Quantifying the Effects of Measures to Control Highly Pathogenic Avian Influenza H5N1 in Poultry in Southeast Asia

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Abstract

Despite the ongoing efforts to contain its spread, H5N1 is now considered endemic within poultry in various settings worldwide, threatening both the livelihoods of those involved in poultry production in affected countries and posing a continuous public health risk. The reasons for the varying levels of success in controlling H5N1 in Southeast Asia need to be better understood. In this thesis, various different methods of quantifying the effects of individual control measures, using the types of data available in various different contexts, are discussed and applied. In the first half of this thesis a spatio-temporal survival model is fitted to H5N1 outbreak surveillance data from Vietnam and Thailand using a Bayesian framework in order to account for unobserved infection times. Following vaccination in Vietnam it was found that transmissibility had been successfully reduced but, during a wave of outbreaks in 2007, that this coincided with a reduction in the rate of at which outbreaks were reported following the introduction of infection, limiting the overall impact this reduction in transmissibility had on the total epidemic size. In Thailand, active surveillance was found to be successful in contributing to the control of infection. Furthermore, backyard producers, whilst responsible for the majority of outbreaks, were, on average, less likely to transmit infection than those involved in more intensive production. In the second half of the thesis, the use of final size methods to assess the effectiveness of vaccination from trial data is explored. This involved an investigation into the effects of different assumptions regarding the action by which vaccination confers immunity and fitting estimates of transmissibility to data collected from outbreak investigations in the context of a field trial of vaccination in Indonesia, where, making strong assumptions about the underlying infection process, a reduction in both within and between flock transmissibility was detected for outbreaks occurring in areas where vaccination was being carried out.

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Abbreviations

AI	Avian Influenza
AoN	All-or-Nothing Vaccine
ARDS	Acute Respiratory Distress Syndrome
BF	Bayes' Factor
CrI	Credible interval
CJD	Creutzfeldt-Jakob Disease
DAH	Department of Animal Health, Viet Nam
DIC	Deviance Information Criterion
DOC	Day-Old Chick
DIVA	Differentiating Infected from Vaccinated Animals
EU	European Union
FAO	Food an Argriculture Organization of the United Nations
FMD	Foot and Mouth Disease
GETS	Gathering Evidence for a Transitional Strategy
GDP	Gross Domestic Product
GLM	General Linear Model
GSE	General Stochastic Epidemic
HA	Haemagglutinin
HI	Haemagglutination Inhibition
HPAI	Highly Pathogenic Avian Influenza
IP	Infected Premises
LPAI	Lowly Pathogenic Avian Influenza
MCMC	Markov Chain Monte Carlo
NA	Neuraminidase
OIE	World Organisation of Animal Health
ORIHPAI	Operational Research in Indonesia for the effective control of HPAI
PCR	Polymerase Chain Reaction
PDSR	Participatory Disease Surveillance and Response
RNA	Ribonucleic Acid
RRD	Red River Delta
SARS	Severe Acute Respiratory Syndrome
USD	US Dollar
VND	Vietnamese Dong
WHO	World Health Organisation

Chapter 1 Introduction

1.1 Aims and objectives of this thesis

The overall aim of this thesis is to develop and apply techniques in order to quantify the transmission dynamics of the spread of H5N1 in poultry in Southeast Asia, and from that, the effectiveness of different control measures, taking into account the kinds of outbreak data likely to be available.

HPAI H5N1 continues to circulate widely in poultry in Southeast Asia. In Vietnam, the economic costs and level of destruction required to maintain a stamping out approach to disease control in the face of sustained transmission in many parts of the country led to the switch to a policy of mass nationwide vaccination in 2005. Although this resulted in a strong reduction in the number of cases in the short term, outbreaks reoccurred in sustained waves in 2007 and the disease remains endemic.

The first aim of this thesis is to use the surveillance data for outbreaks of H5N1 in Vietnam in an attempt to assess the changes in the dynamics of transmission which may have occurred as the result of this vaccination campaign.

Research Question 1: What was the impact of mass vaccination of poultry on the spread of H5N1 in Vietnam?

Specific objectives:

- Design a model and model fitting procedure appropriate to the spread of H5N1 in Vietnam, taking into account the data available.

- Fit this model to outbreak data for the waves of outbreaks occurring before, during and after the implementation of vaccination.
- Use the results to assess the possible effects of vaccination.

In Thailand, where poultry production is more heavily industrialised, measures to prevent the spread of infected have proved more successful. However, because measures such as a policy of carrying out mass culls of poultry flocks in the vicinity of reported outbreaks, a concerted drive to improve biosecurity and the use of active surveillance continue to be preferred to vaccination, this may have negatively affected the livelihoods of many smallholders.

Research Question 2: To what extent were backyard flocks responsible for the spread of H5N1 in Thailand?

Specific Objectives

- Determine the best way to extend the existing model of the spread of H5N1 transmission in order to incorporate intensive industrial production in Thailand.
- Use this to estimate the extent to which transmission occurred from infected flocks in different sectors of the poultry production system
- Interpret these results to assess where control policies are likely to have had an effect on the spread or reporting of infection.

Thailand and Vietnam are examples of countries where outbreak data were collected routinely on a national scale. Many countries in Southeast Asia lack the veterinary infrastructure to implement such surveillance (Normile 2005; Sims 2007). In the absence of such data, inferences about the transmissibility of infection and the effects of control

measures such as vaccination are generally made using data on flock-level outbreaks, either in an experimental context or as a result of outbreak investigations in the field. In these settings, the final size of an outbreak is often used to quantify the transmissibility of infection, as such data is often the most straightforward to collect.

Research Question 3: How can final size methods be used to quantify the effectiveness of vaccination from trial data?

Specific Objectives

- Assess the implications of assumptions commonly made about the action of the vaccine when analysing transmission experiments and investigate the effects of different experimental protocols.
- Develop and apply suitable final size methods to analyse data collected from a field trial into the effects of vaccination in Indonesia.

1.2 Structure of the thesis

Chapter 2 begins with an introduction into the history and epidemiology of avian influenza, leading to a description of the spread of H5N1 throughout Southeast Asia with particular focus on the poultry production systems and the experiences of H5N1 during the epidemics featured in this thesis. This is then followed with a review of the existing modelling literature.

Chapter 3 describes a spatially explicit transmission model of the spread of outbreaks of HPAI H5N1 which is then fitted to report data for three waves of outbreaks which occurred in northern Vietnam before, during and after the switch to mass vaccination. The results of

this are then used to summarise the transmission dynamics of infection and to assess the potential effects of control measures including vaccination.

Chapter 4 describes various attempts to extend the model in order to incorporate the industrial production sector in Thailand. The relative role of backyard production in the spread of infection, as well as the likely effects of different surveillance strategies on the spread of infection and on the modelled results, are then assessed and discussed.

Chapter 5 reviews the use of final size methods in an experimental context and explores the implications of different assumptions regarding the protocol of such experiments and the action by which a vaccine confers immunity for the accuracy and reliability of estimates of vaccine efficacy. Chapter 6 then describes how final size methods were used to analyse data from outbreak investigations conducted in the context of a field trial of the effects of vaccination in Indonesia.

Chapter 7 summarises the key findings of the thesis and suggests how these contribute to our understanding of the transmission dynamics of H5N1 in Southeast Asia.

1.3 Role of the Author

All work presented in this thesis was developed and carried out by the doctoral candidate Patrick Walker under the supervision of Azra Ghani and Simon Cauchemez. For the modelling work based on outbreak data in Vietnam, the data used were compiled and cleaned with help from Dirk Pfeiffer and Raphaelle Metras from the Royal Veterinary College and Do Huu Dung from the Ministry of Animal Health in Vietnam, all of whom provided helpful feedback and advice on data interpretation. For Thailand the data were obtained and prepared with the help of a technical collaboration with the PhD programme

of Tiensin Thanawat and with Nienke Hartemink and Hans Heesterbeek, from the University of Utrecht. For Indonesia, the data were collected and obtained through collaboration with Christine Jost and Jeff Mariner from the International Livestock Research Institute in Kenya and Muhammed Azhar from the Ministry of Agriculture, Indonesia.

