20. Putative explanatory variables that were not statistically

significant were removed from the model one at a time, beginning with the least significant. Only those explanatory variables that had estimated regression coefficients that were significant at $P < 0.05$ or those that were biologically plausible were retained in the final model. A frailty term was included to account for multiple vaccination intervals per bird and clustering at the individual flock level. All biologically plausible interactions between each of the explanatory variables remaining in the final model were assessed.

The final model is reported in terms of an adjusted time ratio for each explanatory variable. An adjusted time ratio (and its 95% confidence interval) of greater than one indicates that, after accounting for other explanatory variables in the model, exposure increased the length of time taken for a bird to become seronegative following vaccination. An adjusted time ratio (and its 95% confidence interval) of less than one indicates that exposure decreased time taken to become seronegative. Statistical analyses and model development were conducted using the survival package (Therneau & Lumley 2012) in R, version 2.15.2 (R Development Core Team 2012).

5.3 Results

Figure 5.3 is a Lexis diagram showing the age of individual vaccinated birds (n = 2,233) as a function of the calendar date on which they were enrolled in the study, stratified by production type. The median time birds remained in the study was 53 (minimum 1, maximum 103) days. Layer ducks remained in the study for longer periods, though some were shorter lived than others. Broiler ducks remained in the study for the shortest period of time and the age of this subgroup at the time of last observation was generally less than 4 months.

Table 5.1 presents results of the bivariate analyses for the 17 putative explanatory variables. A total of 15 variables that were significant at $P < 0.20$ were selected for inclusion in the multivariate model. These selected variables were: village, period, production type, flock size, age at time of vaccination, vaccinator, movement of poultry, buy new poultry, sick poultry at home, sick poultry at home village, multiple animal species, pigs in the household, visited by poultry traders between two consecutive sampling rounds, purchased feed for poultry between consecutive sampling rounds, and cleaning activities between two consecutive sampling rounds.

Figure 5.4 shows the cumulative proportion of seropositive birds as a function of the number of days since last vaccination, stratified by production type. The shaded areas in Figure 5.4 reflect the interval censored structure of the data set, showing the indeterminate nonparametric maximum likelihood estimators of survival within a given interval (Turnbull 1976). This figure shows that layer ducks took longer to become seronegative, followed by in-contact species. The test of homogeneity of the Kaplan-Meier survival curves was significant at the alpha level of 0.05 (log-rank test statistic 729; 2 *df*; $P < 0.01$).

Table 5.2 presents results of the AFT Weibull regression model quantifying the effect of risk factors on the time taken for H5 HI positive birds to become seronegative following vaccination. After adjusting for the effect of when vaccination was carried out (that is, during a high risk or low risk period), the time for H5 HI positive bird aged more than 365 days to become seronegative was increased, compared with birds aged less than 90 days (time ratio 2.09, 95% CI 1.80 – 2.43). After adjusting for the effect of age at the time of vaccination, the time taken for birds vaccinated during the first high risk period (December 2008 to March 2009) and during second high risk period (December 2009 to March 2010) to become seronegative was decreased by a factor of 0.87 (95% CI 0.75 – 1.01) and 0.85 (95% CI 0.73 – 0.99), respectively, compared with birds vaccinated during the single low risk period (April to November 2009).

Table 5.1: Putative risk factors that influence the probability of individual birds becoming seropositive in the Mekong region, Viet Nam.

Variable	n bird-rounds		P value
	Seropositive	Seronegative	
1. Period:			< 0.01
High risk period [a]	1,184	582	
Low risk period [b]	1,071	428	
High risk period [c]	1,149	374	
2. Village:			< 0.01
Ap 4	280	83	
Thoi Phuoc 2	215	64	
Dong Thanh	451	147	
Tan Lap	570	262	
Phung Quoi A	393	164	
Tra Hat	443	227	
My Trinh	558	223	
My Tuong II	494	214	
3. Production type:			< 0.01
Broiler duck	441	219	
Layer duck	1,434	295	
In-contact species	1,529	870	
4. Flock size (number of birds):			< 0.01
≤ 200	986	496	
201 – 500	1,263	466	
> 500	1,155	422	
5. Age at time of vaccination (days):			< 0.01
≤ 90	1,027	544	
91 – 365	1,389	661	
> 365	988	179	
6. Vaccinator:			< 0.01
From other communes	1,831	753	
From home commune	157	70	
Unclear	1,416	561	
7. Movement of poultry:			< 0.01
No	882	464	
Within their home district	2,182	815	
To other districts	340	105	
8. Purchase of new poultry:			< 0.01
No	1,542	555	
Yes	1,703	759	
Not stated	159	70	
9. Change of poultry population:			< 0.01
No	583	256	
Yes	2,556	988	
Not stated	265	140	
10. Sick poultry at home:			< 0.01

Table 5.1 (continued)

Variable	n bird-rounds		P value
	Seropositive	Seronegative	
No	2,358	996	
Yes	866	343	
Not stated	180	45	
11. Sick poultry at home village:			< 0.01
No	2,172	927	
Yes	301	110	
Not stated	931	347	
12. Keep multiple animal species:			< 0.01
No	635	237	
Yes	2,493	1,038	
Not stated	276	109	
13. Presence of pig:			< 0.01
No	1,289	499	
Yes	1,411	575	
Not stated	704	310	
14. Visited by traders:			< 0.01
No	1,500	616	
Yes	1,312	462	
Not stated	592	306	
15. Visited by other poultry flocks:			0.332
No	2,060	803	
Yes	413	171	
Not stated	931	410	
16. Purchase of feed:			< 0.01
No	1,568	706	
Yes	1,644	622	
Not stated	192	56	
17. Cleaning places kept poultry:			0.02
No	3	0	
Yes	3,158	1,300	
Not stated	243	84	
Total:	3,404	1,384	

[a] December 2008 to March 2009.

[b] April to November 2009.

[c] December 2009 to March 2010.

Table 5.2: Results of the Weibull AFT regression model show the effect of risk factors on the probability of individual birds becoming serogenative in the Mekong region, Viet Nam.

Variable	n^a	Coefficient (SE)	P value	Time ratio (95% CI)	Days (95% CI)^b
Intercept	3,404	3.827 (0.0872)	< 0.01		
Study period:					
High risk period ^{c}	1,184	-0.137 (0.0768)	< 0.01	0.87 (0.75 – 1.01)	40 (29 – 55)
Low risk period ^{d}	1,071	Reference		1.00	46 (39 – 54)
High risk period ^{e}	1,149	-0.158 (0.0776)	< 0.01	0.85 (0.73 – 0.99)	39 (28 – 54)
Age at time of vaccination (days):					
≤ 90	1,027	Reference		1.00	46 (39 – 54)
91 – 365	1,389	0.406 (0.0846)	< 0.01	1.50 (1.27 – 1.77)	69 (49 – 97)
> 365	988	0.736 (0.0763)	< 0.01	2.09 (1.80 – 2.43)^f	96 (72 – 132)

SE: Standard error of regression coefficient; CI: confidence interval.

^{a} Number of bird-rounds with a positive H5HI test.

^{b} Number of days taken for birds to become seronegative.

^{c} December 2008 to March 2009.

^{d} April to November 2009.

^{e} December 2009 to March 2010.

^{f} Interpretation: after adjusting for the effect of the defined risk periods, the median time taken for birds aged greater than 365 days to become seronegative was increased by a factor of 2.09 (95% CI 1.80 – 2.43) compared with birds aged less than 90 days.

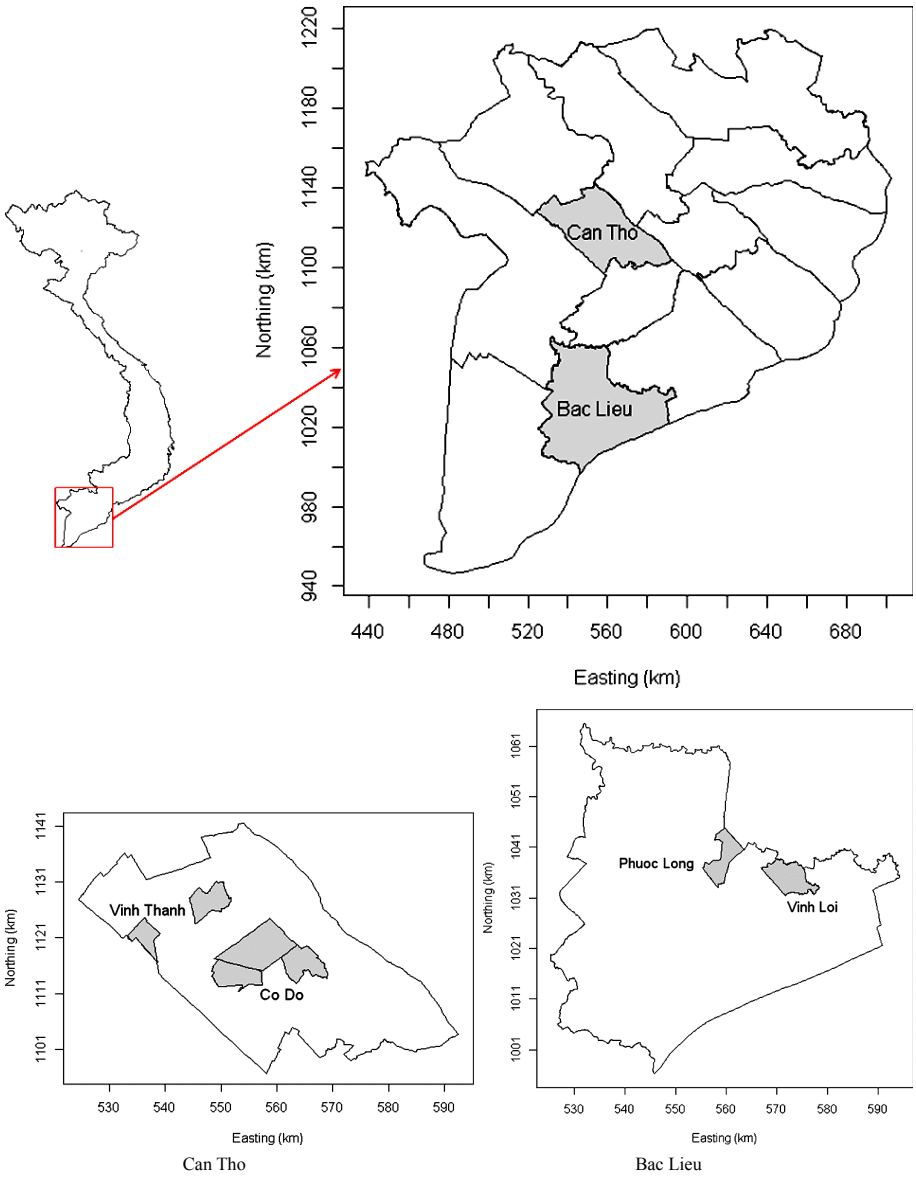


Figure 5.1: Map of Viet Nam showing the study areas. The upper figure shows the Mekong River Delta and the two study provinces (Can Tho and Bac Lieu). The lower figure shows the two study provinces and the shade showing study communes of selected districts (Vinh Thanh and Co Do of Can Tho, Phuoc Long and Vinh Loi of Bac Lieu).

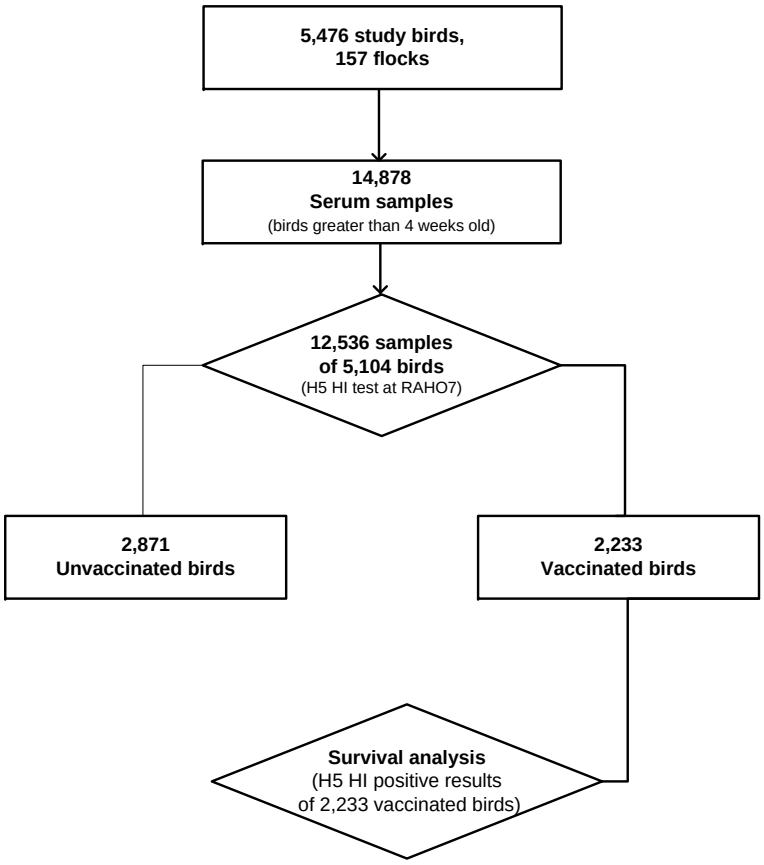


Figure 5.2: Flow chart of sample testing and data analysis. The numbers listed in each box indicating the total number of samples/birds collected and tested over the entire study period.

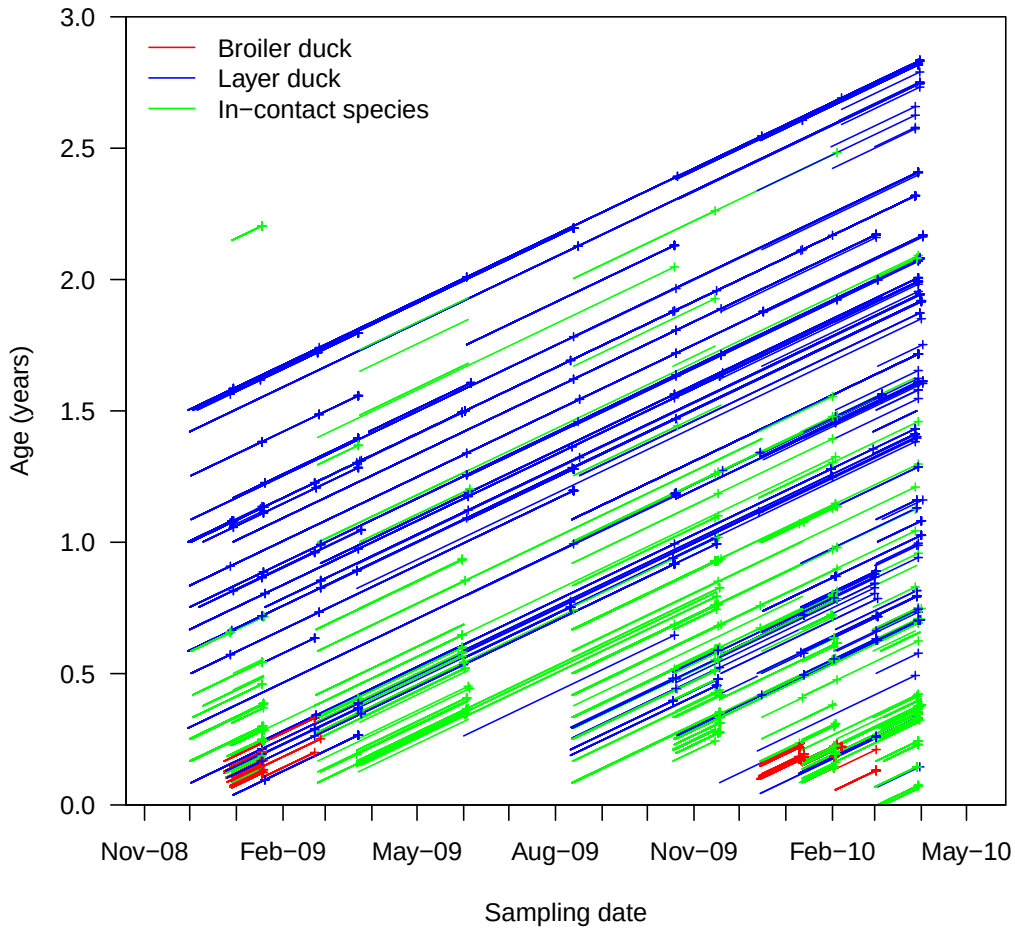


Figure 5.3: Lexis diagram showing the age of individual vaccinated birds (n = 2,233) as a function of the calendar date they were enrolled in the study, stratified by production type.