Chapter 2 Highly Pathogenic Avian Influenza H5N1 and the Role of Mathematical Modelling

The first half of this chapter presents a review of the epidemiology of avian influenza infection in poultry and humans, the impact HPAI H5N1 has had in Southeast Asia and the current options available for controlling its spread. This is followed by a review of the role of mathematical models in quantifying the dynamics of infectious disease transmission, including a description of existing models of avian influenza and an assessment of their application to existing H5N1 outbreak data.

2.1 The biology of Influenza

There are three types of influenza virus: A, B and C, all of the family Orthomyxoviridae. Of these only A and B, isolated in 1933 and 1940 respectively, are associated with significant seasonal disease in humans. These are segmented single-stranded RNA viruses with genomes composed of eight segments (Figure 2-1), two of which code for the hemagglutinin (HA) and neuraminidase (NA) surface proteins. These surface proteins are responsible for the binding and fusion of a virion to a host cell (HA) and for spreading the virus within the host by freeing virus particles from host cells (NA) and are additionally used to characterise subtypes of the virus. HA binds with sialic acid found on the surface of the cell membrane. The virion is then brought into the cell by endocytosis, the process by which cells absorb molecules by engulfing them. This allows the virion to enter the cell without passing through the cell membrane and, by travelling within the endosome (a membrane-bound compartment which transports molecules required for digestion into the cell from the cell

membrane). The HA is then responsible for fusion between the viral envelope and the endosomal membrane which allows nucleoproteins containing viral RNA to enter the cell nucleus (Smith and Helenius 2004). Once within the nucleus the negative-sense viral RNA is transcribed and used for translation of viral proteins. The HA and NA surface proteins are then transported to the cell surface and integrate with the cell membrane, creating a protrusion into which viral RNA and core proteins are imported from the cell nucleus. This buds into a mature virus which is freed from the host cell through NA activity (Webster, Bean et al. 1992).

Once a subtype is established within a population, rising levels of herd immunity act as an evolutionary pressure and randomly generated mutations in the segments of genomes responsible for coding both proteins are accumulatively selected, increasing the ability of the pathogen to spread within a population (de Jong, Rimmelzwaan et al. 2000).

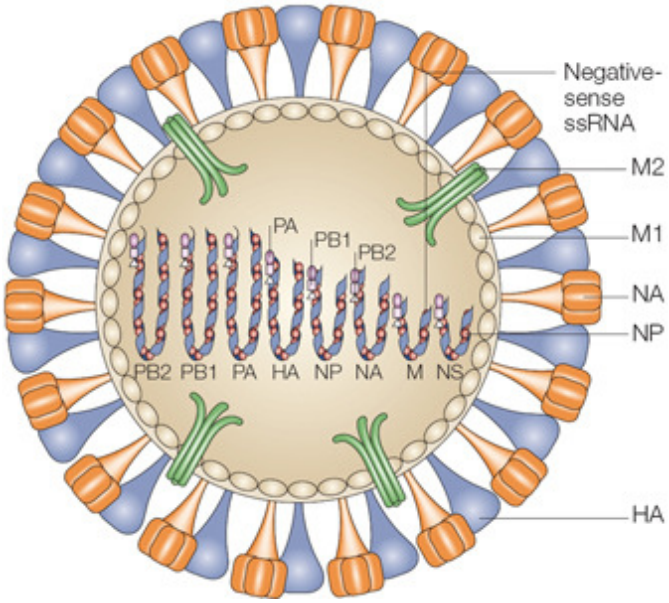


Figure 2-1| Schematic diagram of an influenza A virus virion.
Source: (Horimoto and Kawaoka 2005)
Figure illustrates the structure of an influenza A virus virion and the eight segments of negative-sense single stranded RNA comprising the viruses genome. These include segments responsible for encoding three polymerases PB2, PB1 and PA which localise in the cell nucleus and, amongst other known functions, are responsible for transcription of the viral RNA into positive-sense RNA and a segment which encodes non-structural proteins (NS1 and NS2). Influenza A is differentiated from types B and C according to antigenic differences in matrix proteins (M1 and M2 which link the viral envelope to the virus core) and nucleoprotein (NP) which are encoded by two more segments of the genome. Different subtypes of Influenza A are then characterised according to the antigenicity of the NA and HA surface glycoproteins which are each encoded by separate segments of the genome (Webster, Bean et al. 1992; Horimoto and Kawaoka 2005).

Whilst influenza B has been responsible for sustained epidemics and, prior to the 2001 H1N1 pandemic, due to such ongoing antigenic drift in the interim, tended to become prominent every few years following peaks of influenza A infection, only one subtype of the virus has been identified and the host reservoir of such viruses is almost exclusively human (Hay, Gregory et al. 2001). In contrast, influenza A viruses, whilst primarily of an avian source, can infect a wide variety of mammalian hosts including cats, dogs, swine, whales and horses. The range of recognised subtypes of Influenza A consist of viruses exhibiting combinations of 16 distinct types of HA and 9 types of NA, with the most recent isolated for the first time in 2005. This large host range, combined with the ability of the virus to shift antigenically (as the structure of the virus allows the exchange of entire segments of the

genome when two subtypes are present within the same cell) facilitates the generation of novel strains which may have the potential to cause a global pandemic. Such reassortment events between human-adapted and avian viruses have been suggested as a primary cause of pandemics in 1957 and 1968 (Scholtissek, Rohde et al. 1978; Schafer, Kawaoka et al. 1993; Webster, Sharp et al. 1995) and have been implicated in the development of the 1918 H1N1 pandemic strain (Cox and Subbarao 2000). However the exact cause of this most severe pandemic of modern times remains open to debate (Reid and Taubenberger 2003).

Since the subtyping of virus strains began, only those containing H1, H2 and H3 of the HAs and N1 and N2 of the NAs have been observed to spread widely in humans (Webster, Bean et al. 1992). The relatively small number of human-adapted viruses is largely, but not wholly, ascribed to the fact that avian influenza viruses bind preferentially to the SAα2,3Gal receptor in birds whereas human-adapted influenza viruses bind preferentially to SAα2,6Gal, corresponding to the distribution of receptors in prime virus replication sites within the two species (respectively the intestine and the trachea) (Webster, Bean et al. 1992; Peiris, de Jong et al. 2007). However, both receptors are found abundantly in the trachea of swine, leading to the hypothesis that pigs act as an intermediate host, reassortment within which can lead to the emergence of new strains capable of transmitting to and between humans (Webster, Bean et al. 1992; Ito, Couceiro et al. 1998; de Jong, Rimmelzwaan et al. 2000). This was borne out in 2009 when a virus containing a hitherto unreported combination of gene segments from various swine viruses in the Classical, Eurasian and Triple reassortant lineages, all of which themselves were primarily of an avian source (Figure 2-2), caused the first global pandemic of the 21st Century (Garten, Davis et al. 2009).

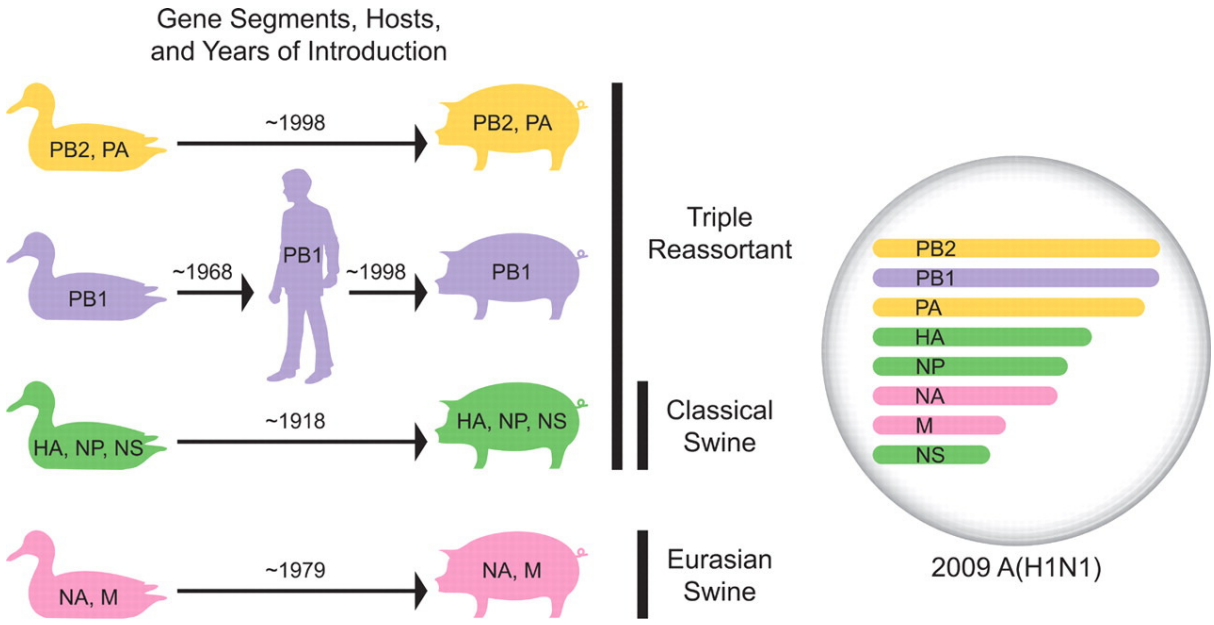


Figure 2-2|Illustration of the avian and swine origins of the individual gene segments of the 2009 H1N1 pandemic influenza subtype. Influenza gene segments have the same abbreviation as in Figure 2-1, dates are approximate estimates of the year in which a virus with the corresponding gene segment of the 2009 H1N1 virus was isolated for the first time in the indicated mammalian host. Source: (Garten, Davis et al. 2009)