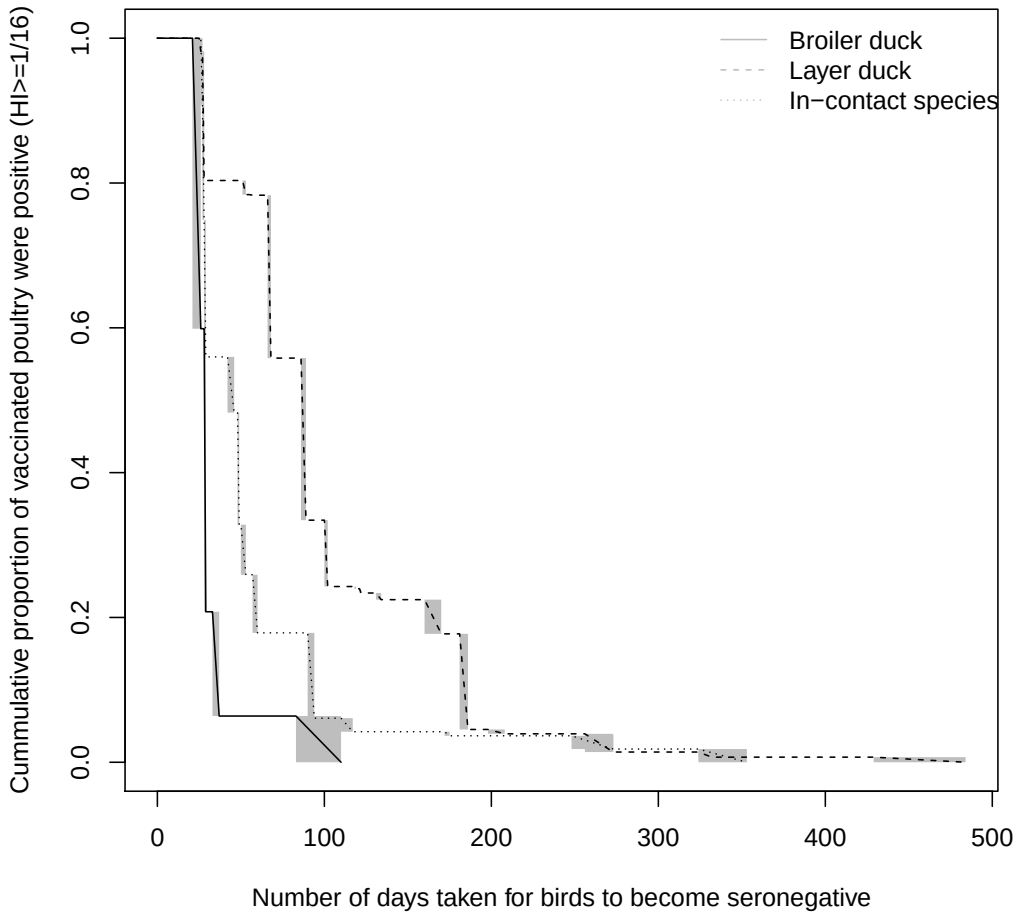


Figure 5.4: Kaplan-Meier survival curves showing the cumulative proportion of seropositive birds stratified by production type (log-rank test statistic 729; 2 *df*; $P < 0.01$).

5.4 Discussion

Vaccination has been used as a tool to control HPAI H5N1 in Viet Nam since late 2005. The efficacy of the HPAI H5N1 vaccine has been regularly evaluated under laboratory conditions through challenge experiments (Tian et al. 2005, Thanh 2007, Pfeiffer et al. 2010, Tian et al. 2010, Tung & Inui 2011). Post-vaccination monitoring under field conditions has been carried out using a series of cross-sectional studies that have estimated the proportion of vaccinated poultry positive to a H5 HI test at single points in time (Taylor & Dung 2007, Long 2008, Diep 2011). Prior to this study, the duration of immune response of vaccinated poultry under field conditions was unknown and likely to be influenced by a number of factors operating at the individual bird, flock and regional levels.

Our results show that, following a vaccination event, the median time taken for H5 seropositive birds to become seronegative was 56 (95% CI 51 – 61) days, considerably shorter than the 10 month period documented in laboratory-based studies (Tian et al. 2005). This difference can be explained by a number of factors including vaccine quality, vaccine administration, the number of vaccination shots given, the age of birds at the time of vaccination, breed and production type. Our AFT model showed that, in agreement with previous studies (Peyre et al. 2009, Hinrichs & Otte 2012), factors such as production type, flock size, and the identity of the individual administering the vaccine (Table 1) did not significantly influence the duration of immunity. Analyses of data from a cross-sectional study (Long 2008, 2009) carried out in the MRD between 2007 and 2009 found that factors operating at the individual household and provincial levels accounted for most of the variation in flock-level protection risk (Diep 2011). When discussing the findings from a longitudinal study carried out in the MRD from May 2008 to May 2009 (Henning et al. 2011) speculated that maintenance of the vaccine cold chain and skill of those administering vaccine to flocks were likely determinants of protection risk in this area of Viet Nam.

Vaccine quality is influenced by cold chain management because commercially available HPAI vaccines are not thermostable (Peyre et al. 2009, Hinrichs & Otte 2012). The cold chain is therefore important, particularly when environmental temperatures are relatively high, as in this area of Viet Nam. For example, in the provinces of Can Tho and Bac Lieu temperatures range between 24 °C and 26 °C during the rainy season (from April to

November), and 28 °C and 33 °C during the dry season (from December to March). From the place of manufacture (Harbin, Heilongjiang province, North China) H5N1 vaccine is transported to selected vaccine suppliers (RTD in Ha Noi and Navetco in Ho Chi Minh City) in Viet Nam. From there it is sent to SDAHs and District Veterinary Stations (DVS) where it is stored in refrigerators. The cold chain from place of manufacture down to the DVS level is likely to be consistent because refrigeration facilities from one SDAH/DVS to the next are of a uniformly high standard (Kien 2009).

We speculate that the cold chain from SDAH/DVS to the individual flock level is not as uniform and this is an area requiring further investigation. In a typical working day, field veterinarians come to SDAH/DVS offices to collect H5N1 vaccine and store it in wet-ice cool boxes until the time of vaccination. The temperature of these cool boxes tends not to be monitored and depletion of ice in these boxes due to melting may negatively impact on vaccine quality, particularly during the hotter, dry season. This explanation is supported by the results of our AFT model which shows that the median time taken for H5 seropositive birds that were vaccinated during the dry season (December 2008 to March 2009 and December 2009 to March 2010) was 39 (95% CI 28 – 55) days, somewhat shorter than the median of 46 (95% CI 39 – 54) days for birds vaccinated during the wet season (from April to November 2009). The descriptive results from the prospective cohort study (of which this study is a component, (Long et al. 2013)) showed that the period prevalence of H5 HI seropositivity of vaccinated birds for the April to November period was greater than that recorded for vaccinated birds in the two December to March periods. (Henning et al. 2011) also found that vaccinated birds had a lower protection rate in November 2007.

In this study, we found no association between the identity of those who administered vaccine and duration of immunity. The most likely reason for this finding was that the field staff who carried out vaccination procedures were trained and continuously supported by provincial SDAH and project staff throughout the course of the study period.

The immune response in vaccinated birds was influenced by age at the time of vaccination (Table 5.2). In particular, the time taken for H5 seropositive birds aged less than 90 days, between 91 and 356 days and more than 365 days to become seronegative was 46 (95% CI 39 – 54), 69 (95% CI 49 – 97) and 96 (95% CI 72 – 132) days, respectively. Reasons for this difference are that birds aged less than 90 days mainly comprised broiler ducks

which were also the youngest birds and are often kept for about three months so that they only had maximum of two vaccine shots over their lifespan. In addition, young birds are known to have a relatively poor level of antibody induction after vaccination (Maas et al. 2011) as their immune system is still developing. In our study, the average HI titre of positive broiler ducks (youngest birds) was 6log2. In contrast, layer ducks aged more than 365 days had an average of 3 vaccination rounds (equivalent to 6 vaccine shots) and given that they were older, it is likely that they had mature immune systems. The booster vaccinations or natural exposure to virus (Long et al. 2013) is likely to have played a central role in the strong immune response of vaccinated layer ducks (the average HI titre was 7log2 in our study). Our AFT model shows that, after adjusting for the effect of risk period, time taken for birds aged more than 365 days to become seronegative was increased by a factor of 2.09 (95% CI 1.80 – 2.43) compared with birds aged less than 90 days. In-contact species, comprised mainly of chickens, also had a stronger immune response (the average HI titre was 6.5log2) and a longer duration of immunity of 46 (95% CI 25 – 75) days compared with broiler ducks. This finding is consistent with previous studies which found that antibody responses in ducks could be lower than chickens under laboratory and field conditions (Tumpey et al. 2004, Webster et al. 2006) in Viet Nam (Taylor & Dung 2007, Diep 2011).

One of the strengths of this study was that our analyses respected the interval censored structure of the data. Ignoring the presence of interval censoring produced estimates of immune duration that were at least twice that of our estimates where interval censoring was accounted for. In this respect, the estimates of immune duration presented in this paper are conservative. A limitation of this study is that our results are only applicable to poultry in the MRD of Viet Nam. It is possible that in other areas of the country the effect of season and age at the time of vaccination as determinants of immune duration could vary. Our results should be interpreted accordingly. A second limitation relates to the number of bird-vaccination intervals available for analysis. Other, more subtle, influences on immune duration could have been detected if a larger study population was available.

5.5 Conclusions

Under field conditions the overall median time taken for H5 vaccinated, seropositive birds to become seronegative was 56 (95% CI 51 – 61) days, considerably shorter than the 10 month period documented in laboratory-based studies. Age of poultry at the time of vaccination and time of the year when vaccination was carried out significantly influenced duration of immunity in vaccinated birds. These two factors should be taken into account if the current vaccination strategy is to be continuously practiced in Viet Nam.

CHAPTER 6

Risk factors for influenza Type A virus infection in poultry in the Mekong River Delta of Viet Nam, 2008 – 2010

Abstract – A prospective cohort study was carried out to quantify the relative contribution of bird, flock and village-level influences on the risk of influenza Type A virus infection in field running ducks (broiler and layer) and in-contact species (predominantly chickens) in eight villages in the Mekong region of Viet Nam from December 2008 to April 2010. A total of 17,968 oropharyngeal and cloacal swab samples were taken from 5,476 birds within 157 flocks throughout the follow-up period. These were then tested using RRT-PCR to detect the matrix (M) gene of avian influenza (AI) viruses. A multilevel logistic regression analysis was carried out to quantify risk factors for influenza Type A infection and to estimate the relative contribution of unmeasured flock- and bird-level factors on influenza Type A infection risk.

The overall proportion of positive individual birds was 0.10 (95% CI 0.09 – 0.11). Fifty eight *percent* (91 of 157) study flocks from all eight study villages were identified as infected with influenza Type A viruses throughout the follow-up period. The proportions of flock rounds influenza Type A virus positive were 0.20 (95% CI 0.17 – 0.23). Our multilevel analyses show that, after adjusting for the other variables included in the model, the odds of influenza Type A virus infection in broiler ducks was 28.2 (95% CI 5.93 – 133) times the odds of influenza Type A virus infection in in-contact species. The proportions of variance at the village, flock and bird level were 5%, 48% and 47%, respectively. Most of the significant fixed-effects in our model were flock-level exposures. The findings from this study support a feasible policy of targeting influenza Type A intervention measures at the individual bird and individual flock level.

Long, N.V., Stevenson, M., Schauer, B. and Diep, N.T. (2013) Risk factors for influenza Type A virus infection in poultry in the Mekong River Delta of Viet Nam, 2008 – 2010.

6.1 Introduction

Avian influenza (AI) viruses include the highly pathogenic avian influenza (HPAI) subtypes such as H5 and H7, which have the potential to produce severe disease not only in animals but also in humans (OIE 2012, WHO 2012). Influenza Type A viruses, based on their replication characteristics, can infect a number of mammalian and poultry species and can cross species barriers with relative ease (Webby et al. 2002). Aquatic birds, including domestic ducks, are the natural reservoir of many subtypes of influenza viruses (Webster et al. 1992, Monto 2000). A better understanding of factors influencing the way in which influenza Type A viruses circulate in animals is important because it provides the opportunity to better understand and therefore predict the way in which HPAI might behave in previously unexposed populations.

In Viet Nam, multiple influenza Type A subtypes, including the low and high pathogenic viruses have been identified in apparently healthy geese and domestic ducks (Nguyen et al. 2005, Nomura et al. 2011, Hotta et al. 2012, Long et al. 2013). The HPAI H5N1 virus that caused tremendous losses in poultry populations since late 2003 (Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, MARD 2011) has mainly been confined to areas of intensive poultry production, such as the Mekong River Delta (MRD) and Red River Delta (RRD). In these areas, the concurrent circulation of multiple AI viruses and a large reservoir population is believed to provide conditions favourable for virus reassortment (Hulse-Post et al. 2005, Webster et al. 2007). The epidemiology of AI may be further complicated as AI viruses have been shown to circulate widely in vaccinated poultry populations (Nomura et al. 2011, Long et al. 2013).

A number of studies have been carried out to identify risk factors for HPAI H5N1 virus infection in poultry in Viet Nam (Henning et al. 2009, Minh 2010, Desvaux et al. 2011, Nomura et al. 2011, Long et al. 2013). A matched case-control study was carried out using epidemic data collected between December 2006 and January 2007 in the MRD to estimate household- and flock-level risk factors for HPAI H5N1 outbreaks (Henning et al. 2009). This study found that there was a significant increase in the risk of HPAI H5N1 infection in poultry flocks that were either not vaccinated or those that received only a single vaccine shot. Another case-control study (Minh 2010) carried out in the same area between January and September 2009 identified risk factors for HPAI H5N1 outbreaks

at the individual village and household level. Minh's study found that households with large flocks and those with field running ducks had the greatest risk of HPAI H5N1. In the Red River Delta (RRD) a case-control study showed that villages with traders and high proportions of broiler duck flocks were at an increased risk of disease (Desvaux et al. 2011). At the individual household level the risk of disease was increased when multiple animal species were present, where there were large numbers of birds and where there was ready access to pond water.

Active surveillance for HPAI H5N1 has been undertaken throughout Viet Nam since 2005 (Taylor & Dung 2007, Long 2008, Wan et al. 2008, Long 2009, Nguyen et al. 2009, 2012). Twice yearly Department of Animal Health (DAH) staff collect swab samples from the cloaca of live birds in markets and poultry-owning households. At the same time information about bird production type, age, flock size and location is collected from the owners of sampled birds. The main of objective of this program is to estimate the prevalence of HPAI H5N1 infection. A secondary objective is to re-confirm the presence or absence of previously identified risk factors for infection. Finally, documentation of the H5N1 clades responsible for clinical disease (which can vary both temporally and geographically) determines which of the HPAI H5N1 candidate vaccines is appropriate for the national vaccination campaign to be carried out in the following year.

While each of the observational epidemiological studies and the on-going, active surveillance program described above have provided useful information to inform the development of HPAI H5N1 control strategies in Viet Nam, a key limitation is that they provide little indication of risk factors for disease that may change over time. To address this knowledge gap a prospective cohort study of AI infection in field running ducks (both broilers and layers) and in-contact species in the MRD was carried out between December 2008 and April 2010. The objective of the cohort study was to estimate the period prevalence and incidence rates of AI virus infection in poultry populations (Long et al. 2013) and to monitor the course of antibody response following vaccination (Long, Stevenson, Schauer & Thuy 2012). This third paper, using data from the prospective cohort study, uses multilevel logistic regression to quantify risk factors for influenza Type A virus infection.

6.2 Materials and methods

6.2.1 Study area and study population

A prospective cohort study was conducted between 1 December 2008 and 3 April 2010 to identify risk factors for AI virus infection of poultry flocks. The study population was comprised of a total of 5,476 poultry selected at random from 157 flocks kept within 97 households within eight villages in four districts within two provinces (Can Tho and Bac Lieu) in the MRD of Viet Nam (Figure 6.1). In brief, poultry flocks were visited repeatedly throughout the study period with enrolled birds sampled on each occasion. Further details of the study design and sampling procedures are provided by (Long, Minh, Schauer, Diep, Phuong & Thuy 2012, Long et al. 2013).

6.2.2 Laboratory procedures

At each sampling round a single oropharyngeal and cloacal swab sample was taken from each study bird. These swab samples were mixed, providing a total of 17,968 samples from 5,476 birds over the entire study period. Samples were tested for the presence of influenza Type A viruses using the Matrix (M) gene RRT-PCR

6.2.3 Data management

Details of possible risk factors for influenza Type A virus infection were recorded at the individual bird and household level using two types of questionnaire written in Vietnamese. A baseline questionnaire was completed at the time a flock was first enrolled into the study. This questionnaire was comprised of 51 questions related to household contact details, poultry and animal production within the household, biosecurity and the use of control measures.

A follow-up questionnaire was administered at each of the subsequent sampling rounds. Sampling rounds were carried out monthly during the two high risk periods (from December 2008 to March 2009 and from December 2009 to April 2010) and every two months during the single low risk period (from April to November 2009). The follow-up questionnaire was comprised of 32 questions divided into four main sections. The first section

provided the household owner with the opportunity to update their contact details. The second provided details of current poultry and animal numbers within the household as well as details of changes in flock size that occurred since the last sampling visit. The third section was comprised of three questions seeking information about duck raising, duck movements and details of the contact that ducks may have had with other poultry populations since the last sampling visit. The fourth section dealt with disease prevention and control measures applied since the last sampling visit, including details of administered vaccines, the current disease situation in the home village, the presence or absence of health issues in the flock, and treatment and management of sick and dead birds.

Three trained field staff (provincial Sub-department of Animal Health and/or district veterinarians) were assigned to each study village, with assistance provided by one commune veterinarian and one village headman. These individuals were continuously supervised by project staff throughout the study. At each sampling visit, one member of the field staff team conducted a face to face interview with the flock owner while the two other members of the team carried out the sampling. Field staff filled in two separate recording forms: the sampling form used to collect information about the samples and the follow-up questionnaire, described above.

Unique identifiers were assigned to each study bird and each study flock at each sampling round. Questionnaire responses were entered directly into a relational database by assigned project staff. Pooled and individual M gene RRT-PCR results were entered into a spreadsheet package by laboratory staff. Following each sampling round these data were imported into the relational database by assigned project staff. Laboratory and questionnaire data were linked within the relational database using the bird, flock, household and sampling round identifiers.

6.2.4 Statistical analyses

In this study the outcome of interest was the presence or absence of influenza Type A virus infection at the individual bird level. A bird was considered influenza Type A positive if at least one swab sample out of all samples collected throughout the study period was positive to the M gene RRT-PCR. Multilevel logistic regression analyses were carried out to estimate the contribution of the hierarchical structure of the data (that is, study bird

within flocks and study flocks within villages) to the total variation in the probability of individual poultry being influenza Type A virus positive. The estimate of the proportion of variation at each level of the data were computed assuming the level one (that is, bird) variance on the logit scale was $\pi^2/3$ (Dohoo et al. 2001, Snijders & Bosker 2011).

Each of the 19 putative explanatory variables derived from the questionnaire responses were selected in turn as the explanatory variable in a univariate logistic regression model. These explanatory variables were broadly grouped into those related to geographical location (e.g. the village in which a flock was located), the study period (the two high and one low risk periods) and bird (production type, flock size, age of birds). Other, time varying covariates, included whether or not birds were vaccinated, who were vaccinator, whether or not birds were moved out of their home district, the origin of birds introduced into a flock, whether or not newly purchased birds were separated, the presence of multiple animal species in the household, the presence of pigs in the household, the presence of sick birds in the household between sampling rounds, the presence of sick birds in the home village between sampling rounds, whether or not the household was visited by poultry traders between two consecutive sampling rounds, whether or not the household flock was visited by other poultry flocks (shared rice fields) between two consecutive sampling rounds, and the presence or absence of cleaning activities between two consecutive sampling rounds, changes in flock size, whether the flock owner purchased feed for poultry between consecutive sampling rounds. Continuously distributed variables, for example age at the time of vaccination (expressed in days) and flock size were re-coded as categorical variables.

The Wald test (Agresti 2007) was used to test the significance of each explanatory variable as a predictor of the probability of a bird being influenza Type A positive. All explanatory variables associated with the study outcome at an alpha level of less than 0.2 were then entered into a multivariable logistic regression model. Non-significant explanatory variables were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all of the retained variables were significant at $P < 0.05$. Biologically plausible two-way interactions were included and retained in the final model if they were significant at $P < 0.05$. Random effect terms were then included to account for the unmeasured effects of flock and village on the probability of being influenza Type A positive (a mixed-effects logistic regression model). Using this

approach the logit transform of the probability (p) a bird i being positive with influenza Type A virus in flock j in village k, p_{ijk} was modelled as a linear function of a set of m fixed effects β_1, ..., β_m and unmeasured influences at the village F_j and village V_k level:

$$log\left[\frac{p_{ijk}}{1-p_{ijk}})\right] = \beta_0 + \sum_{i=1}^{m} \beta_m X_{mijk} + F_j + V_k + \epsilon_{ijk} \quad (6.1)$$

The results of the final model are reported in terms of adjusted odds ratios for each explanatory variable. An adjusted odds ratio (and its 95 per cent confidence interval [CI]) of greater than 1 indicates that, after adjusting for other variables in the model, exposure to the explanatory variable increased the risk of poultry being positive with influenza Type A viruses. An adjusted odds ratio (and its 95 per cent CI) of less than 1 indicates that exposure to the explanatory variable was positive, and an odds ratio of 1 indicates that the variable had no influence on incidence risk.

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 AI virus infected and non-infected poultry. The greater area under the ROC curve the better the model's discriminatory power (Hosmer & Lemeshow 2000). Statistical analyses were performed using the lme4 package (Bates et al. 2012) in R version 2.15.2 (R Development Core Team 2012).

6.3 Results

6.3.1 Descriptive analyses

Table 6.1 represents a summary of the hierarchical structure of the data. A total of 5,476 birds from 157 flocks were selected from 97 households within 8 villages. The average number of swab samples per bird was 3 (range 1 to 14 samples). The average number of birds per flock was 35 (range 10 to 114 birds). The average number of flocks per household was 2 (range 1 to 5 flocks). The average number of households per village was 12 (range 7 to 15 households). Sixty eight *percent* of households kept flocks comprised

of only one production type while 25% (24 of 97) and 7% (7 of 97) kept flocks comprised of two production types and three production types, respectively.

The overall proportion of pools and individual birds positive with influenza Type A viruses was 10% (95% CI 9 – 11). Fifty eight *percent* (91 of 157) of the study flocks were identified as influenza Type A virus positive throughout the study period. The proportion of flock rounds (n = 183) influenza Type A positive was 20% (95% CI 18 – 23).

One hundred *percent* and 99% of flock owners responded to the baseline questionnaire (n = 97) and the follow-up questionnaires (n = 808), respectively. Information about putative risk factors was recorded for a total of 905 flock rounds.

6.3.2 Univariate analyses

A total of 19 putative explanatory variables were assessed in the bivariate logistic regression analyses (n = 5,476). Fifteen of these were significant at $P < 0.20$ (Table 6.2): risk period (as described earlier), village, production type, flock size, age category, presence or absence of movement of the study poultry flocks to other districts, presence or absence of purchasing new birds, presence or absence of separation of newly purchased birds, presence or absence of other animal species within the study household, presence or absence of sick bird within home village, presence or absence of sick bird within study household, presence or absence of a visit by poultry traders, presence or absence of a visit by other poultry flocks, changes in flock size and, presence or absence of purchasing poultry feed between consecutive sampling rounds. These risk factors were the selected for inclusion in the multivariable analyses.

6.3.3 Multilevel analyses

Table 6.3 provides the estimated regression coefficients for each of the explanatory variables included in the final mixed-effects logistic regression model. After adjusting for the other variables in the model, the odds of influenza Type A virus infection in broiler ducks was 28.16 (95% CI 5.93 – 133.65) times the odds of influenza Type A virus infection in in-contact species. For broiler flocks with newly purchased birds there was a modest increase in the odds of infection to 29.74 (95% CI 15.16 – 44.33, that is

$\exp^{[3.338-0.8859+0.9407]}$). In contrast, the interacting effect of newly purchased birds into layer flocks substantially increased the odds of infection to 33.86 (95% CI 21.52 – 37.97, that is $\exp^{[2.8641-0.8859+1.5440]}$).

In the mixed-effects model the level one (that is, bird) variance was constrained to unity. The estimates of the proportion of variance at the level two (flock) and level three (village) levels were computed by assuming the level one variance on the logit scale was $\pi^2/3$. The variance estimates at the village and flock levels were 0.33 and 3.37 (respectively) giving rise to the total variance in the data as 6.99 (0.33 + 3.37 + $\pi^2/3$). The proportions of variance at the village, flock and bird level were 0.05 (0.33 $\div$ 6.99), 0.48 (3.37 $\div$ 6.99) and 0.47 ($\pi^2/3 \div$ 6.99), respectively.

Figure 6.3 is a box and whisker plot showing the point estimate and 95% confidence intervals for the estimated regression coefficients for each of the explanatory variables in the fixed-effect and mixed-effect models. A caterpillar plot of the flock-specific mean log odds of being influenza Type A positive (Figure 6.4) was generated to evaluate how infection risk varied across flocks. The overall average log odds of influenza Type A infection at the flock level was 0.09 (range -3.33 to 3.85).

The predictive power of the final mixed-effects model, as measured by the area under Receiver Operating Characteristic curve was 0.82 indicating that the model had a good discriminatory ability to predict the flock influenza Type A virus infection status.

Table 6.1: Risk factors for influenza Type A infection of poultry flocks in the MRD of Viet Nam, December 2008 to April 2010. Description of the hierarchical structure of the data.

Level	Number	Average number per unit at next-higher level[α]	Range
Village	8	–	–
Household	97	12	7 – 15
Flock	157	2	1 – 5
Bird	5,476	35	10 – 114
Sample	17,968	3	1 – 114

[α] Each village had, on average, 12 (range 7 – 15) households. Each household had, on average, 2 (range 1 – 5) flocks. Each flock had, on average, 35 (range 10 – 114) birds. Each bird had, on average, 3 (range 1 – 14) swab samples.

Table 6.2: Risk factors for influenza Type A infection of poultry flocks in the MRD of Viet Nam, December 2008 to April 2010. Descriptive statistics of the explanatory variables that were selected to be included in the fixed-effects and mixed-effects models.

Variable	*n* flock positive	*n* flock tested	OR (95% CI)	P value
1. Period:				
High risk period [a]	343	2,493	1.45 (1.19 – 1.77)	<0.01
Low risk period [b]	164	1,658	1.00	Reference
High risk period [c]	92	1,325	0.68 (0.52 – 0.88)	<0.01
2. Village:				
Ap 4	52	551	2.54 (1.56 – 4.25)	< 0.01
Thoi Phuoc 2	84	557	4.34 (2.75 – 7.07)	< 0.01
Dong Thanh	24	610	1.00	Reference
Tan Lap	69	780	2.37 (1.49 – 3.89)	< 0.01
Phung Quoi A	86	632	3.85 (2.45 – 6.26)	< 0.01
Tra Hat	111	764	4.15 (2.68 – 6.69)	< 0.01
My Trinh	62	819	2.00 (1.25 – 3.30)	< 0.01
My Tuong II	111	763	4.16 (2.68 – 6.70)	< 0.01
3. Production type:				
Broiler duck	246	800	24.52 (18.09 – 33.85)[d]	< 0.01
Layer duck	301	1,752	11.46 (8.56 – 15.64)	< 0.01
In-contact species	52	2,924	1.00	Reference
4. Flock size (number of birds):				
$\leq$ 200	128	1,901	1.00	Reference
$>$ 200	471	3,575	2.10 (1.72 – 2.59)	< 0.01
5. Age categories (days):				
$\leq$ 90	499	4,998	1.00	Reference
91 - 365	70	369	1.94 (1.46 – 2.53)	0.03
$>$ 365	30	82	5.20 (3.25 – 8.17)	< 0.01
6. Poultry moved to other districts:				
No	445	4,865	1.00	Reference
Yes	154	611	3.35 (2.72 – 4.11)	< 0.01
7. Buy new bird:				
No	120	1,750	1.00	Reference
Yes	479	3,726	2.00 (1.63 – 2.48)	< 0.01
8. Separation of new birds:				
No	562	4,904	1.87 (1.34 – 2.68)	< 0.01
Yes	37	572	1.00	Reference
9. Presence of other animal species:				
No	118	802	1.65 (1.31 – 2.05)	< 0.01
Yes	362	3,820	1.00	Reference
Not stated	119	854	1.55 (1.23 – 1.93)	< 0.01
10. Presence of sick birds at village:				
No	575	5,111	1.00	Reference
Yes	24	365	0.56 (0.35 – 0.83)	< 0.01
11. Presence of sick birds at study household:				

Table 6.2 (continued)

Variable	*n* flock positive	*n* flock tested	OR (95% CI)	P value
No	541	4,454	1.00	Reference
Yes	58	931	0.49 (0.37 – 0.65)	< 0.01
12. Visited by poultry traders:				
Yes	262	2,955	1.00	Reference
No	337	2,521	1.59 (1.34 – 1.88)	< 0.01
13. Visited by other poultry flocks:				
No	577	4,993	1.00	Reference
Yes	22	483	0.37 (0.23 – 0.55)	< 0.01
14. Changes of poultry flock size:				
No	401	2,394	1.00	Reference
Increased	97	1,538	0.33 (0.26- – 0.42)	< 0.01
Decreased	101	1,544	0.35 (0.28 – 0.44)	0.22
15. Buy poultry feed				
No	168	2,519	1.00	Reference
Yes	431	2,957	2.39 (1.98 – 2.88)	
Total:	599	5,476		

n number of flocks positive and flocks tested; OR = Odds ratio; CI: confidence interval.

[a] December 2008 to March 2009.

[b] April to November 2009.

[c] December 2009 to March 2010.

[d] Interpretation: The odds of influenza Type A virus infection in broiler ducks was 24.52 (95% CI (18.09 – 33.85) times the odds of influenza Type A virus infection in in-contact species.

Table 6.3: Risk factors for influenza Type A infection of poultry flocks in the MRD of Viet Nam, December 2008 to April 2010. Regression coefficients and their standard errors for the final mixed-effects logistic regression model.

Variable	Coefficient (SE)	t-value	OR (95% CI)	P value
Fixed effects:				
Intercept	-6.1767 (0.6296)	-9.810		< 0.01
Production type:				
Broiler duck	3.3378 (0.7946)	4.201	28.16 (5.93 – 133.65)[a]	< 0.01
Layer duck	2.8641 (0.5645)	5.074	17.53 (5.80 – 53.01)	< 0.01
In-contact species	Reference		1.00	
Flock size (number of birds):				
≤ 200	0.0653 (0.3296)	0.198	1.07 (0.56 – 2.04)	0.84
> 200	Reference		1.00	
Buy new bird:				
No	Reference		1.00	
Yes	-0.8859 (0.4128)	-2.146	0.41 (0.18 – 0.93)	< 0.03
Separation of new birds:				
No	1.2120 (0.2878)	4.212	3.36 (1.91 – 5.91)	< 0.01
Yes	Reference		1.00	
Presence of other animal species:				
No	0.1363 (0.4402)	0.310	1.15 (0.48 – 2.72)	0.37
Yes	Reference		1.00	
Not stated	0.2863 (0.3197)	0.896	1.33 (0.71 – 2.49)	< 0.01
Visited by other poultry flocks:				
No	Reference		1.00	
Yes	-0.5873 (0.3043)	-1.930	0.56 (0.31 – 1.01)	0.05
Changes of poultry flock size:				
No	Reference		1.00	
Increased	-0.6941 (0.2189)	-3.173	0.50 (0.33 – 0.77)	< 0.01
Decreased	-0.8662 (0.2093)	-4.139	0.42 (0.28 – 0.63)	< 0.01
Interaction terms:				
Broiler duck $\times$ buy new birds[b]	0.9407 (0.7999)	1.176		0.24
Layer duck $\times$ buy new birds[c]	1.5440 (0.4570)	3.379		< 0.01
Size $\leq 200 \times$ other animals	1.6831 (0.6363)	2.645		< 0.01
Size $> 200 \times$ other animals	0.7944 (0.6223)	1.277		0.20
Mixed effects:	Variance			
Flock : Village	3.3678			
Village	0.3345			

SE: Standard error of regression coefficient. OR: Odds ratio. CI: confidence interval.

[a] Interpretation: After adjusting for the other variables in the model, the odds of influenza Type A virus infection in broiler ducks was 28.16 (95% CI 5.93-133.65) times the odds of influenza Type A virus infection in in-contact species. [b] For broiler flocks with newly purchased birds there was a modest increase in the odds of infection to 29.74 (95% CI 15.16 – 44.33, that is $\exp^{[3.338-0.8859+0.9407]}$). [c] The interacting effect of newly purchased birds into layer flocks substantially increased the odds of infection to 33.86 (95% CI 21.52 – 37.97, that is $\exp^{[2.8641-0.8859+1.5440]}$).

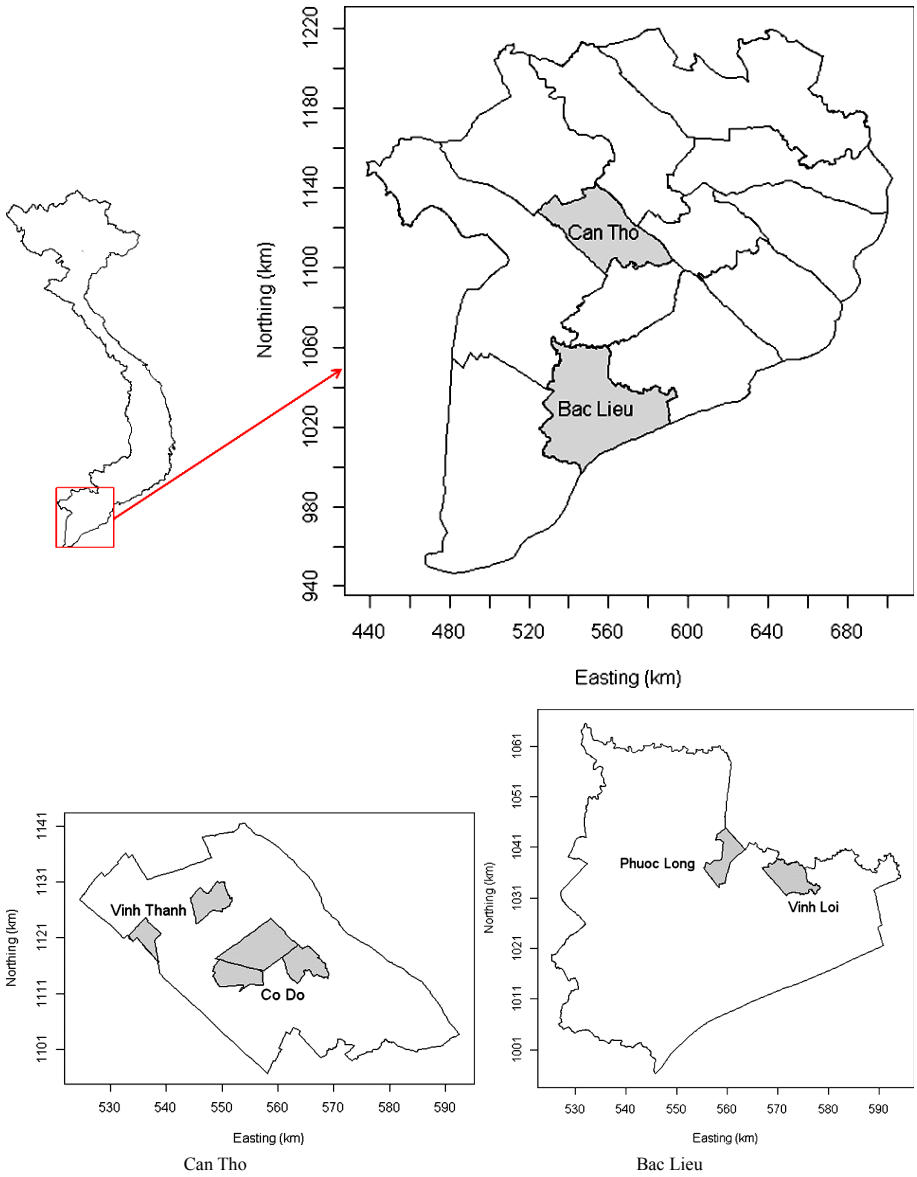


Figure 6.1: Map of Viet Nam showing the study areas. The upper figure shows the Mekong River Delta and the two study provinces (Can Tho and Bac Lieu). The lower figure shows the two study provinces and the shade showing study communes of selected districts (Vinh Thanh and Co Do of Can Tho, Phuoc Long and Vinh Loi of Bac Lieu).