Direct transmission of avian influenza viruses to humans does occasionally take place. In addition to the human cases of H5N1 which are currently accumulating globally, human cases of H9N2, H10N7, H7N2, H7N3 and H7N7 have all been documented in the past decade (Kalthoff, Globig et al. 2010) and there is evidence that the viruses involved in cases of H5N1 and various H7 subtypes may have undergone mutations allowing them to bond more effectively to the SAα2,6Gal receptors in the upper respiratory tract (Yamada, Suzuki et al. 2006; Belser, Blixt et al. 2008). However, with the possible exceptions of highly pathogenic H7N7 and H5N1 where the existence of clusters of cases have been the subject of much debate (Meijer, Wilbrink et al. 2004; Ungchusak, Auewarakul et al. 2005; Pitzer, Olsen et al. 2007; Uyeki and Bresee 2007; Yang, Halloran et al. 2007), there has been negligible evidence of human-to-human transmissibility, though it has been suggested that the 1918 pandemic itself was caused by a virus entirely of avian origin (Belshe 2005).

2.2 The spread of H5N1

In May 1997, a young boy in Hong Kong was confirmed to have died from infection with highly pathogenic avian influenza H5N1. This form of the virus was first detected in geese in Southern China in 1996 (Xu, Subbarao et al. 1999), following an outbreak within chickens in neighbouring farms (Claas, Osterhaus et al. 1998; Subbarao, Klimov et al. 1998). This was the first occasion that an avian influenza virus had been isolated from a human with respiratory infection (Shortridge, Gao et al. 2000), adding weight to the suggestion that animal influenza were a potential source of human pandemic strains (de Jong, Claas et al. 1997). This was followed by a further 17 cases between November and December of the same year, five of which were fatal. All of these cases had visited or been within the vicinity of live poultry markets, within which, it was later established, the prevalence of the virus in chickens had reached around 20%, with lower prevalence in geese and ducks (Shortridge 1999). This outbreak was eradicated by culling around 1.5 million poultry including the emptying of all live bird markets in Hong Kong and by imposing a ban on poultry importation (Shortridge, Gao et al. 2000). However, the virus continued to circulate and re-assort within geese and duck populations (Cauthen, Swayne et al. 2000; Guan, Peiris et al. 2002; Chen, Deng et al. 2004), until, in early 2003, further human cases were reported in Hong Kong (Peiris, Yu et al. 2004).

Different genotypes of H5N1 are characterised by observed mutations in any of eight viral gene segments. Currently the most prevalent of these are descendents of the "Z" genotype, which was also the genotype isolated from the human cases in Hong Kong in 2003 (Li, Guan et al. 2004). This strain spread rapidly throughout Southeast Asia (Martin, Sims et al. 2006; Webster, Peiris et al. 2006) (Figure 2-3), with China, Vietnam, Thailand, Indonesia and

Cambodia all reporting multiple human cases during the years 2003-2005 (WHO 2010). This became the predominant H5N1 genotype globally and remained so until 2005 when three divergent subgenotypes of virus were distinguished: subgenotype Z.1 which is responsible for causing outbreaks in many areas of the world including Africa and Europe, subgenotype Z.2 which has generally been isolated in Thailand and Vietnam and subgenotype Z.3 which has been mostly isolated in Indonesia (Neumann, Green et al. 2010).

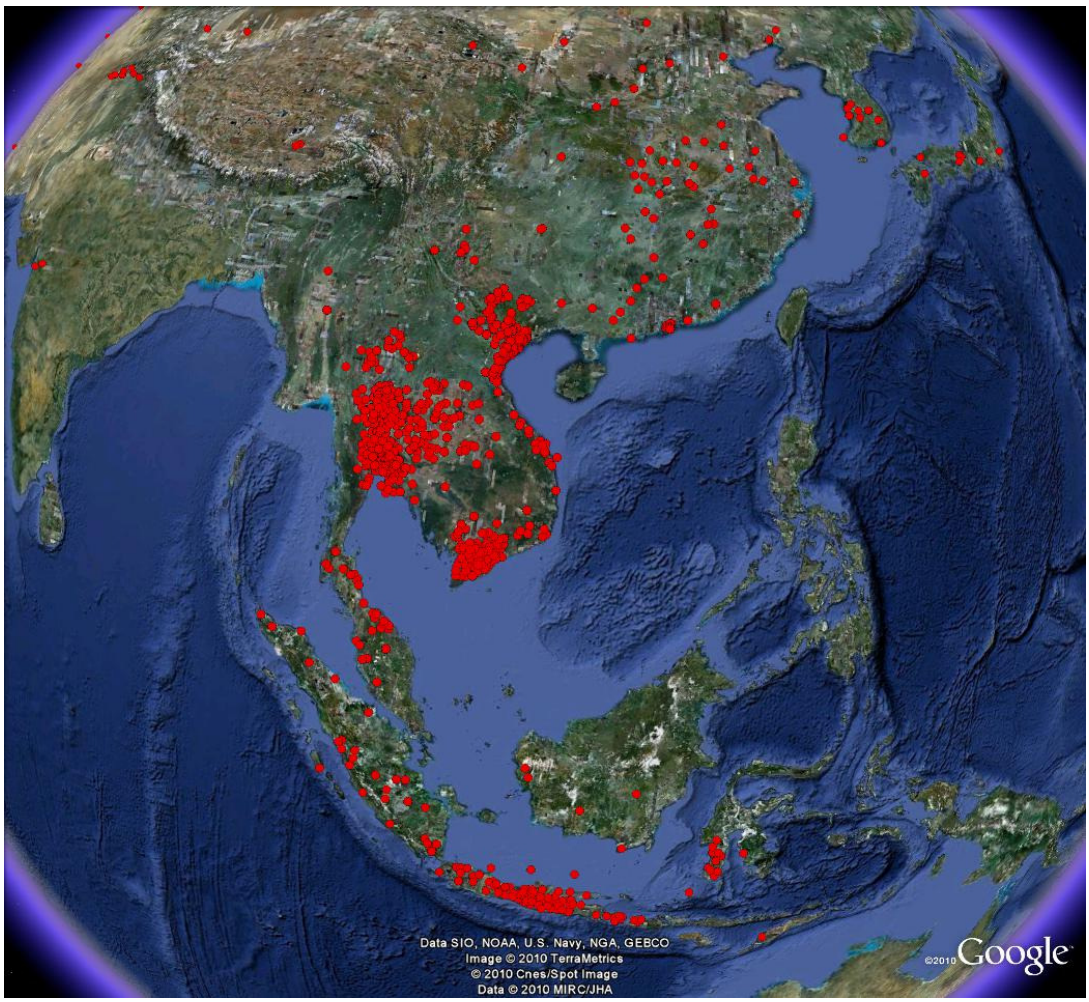


Figure 2-3| Outbreaks of H5N1 in poultry in Southeast Asia 2003-2006
Map created using a "mashup" (a term referring to the compilation of data from different sources into a new service) of avian influenza outbreak locations (Butler 2006).

To date 63 countries have experienced outbreaks of H5N1, with human cases reported in 16, and the disease remains enzootic in much of Southeast Asia (Eagles, Siregar et al. 2009)

and other countries such as Egypt (Kim, Kayali et al. 2010) and Bangladesh (Loth, Gilbert et al. 2010). Sporadic cases of infection in poultry and wild birds within other parts of Asia and Eastern Europe have also been reported. However, the virus, so far, has never transmitted in a sustained chain between humans (Ungchusak, Auewarakul et al. 2005; Yang, Halloran et al. 2007).

2.3 Avian influenza infection in humans

Human cases of avian influenza arising from low-pathogenic avian influenza (LPAI) subtypes have, to date, proved uniformly non-fatal (Kalthoff, Globig et al. 2010) . Cases of LPAI H9N2 and LPAI H10N7 have only been associated with mild respiratory symptoms and fever (Lin, Shaw et al. 2000; Butt, Smith et al. 2005), whereas the majority of cases of infection with LPAI H7 subtypes are characterised by conjunctivitis (Meijer, Wilbrink et al. 2004; Nguyen-Van-Tam, Nair et al. 2006). Indeed, with the exception of a single fatality caused by infection with HPAI H7N7 where a Dutch veterinarian developed pneumonia followed by acute respiratory distress syndrome (ARDS) (Fouchier, Schneeberger et al. 2004), HPAI H5N1 is the only recorded avian influenza subtype to cause severe disease within humans.

The majority of human cases of H5N1 have a history of handling sick or dead poultry (Abdel-Ghafar, Chotpitayasunondh et al. 2008) and matched case-control studies have consistently identified this as a risk factor associated with H5N1 infection. Specific practices associated with a high risk of infection include slaughtering, defeathering, handling fighting cocks, preparing sick poultry for cooking and consuming raw and undercooked poultry products (Areechokchai, Jiraphongsa et al. 2006; Dinh, Long et al. 2006; Van Kerkhove, Mumford et al. 2011). In some instances, environmental transmission, as a result of visiting live bird markets, has also been suggested (Mounts, Kwong et al. 1999; Yu, Feng et al. 2007).

Following exposure, cases generally become symptomatic after around 2-4 days (Yuen, Chan et al. 1998), although incubation periods of up to 8 days have been reported (Yuen, Chan et al. 1998; Hien, Liem et al. 2004; Chotpitayasunondh, Ungchusak et al. 2005). Early disease symptoms are non-specific and include fever, cough, shortness of breath and pneumonia, leading to a large level of initial misdiagnosis. Cases with pneumonia often progress to ARDS and multiple organ failure. When infection proves fatal, the median duration of time between the onset of disease and death is around 9 to 10 days (Abdel-Ghafar, Chotpitayasunondh et al. 2008; Peiris 2009).

It has been suggested that some human cases in South-East Asia are not reported, particularly within rural areas (Normile 2005; Sims 2007). This may be due to global surveillance for cases focusing primarily on hospitalised patients with respiratory symptoms who report being in contact with sick or dead poultry and thus missing those that do not access tertiary healthcare. As a result, the distribution of mild or asymptomatic infection is not well known (Uyeki 2008). However, sero-surveys of rural villagers in Cambodia (Vong, Coghlan et al. 2006; Vong, Ly et al. 2009), poultry workers in Nigeria (Ortiz, Katz et al. 2007) and healthcare workers in Vietnam (Liem and Lim 2005) and Thailand (Apisarnthanarak, Erb et al. 2005) suggest that mild or asymptomatic H5N1 infection is uncommon.

2.3 Pathogenicity and transmissibility of HPAI in birds

Clinical signs of HPAI infection in chickens include severe respiratory distress, cyanosis (blue or purple discolouration due to a lack of oxygen in the cells near the surface of the skin) of the wattles, combs and shanks, oedema of the head and eyelids, diarrhoea, eggs without shells, loss of the ability to stand and paralysis (Shortridge, Zhou et al. 1998; Martin, Forman et al. 2006). Following infection with H5N1 and a short incubation period, virus is shed for

an infectious period of around 1-3 days (negatively correlated with infectious dose received) with death generally occurring 2-3 days following the onset of infection (Shortridge, Zhou et al. 1998; Spickler, Trampel et al. 2008; Bouma, Claassen et al. 2009). Flock-level mortality often reaches 100% (Alexander 2000). However, as infection often occurs in the context of high background mortality, upper limits of the time it takes to recognize an outbreak within an epidemic setting are hard to define. Outbreaks previously have been known to take periods of up to 14-21 days to be detected (Nguyen 2005; ACI 2006).

The virus is also highly pathogenic to other species of domestic poultry such as turkeys and quails (Alexander 2000). However, the pathology of infection within ducks and other waterfowl is more complicated. HPAI viruses rarely cause severe disease in ducks, as the virus typically replicates in the intestine rather than in the upper respiratory tract (Webster, Yakhno et al. 1978), and prior to 2002, virus isolates of H5N1 failed to replicate effectively. However, a variant which emerged in Hong Kong in December 2002 showed increased pathogenicity to waterfowl with the virus causing systemic viraemia and neurologic disease (Ellis, Bousfield et al. 2004; Sturm-Ramirez, Ellis et al. 2004). Moreover, Clade 2.2 H5N1, a further new variant first detected during the mass die-offs of waterfowl at Qinghai Lake in China in 2005 (Chen, Li et al. 2006), has proven more lethal and infectious in a wide range of wild birds (Chen, Smith et al. 2005). Commercial ducks are also susceptible to severe disease (Vascellari, Granato et al. 2007). However, in experimental settings, a continuum of disease responses has been found, resulting in lower overall flock mortality than in other species of bird and the asymptomatic transmission of infection (Sturm-Ramirez, Hulse-Post et al. 2005). The pathogenicity of both pre and post-Qinghai Lake variants has also been shown to be age-dependent (Pantin-Jackwood, Suarez et al. 2007; Londt, Nunez et al. 2008; Saito,

Watanabe et al. 2009). A surveillance study in Thailand found a high level of asymptomatic infection within sampled ducks (Songserm, Jam-on et al. 2006), suggesting that the silent spread of infection may be contributing to the global circulation of the virus (Kim, Negovetich et al. 2009).

Although aerosol transmission cannot be ruled out, transmission experiments have shown that, when contact birds are caged directly adjacent to cages of infected birds, transmission between cages rarely occurs. In contrast, three out of four contact birds caged directly below infected chickens, which allowed faeces to fall into the lower cage, were infected and died, which may indicate that the majority of direct infection takes place in the form of faecal-oral transmission (Shortridge, Zhou et al. 1998). In addition, wild and migratory birds have been identified as carriers of infection but their overall role in transmission between flocks remains unclear (Webster, Peiris et al. 2006). Transmission can also take place indirectly with the virus surviving for long periods in manure, contaminated feed and water (Webster, Hulse-Post et al. 2007; Stallknecht and Brown 2009). As a result, transmission is likely to be possible over long distances via fomites (Webster, Peiris et al. 2006).

2.4 Poultry production in Southeast Asia prior to H5N1

Poultry rearing is part of everyday life for a large proportion of people throughout Southeast Asia. By far the most common form of poultry production, in terms of the number of flocks, is that of informal “backyard” poultry (Rushton, Viscarra et al. 2005). This is especially the case in rural areas where around 60-80% of all households keep poultry of this type (Dolberg, Guerne-Bleich et al. 2005). Generally, such flocks are defined as comprising of up to 100 birds which usually belong to a breed indigenous to the region (as opposed to commercial produced birds which are often bred from non-indigenous layer and broiler

parent stock). These poultry are generally bought cheaply and locally as chicks, are allowed to wander freely, are not monitored closely and are fed on household leftovers or other cheap and other easily available local inputs or left to scavenge during the day and only caged at night (ACI 2006). Poultry tend to either be chicken or ducks, with ducks constituting around 10-15% of backyard production in South-east Asia (Dolberg, Guerne-Bleich et al. 2005).

As a result, backyard poultry are susceptible to numerous diseases and experience an extremely high level of background mortality with reported annual rates of around 50-60% (Rushton, Viscarra et al. 2005). Poultry produced in this way are often consumed directly; however poorer households often sell birds or eggs in order to purchase cheaper food items such as rice and vegetables, clothing or education (Burgos, Hong Hanh et al. 2007).