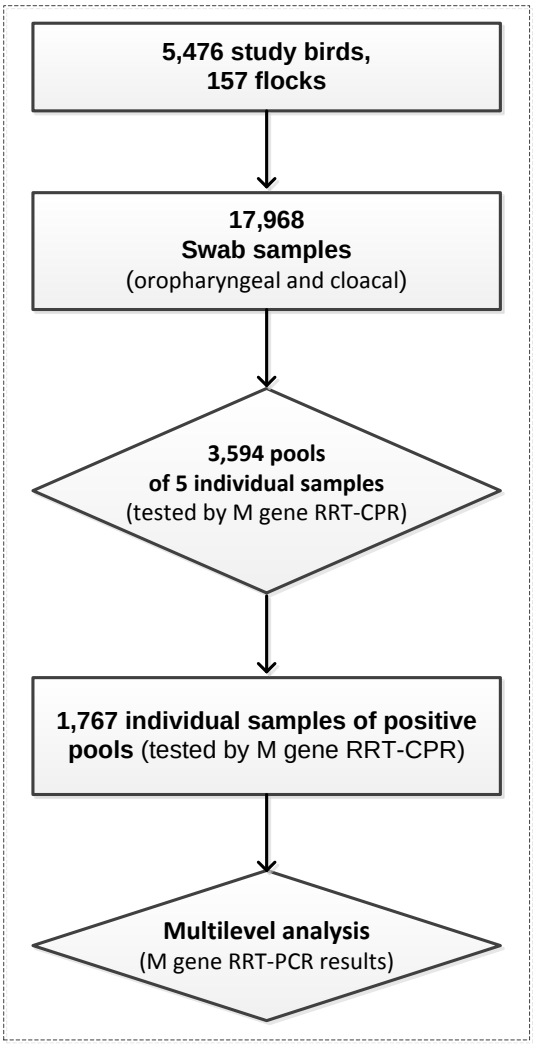


Figure 6.2: Risk factors for influenza Type A infection of poultry flocks in the MRD of Viet Nam, December 2008 to April 2010. Flow chart showing the sample testing procedures. Numbers listed in each box indicating total number of samples that were collected and tested at individual or pool level. The dash boundary indicates samples and results are presented in this paper.

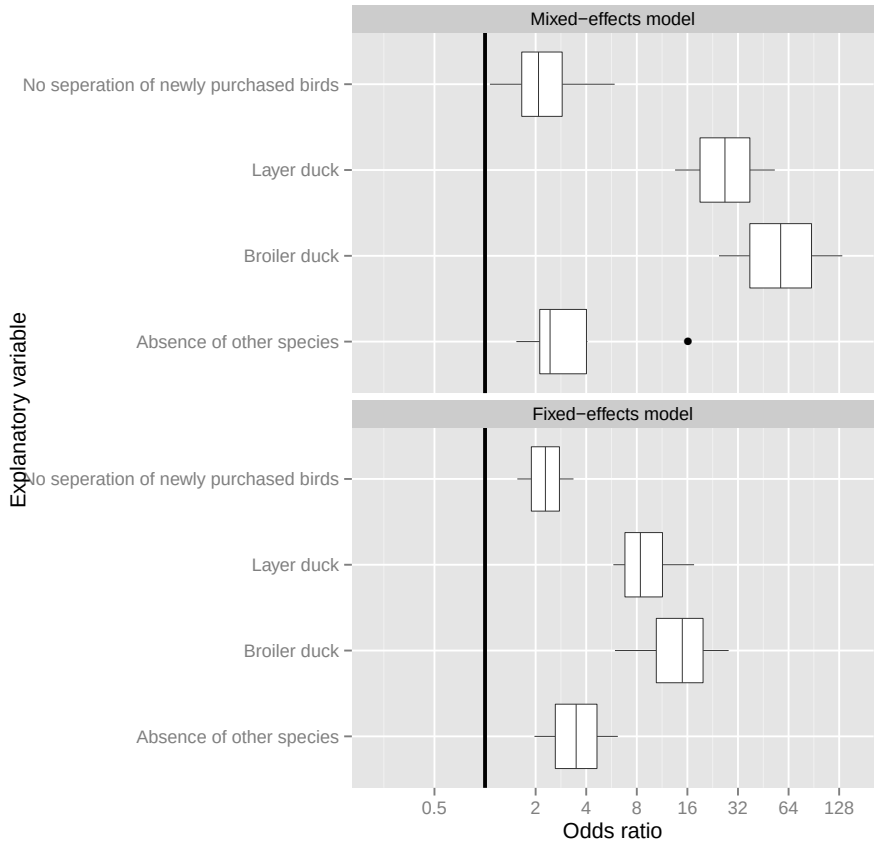


Figure 6.3: Risk factors for influenza Type A infection of poultry flocks in the MRD of Viet Nam, December 2008 to April 2010. Box and whisker plot showing the point estimate and lower and upper bounds of the odds ratios for each of the explanatory variables included in the fixed-effects and mixed-effects models.

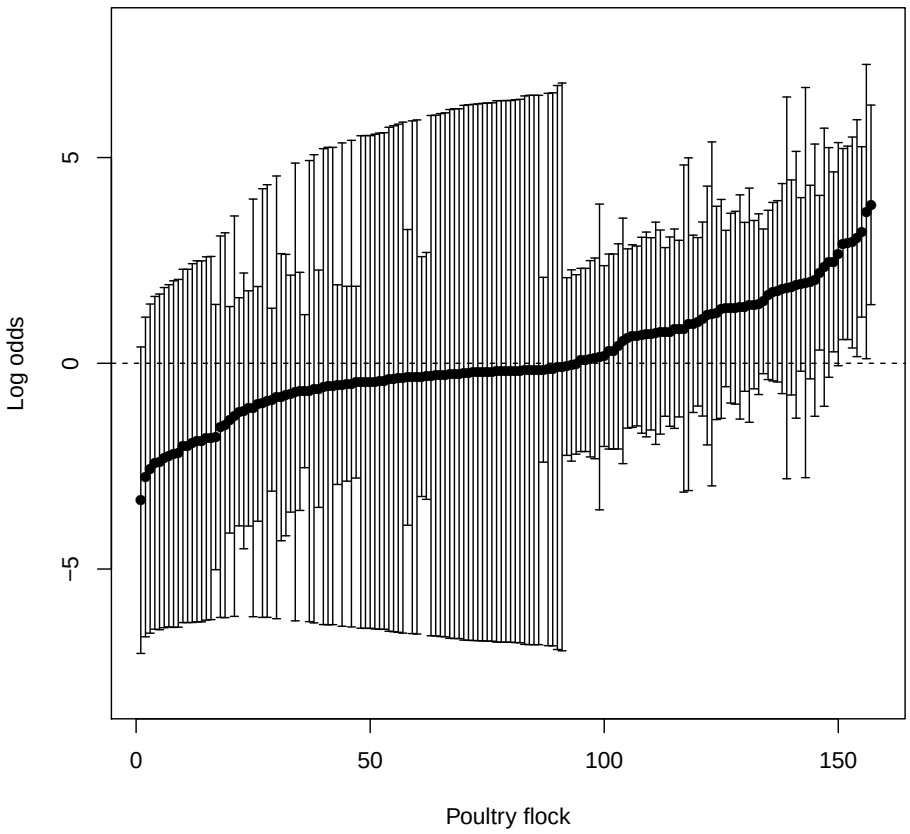


Figure 6.4: Risk factors for influenza Type A infection of poultry in the MRD of Viet Nam, December 2008 to April 2010. Caterpillar plot showing the flock-level log odds of being influenza Type A positive as a function of flock identifier.

6.4 Discussion

In this study we quantified the relative contribution of bird, flock and village-level risk factors for influenza Type A virus infection in the MRD of Viet Nam using a multilevel modelling approach. A distinguishing feature of this work is that the outcome of interest was the presence or absence of influenza Type A virus infection, in contrast to previous studies that have investigated risk factors for clinical HPAI H5N1 (Henning et al. 2009, Minh 2010, Desvaux et al. 2011). Besides HPAI H5N1, other Type A influenza viruses, notably H7 and H9, have been detected at a relatively high prevalence in poultry in the MRD (Nomura et al. 2011, Long, Minh, Schauer, Diep, Phuong & Thuy 2012). Acknowledging that some low pathogenic strains of Type A influenza viruses can mutate to highly pathogenic strains, a knowledge of characteristics that render a flock more likely to be infected with Type A virus provides information that will allow control and surveillance measures to be better targeted.

Of the three levels of hierarchy in our data (that is, bird, flock and village) only 5% of the variation in influenza Type A infection risk was attributable to the village in which flocks were located. Unmeasured flock- and bird-level characteristics accounted for 48% and 47% of the total variation in infection risk, respectively. These findings are consistent with the findings of (Minh 2010) who carried out a case-control study of risk factors for clinical HPAI H5N1 in the MRD during the same time frame. These authors found that significant risk factors for clinical H5N1 operated predominantly at the individual household (as opposed to village) level. Care needs to be taken when making comparisons across studies because of differences in study design, the outcome of interest and analytical approach. Our findings indicate that practices applied at the individual flock and bird level, such as sourcing of replacement birds, biosecurity and disease prevention measures such as vaccination are likely to have the greatest impact on reducing influenza Type A virus infection (and therefore clinical disease) risk in this area of Viet Nam.

The odds of influenza Type A infection was significantly greater in flocks comprised of broiler ducks and layer ducks, compared with those comprised of in-contact species (OR 28.16, 95% CI 5.93 – 133.65 and OR 17.53, 95% CI 5.80 – 53.01, respectively, Table 6.3). Our explanation for this finding is that flocks comprised of in-contact species (predominantly chickens) in this area of Viet Nam are often managed as isolated groups

within a household and are therefore less likely to make contact with other poultry species such as field running ducks that can asymptomatically carry influenza viruses (Henning et al. 2011, Long et al. 2013). In addition, for the period 2007 to 2009 (Diep 2011) showed that throughout Viet Nam chickens were more likely to show evidence of seroconversion as a result of HPAI H5N1 vaccination compared with ducks.

Our regression analyses show a significant interaction between production type of the flock and the presence or absence of newly purchased birds (Table 6.3). The odds of influenza Type A infection in broiler flocks was 28.16 (95% CI 5.93 – 133.65) times the odds of infection in in-contact species flocks. The equivalent measure for layer flocks was 17.53, (95% CI 5.80 – 53.01). For broiler flocks with newly purchased birds there was a modest increase in the odds of infection to 29.74 (95% CI 15.16 – 44.33, that is $\exp^{[3.338-0.8859+0.9407]}$). In contrast, the interacting effect of newly purchased birds into layer flocks substantially increased the odds of infection to 33.86 (95% CI 21.52 – 37.97, that is $\exp^{[2.8641-0.8859+1.5440]}$). We hypothesise that the reason for this increase in infection risk associated with new introductions is that layer ducks are more likely to be older at the time of sale and are therefore more likely to be carrying influenza Type A virus when introduced into new layer duck flocks (Long et al. 2013).

Of the explanatory variables included in our multilevel regression analyses, six of the eight fixed effects (flock size, the purchasing of new birds, the separation of new birds following purchase, the presence of other animal species, whether or not the flock had contact with other poultry flocks and changes in flock size, Table 6.3) were flock-level characteristics. These associations, in addition to the non-negligible influence of unmeasured flock-level effects (as quantified by the flock-level random effect term) indicate that strategies to reduce infection risk should focus on modification of the way in which individual flocks are managed. Disease awareness and education campaigns emphasising 'good farming practice' would be a logical way to achieve these objectives.

6.5 Conclusions

This prospective cohort study quantified the relative contribution of bird, flock and village-level influences on the risk of influenza Type A virus infection in poultry in the MRD of Viet Nam for the period December 2008 to April 2010. Our analyses indicate that after

controlling for the fixed effects included in the model, the relative contribution of unmeasured flock- and bird-level factors on influenza Type A infection risk were approximately equal. Most of the significant fixed-effects in our model were flock-level exposures. The findings from this study support the idea that interventions to reduce the maintenance and transmission of influenza Type A virus should be applied at the individual flock level.

CHAPTER 7

Patterns of poultry movement in the south of Viet Nam, 2009 – 2010

Abstract – A cross-sectional study was carried between 1 September 2009 and 30 June 2010 to record poultry movement data from 52 animal quarantine stations in 19 provinces in the south of Viet Nam.

A total of 26,490 commune-to-commune movement events involving approximately 21 million poultry were documented throughout the study period. Movement event details were recorded from 850 of the 2,479 communes (34%) within the study area. The most frequently cited reason for moving poultry was to shift birds to alternative places for grazing (46%) followed by movements to live bird markets (35%), slaughterhouses (16%) and for other purposes (3%). The number of duck movements and the total number of individual ducks moved were 5 to 6 times greater (respectively) than that recorded for chickens. The number of directed links decreased over the study period from 8,766 (September 2009 to November 2009), to 6,741 (December 2009 to February 2010) and then to 3,843 (March 2010 to June 2010). The largest number of directed links during the September 2009 to November 2009 period is consistent with large numbers of ducks (12.5 million) moved to graze in other places at the end of the rice harvest. Poultry were more likely to be moved between communes with provincial roads and communes with relatively large numbers of poultry-owning households. In contrast, communes with large numbers of people were less likely to be connected by poultry movement events. Assuming a causal relationship exists between a commune's connectivity within a poultry movement network and HPAI H5N1 risk, communes identified as being likely to be highly connected within a network should be targeted for disease control and surveillance.

Long, N.V., Stevenson, M. and Dong, P.V. (2013) Patterns of poultry movements in the south of Viet Nam, 2009 – 2010.

7.1 Introduction

Poultry are the most common amongst animal species in Viet Nam. In 2011 it was estimated that 57% of the total number of households with animals in 94% (10,441 of 11,121) communes throughout the country kept poultry (GSO 2012). Of those households with poultry, approximately 90% keep less than 50 birds whereas only 3% of households have more than 100 birds indicating that backyard rearing is the predominant form of poultry production. The south of Viet Nam includes the Mekong River Delta (MRD) which is one of the most important regions for poultry production in the country. Estimates from the 2011 national animal census (GSO 2012) showed that approximately 40% of the country's total duck population (of approximately 80 million) is located in the MRD. A characteristic feature of poultry production in the MRD is that duck flocks are itinerant (vịt chạy đồng, in Vietnamese) meaning that they are frequently moved from one location to another so they can freely scavenge in rice fields to glean grains that fall to the ground during harvesting (Men 2010, Minh et al. 2010, Henning et al. 2012).

The movement of poultry in Viet Nam is complex because most poultry flocks are comprised of relatively small numbers of birds and the Vietnamese consumer prefers birds that are either live or freshly slaughtered at the time of sale. For these reasons, throughout Viet Nam, poultry are typically bought and sold on a daily basis in local live bird markets. Poultry are transported to market via road and river networks. Movement along road networks can be monitored as it is necessary for poultry transporters to obtain a certificate of quarantine to allow them to move birds from one commune to another. In contrast, movement via rivers is an activity that is more difficult to monitor. In addition to movement related to trade, the movement of field running ducks is difficult to monitor and provides favourable conditions for the transmission of infectious disease agents such as highly pathogenic avian influenza (HPAI) caused by H5N1 virus which has been endemic in Viet Nam since 2006 (MARD 2011, Minh et al. 2011, DAH 2012). Cocks are also frequently moved from one location to another for fighting competitions (Duc & Long 2008). The situation is further complicated in border areas where relatively large volumes of poultry and poultry product are moved into Cambodia on a daily basis (Van Kerkhove et al. 2009). Throughout the year the frequency of poultry movement events tends to be greatest over the November to February period because of the rice harvest and an increase

in poultry consumption due to the Têt holiday period.

A number of studies have investigated the association between poultry movement and the presence of HPAI H5N1 in domestic poultry in a number of Asian countries (Van Kerkhove et al. 2009, Magalhães et al. 2010, Martin et al. 2011, Magalhães et al. 2012, Poolkhet et al. 2012). In a study carried out in Cambodia from November to December 2006 and November to December 2007 (Van Kerkhove et al. 2009) speculated that poultry traders ('middlemen') were hubs for the transmission of HPAI H5N1. These authors concluded that these middlemen should be targeted for H5N1 surveillance (Van Kerkhove et al. 2009). In Thailand network analyses of cross-sectional movement data led (Poolkhet et al. 2012) to conclude that farmers who raise consumable chickens, those who raise both consumable chickens and fighting cocks, and the owners and observers of fighting cocks should be monitored for disease control purposes (Poolkhet et al. 2012). In China a longitudinal study was carried out monthly in four wholesale live bird markets in Hunan and Guangxi provinces from January to April 2010. This study identified an increase in the amount of live poultry trade coincident with the timing of Chinese New Year festivities (February 2010) which was then hypothesised to be associated with a higher HPAI H5N1 infection risk in humans and poultry in the same region (Magalhães et al. 2012).

While each of the aforementioned studies provides insight into the frequency and volume of poultry movement within individual countries none have identified a causal relationship between poultry movement and HPAI H5N1 risk. This is because these studies have mostly been based on cross-sectional surveys which have entailed the recording of movement events in defined geographic areas for limited periods of time. The ability to confidently extrapolate the findings from these studies to target populations is questionable. In addition, in most studies, the timing of movement data collection did not coincide with the occurrence of HPAI H5N1 outbreaks and there has been a tendency (see, for example (Magalhães et al. 2010, Martin et al. 2011, Magalhães et al. 2012) to draw conclusions based on associations between contemporary movement information and historic HPAI H5N1 outbreak details.

Because of the complexity of poultry movement in Viet Nam surveys to capture complete movement event information require considerable resources and even if a complete network study could be carried out it is likely that it would soon be out of date given the frequent changes that occur in poultry demographics, economics, and trading activities.

Acknowledging these issues we propose that cross-sectional movement studies in such populations are best carried out to identify the characteristics of nodes within a given network (typically the commune in a Vietnamese context) that render them more likely to receive or distribute poultry. In this way, the network behaviour of nodes (i.e. communes) that were not part of the original study can be predicted. Making the biologically plausible assumption that a causal relationship exists between a commune's behaviour within a poultry movement network and HPAI H5N1 risk, communes that are expected to be highly connected (i.e. communes that send and receive large volumes of poultry) can then be targeted for surveillance and monitoring. This is important as a real-time monitoring system for poultry movement will take time to be established in Viet Nam.

With this background our aims in this paper were to: (1) provide a descriptive analysis of poultry movement event data from communes in the south of Viet Nam over the period from September 2009 to June 2010, and (2) identify the geographic and demographic characteristics of individual communes that can be used to predict their network behaviour. This study provides information to justify allocation of resources for risk-based surveillance and development of disease control measures not only limited to HPAI H5N1.

7.2 Materials and methods

7.2.1 Movement data

In Viet Nam, the animal quarantine system currently operates at three main levels including animal quarantine stations (AQS) located at international borders (e.g. airports, seaports and inland border crossings), provincial AQSs located on national roads connecting provinces, and district AQSs positioned on provincial roads connecting districts (Figure 7.1). Within a given AQS, three to seven government veterinary officers are assigned to work on animal quarantine activities include checking documents for transportation of animals and animal products, physical and biological condition of transportation facilities and animals, granting certificates for those animal transportations that meet government regulations, and recording information about animal transportation using a standardised recording notebook (log book). Those transporting livestock need to stop at all AQSs po-

sitioned along their elected transportation route. An animal quarantine certificate from the previous AQS is required to be produced in order for a transporter to receive a subsequent AQS certificate.

A cross-sectional study was carried from 1 September 2009 to 30 June 2010 to collect movement data (see Appendix D) from 52 AQSs within 19 provinces in the south of Viet Nam (Figure 7.1). In this region, which includes the MRD, poultry are transported from their home communes to other communes for either further raising or to live bird markets or slaughterhouses. Transportation occurs via three main routes: trucks and motorbikes, boats on rivers and by foot where ducks freely graze on rice fields as they are moved from one location to another. The data for this study were comprised of records of birds moved by either truck or motorbike via roads. Local veterinary services do not have facilities to issue quarantine certificates for poultry moved via rivers or on foot and, for this reason, it is likely movements that occur via these routes are over short distances (such that quarantine certificates do not need to be shown when poultry reach their final destination).

In August 2009 a one-day workshop was organised for all AQS staff of the Sub-Department of Animal Health (SDAH) within the 19 provinces that took part in this study. The purpose of the workshop was to inform participant staff of the study objectives, familiarise them with the specific details of data collection and other details necessary to allow the accumulated data to be electronically transferred to project staff in Ha Noi.

Quarantine regulations set by the Vietnamese Ministry of Agriculture and Rural Development (MARD 2005*b*, 2009) stipulate that the following information must be recorded for livestock transported outside of their home commune: the source and destination commune, the date when poultry passed through an inspecting AQS, the species and numbers of birds being transported (chickens, ducks and others which included Muscovy ducks, geese, fitting cocks, and quails) and the reason for transportation. These details are recorded into a log book in a format common across all AQSs. As part of this study AQS staff were asked to transfer the movement details described above to a spreadsheet. These spreadsheets (as printed copies and in electronic format) were then sent to the Department of Animal Health (DAH), Ha Noi where they were aggregated into a relational database. The validity of the accumulated data was assessed by carrying out a series of cross-checks on the details recorded by 15 AQSs selected at random from the 52 AQSs

that took part in the study. DAH staff travelled to each of these 15 AQSs and compared details in each AQS log book with the details recorded in each spreadsheet.

In this study, poultry transported directly (without stopping at any other place) from their home commune to another commune were classified as direct movements. Poultry that were transported with a stop-over at some location before reaching their final destination were classified as indirect movements.

7.2.2 Demographic and geographic data

For each commune details of human population counts were extracted from the 2011 Vietnamese national census (GSO 2012). Poultry population counts (including the number of households that keep poultry and the number of poultry species present) were extracted from the national agriculture census that was carried out in the same year (GSO 2012).

The geographic data for this study including the boundaries of communes, districts and provinces, the distribution of national and regional roads, and urban-rural classification of communes and districts was obtained in digital format from the Vietnamese Publishing House of Natural Resources, Environment and Cartography (NARENCA 2012).

7.2.3 Statistical analyses

The unit of interest for this study was the commune (n = 2,479). Descriptive social network analyses were carried out for the commune-to-commune poultry movements. Here, each commune comprised the vertices (nodes) of the network and the recorded movement events that occurred between two communes formed the edges (ties) between two nodes. A movement event was defined as the transportation of at least one domestic poultry from a given commune of origin to a destination commune where details of the movement were recorded by an AQS. Poultry that were transported within a given commune were classified as internal movements whereas poultry transported between communes were classified as external.

The distance of each external movement event was estimated as the Euclidean (straight line) distance between the centroid coordinates of two communes in which poultry are

moved out and moved in, respectively. The distances of internal movements were not estimated because of the centroids of origin and destination commune were the same.

Descriptive social network analyses were carried using the igraph package (Csardi & Nepusz 2006) in R version 2.15.2 (R Development Core Team 2012). Poultry movements were summarised in terms of the number of individual movement events and the number of birds moved, stratified by three periods: 1 September 2009 to 30 November 2009 (the first low risk period for HPAI H5N1), 1 December 2009 to 28 February 2010 (the high risk period), and 1 March to 30 June 2010 (the second low risk period). These period classifications were based on previous studies which identified the November to February period as the high risk period for avian influenza in Viet Nam (Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009, Long et al. 2013).

Records of movement events between origin and destination communes were used to construct a directed (asymmetrical) network for each of the three defined HPAI H5N1 risk periods. Each network was summarised in terms of size, measures of centrality (in- and out-degree, in- and out-degree centralization, betweenness and betweenness centralization scores), and cohesion (network density, summaries of geodesic distances and clustering coefficient).

An exponential-family random graph modelling approach was used to quantify the influence of commune-level factors on the probability of a connection between two communes using the ERGM package (Hunter et al. 2008) in R. Five commune-level explanatory variables were hypothesised to influence a commune's position in the poultry movement network: (1) the type of district in which the commune was located (urban, rural); (2) the classification of roads entering the commune (communal, provincial and mixed); (3) human population density expressed as the number of humans per square kilometre; (4) the number of poultry raising households present; and (5) the total number of domestic poultry present as estimated from the national agriculture census carried out in 2011 (GSO 2012). The three continuous variables human population density, the number of poultry raising households and the total number of domestic poultry present were not normally distributed and were subject to a natural logarithmic transformation.

Each of the five putative explanatory variables (district type, road classification, human population density, number of poultry households and number of domestic poultry) were selected in turn as explanatory variable in an exponential-family random graph model.

The Wald test (Agresti 2007) was used to test the significance of each variable as a predictor of the probability of a connection being present between two communes. All variables associated with a commune being connected at an alpha level of less than 0.2 at the bivariate level were entered into a multivariable ERGM. Non-statistical significant explanatory variables were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all the retained variables were significant at $P < 0.05$.

The results of the final model are reported in terms of adjusted odds ratios for each explanatory variable. An adjusted odds ratio (and its 95 per cent confidence interval [CI]) of greater than one for a given explanatory variable means that, after accounting for other variables in the model, communes were more likely to be connected to other communes in the same category. Conversely, an adjusted odds ratio of less than one indicates that after accounting for other variables in the model, communes were less likely to be connected to other communes in the same category.

7.3 Results

A total of 26,490 commune-to-commune movement events via roads were recorded throughout the study period. This involved a total of 3.15 million ducks and 18.14 million chickens. Throughout the study period 850 of the 2,479 communes (34%) in the south of Viet Nam (Table 7.1). were either the source or destination of a recorded poultry movement event. In the northern part of the study region the number of duck movements steadily decreased with a sudden drop in movement numbers starting from March 2010. In the northern part of the study region the number of chicken movements remained constantly high from September 2009 to March 2010 then, similar to the duck movements, decreased Figure 7.2). The total number of duck movements in the north and south of the study region were similar. In contrast, the total number of chicken movements was greater in the north compared with the south. The most frequently cited reason for moving poultry was to shift birds to alternative places to be raised (46%) followed by movements to live bird markets (35%), to slaughterhouses (16%) and for other purposes (3%). The number of duck movements and the total number of individual ducks moved were 5 to 6 times greater (respectively) than that recorded for chickens (Table 7.1 and Figure 7.3).

Table 7.2 provides descriptive statistics for each of the social networks computed for the periods September 2009 to November 2009, December 2009 to February 2010 and March 2010 to June 2010. The number of nodes (communes) and network size remained constant throughout the 9-month study period but the total number of directed links varied. The number of directed links decreased from 8,766 (September 2009 to November 2009) to 6,741 (December 2009 to February 2010) and then to 3,843 (March 2010 to June 2010). The largest number of directed links during the September 2009 to November 2009 period is consistent with the large number of ducks (12.48 million) that were moved to other places for alternative raising as result of rice harvesting.

Median commune in-degree scores were small. Maximum in-degree scores were highest during the September 2009 to November 2009 period (2,298), followed by March 2010 to June 2010 (1,689) and December 2009 to February 2010 (1,315). The median commune out-degree scores were small and maximum out-degree scores decreased throughout the study period. Across all periods the in-degree centralisation scores were greater than the out-degree centralisation scores indicating that there was a greater deviation in the number of in-coming contacts to a commune compared with the deviation of the number of out-going contacts.

Table 7.3 presents the results of the multivariable ERGM quantifying the association between selected geographic and demographic characteristics of the study communes and the odds of a commune-to-commune connection arising from the movement of poultry. After accounting for the confounding effect of road type, human population size, the number of poultry raising households within the commune and commune poultry population size the odds of a connection between two communes within a district classified as urban was 1.19 (95% CI 1.10 – 1.30) times the odds of a connection between communes within rural districts. This indicates that poultry were more likely to be moved between urban communes. Poultry were more likely to be moved between communes with provincial roads compared with those with communal roads only (OR 1.49, 95% CI 1.38 – 1.62). As the number of humans per commune increased the odds of a commune-to-commune connection decreased. As the number of poultry-owning households per commune increased the odds of a commune-to-commune connection increased.

Table 7.1: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Counts of movement events and total number of poultry moved, stratified by period and movement reason.

Reason for movement	Sep 2009 – Nov 2009	Dec 2009 – Feb 2010	Mar 2010 – Jun 2010	Total
To live bird markets:				
Number of movement events:				
Chickens	579	84	3	1,666
Ducks	3,455	2,076	3,014	8,545
Total	4,034	2,160	3,017	9,211
Number of birds moved:				
Chickens	92,678	11,973	700	105,351
Ducks	2,321,697	1,331,293	2,211,586	5,864,576
Total	2,414,375	1,343,266	2,212,286	5,969,927
To slaughterhouse:				
Number of movement events:				
Chickens	145	2,106	576	2,827
Ducks	636	664	176	1,476
Total	781	2,770	752	4,303
Number of birds moved:				
Chickens	501,525	935,315	360,278	1,797,118
Ducks	333,087	239,212	98,084	670,383
Total	834,612	1,174,527	458,362	2,467,501
To be raised in other places:				
Number of movement events:				
Chickens	254	232	219	705
Ducks	5,673	3,383	2,753	11,809
Total	5,927	3,615	2,972	12,514
Number of birds moved:				
Chickens	340,127	343,840	318,680	1,002,647
Ducks	5,897,637	3,563,894	2,018,721	11,480,252
Total	6,237,764	3,907,734	2,337,401	12,482,899
For other purposes:				
Number of movement events:				
Chickens	235	101	10	346
Ducks	13	10	93	116
Total	248	111	103	462
Number of birds moved:				
Chickens	170,920	65,457	7,694	244,071
Ducks	8,652	9,800	103,838	122,290
Total	179,572	75,257	111,532	366,361
Total:				
Number of movement events:				
Chickens	1,213	2,523	808	4,544
Ducks	9,777	6,133	6,036	21,946
Total	10,990	8,656	6,844	26,490
Number of birds moved:				
Chickens	1,105,250	1,356,585	687,352	3,149,187
Ducks	8,561,073	5,144,199	4,432,229	18,137,501
Total	9,666,323	6,500,784	5,119,581	21,286,688

Table 7.2: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Descriptive statistics of network size, measures of centrality and cohesion for the three social networks constructed for September 2009 to November 2009, December 2009 to February 2010, and March 2010 to June 2010.

Parameter	Sep 2009 – Nov 2009	Dec 2009 – Feb 2010	Mar 2010 – Jun 2010
Network size:			
Number of nodes[a]	850	850	850
Number of directed links[b]	8,766	6,741	3,843
Size[c]	721,650	721,650	721,650
Diameter[d]	6	7	8
Size of giant strong component[e]	1	4	6
Size of giant weak component[f]	602	588	377
Measure of centrality:			
In-degree (range)[g]	0 (0 – 2,298)	0 (0 – 1,315)	0 (0 – 1,689)
Out-degree (range)[h]	0 (0 – 499)	0 (0 – 416)	0 (0 – 201)
In-degree centralisation[i]	1,944,534	1,111,009	1,431,807
Out-degree centralisation[j]	415,384	346,859	167,007
Normalized betweeness (range)[k]	0 (0 – 246)	0 (0 – 301)	0 (0 – 357)
Betweeness centralisation[l]	0.000	0.000	0.000
Measure of cohesion:			
Density (directed)[m]	0.012	0.009	0.005
Geodesic distance (mode)[n]	1.722	1.870	2.537
Clustering coefficient[o]	0.028	0.025	0.037

[a] Number of nodes: the total number of network members.
[b] Number of directed links: the total number of connections between nodes.
[c] Size: the total possible number of unique pairs of nodes.
[d] Diameter: the number of links in the largest path between two nodes.
[e] Strong component: sections of the network where every commune can be reached from every other commune by a directed path.
[f] Weak component: sections of the network where every commune can be reached from every other commune by an indirected path.
[g] In-degree: the number of contacts to a node (i.e. movements into the communes).
[h] Out-degree: the number of contacts from a node (i.e. movements out of the communes).
[i] In-degree centralisation: an estimate of the deviation of the largest values of in-degree from the value computed for all other nodes in the network.
[j] Out-degree centralisation: an estimate of the deviation of the largest values of out-degree from the value computed for all other nodes in the network.
[k] Betweenness: the frequency with which a node falls between pairs of other nodes on the path connecting them. Betweenness provides an indication of the amount of flow within the network that is 'controlled' by a node.
[l] Betweenness centralisation: an estimate of the deviation of the largest values of betweenness from the value computed for all other nodes in the network. Higher values indicate the presence of large numbers of nodes that act as mediators.
[m] Density: the proportion of all contacts that could be present that actually are.
[n] Geodesic distance: the shortest path between two nodes.
[o] Clustering coefficient: gives the average probability of individual nodes being directly connected to another node in the network. The possible maximum value of 1 indicates that every node is directly connected to all other nodes in the network.

Table 7.3: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Estimated regression coefficients and their standard errors from the exponential-family random graph model described in the text.

Explanatory variable	Coefficient (SE)	OR (95% CI)	P-value
Intercept	-9.3539 (0.1088)		< 0.01
Type of district:			
Rural	Reference	1.00	
Urban	0.1773 (0.0422)	1.19 (1.10 – 1.30)	< 0.01
Type of road:			
Communal road	Reference	1.00	
Provincial road	0.3996 (0.0412)	1.49 (1.38 – 1.62)[a]	< 0.01
Mixed (communal and provincial)	0.2248 (0.0391)	1.25 (1.16 – 1.35)	< 0.01
Number of humans per commune:			
$\leq$ 10,000	Reference	1.00	
10,000 – 20,000	-0.0661 (0.0327)	0.94 (0.88 – 1.00)	0.04
$>$ 20,000	-0.3153 (0.0617)	0.73 (0.65 – 0.82)[b]	< 0.01
Number of poultry households per commune:			
$\leq$ 1,000	Reference	1.00	
1,000 – 2,000	0.5771 (0.0405)	1.78 (1.64 – 1.93)	< 0.01
$>$ 2,000	0.6567 (0.0530)	1.93 (1.74 – 2.14)	< 0.01
Number of poultry per commune:			
$\leq$ 10,000	0.3238 (0.0615)	1.38 (1.23 – 1.56)	< 0.01
10,000 – 50,000	0.2679 (0.0374)	1.31 (1.21 – 1.41)	< 0.01
$>$ 50,000	Reference	1.00	

SE: standard error; OR odds ratio; CI confidence interval.

[a] Interpretation: After accounting for other variables in the model, the odds of a connection between two communes with provincial roads was 1.49 (95% CI 1.38 – 1.62) times the odds of a connection between communes with communal roads only.

[b] Interpretation: After adjusting for the other variables in the model, the odds of a connection between two communes with more than 20,000 people was 0.73 (95% CI 0.65 – 0.82) times the odds of a connection between communes with less than 10,000 people.

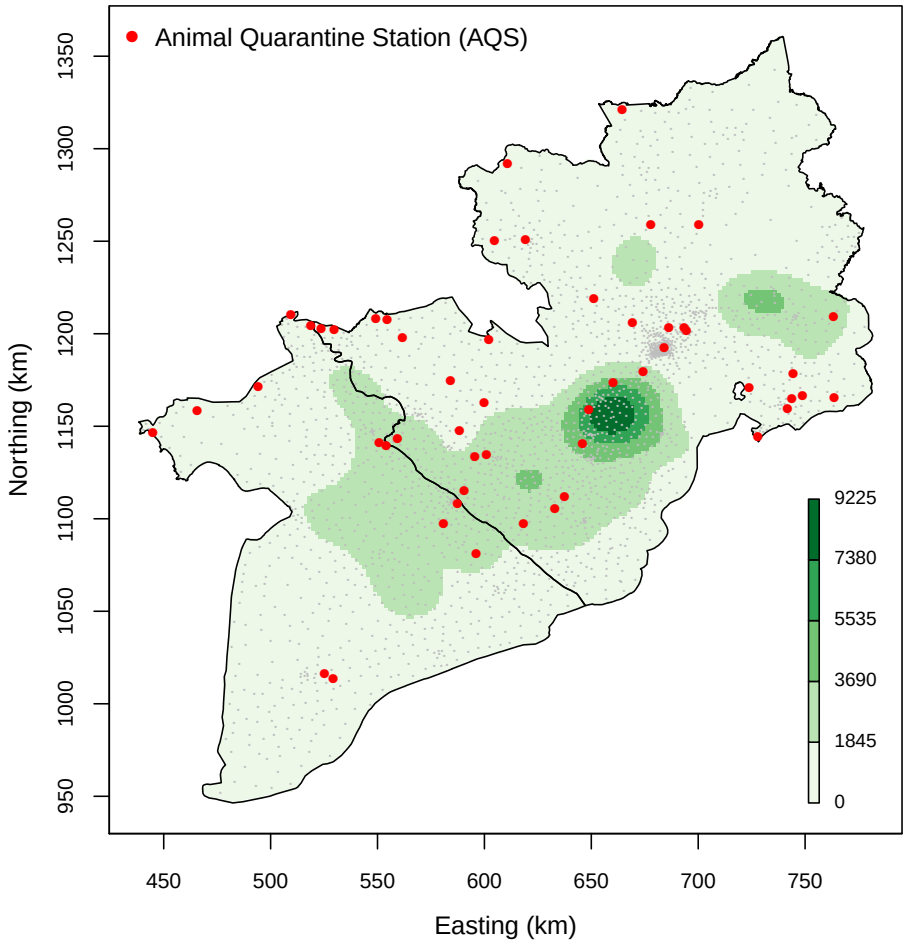


Figure 7.1: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Image plot of the 19 study provinces included in the study area showing the number of poultry per square kilometre. Superimposed are points showing the location of Animal Quarantine Stations (red dots) and commune centroids (gray dots).