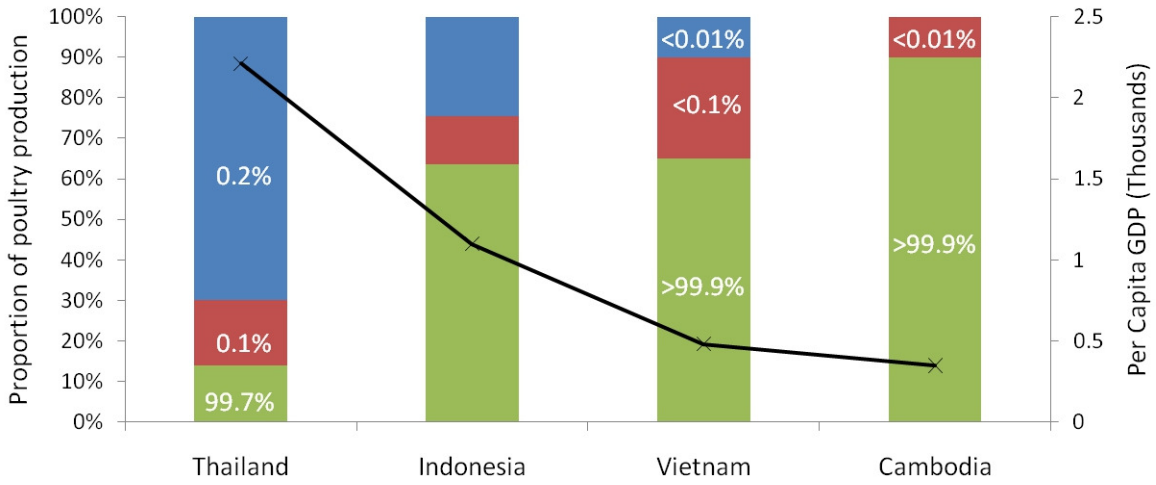


Figure 2-4|Composition of poultry production and GDP in selected countries in Southeast Asia.
Bar represent proportion of poultry production by production type with industrial and large-scale commercial farms (blue segment), mid-to-small scale commercial farms (red segment), backyard flocks (green segment). Percentage of overall number of producers by production type in white type. Black line shows the per-capita GDP of each country (data on number of backyard flocks in Indonesia not available).
Source: Poultry production data adapted from (Rushton, Viscarra et al. 2005), per capita GDP data from the World Bank.

Whilst this is uniformly the predominant form of poultry in terms of the number of producers, the extent to which backyard production contributes to overall national poultry

production varies from country to country and is correlated with various indicators of overall development such as GDP and urbanisation (Dolberg, Guerne-Bleich et al. 2005) (Figure 2-4). In less developed countries such as Laos and Cambodia backyard systems constitute around 80-90% of all production, in the absence of large industrial producers the only other type of production comes in the form of small scale commercial broiler (chicken raised specifically for meat production), layer (chicken raised specifically for egg production), duck and hatchery operations which, whilst only representing a tiny fraction of overall producers, are responsible for around 10% of the total poultry produced (Rushton, Viscarra et al. 2005; Otte, Hinrichs et al. 2008).

In Vietnam, the GDP of which roughly represents a median value for the region, backyard flocks represent around two-thirds of overall production. Nearly all poultry are purchased from local sources, with a Vietnamese government study finding that 90% of farmers surveyed obtained their poultry within the district and that all poultry were purchased within the province (GSO-Vietnamese General Statistics Office 2004). If birds are sold, this predominantly occurs locally with nearly all sales in the government study talking place within the district, half at the farm gate (GSO-Vietnamese General Statistics Office 2004). However, prior to the emergence of H5N1, there did exist supply chains of small-scale farmers, traders and market vendors which saw the movement of poultry from the farm-gate to wholesale markets in Hanoi or Ho Chi Minh City (ACI 2006). Birds are even traded across the border in Cambodia (Van Kerkhove, Vong et al. 2009). Vietnam also has the highest concentration of waterfowl in the region especially in low lying regions such as the Mekong delta (Dolberg, Guerne-Bleich et al. 2005) where distinct cyclic production sub-

systems exist with ducks allowed to scavenge on paddies following rice harvests in order to remove lost grain (Men, Ogle et al. 2002).

The remainder of poultry production takes place in a commercial setting. A handful of large-scale, relatively bio-secure, producers and state breeding farms exist, which tend to sell directly to large retailers or hotels and restaurants. However, consumers report a preference for local breeds (Ifft, Otte et al. 2007) and the majority of commercial producers operate on a small-scale of up to 2000 birds where day old chicks (DOCs) are purchased from hatcheries and then reared in a relatively low bio-secure setting (either roaming free or with some rudimentary shelter). The majority of these are then marketed, either taken to live markets or sold to buyers at the farm gate. Buyers may also be live poultry traders who collect poultry from various farms, often mixing birds from multiple different communes and transporting them to and from live bird markets (Agrifood Consulting International 2006; Magalhaes, Ortiz-Pelaez et al. 2010).

Both Indonesia and Thailand, with respective GDPs approximately twice and four times that of Vietnam, have more industrialised production systems. Despite incurring heavy losses during a financial crisis in 1997, commercial production in Indonesia had recovered and was growing prior to the advent of H5N1 (Katial-Zemany and Rangkuti 2003), partly helped by stringent import restrictions sustaining domestic poultry prices at a level 55 percent higher than the world price (Fabiosa, Jensen et al. 2004). Indonesia also has a small industrial production sector, primarily involving “contract farming”, where a larger company sends DOC to a series of smaller farms (Katial-Zemany and Rangkuti 2003). These farms then raise the birds for a set price and then return them to the producer. Approximately ten percent of poultry in Indonesia are raised this way (Rushton, Viscarra et al. 2005).

In contrast, around 70% of poultry in Thailand is produced industrially. Whilst this sector was in development, contract farming proved a mutually beneficial form of production, giving large poultry farms the flexibility to adjust production volumes to suit demand. The fixed price and size of the contract, a legally binding agreement, also protected the smaller producer from price and supply fluctuations. However, in order to meet stringent international import requirements for food safety and animal welfare and due to technological advances such as evaporative-cooling systems which save on production costs, large poultry integrators were, even prior to the introduction of H5N1, progressively heading towards vertical integration, with all aspects of production increasingly brought in-house (NaRanong 2007; Burgos, Otte et al. 2008).

2.5 Controlling the spread of H5N1 in Poultry

The effectiveness of attempts to control the spread of avian influenza depends on two factors: the extent to which outbreaks are prevented and the extent to which outbreaks, when they are occur, are prevented from spreading infection onwards. As the early outbreaks of H5N1 in Hong Kong and the rapid global proliferation of the "Z" type strain in 2003 both constituted relatively unexpected events, control strategies were necessarily forced to focus on the latter. These involved reactive measures such as mass culling and movement restrictions aimed towards limiting spread once infection had become established within a locality coupled with the development of surveillance capacity. However, over time, the wave-like nature of the spread of infection during the first few years of the epizootic, has allowing a re-evaluation of approaches to controlling infection. The development of effective vaccines and the realisation that, in many settings, H5N1 is unlikely to be eradicated in the short term, has seen national control policies evolve towards

more proactive approaches such as the adoption of mass vaccination and the drive towards improving bio-security.

2.5.1 "Stamping out" H5N1

The "stamping out" of infection is recognised by the FAO as a proven strategy for the rapid elimination of an emergency livestock disease. Implementing this strategy involves the designation of infected zones, intense disease surveillance within these zones, the immediate slaughter of animals infected, those identified as dangerous contacts or, if deemed necessary, all animals regardless of infection status, followed by the disinfection of the area, quarantining of remaining animals and biosecure disposal of those slaughtered. Prior to the spread of H5N1, this was a recognised strategy for rapid elimination of a livestock disease newly introduced into an area (Geering, Penrith et al. 2001). The overall aim of this strategy is to eliminate infection from a compartment, region or country and to reduce the number of infected or susceptible animals within a population (Sims 2007).