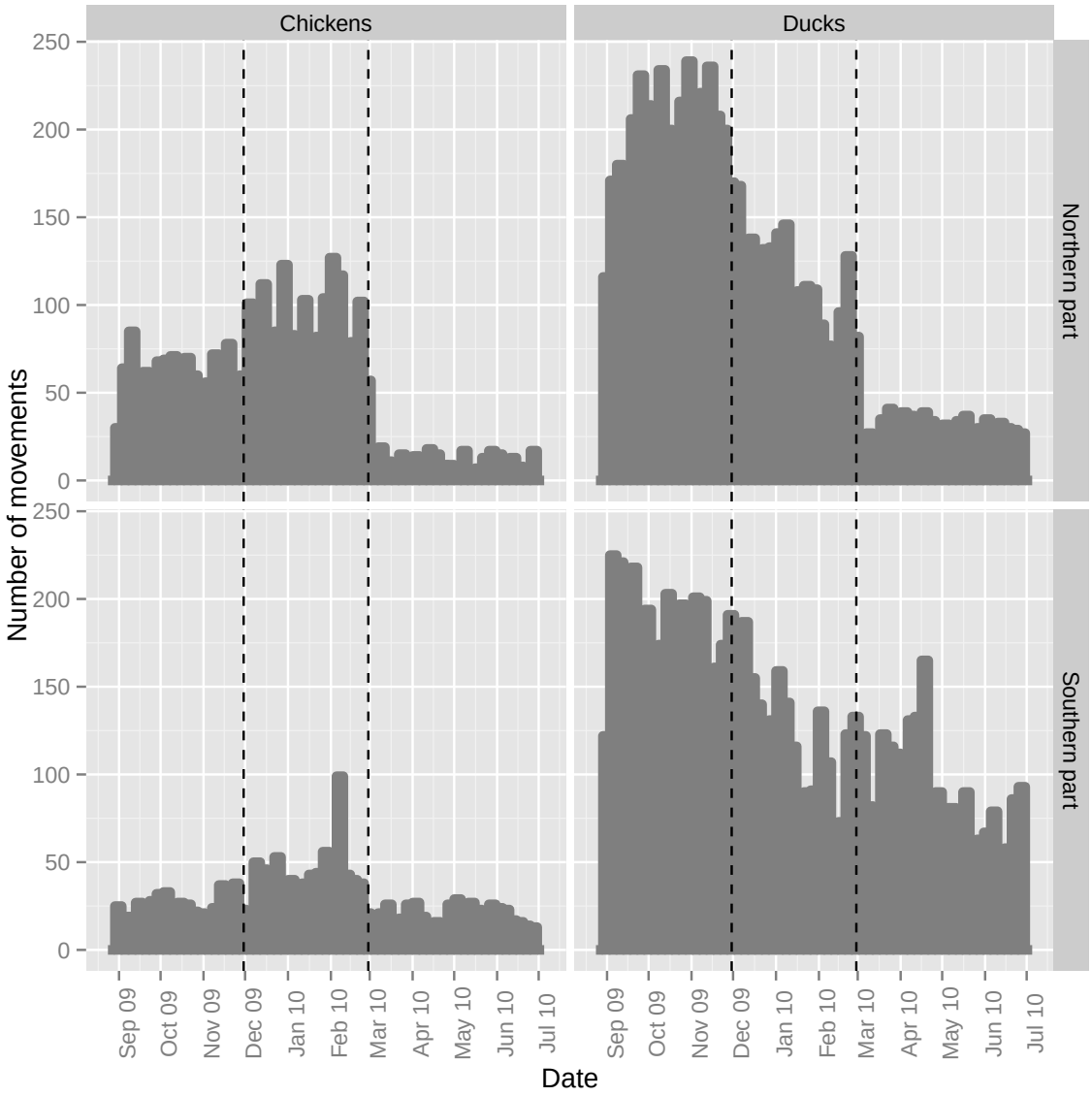
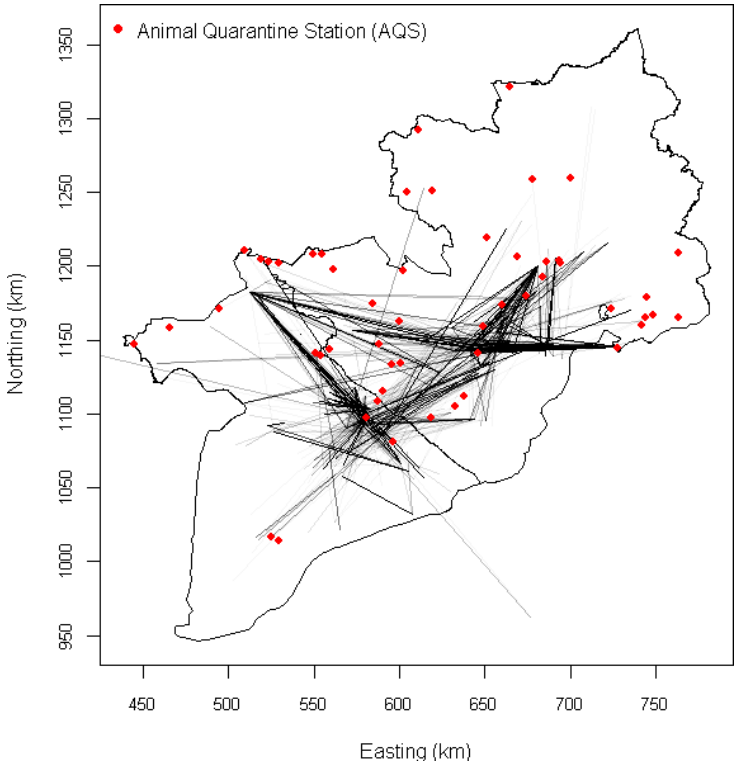
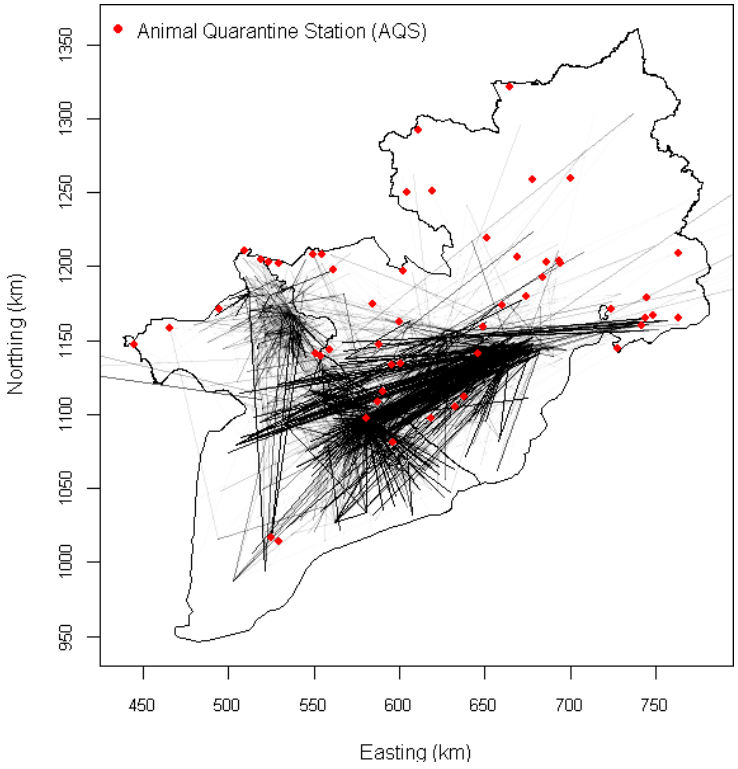


Figure 7.2: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Frequency histograms showing the total number of movement events per month as a function of calendar date, stratified by species and study area. The dashed vertical lines in each plot indicate the periods September 2009 to November 2009, December 2009 to February 2010 and March 2010 to June 2010.



(a) Network of chicken movements



(b) Network of duck movements

Figure 7.3: Commune-to-commune movements of poultry in the south of Viet Nam from 1 September 2009 to 30 June 2010. Maps showing the commune-to-commune connections arising from the movement of (a) chickens and (b) ducks.

7.4 Discussion

In this study we monitored movement events that were routinely recorded by officers of the AQSs with only 34% of the total number of communes in the study area recorded as being involved in a movement event throughout the 9-month study period (Figure 7.3). A number of explanations can be provided to account for the apparent incompleteness of our movement data. Firstly, our study design was such that we focussed only on movements that occurred via road networks; movements of poultry by other means (for example by foot or via rivers) were not recorded. Secondly, AQS offices are located predominantly on the main roads connecting districts and provinces so that poultry movements that occurred between adjacent communes or communes situated a long distance from major roads were unlikely to be recorded. Incomplete network enumeration is a feature of this and other similar studies that have investigated poultry movement patterns in Cambodia (Van Kerkhove et al. 2009), China (Martin et al. 2011) and Thailand (Poolkhet et al. 2012). In developed countries with well established infrastructures to record the identity of individual livestock of relatively high market value (e.g. sheep and cattle) complete or near-complete network enumeration is possible (see, for example (Webb 2005, Christley et al. 2005, Kiss et al. 2006, Natale et al. 2009). The same cannot be said for shorter-lived animals of relatively low market value such as poultry where data to inform social network analyses are typically collected using cross sectional surveys, similar to the techniques applied in the above mentioned studies (see, for example (Lockhart et al. 2010, Nickbakhsh et al. 2011). Incomplete enumeration of contact data represents a form of selection bias and its effect on the validity of network statistical measures (representativeness of the actual situation) is difficult to quantify (Borgatti et al. 2006, Lockhart et al. 2010). In the context of this study it would be reasonable to assume that direction and magnitude of this bias was similar across each of the three evaluated time periods and, for this reason, we believe that appropriate inferences can be made by focussing on how the network parameters vary in relative rather than absolute terms.

The ERGM analyses described in this study allowed us to identify commune-level characteristics that rendered them more likely to receive or distribute poultry. Given the mechanism of the association between each of the commune-level explanatory variables and the outcome was likely to operate in all communes, not just those that were included

in this study, we believe that the associations quantified by our ERGM analyses can be used to predict the network behaviour of communes in the south of Viet Nam that were not involved in this study (Elwood 2007). Making the biologically plausible assumption that a causal relationship exists between a commune's behaviour within a poultry movement network and HPAI H5N1 risk, communes that are expected to be highly connected (i.e. communes that send and receive large volumes of poultry) can then be targeted for surveillance and monitoring. This is important as a real-time monitoring system for poultry movement will take time (if ever) to be established in Viet Nam.

Throughout the entire study period the frequency of duck movements outnumbered chicken movements by a factor of 5 (Table 7.1). In both the north and south of the study area the frequency of duck movements decreased throughout the follow-up period. In contrast, the number of chicken movements decreased suddenly from March 2010 (Figure 7.2). The high frequency of duck movements from September to February and the sudden decrease in movement frequency after February is consistent the decrease in consumer demand for poultry following the Têt holiday period (from 15 to 18 February 2010) as well as completion of the autumn rice harvesting period. These findings are consistent with those of (Men 2010) and (Minh et al. 2010). We speculate that the high frequency of duck and chicken movements during the late dry period is a contributing factor to the relatively high incidence risk of HPAI H5N1 that typically occurs in Viet Nam at this time of the year (Pfeiffer et al. 2007, Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009).

The results of our ERGM analyses show that communes in which there were provincial roads had 1.49 (95% CI 1.38 – 1.62) times the odds of having a connection mediated by movement of poultry compared with those communes without provincial roads (Table 7.3). The most likely reason for this finding is that the most common means by which ducks are transported from one location to another to graze rice paddies is by road. Because flock owners are required to pay for rice paddy grazing rights, it is likely that an individual flock owner will secure grazing at a lower price if he/she is prepared to transport their flock over longer distances. This being the case, transportation of birds is likely to occur via provincial roads. These findings are consistent with our descriptive analyses which show that the most frequent reason for moving poultry was for grazing (Table 7.1).

Commune-to-commune connections were more likely to occur between communes lo-

cated within urban districts compared with those in rural districts (Table 7.3). One possible reason for this finding is that movements to markets, which comprised a significant proportion of all movement reasons in this study (35%) were more likely to be located in urban communes and the demand for poultry is likely to be higher in urban communes compared with rural communes. We acknowledge that our study design, by using AQS inspection points (themselves predominantly located in urban communes) is likely to have resulted in a data set biased towards urban communes. Extension of this study with special attention on data collection in rural areas should allow this relationship to be characterised in greater detail.

Human population counts, poultry population counts and the number of poultry raising households per commune were significantly associated with the odds of a commune-to-commune connection arising from poultry movement. As the number of humans per commune increased the odds of a poultry movement connection being present decreased. This result implies that communes with large numbers of people (for example, those in city areas) were less likely to be connected via poultry movements. In contrast, with increases in the number of poultry-owning households per commune, the larger the odds of a connection. Larger numbers of poultry-owning households logically means that the probability of individual flock owners wishing to move birds for either trade or grazing increases.

7.5 Conclusions

This study documented poultry movement patterns in the south of Viet Nam from 1 September 2009 to 30 June 2010. The most frequently cited reason for moving poultry was to shift birds to alternative places to be raised (46%) followed by movements to live bird markets (35%), to slaughterhouses (16%) and for other purposes (3%). The number of duck movements and the total number of individual ducks moved were 5 to 6 times greater (respectively) than that recorded for chickens. Poultry were more likely to be moved between communes with provincial roads and communes with relatively large numbers of poultry-owning households. In contrast, communes with large numbers of people were less likely to be connected by poultry movement events. Assuming a causal relationship exists between a commune's connectivity within a poultry movement net-

work and HPAI H5N1 risk, communes identified as being likely to be connected within a network should be targeted for disease control and surveillance.

CHAPTER 8

General discussion

This thesis has applied a number of investigatory techniques to highly pathogenic avian influenza including observational studies (Chapter 4, Chapter 5 and Chapter 6), the analysis of animal movement data (Chapter 7) and the design and implementation of an animal health decision support system (Chapter 3). The findings presented in each of these chapters have broadened our knowledge of the epidemiology of not only the HPAI H5N1 subtype, but influenza Type A viruses in poultry in general. It should be stressed that the methodological techniques that have been used in this thesis can be applied to a wide range of animal health issues, not just HPAI H5N1.

The observational studies described in this thesis have provided critical insights into the epidemiology of HPAI H5N1 in the MRD of Viet Nam. The prospective cohort study described in Chapter 4 is, to the best of our knowledge, the first study of its type to characterise the epidemiology of both low pathogenic and high pathogenic avian influenza in the field and to compare H5 virus and Type A virus activity. In this study, the overall incidence rate of influenza Type A and H5N1 virus infection was shown to be relatively high over the entire follow-up period. AI viruses were circulating widely in village poultry implying that interventions such as vaccination, movement controls and biosecurity measures need to be carried out continuously rather than only focusing on the established high risk periods. Broiler ducks had an incidence rate of influenza H5 virus infection that was approximately four times greater than that of layer ducks and in-contact species indicating that broiler ducks are likely to be the main entry route for H5 virus into poultry flocks in the MRD.

Our survival analyses (Chapter 5) showed that the duration of immunity in vaccinated poultry under field conditions was considerably shorter than the duration of immunity

documented in laboratory studies (Tian et al. 2005, 2010). The age of poultry at the time of vaccination and the time of the year when vaccination was carried out significantly influenced the duration of immunity in vaccinated birds. These are important findings if the Department of Animal Health in Viet Nam intends to keep using vaccination as a tool to control HPAI H5N1.

Possible modifications to the current HPAI H5N1 vaccination protocol are as follows. The current vaccination strategy which is based on two rounds (April-May and November-December) should be changed to an on-going individual flock-based campaign. With this approach flock owners will be required to keep HPAI H5N1 vaccine on hand and vaccinate birds at 2 weeks of age and then a give a follow-up shot up to 4 weeks later. Individual birds (for example, purchased birds) should be vaccinated at the time of entry into a flock. The advantage of this approach is that it is likely to increase vaccination coverage within individual flocks. The key disadvantage is that this strategy places most of the responsibility for vaccination in the hands of individual flock owners and to be effective, time and effort is required to educate flock owners regarding storage and handling of vaccine and correct vaccination technique. In addition, we note that vaccinated birds had a non-negligible incidence of H5 virus infection (Chapter 4, Table 4.2) indicating: (1) the strains included in the vaccine in use did not match current field strains; (2) vaccine storage and delivery methods were less than optimal; and/or (3) a combination of items (1) and (2). These findings indicate that regular monitoring of field virus strains is important to ensure that the vaccines selected for regional campaigns are appropriate for identified contemporary field strains.

The multilevel analyses presented in Chapter 6 showed that most of the significant fixed-effects influencing the risk of influenza Type A virus infection were all flock-level exposures including production type, flock size, purchasing poultry, not separating newly purchased birds, the presence of other animal species within the household, the presence of visits by other poultry flocks and changes in flock size between consecutive sampling. Our analyses showed that the relative contribution of unmeasured flock- and bird-level factors on influenza Type A infection risk were approximately equal. The findings from this study support the argument that interventions to reduce the maintenance and transmission of influenza Type A virus should be applied at the individual bird and individual flock level. These conclusions are consistent with studies that investigated risk factors

for the presence of clinically apparent HPAI H5N1 in poultry flocks in the same region during the same time period (Minh 2010). Flock owners have been the recipient of awareness campaigns about HPAI H5N1 starting from when the first outbreaks occurred in late 2003. We propose that these need to be extended to allow flock owners to receive education on specific details of flock-level biosecurity practices to reduce virus transmission risk. These education campaigns should be focused on duck flock owners because duck flocks are frequently moved out of their home communes for grazing (Chapter 7), increasing the likelihood of spreading AI viruses at the same time. These education campaigns could be coupled with the change in vaccination campaign policy outlined above which would help to ensure that duck flocks were adequately vaccinated well before the time of movement (as opposed to the current system in which vaccination coverage is entirely reliant on the timing of the two vaccination rounds).

A further long-term strategy would involve restructuring Vietnamese poultry production to eliminate or at least reduce the number of small poultry holdings, including field running duck flocks. This would encourage owners to invest more in individual flocks and would allow the government to concentrate their limited recourses on flocks that were of larger size. At the time of writing it is our assessment that this strategy would be difficult to implement. We note however, that if successful, it would have positive impacts on other infectious diseases of poultry, not only HPAI H5N1.

Our social network analyses (Chapter 7) showed that in the south of Viet Nam in 2009-2010 the number of duck movements and the total number of individual ducks moved were 5 to 6 times greater (respectively) than that recorded for chickens. Poultry were more likely to be moved between communes with provincial roads and communes with relatively large numbers of poultry-owning households. In contrast, communes with large numbers of people were less likely to be connected by poultry movement events. Assuming a causal relationship exists between a commune's connectivity within a poultry movement network and HPAI H5N1 risk, we conclude that communes identified as being likely to be connected within a network should be targeted for disease control and surveillance. In contrast to the policy implications arising from our survival and multilevel analyses (Chapter 5 and Chapter 6), our social network analysis provide an explicit set of guidelines that can be used by DAH to identify communes that are more likely to be involved in poultry movement transactions and therefore more at risk of receiving or dis-

tributing infection. Tabulating, for each commune in a given jurisdiction, the rural-urban classification of the district in which the commune was located, the presence or absence of provincial roads, human population size, poultry population size and the number of households with poultry will allow communes more likely to participate in poultry movement transactions to be listed. Once identified surveillance attention can then be focussed on these communes, or following on from the conclusions from Chapters 5 and 6, flock owners in these communes can be targeted to receive education campaigns designed to enhance flock-level biosecurity. In this way a finite level of resource can be more effectively distributed amongst the commune population at risk. Rather than DAH trying to develop and carry out education campaigns in every commune, we have shown how potentially 'risky' communes can be identified and specifically targeted for intervention. This classification needs to be flexible and should be reviewed and updated frequently. These ideas are consistent with the Progressive Control Pathway principles recently developed by the Food and Agriculture Organization of the United Nations (FAO 2011, Sumption et al. 2012) and in many ways the HPAI H5N1 risk management proposals outlined here could fit well within this framework.

8.1 Epidemiological research studies

In epidemiological research studies using a prospective cohort approach study subjects can be either individuals (e.g. individual birds) or groups of individuals (e.g. poultry flocks) that are followed over time and monitored for a defined outcome of interest. A similar study design is a repeated cross-sectional study, whereby observations about a population are carried out at either regular or irregular intervals throughout a follow-up period. The key difference between a repeated cross-sectional study and that of a prospective cohort study is that a repeated cross-sectional study does not require individuals within the population to be explicitly identified: the incidence or prevalence of the outcome of interest at a given time of observation is simply the identified number of outcomes divided by the size of the population at risk. For this reason, repeated cross-sectional studies are generally easier to administer than prospective cohort studies because individual study subjects do not have to be identified and followed-up repeatedly over time. The main advantage of the prospective cohort study design in the context of the work presented in

this thesis is that it allowed us to identify factors influencing the time taken for an event to occur such as the duration of immunity following HPAI H5N1 vaccination (Chapter 5). On reflection, a repeated cross sectional study could have been used to achieve the objectives of the descriptive study presented in Chapter 4 and the multilevel analyses presented in Chapter 6. This would have simplified the overall project design and would have been significantly cheaper. However, if this approach was taken it would have eliminated our ability to document the duration of immunity following vaccination. Given the cost of the HPAI H5N1 vaccination program in Viet Nam (estimated to be in the order of USD 50 million in 2011) and given the on-going existence of HPAI H5N1 outbreaks in the presence of vaccination our ability to critically evaluate the field performance of vaccine provides key information that can be used to appropriately adjust vaccination policies. In this respect the expense of the prospective cohort study was well justified.

Implementation of the prospective cohort study provided a number of good lessons for the Vietnamese DAH in terms of the design of observational epidemiological studies of this type that may be carried out in future. The prospective cohort study was carried out over a period of 17 months and during that time frame a large percentage of enrolled poultry flocks (59% layer and 50% in-contact species) were replaced by new flocks. The main reason for flock loss was that the price of eggs decreased sharply making it necessary for many flock owners to sell their birds. The key learning point here is that when carrying out this type of research in poultry populations of this type in Viet Nam allowance has to be made for these types of unforeseen events. Failure to do so reduces the total number of subjects available for analysis and a subsequent loss of study power. We recommend that in prospective cohort studies of this type carried out in future that the calculated number of enrolled flocks to meet the design objectives should be increased by a factor of at least 1.5.

In addition, the prospective cohort study involved a relatively complex sequence of sample collection which in turn required good logistic preparation. For example, poultry flock owners were invited to take part in this study after a careful introduction whereby DAH staff presented them with the study objectives and outlined the duties and responsibilities of flock owners if they decided to take part. Once a flock owner agreed to take part a contract was signed to ensure that flocks would be made available for sampling on each of the pre-defined sampling dates. It was then essential to provide field staff with appropriate

training to allow them to carry out each of the study activities such as collection of primary information about the study flocks, collection, storage and transportation of samples from the field to the laboratory, and collection of information about factors influencing the study outcomes. Provision of feedback to field staff such as the number of completed questionnaires and the number of samples delivered to the laboratory in a satisfactory state had a positive impact on field activities as indicated by a steady increase in the proportion of completed questionnaires and a reduction in the number of spoiled samples submitted to the laboratory as the study progressed.

A pilot study was carried out in October 2008 in two districts within the two study provinces (Can Tho and Bac Lieu) to validate aspects of the sample collection and testing procedures. Lessons learned from the pilot study were then used to modify the standard operating procedures set out for the prospective cohort study. In the pilot study three types of swab samples (mixed cloacal-oropharyngeal, cloacal and oropharyngeal) were tested using M gene RRT-PCR. Swab samples were pooled into groups of three and five and the M gene RRT-PCR results for each of the six sample type – pool combinations compared. The results showed that there was no clear benefit provided by pooling swab samples by threes compared with five. The results from the mixed cloacal-oropharyngeal samples were no different to either the cloacal or oropharyngeal samples. Based on these findings it was decided to mix oropharyngeal and cloacal swabs in one tube and then pool the swab samples into groups of five for M gene RRT-PCR testing.

8.2 Data collection and management

Although Viet Nam, in comparison to other countries, is limited in terms of physical resources, it is rich in veterinary human resource which allows the DAH to collect a range of animal health information at relatively low cost. For example, poultry movement data routinely recorded in the daily recording books at all animal health quarantine stations were used for our social network analyses described in Chapter 7. In this chapter, the aim of the study was to describe and model the patterns of poultry movement in the south of Viet Nam for the period 1 September 2009 to 30 June 2010. A large number of individual poultry movement records (appropriately 20,000) were submitted by the animal health quarantine stations throughout the 9-month study period. Setting up the study was

relatively straightforward. A single workshop was carried out in August 2009 (one month before the study start) where animal health quarantine station staff were instructed on the objectives of the study and how to record the required information and present it in a format ready for analysis. This study provides an example of how, in a Vietnamese context, a relatively large amount of useful information can be collected over a relatively short period of time. Looking to the future, a system needs to be established allowing data from the field to be recorded and then seamlessly transferred to a national database (using, for example, smart phone applications, Aanensen et al., 2009, Robertson et al., 2010) ready for analysis.

While the Vietnamese DAH is rich in terms of the number of staff available to carry out and administer field work, problems still exist in terms of inconsistencies in: (1) disease event recognition and recording, and (2) the completeness of data capture once disease events have actually been detected. The presence of these issues represents an on-going obstacle to the provision of timely analyses of animal health data that can be used to support decision making. For example, although poultry are kept in approximately 94% of communes throughout the country (GSO 2012) poultry movement data were only recorded from 35% (850 of 2549) of communes in the poultry movement study described in Chapter 7, raising questions about the completeness of the poultry movement data that was actually recorded. In a study of the epidemiology of HPAI H5N1 in Viet Nam (Minh, Morris, Schauer, Stevenson, Benschop, Nam & Jackson 2009) it was necessary for the authors to seek clarification from DAH field staff on approximately 20% of recorded outbreak details. These issues are not unique to Viet Nam and are a common problem in virtually all countries where food is produced from livestock. A recent study by Madin (2011) found that foot-and-mouth disease outbreak event details were not consistently collected and reported across south-east Asian countries during the period 2000 to mid-2010. This study identified, using details entered into the ASEAN Region Animal Health Information System (ARAHIS), a considerable number (approximately 43%) of the total number of outbreak reports lacked basic demographic information such as the size of the animal population at risk, the number of affected animals and the serotypes of FMD virus involved.

To address the issues outlined above it is important for animal health authorities such as the Vietnamese DAH to categorise information recorded at the time of identified out-

breaks as either essential or non-essential. Once this is done, the emphasis of field staff training can be on collection of essential outbreak information such as date of onset, location details, size of the animal population at risk, the number of infected animals at the time of examination and the number of deaths. At the national level there needs to be consistency across provinces in terms of disease event recognition and the completeness of details recorded for each identified outbreak. Again, at the national level, staff epidemiologists need to carry out descriptive analyses of outbreak data and then regularly feed that information back to field staff so that they can get a better sense of where their jurisdiction fits into 'the bigger animal health picture'.

At the time of writing the Vietnamese DAH runs a predominantly paper-based recording and reporting system. We propose (as outlined in Chapter 3) that this is progressively replaced by a national on-line decision support system. To be effective the system needs to allow users to enter data into the system in both online and offline modes. An offline mode is essential, particularly for staff working in remote areas of the country where Internet connection is either slow or intermittent.

8.3 Conclusions

This thesis has addressed various topics related to the epidemiology of highly pathogenic avian influenza H5N1 in the Mekong River Delta of Viet Nam. In light of the findings of the studies presented we provide the following conclusions and recommendations:

1. Chapter 3 of this thesis described the design and operation of an animal health decision support system for use in Viet Nam. We believe that this system, if properly deployed within central and regional DAH offices and if appropriately used by DAH staff will overcome many of the data management and analysis issues that currently exist in Viet Nam. Ultimately this will allow DAH to identify and respond to existing and emerging threats to animal health in a timely and cost-effective manner.

2. The overall incidence rate of influenza Type A and H5 virus infection within village poultry was relatively high throughout the follow-up period of the prospective cohort study (from December 2008 to April 2010). This implies that interventions such as vaccination, movement controls and biosecurity measures need to be carried

out continuously throughout the year rather than focusing only on the established high risk periods (from December to February and May to July within a given year). Broiler ducks had an incidence rate of influenza H5 virus infection that was approximately four times greater than that of layer ducks and in-contact species indicating that broiler ducks should be the focus of disease surveillance and control strategies.

3. The duration of immunity in vaccinated poultry under field conditions was considerably shorter than the duration of immunity that has been documented in laboratory studies. The age of poultry at the time of vaccination and the time of the year when vaccination was carried out significantly influenced the duration of immunity in vaccinated birds. These findings indicate that the HPAI H5N1 vaccination protocol (currently delivered as two vaccination rounds carried out in April-May and November-December within a given year) should be modified to an individual, flock-based campaign. If this approach is taken it will be necessary to make HPAI H5N1 vaccine readily available to flock owners so that birds can be vaccinated on the basis of age and whenever new birds are introduced into individual flocks.

4. We propose that the current community-based HPAI H5N1 awareness campaigns should be re-focussed and expanded to allow individual (duck) flock owners to be educated on the correct application of biosecurity practices. These campaigns should be carried out in conjunction with the change in vaccination campaign policy outlined above.

5. Communes and districts need to be classified in terms of their perceived capacity to spread disease (not only HPAI H5N1) using multiple criteria, including (but not restricted to) the findings derived from social network analyses of the type presented in Chapter 7.

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APPENDIX A

Baseline questionnaire for the prospective cohort study of HPAI and LPAI in the Mekong River Delta

Baseline questionnaire for the prospective cohort study of HPAI and LPAI in the Mekong River Delta

Date of investigation	
Code of investigator	
Name of investigator	

Good morning/afternoon/evening. I am ……, a veterinarian of.……

We are planning a study between November 2008 and March 2010 to identify potential risk factors of avian influenza. The information will help finding appropriate control and prevention strategies for HPAI in Vietnam.
For this study, twenty birds from each of the selected flocks will be individually identified. Every one to three months, you will need to catch these birds so that vets can take swab samples and sometimes blood samples.

Today, we are conducting a small trial study for which you were selected as one of the participants. Before the start of the main study, a commune vet will visit you to officially sign a contract between you and the Department of Animal Health.
First, we would like to ask you background information on you and your poultry production. The interview will take approximately 20 minutes. Can I speak to the person who is mainly responsible for poultry raising in this household? If at any time during the interview you are not clear about the question, be sure to ask me.

Address

Name of village	
Name of poultry owner	
Position of interviewee (owner, wife, etc.)	
Flock Identification Number	
Household Identification Number	

I. General information

For the interviewer: Is the respondent ☐ male or ☐ female?

1. How old are you?

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

2. What ethnic group do you belong to?

   Kinh: ____% Khmer: _____% Other (please specify):_____

3. Are you a member in any social association (e.g. women union, farmer union, …)?

   ☐ Farmer Organization ☐ Farmer Union

   ☐ Women's Union ☐ None

   ☐ Others, please specify ……

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

   ☐ None ☐ Secondary school

   ☐ Primary school ☐ High school

   ☐ Other (specify) _____
   _____

5. How many men, women and children live in your household today?

   Men (> 15 years): ……

   Women (>15 years): ……

   Children (6 to 15 years of age): …… (if no children → skip to question 16)

   Children (0 – 5 years of age): …… (if no children → skip to question 16)

6. Do your children go to school?

   ☐ Yes ☐ No

7. What are the main occupations of people living in this household?

   ……

8. Do you grow rice?

☐ Yes ☐ No → (Skip to **Question 10**)

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

9. How big is the size of your rice field? …… m2 or …… ha

10. What means of transport do you have?

☐ Boat ☐ Motorbike

☐ Bicycle ☐ None

☐ Others, please specify ……

11. Do you have electricity?

☐ Yes ☐ No

12. Do you have fresh water supply?

☐ Yes ☐ No

13. Have you borrowed money in the last 12 months to invest in your farm?

☐ Yes, please specify what you invested the money in: ……

☐ No

14. Which other animal species than poultry do you own today?

Species	Number	Species	Number
☐ Pigs		☐ Cattle	
☐ Buffalo		☐ Dogs	
☐ Other: Please specify: ……			

II. Poultry production

Now, we want to ask you some information about your poultry.

15. How many of each poultry species do you have? 1 Please specify ages of your poultry flocks. (Fill in all rows; write "0" if not applicable)

Species	**Number of poultry**	**Age in months** [2]
Chickens		
Ducks		
Muscovy ducks		
Geese		
Fighting cocks		
Quail		
Pigeons		
Pet birds		
Other (specify) ……		

16. Where do you often purchase your poultry?

Purchase live poultry	Rank
☐ Markets in your village	
☐ Markets outside your village	
☐ Hatchery in your village	
☐ Hatchery outside your village	
☐ Other farmers in your village	
☐ Traders from the same commune	
☐ Traders from outside your commune	
☐ Other (please specify: ……)	
☐ Not sure	

[1] For cases: total population before the outbreak

[2] 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 i) 2-3 months; ii) 6 months; and iii) more than 18 months), OR write "mixed"

17. Where do you often sell live poultry or poultry products? If you select more than one category, please rank them according to 1= most frequent, 2 = second most frequent.

Sale of live poultry		Rank
☐ Markets	☐ Markets in your village	
	☐ Markets outside your village	
☐ Neighbours	☐ Other farmers in your village	
☐ Traders	☐ Traders from the same commune	
	☐ Traders from outside your commune	
☐ Other (please specify: ……)		
☐ Not sure		

18. Has the size of your chicken or duck flock(s) increased or decreased compared to five years ago; please roughly indicate the timing and size of changes?

☐ Increase: In (*specify time*) ……, by ……%

☐ Decrease: In (*specify time*) ……, by ……%

☐ No change

19. Are you the only person in this household who is responsible for raising poultry?

☐ Yes

☐ No

If no, please specify responsibilities of other household members: a) responsible for which poultry group (species, production type); b) what activities (e.g. feeding, cleaning, moving, buying, selling …):

Person 1: ……

Person 2: ……

Person 3: ……

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

☐ Yes

☐ People from the same village

☐ People from outside the village

☐ No

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

Category	Chickens	Ducks	Muscovy ducks
Confined with roof	☐	☐	☐
Confined by fence (with no roof or partly roofed) with access to yard only	☐	☐	☐
Confined by fence (with no roof or partly roofed) with access to yard and waterways	☐	☐	☐
Free range on your own property	☐	☐	☐
Free range on your own and neighbour's yards	☐	☐	☐
Free range with access to waterways	☐	☐	☐
Not raising	☐	☐	☐

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

Category	Chickens	Ducks	Muscovy ducks
Confined with roof	☐	☐	☐
Confined by fence (with no roof or partly roofed) with access to yard only	☐	☐	☐
Confined by fence (with no roof or partly roofed) with access to yard and waterways	☐	☐	☐
Keep freely on your own property	☐	☐	☐
Keep freely on your own and neighbour's yards	☐	☐	☐
Keep freely with access to waterways	☐	☐	☐
Not raising	☐	☐	☐

23. Do you purchase feed for your poultry?

☐ Yes ☐ No

If yes, please estimate the percentage of total feed you buy usually buy for your poultry:…… % ☐ Not sure

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

☐ Yes, _____

☐ No

☐ Not known

III. Duck production (if the household does not keep ducks, skip to question)

Next we want to ask you some questions about your ducks.