In countries where the introduction of H5N1 was detected before infection became widespread such as in Japan, South Korea and Malaysia, where the predominantly intensive poultry production systems make it relatively straightforward to implement the strategy promptly and properly, it was possible to eradicate the spread of infection before the disease became widespread (Peiris, de Jong et al. 2007; Sims 2007). Before the return of infection in 2002, the H5N1 strain which surfaced in Hong Kong in 1997 was also successfully controlled by extensive stamping out measures (Sims, Ellis et al. 2003). However, stamping out as a strategy to control the spread of infection where the disease has already become established, and in countries where extensive poultry production provides large reservoirs of infection, has proven less effective. Despite mass culling campaigns, countries such as

Vietnam, Thailand and China experienced huge waves of outbreaks and infection often returned within a short period of time following the end of an epidemic (Sims 2007). The costs of sustaining such a strategy, which have involved culling millions of poultry, is also restrictively high (Capua and Marangon 2006). In addition to the resources needed to carry out culling on such a widespread scale, poultry owners need to be compensated for their losses in order to ensure infection is readily reported and that poultry are not moved illegally, which could otherwise even lead to culling becoming counterproductive (Johansen and Penrith 2009). In 2005, experts at a joint meeting of the FAO and OIE concluded that, due to economic, ethical and ecological considerations, the mass culling of poultry was no longer an acceptable strategy to contain the spread of avian influenza in developing countries and recommended that vaccination be considered as an alternative (Butler 2005).

2.5.2 The development and use of H5N1 vaccination

An ideal vaccine should be safe, induce sterilising immunity whereby infection is completely prevented during subsequent challenge by a dose of a wild-type of the pathogen, provide long-lasting immunity, be administered via a single dose and be affordable and stable at room temperature for the setting in which it is required (Ada 1994). No H5N1 vaccine currently licensed for use in the field satisfies all these criteria (Peyre, Fusheng et al. 2009).

H5N1 poultry vaccines fall into two categories: Inactivated whole virus vaccines and subunit recombinant vaccines (Suarez and Schultz-Cherry 2000; Peyre, Fusheng et al. 2009). Inactivated vaccines are produced by growing a whole avian influenza virus subtype within an embryonic chicken egg which is then chemically inactivated and oil-emulsified. The specific subtype can either be homologous (i.e. inactivated H5N1 vaccine), which means the vaccine has the advantage that the grown strain matches the field strain but does not allow

for detection of infection within a flock, or heterologous where infection can be diagnosed through the detection of antibodies against the field strain-specific NA (van den Berg, Lambrecht et al. 2008). However, in the case of the current strain of H5N1, the virus is too pathogenic to grow to sufficient titres within the embryo, necessitating the use of reverse genetics, whereby plasmids containing the HA and NA genes of the H5N1 strain and plasmids of the six remaining genes from a low-pathogenic high growth strain are inserted into animal cells. These are then used to generate a reassortant virus with the requisite HA and NA genes which can grow to sufficiently high titres (Chen 2009). Both inactivated and recombinant H5N1 vaccines have been used to vaccinate poultry in China, Hong Kong, North and South Korea, Vietnam, Indonesia and Egypt (Peyre, Fusheng et al. 2009; Kim, Kayali et al. 2010). Other recombinant vaccines which have been licensed for use include recombinant fowlpox vaccines, where a gene coding for the HA of an H5 strain is inserted into the fowlpox virus, and a Newcastle disease (ND) vaccine which can be used to immunise against AI and ND simultaneously (Swayne, Suarez et al. 2003; Veits, Wiesner et al. 2006).

All H5N1 vaccines have been tested within a laboratory setting to ensure that they meet a minimum efficacy standard of the level of protection against AI disease in chickens (haemagglutination inhibition (HI) titres > 4 $\log_2$) and significantly reduce viral shedding and transmissibility following challenge with infection (Tian, Zhang et al. 2005; Swayne, Lee et al. 2006; Bublot, Pritchard et al. 2007; Ge, Deng et al. 2007; Bouma, Claassen et al. 2009; Peyre, Fusheng et al. 2009). However, with the exception of the fowlpox-derived vaccine which only requires a single dose but has the disadvantages of not being effective in poultry with a history of fowlpox infection as well as being less effective in turkeys and ducks (Swayne,

Beck et al. 2000; Steensels, Van Borm et al. 2007), two doses of vaccine are often required to achieve adequate protection, especially in longer-living poultry and ducks (Swayne 2006; van der Goot, van Boven et al. 2007). Moreover, in order to ensure the vaccine remains potent, a cold chain of vaccine delivery is required. As a result, translating efficacy within a laboratory setting to effectiveness in a field setting is not straightforward (Swayne and Kapczynski 2008; Peyre, Fusheng et al. 2009). There is also evidence that current vaccines may provide less effective protection from recently evolved strains of H5N1 isolated in Southern China compared to the protection provided from older strains (Tian, Zeng et al. 2010).

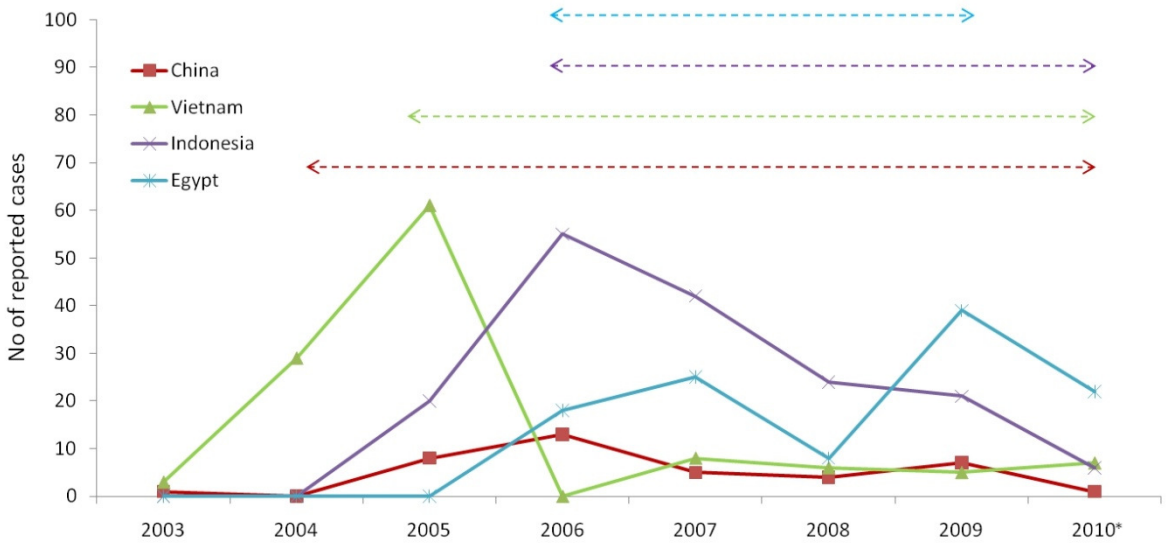


Figure 2-5| Annual number of reported human cases in countries using mass vaccination.
Solid lines and markers show number of cases reported to the WHO, dashed arrows show years during which mass vaccination was carried out.
*Number of cases as of 31 August 2010.
Source (WHO 2010).

Despite this, field trials of vaccination in Indonesia, Vietnam, Hong Kong and China have shown promise in controlling H5N1 outbreaks (Ellis, Leung et al. 2004; To, Bui et al. 2007; Bouma, Muljono et al. 2008). Following this, mass vaccination campaigns were initiated in Vietnam, China, Indonesia and Egypt and emergency vaccination has been incorporated into

outbreak response protocols in Hong Kong (Ellis, Leung et al. 2004; Peyre, Fusheng et al. 2009). However, whilst the number of human cases in China and Vietnam has subsequently declined, the disease has remained entrenched in all of these countries (Figure 2-5). The experiences of Vietnam and Indonesia will be discussed further later, whilst in China, despite the use of over 40 billion doses of vaccine, rates of protection in waterfowl and backyard chicken remain relatively low (Chen 2009). The expense of maintaining nationwide campaigns, coupled with the recognition that vaccination is not likely to eradicate infection in the short-to-medium term is increasingly leading to the re-evaluation of such strategies. In Egypt the widespread presence of maternal antibodies was also identified as a contributing factor to the failure of a vaccination campaign which had no positive impact on HPAI incidence even after the administration of three doses of vaccine (Peyre, Samaha et al. 2009; Kim, Kayali et al. 2010). As a result, in July 2009 the campaign was suspended indefinitely (FAO 2010).

Indeed, it has been suggested that without concerted efforts to improve surveillance capacity, especially as incomplete coverage or improperly administered vaccination may make circulating disease more difficult to detect (Savill, St Rose et al. 2006), vaccination will never successfully eradicate infection in countries where the extensive production of poultry is widespread (Marangon, Cecchinato et al. 2008; Peyre, Fusheng et al. 2009).