25. Do you own your duck flock?

    ☐ Yes ☐ No, I am employed to look after the ducks

26. If household owns layer flocks: At what age (of ducks) do you usually replace a layer flock? ……...……

27. If household owns broiler flocks: Please list the periods when you usually raise broiler flocks (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

    Comments ……..……...
    ……...

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

    ☐ Yes ☐ No ☐ Not known

29. Do you run ducks on rice fields?

    ☐ Yes ☐ No → (Skip to question 36)

30. Does any of your duck flocks move outside the village?

    ☐ Yes

    ☐ No → (Skip to **Question 36**)

31. Who is responsible for moving ducks if they are moved outside the village? ……...

32. Where do you move duck flocks to if they are moved outside the village?

    ☐ To other villages in the same commune

    ☐ To other communes in the same district

    ☐ To other districts in the same province

    ☐ To other provinces; please specify province names ……..

33. Please list the time periods when you often move ducks outside the village (lunar calendar)

☐ To other villages in the same commune……...…...

☐ To other communes in the same district……..…

☐ To other districts in the same province……

☐ To other provinces …….

…….…….

34. Do you think you can always inform sampling teams of the location of the flock if it moves outside the village?

☐ Yes ☐ No (skip to question **Question 36**)

35. Why do you think you can always inform sampling teams of the location of the flock?

☐ The duck herder can always be contacted by phone

☐ Flock movements are planned in advance

☐ The flock is always staying at the same places

☐ One household member always knows where the flock is

☐ Others, please specify: ……

After asking this question, skip to question 36

IV. Biosecurity and avian influenza control

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

☐ sweeping ☐ disinfect ☐ other:_____ ☐ Not done

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

☐ daily ☐ weekly ☐ monthly

☐ after selling poultry ☐ other:_____ ☐ never

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

☐ weekly ☐ monthly ☐ quarterly

☐ during HPAI vaccination ☐ other:_____ ☐ never

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

☐ Yes ☐ No

40. Do you wash your hands after handling poultry?

☐ Usually ☐ Occasionally ☐ Rarely ☐ Never

41. 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

42. 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 ☐ Not known

43. 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 ☐ Not known

44. What do you do with poultry manure?

45. Have you vaccinated your current poultry with HPAI vaccine?

☐ Yes ☐ No *(> finish interview)*

- 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

46. 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

V. Information important for the study

Next, we want to ask you some information, which will be relevant for our study.

47. Do you have a mobile phone?

☐ Yes. Can you give me your number? ……..……

(*For interviewer: Please ring number to check it is correctly recorded*)

☐ No

48. What is the best alternative way to contact you other than by mobile phone? (*Select one or many)*

☐ By mail; please specify address ……

☐ By phoning relatives or neighbours in the same village; please indicate the name ……, phone number …… and relationship (relative, neighbour……)

☐ By phoning the village headman

☐ Only by visiting the village in person

☐ Other methods: Please specify……

☐ Don't know

49. For this study, twenty birds of your flock will be individually identified, and you will have to recapture the same birds every 1 to 3 months. Do you think you will manage to recapture individually marked birds out of the flock and hold them somewhere until sampling team arrives?

☐ Yes

☐ No, please specify why

☐ To difficult to catch

☐ Nowhere to hold the birds

☐ Others, please specify: ……...……

50. Where can you keep twenty captured birds until sampling team arrives?

☐ House ☐ Fenced area

☐ Cage ☐ No need; easy to catch

☐ Nowhere ☐ Haven't thought about it yet

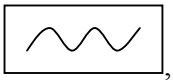
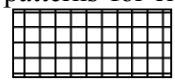
☐ Others: Please specify: ……

51. What are reasons why you want to participate in the study? *(Chọn một hoặc nhiều)*

☐ To help in general ☐ To earn some extra money

☐ To help understand avian influenza ☐ Not sure

☐ Others, please specify: ……

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

Do you have any further comments?

_____

_____

_____

_____

_____

Thank you very much for your cooperation. Do you have any questions?

APPENDIX **B**

Follow-up questionnaire for the prospective cohort study of HPAI and LPAI in the Mekong River Delta

Follow-up questionnaire for the prospective cohort study of HPAI and LPAI in the Mekong River Delta

Study objective:

1. To identify transmission and maintenance mechanisms for LPAI and HPAI viruses in field running duck and other in-contact species flocks;
2. To identify risk factors for AI virus transmission associated with field running duck farming;
3. To monitor the course of antibody response following vaccination and/or natural infection and its effect on virus shedding and transmission;
4. To provide input data for a decision support model that provides epidemiological guidance on selection and implementation of appropriate surveillance strategies for avian influenza.

Purpose of this questionnaire: To collect information for assessment of risk factors that may relate to the transmission and maintenance of avian influenza viruses among poultry population. This is a follow-up questionnaire that is used during sampling rounds 2 to 14, from November 2008 to March 2010. This questionnaire takes about 20 minutes for interviewing.

Implementing organisations:

1. DAH, MARD.
2. RAHO6 and RAHI7.
3. Study SDAHs, DVSs, local animal health workers in Can Tho and Bac Lieu.
4. EpiCentre, Massey University.
5. Reference laboratory, AAHL, Australia.

All information that is collected based on this questionnaire is critically important for the project's objectives mentioned above. The overall goal of the study is helping the government of Vietnam and international community to develop more effective control measures for avian influenza, leading to effective national control of H5N1 infection in poultry and protection of humans against exposure to H5N1. Therefore, the contribution of both farmers and implementing organisations is very important, and we need to make sure that the information collected is correct.

Thank you very much for your kind cooperation.

BASIC INFORMATION OF INTERVIEWER

Date of interview	……/……/……
Interviewer's ID	
Interviewer's name	

PART 1: BACKGROUNDS OF POULTRY HOUSEHOLD

Village name	
Owner name	
Interviewee (owner, husband, wife,...)	
Flock ID	
Location of poultry production area (N = Longitude):	
Location of poultry production area (E = Latitude): *(Geographical coordinates (X, Y) of households)*	

PART 2: POULTRY PRODUCTION

Hello, may I ask some questions about your poultry production as follows:

1. At this moment, how many poultry do you have? Please let us know detailed information as described in the following table.

Poultry species	Number of birds	Breed	Estimate of age of current birds (in days)	How long have they been raised? (in months)
Broiler duck				
Layer duck				
Chicken				
Muscovy duck				
Fighting cock				
Quail				
Geese				
Others (specific)…..				
Total				

2. Do you have any other animals in your house?

Species	Number of animals	Species	Number of animals
□ Pig		□ Cattle	
□ Buffalo		□ Dog	
□ Other: Please give specific name and number of animals.:……			
□ No, I do not have any other animals			

3. Please let us know what following activities (in below table) occurred from the last sampling round? Tick one square box in each row please.

Main activities	Yes	No	Unknown
My poultry contacted with other neighbour poultry	□	□	□
My poultry contacted with other village poultry	□	□	□
My poultry contacted with wild birds	□	□	□
Local veterinary staff visited my poultry flock	□	□	□
Local poultry traders visited my poultry flock	□	□	□
People from my home village visited my poultry flock	□	□	□
People from my other villages visited my poultry flock	□	□	□
My family member came back from working in other villages	□	□	□

4. Did you buy any new poultry from the last sampling round?

□ Yes, please continue with question 5.

□ No, please move to question 7.

5. Where did you buy new poultry? Tick square box in one to many rows please with filling number of bird please.

Buying live birds from	**Number of birds**
□ Markets of home village	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Market of other villages	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Hatchery in home village	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Hatchery in other village	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Households of the home village	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Traders of the home commune	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Traders of other communes	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..
□ Other, please specific:……	Total:……...; Chicken;……..…; Broiler duck:……...; Layer duck……...; M.duck….:…… Others:……..

6. If you got newly bought birds into your flock, did you separate these birds from other existing birds for a while and then mixed them?

□ Yes, I have kept new birds separate for…… days by keeping them (please specify location you used to separate birds)…….

□ No

7. Did you buy feed from outside since the last sampling round?

□ Yes, please specify as in following table

Type of feed	***For species***	***Number of kg feed***	***How were feed made***
	Broiler duck		
	Layer duck		
	Chicken		
	Muscovy duck		
	Quail		
	Others (specific)…..		
	Total		

□ No, I used homemade feed, what they are?:…….....

8. Have you either planted or harvested rice from your field since last sampling round?

□ Yes, please tell us:

□ Plant, when? Please specific day (lunar calendar):……..…….....

□ Harvest, when? Please specific day (lunar calendar):…..……..……..…….

□ No

9. Have you seen any field running ducks from outside the village visit your rice field since the last sampling?

□ Yes, when? Please specific from when to when:…..…….....

□ How many duck herders _____

□ No □ Not sure

PART 3: DUCK PRODUCTION

Interviewers: Please ask these questions if household owns ducks (even if ducks were not contracted).

10. Could you please provide us information about locations where your duck flocks had been to over last month (from the last sampling round to date)?

□ No movement of duck flocks, please tick one of these two square boxes and then move to Question 13, Part 4.

□ Just stayed at home □ Ở Just stayed within home village

□ Yes, I moved my duck flock to other villages. *Please provide details* as following table*:*

Date of movement	Village	Commune	District	Province

11. Did you duck flock contact with other poultry flock during their movements?

□ Yes, please provide details as following table:

Species of contacted poultry	***Number of contacting times***	***Locations where your duck flock contacted with other poultry flocks***

□ No □ Unknown

□ My duck flock was kept at places where other flocks were also kept there, but they moved to other places.

12. If you moved your duck flocks to outside of your village, how did you move your duck flock to other places? (*Please tick one or many following boxes*)

□ Moved to other villages, communes by let duck flock run on the rice field and move to other villages, how far……..….? And when……..?

□ Moved to other districts, provinces by:

□ Truck □ Boat □ Other, specific:…….

□ Can you estimate how much money did you spend on moving duck flocks to other location?.....…… and how much did you pay for rice fields?……...……

PART 4: PRVENTION, CONTROL MEASURES AND DISEASE SITUATION

13. Since the last sampling round, how did you clean and disinfect production areas?

□ Clean □ Disinfection □ Other:…….. □ Nothing

14. How often did you clean, disinfect production areas and waste materials?

□ Everyday □ Every week □ Never

□ After selling poultry □ Other, specific:……

15. If you buy poultry for meat consumption from outsides, how did you get rid of slaughtering waste (feather, redundant parts of intestine system,….)?

……

……

16. Did you vaccinate your poultry since the last sampling round?

□ Yes □ No (please move to Question 18)

- Please let us know:
  - Certificate of vaccination: □ Available □ Not available
  - Most recent vaccination date:_____ □ Unknown
  - Number of poultry at vaccination date

    Chicken:_____ □ Unknown □ No chicken

    Duck:_____ □ Unknown □ No duck

- ***Note:*** *In case, farmers cannot recall or do not have any vaccination information for their poultry flocks, it is requested local animal health workers and district veterinarians to check their own vaccination recording sheets for further information?*

17. If your poultry had vaccination, please let us know who vaccinated your poultry? Please tick below box

□ Family members □ Village animal health workers (AHW)

□ AHWs from other villages □ AHW from other communes

□ Unknown

18. Did egg trader visited poultry flocks since the last sampling round (to date)?

□ Yes, how many times?:……...

□ No

□ Unknown

19. Did you hear about or know any disease in poultry or poultry deaths within your village since the last sampling round?

□ Yes When did you hear about that?……

Poultry species:……

Main clinical signs:……

……

□ No □ Unknown

20. Have you seen any signs of disease in your poultry since the last sampling round?

□ Yes □ No (please move to Question 28) □ Unknown

21. If yes (Question 20), when did you see that?

□ Date: day/month:…… □ Unknown

22. If yes (Question 20), please let us know specific information as follows?

Species	Total population	Scale of age (in months)	Number of cases	Number of deaths
Broiler duck				
Layer duck				
Chicken				
Muscovy duck				
Other, specific….				

23. If you think your poultry had some problems, so which below abnormalities did you observe? Please tick one or many boxes.

□ Sudden deaths

□ Showing clinical signs of avian influenza or similar signs

□ High mortality □ Reduction of egg production

□ Reduction of feed consumption □ Unknown

□ Or other clinical signs? ……..

24. When you saw your poultry having some problems, what did you do with them? Please tick one or many boxes.

□ Separate them from others □ Slaughter for meat consumption

□ Made notification to local vets □ Selling

□ Made notification to village chief □ Did nothing

□ Used some medicines for treatment □ Unknown

25. What did you do with dead birds? Please tick one or many boxes.

□ Made notification to local vets
□ Made notification to village chief
□ Throw them to river, lake, rice field
□ Slaughter for meat consumption
□ Selling
□ Burn/bury
□ Did nothing
□ Not sure

26. With your experiences, which possible following factors that you think they may contributed to your poultry problems? Please tick one or many boxes.

Poultry movements	□ Yes	□ No	□ Not sure
Movement of animal products	□ Yes	□ No	□ Not sure
Wildlife contact	□ Yes	□ No	□ Not sure
Fomites (vehicle of poultry traders, possibly contaminated material…)	□ Yes	□ No	□ Not sure
People (visitors, farmers…)	□ Yes	□ No	□ Not sure
Other (specify)…….			

27. What did you do with manure wastes? Please tick one or many boxes.

□ Collect for burning/burring
□ Selling
□ Use for fishes, spawn
□ Other measures, please specific:……
□ Use for home trees
□ Use for rice production
□ None of use
□ Not sure

28. Are you planning to sell the study flocks?

□ Yes: day/month:……, where are you selling?:……..
□ No
□ Not sure

29. How many birds did you sell, slaughter, give away to someone else or other purposes?

Species	Sold	Slaughtered	Gave away	Other (specify)
Chicken				
Duck				
Muscovy duck				
Other (specify)				

30. Are you planning to move your study flocks to somewhere else?.

□ Yes: day/month:……, where are you moving?:……..
□ No
□ Not sure

31. What will you do with your flocks that you will move them to other places?

□ Sell for consumption when bird are growth enough

□ Sell during the movements

□ Bring them back to home.

□ Not sure at this stage

32. Are you planning to get any new poultry in near future?

□ Yes, please specific as following table:

Poultry species	***Number of birds***	***Proposed date of buying***	***Purpose (for meat consumption, raising,…)***
Broiler duck			
Layer duck			
Chicken			
Muscovy duck			
Quail			
Others (specific)…..			
Total			

□ No □ Unknown

We sincerely thank you very much for your time and your support to this study.

APPENDIX C

Sampling form for the prospective cohort study of HPAI and LPAI in the Mekong River Delta

SAMPLING FORM FOR THE PROSPECTIVE COHORT STUDY OF HPAI AND LPAI IN THE MEKONG RIVER DELTA

Date of sampling: ....

Sampling round:…………………………………...

Village:... Household ID: .... Household name: ....

Name of sample recorders: .... Flock ID: ……………………………………………

Time of samplinge: Start at: ……………………………………Finish at:……………………………… Temperature of cooling box:……………………

UNVACCINATED POULTRY (SENTINEL) – Table 1

New poultry	Poultry sampled last rounds	Number of leg ban (right leg)		Number of leg ban (left leg)		Type of sample			Poultry species					Month of age	Health condition of poultry
		Current number of leg band	New number of leg band (if place is needed)	Current number of leg band	New number of leg band (if place is needed)	Blood	Oropha-ryngeal swab	Cloacal swab	Broiler duck	Layer duck	Chicken	Mus. Duck	Other (specify)		
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Clinical sign observed

Number of leg ban (right leg)	**Number of leg ban (left leg)**	**Summary of clinical signs**

Flock ID: …………………………………………

VACCINATED POULTRY - Table 2

New poultry	Poultry sampled last rounds	Number of leg ban (right leg)		Number of leg ban (left leg)		Type of sample			Poultry species					Month of age	Health condition of poultry
		Current number of leg band	New number of leg band (if place is needed)	Current number of leg band	New number of leg band (if place is needed)	Blood	Oropha-ryngeal swab	Cloacal swab	Broiler duck	Layer duck	Chicken	Mus. Duck	Other (specify)		
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Clinical sign observed

Number of leg ban (right leg)	**Number of leg ban (left leg)**	**Summary of clinical signs**

APPENDIX D

Data collection form for the poultry movement study in the south of Viet Nam

Data collection form for the poultry movement study in the south of Viet Nam
(To collect data from 01 September 2009 to 30 June 2010)

Form 1: To collect information about poultry which **moved into** the commune.

Day/Month/Year	Name of origin			Name of destination			Chicken					Duck					Note
	Commune	District	Province	Commune	District	Province	Total number of chicken flocks	Total number of chicken	Total number of flocks move to live bird markets	Total number of flocks move to other places for raising	Total number of flocks move for other purposes	Total number of duck flocks	Total number of ducks	Total number of flocks move to live bird markets	Total number of flocks move to other places for raising	Total number of flocks move for other purposes	
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01 Nov-2009																	
01 Dec-2009																	
01 Jan-2010																	
01 Feb-2010																	
01 Mar-2010																	
01 Apr-2010																	
01 May-2010																	
30 June-2010																	

Form 2: To collect information about poultry which **moved out** the commune.

Day/Month/Year	Name of origin			Name of destination			Chicken					Duck					Note
	Commune	District	Province	Commune	District	Province	Total number of flocks	Total number of chicken	Total number of flocks move to live bird markets	Total number of flocks move to other places for raising	Total number of flocks move for other purpose	Total number of flocks	Total number of ducks	Total number of flocks move to live bird markets	Total number of flocks move to other places for raising	Total number of flocks move for other purpose	
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01 Dec-2009																	
01 Jan-2010																	
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01 Mar-2010																	
01 Apr-2010																	
01 May-2010																	
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