2.5.3 Improving surveillance capacity

The high levels of mortality associated with the presence of HPAI H5N1 within a flock has led to the proposal of various mortality thresholds as a fast and simple measure of the presence of infection, especially within a commercial flock (Elbers, Fabri et al. 2004; Savill, St Rose et al. 2008). However, the high levels of background mortality and small flock sizes in the

context of backyard poultry production and the potential for disease to circulate silently in waterfowl, live bird markets and vaccinated poultry, necessitate detection methods which, though requiring more time and resources, are more sensitive and specific than simple mortality thresholds (Sims 2007).

Traditional methods of assaying for the presence of haemagglutinating agents in inoculated chicken embryos require animal facilities and supplies of reference antisera and can take up to 2 weeks to complete. However, the development of real-time Polymerase Chain Reaction (RT-PCR) methods where H5 specific nucleic acids can be identified directly, allowing detection of H5 influenza in as little as 24 hours (Pasick 2008). Subsequently, RT-PCR assays have been designed to detect HPAI H5N1 specifically (Hoffmann, Harder et al. 2007). Moreover, the recent development of rapid antigen detection kits, whilst both less specific and sensitive, now allows the detection of the presence of Influenza A virus within a flock in less than an hour without the requirement of laboratory equipment and, whilst the result should always be confirmed by other methods, provide an effective early indicative measure of the presence of H5N1 in the field (Marche and van den Berg 2010).

In countries such as Vietnam and Thailand, improvements in veterinary infrastructure and allocation of resources to compensation schemes has seen an increase in surveillance capacity (Sims 2007). Moreover, countries such as Thailand and China, have invested significant resources in active surveillance, whereby apparently healthy flocks are sampled for signs of infection (Tiensin, Nielen et al. 2007; Chen 2009). However, in many countries including Vietnam and Indonesia, veterinary services remain highly decentralised. As a result key policy directives can become diluted, the flow of information between local authorities is often poor and surveillance resources and effort can vary within countries and even

provinces (Sims 2007). Movement restrictions and culling of infected birds constitute financial disincentives for farmers to report infection within their flock. Thus, in order to ensure timely reporting of outbreaks, they should be compensated for these losses. However, compensation schemes, where they exist, usually only pay a fraction of the market value and accessing compensation is often highly time consuming and bureaucratic (Kanamori and Jimba 2007; Nguyen 2007). The occurrence of human cases in areas where no outbreaks of infection in poultry have been reported, even in countries such as China, Vietnam, Thailand and Indonesia where compensation schemes have already been implemented, highlight the extent of this problem (Sims 2007). There have also been instances where even suspected human cases have not been reported (Normile 2005).

2.5.4 Enhancing biosecurity

Biosecurity refers to the implementation of measures designed to reduce the risk of the introduction of infection within a flock and to prevent the spread of infection in the situations where an outbreak does occur (FAO, World Bank et al. 2007). These consist of three components segregation, cleaning and disinfection. The first involves the creation of barriers in order to prevent any opportunities for infected poultry or materials to come into contact with the flock, whilst the second and third should be applied to all materials which enter or leave a site in order to ensure that any virus is removed (Domenech, Honhold et al. 2008).

Whilst this assumption has recently been questioned (Otte, Pfeiffer et al. 2006), it is generally thought that industrial production is relatively bio-secure as it is strongly in the interests of producers to keep their flocks free from infection using measures such as closed housing and disinfectants. However, for small-scale backyard farmers, the scavenging of

birds for feed plays an important role in making the operation viable. As a result, in countries where such extensive farming is widespread, biosecurity remains poor (Rushton, Viscarra et al. 2005; Capua and Marangon 2006).

Calls to eliminate scavenging poultry in such countries have so far been resisted due to the serious socio-economic impact this would have. However control policies aimed towards reducing the consequences of such low biosecurity often involve preventing the movement of poultry from such small-holdings, either through the selective application of movement restrictions or closure of markets to small-holders. Whilst such measures may result in a net improvement in biosecurity, they also serve to make the environment in which small-holders rear poultry even tougher (Otte, Pfeiffer et al. 2006; Sims 2007; Safman 2010).

2.6 The Impact of HPAI H5N1 in individual countries

2.6.1 H5N1 in Vietnam and the switch to mass vaccination

Avian influenza was first recognized in Vietnam on December 23, 2003, when large poultry die-offs were reported in the provinces of Tien Giang and Long An and on January 8, 2004 Vietnam became the first country to officially declare the epidemic. Within 6 weeks, 57 of the 64 Vietnamese provinces had been affected and during the 2 months it took to bring the outbreaks under control, 44 million birds had been killed, either as a result of infection or culling. During this wave of outbreaks fifteen people died as a consequence of human infection (Nguyen 2005), with outbreaks centring around two distinct foci, the Mekong delta in the South of Vietnam and the Red River delta in the North (Pfeiffer, Minh et al. 2007).

This first wave of H5N1 was the largest that Vietnam has experienced, causing an estimated direct National loss of US$ 200 million and creating a significant reduction in the overall size

of the poultry industry due to a decreased demand for poultry (during the height of the epidemic poultry prices fell by around 50 to 60 percent) (ACI 2006).

The economic effects of the outbreaks were felt most sharply by the smallholders of poultry. A case study of a village in North Vietnam found that smallholders affected by outbreaks suffered losses of between 69 USD and 108 USD (Dolberg, Guerne-Bleich et al. 2005). As around two-thirds of households in Vietnam earn less than US$2 per capita per day, such losses often have serious economic implications for those involved (GSO-Vietnamese General Statistics Office 2004; Rushton, Viscarra et al. 2005; ACI 2006).

Subsequently there have been four more major waves of outbreaks, all on a smaller scale but, at a micro level, having a similar impact upon those affected. The second wave began a year after the first in December 2004, a time of year during which the Vietnamese festival of Têt is celebrated, representing a yearly peak in the consumption of poultry. This wave once again centred around the same distinct foci in the North and South and resulted in the destruction of around two million poultry. The third wave differed in its seasonality, with the first outbreaks occurring in October 2005 and the final outbreaks occurring in December, a month before the beginning of Têt. This wave centred around the North Red River only, affecting 21 provinces and causing the destruction of over 4.2 million poultry (Pfeiffer, Minh et al. 2007; Tuan 2007).

Following this third wave of outbreaks, and an extensive vaccination campaign, Vietnam remained disease free for over a year until, in December 2006, outbreaks occurred, primarily within duck farms in the southern Mekong provinces and in May 2007 a fifth wave occurred primarily in the North. Since the final wave in 2007 sporadic outbreaks have continued to occur throughout the remainder of 2007 and into 2008 (FAO 2009)

Since the beginning of 2008, sporadic outbreaks have been responsible for 18 human cases, resulting in 12 fatalities, bringing the total number of confirmed human infections to 119, 59 of which have been fatal (WHO 2010).

2.6.1.1 Control policies implemented prior to vaccination

Before the first outbreaks in 2003, Vietnam had no systematic surveillance or program to monitor the circulation of HPAI and no formal plan as to how to respond to outbreaks (Nguyen 2005). A steering committee was established in January 2004 which recommended movement restrictions, the establishment of checkpoints, disinfection of premises, export and import quarantine, heightened biosecurity measures in intensive poultry farms and the culling of all poultry within a 3km radius (The National Steering Committee for Avian Flu Prevention and Control 2005). However, these measures could not be applied until a state of emergency was declared within a province. As a result the timeliness of the application of control measures varied between provinces (Nguyen 2005).

The lack of compensation for culled birds at the beginning of the outbreak is also likely to have increased the likelihood that outbreaks went unreported. Later in the outbreak the government announced the initiation of such a compensation policy. However, the level of compensation (around 10 percent of market value) and the inconsistent application of provinces is likely to have limited its success in improving reporting and to have encouraged the movement of poultry between infected and uninfected, or more effectively compensated, areas (Hulse-Post, Sturm-Ramirez et al. 2005; ACI 2006).

In response to the size of the first wave of outbreaks, and the level of destruction necessary to maintain a 3km stamping-out policy, outbreaks between late 2004 and 2005 were controlled by more focussed culling. The scope of such culls varied between provinces but

generally involved the destruction of all poultry within a 1km radius (ACI 2006). Guidelines distributed to para-veterinary professionals advised that all infected flocks should be disposed of within 24 hours of an outbreak being reported (FAO 2005).

In June 2005, between the second and third wave of outbreaks, the level of compensation for culled flocks was trebled to represent half of market value and compensation for flocks volunteered for culling was introduced (ACI 2006; Tuan 2007). However, for many farmers, gaining approval for compensation was a complicated procedure and disbursement of payment generally took a period of 2-6 months(Tuan 2007). The time taken to restock depleted flocks completely has been estimated to be 12 months within a backyard setting and 5-8 months for commercial farms (Dolberg, Guerne-Bleich et al. 2005).

2.6.1.2 The vaccination campaign

A field trial of two vaccines, an inactivated H5N2 vaccine previously used to control outbreaks in Hong Kong and an inactivated H5N1 vaccine from China, was carried out in 2005 (To, Bui et al. 2007; Nguyen 2008). The latter vaccine was selected as it was found to give effective protection to both chickens and domestic ducks, although not to Muscovy ducks which constitute about 20% of the duck population (Men, Ogle et al. 2002). In August 2005 a pilot vaccination campaign was carried out in two trial provinces, Tien Giang in the South Mekong Delta Region and Nam Dinh in the North Red River region. In November and December 2005, occurring contemporaneously with the third wave of outbreaks in the North, the first round of an extensive vaccination campaign was carried out throughout the entire country, with the exception of a few remote regions. The aim of this campaign was to deliver two shots of vaccine, four weeks apart, to all chickens over 8 days old and all ducks over 15 days old, although the vaccination of ducks and chickens due for slaughter in less

than 70 days was only undertaken on a voluntary basis. Post-vaccine surveillance was carried out by randomly selecting one backyard chicken flock, one commercial chicken flock and one commercial duck flock in three randomly selected districts in each province. From this it has been estimated this campaign achieved a coverage of around 80 percent of chickens and 65 percent of ducks in Vietnam. Blood samples of thirty birds from each flock (if the backyard flock was too small these were collected from multiple flocks) were also collected and it was estimated that effective protection, defined as having a HI titre >1/16, had been achieved in approximately 55 percent of each species(Taylor and Dung 2007). Subsequent rounds of mass-vaccination were then carried out during March and September of 2006 and 2007 (To, Bui et al. 2007; Tuan 2007; Nguyen 2008).

Following the implementation of the vaccination campaign there was also a change in policy regarding the response to outbreaks of avian influenza, moving from ring culling to a combination of the culling of infected flocks and ring vaccination around any infected premises (Ministry of Agriculture and Rural Development 2006). Policies aiming to improve bio-security by restructuring the poultry industry, including the upgrading of farming practices to more industrialised production and restrictions on free-grazing ducks, were also initiated (Ministry of Agriculture and Rural Development 2006; Men 2007).

Vaccination proved successful at preventing outbreaks in the short term, with no outbreaks reported for a year following the first round of the campaign, and has substantially reduced the number of reported outbreaks and human cases relative to the scale of infection during the first years of the epidemic. However, with the disease still remaining widespread and firmly entrenched within poultry populations, it is increasingly being recognised that the high costs and resources associated with maintaining twice annual nationwide vaccination,

as well as the requisite support from smallholders, are not sustainable in the long term. As a result, alternative vaccination strategies are now being trialled in Vietnam as part of the Gathering Evidence for a Transitional Strategy (GETS) for HPAI H5N1 Vaccination in Vietnam (USAID 2010). This involves targeted vaccination of identified high risk populations such as mobile duck flocks in five trial provinces, judged to be at varying levels of disease risk. These will then be monitored closely, including active sampling to assess whether infection is circulating silently, in order to assess the extent to which the protection this affords each province is cost-effective.

2.6.2 The Control of Avian Influenza in Thailand

The first confirmed outbreak of H5N1 in domestic poultry in Thailand occurred in late January 2004, although subsequent analyses have concluded that the disease had been circulating from late 2003 (Tiensin, Chaitaweesub et al. 2005). This led to a large wave of outbreaks spreading throughout the country, emanating from an epicentre situated in Central Thailand where the majority of poultry is produced (Tiensin, Nielen et al. 2007). Both in terms of timing and size this wave was comparable to those occurring in other Southeast Asian countries such as China and Vietnam.

The first wave of outbreaks, lasting until early 2004, was followed by a second large wave beginning in mid-2004 which, in terms of the number of outbreaks reported and the duration was much larger than any other wave in either Thailand or Vietnam (Gilbert, Xiao et al. 2008). The consequences of outbreaks in poultry for both smallholders and large-scale producers were severe. Thailand's main export markets, the EU and Japan, banned the importation of Thai poultry products and domestic demand for poultry fell dramatically, causing the prices of poultry to drop (Safman 2010). This, coupled with the costs arising

from a “stamping-out” policy which involved the culling of all birds within a 5km radius of the source of infection and the destruction of all contaminated housing, contributed to losses in the private poultry sector which have been estimated at 100 billion Baht (≈ US$3 billion) during 2004 alone (Burgos, Otte et al. 2008).

Despite pressure from smallholders (Safman 2010), vaccination of poultry has remained illegal in Thailand throughout all outbreaks, a decision which was supported by large export poultry producers, due to Japan and the EU’s refusal to accept poultry products from vaccinated birds. Instead a policy of upgrading biosecurity was implemented. Industrial producers who sent birds to be reared in contracted farms made it a requirement that these farms upgrade their poultry housing to closed houses and many switched to more vertically integrated production, a pre-existing trend which was intensified by the onset of H5N1. The Thai government also announced that chickens should only be raised in closed farms (NaRanong 2007; Safman 2010).

Another noteworthy characteristic of Thailand’s response to H5N1 was that of intense active ‘X-ray’ surveillance (Otte, Pfeiffer et al. 2006; NaRanong 2007; Tiensin, Nielen et al. 2007; Safman 2010). These were nationwide campaigns, the first beginning in October 2004 and repeated three times in 2005, of 3-4 weeks duration, where thousands of inspectors where recruited to search door-to-door with the aim of assessing the infection status of every flock in the country and thus obtaining a clearer picture of the scale of outbreaks occurring and reducing the opportunity for infection to spread.

Following the implementation of these measures, a third wave of outbreaks in 2005 was much smaller in scale and, since then, outbreaks have been restricted to isolated cases, with the last human case reported in 2006 (WHO 2010) and the last reported outbreak in poultry

in late 2008 (Pickelsimer and Preechajarn 2008). However, faced with a much tougher environment within which they had to operate as a result of the pressure to upgrade, many smallholders were forced to leave the industry altogether (NaRanong 2007).

2.6.3 Endemic spread in Indonesia

In Indonesia, the disease was first detected in poultry in Java in August 2003 and by January 2004 had spread to Bali, Kalimantan and southern Sumatra. By mid-2008 all but two of 33 provinces in Indonesia had been affected (Forster 2010), with the disease considered enzootic in Java, Sumatra, Bali and Sulawesi (FAO 2010). The control strategy implemented during the early stages of the epidemic focussed upon culling and compensation. Initially this only applied to infected birds but was subsequently extended to include all poultry within a 3km radius and testing within 20km. By the end of January 2004, when the first wave of outbreaks reached its conclusion, 17 million birds had been killed, either as a result of infection or culling, and this was followed by a sharp drop in the price of chicken due to a lack of demand due to worries about food safety (Rushton, Viscarra et al. 2005).

Large commercial enterprises began vaccinating their poultry privately from an early stage in the epidemic using a range of imported and locally produced vaccines (Domenech, Dauphin et al. 2009). A mass vaccination campaign was instigated by the government in June 2004, with the aim of providing 300 million doses of vaccine free of charge to smallholders and backyard flocks nationwide (Forster 2010). However, only 16 million doses of vaccine were distributed between 2004 and 2005 (Santhia, Ramy et al. 2009). Due to the limited vaccine supply, the decision was taken in mid 2006 to instead focus mass vaccination in 12 high-risk provinces with the use of reactive vaccination in other areas (Forster 2010). However, with problems such as the de-centralised, locally autonomous Indonesian

veterinary infrastructure; wide geographical area and extensive farming system; large number of species and resource and budget limitations (Siregar, Darminto et al. 2007; Peyre, Fusheng et al. 2009) this failed to bring the epidemic under control. In 2009 Indonesia reported 21 human cases, 19 of which were fatal (WHO 2010), and more than one thousand outbreaks in poultry, higher than any other country (FAO 2010).

One factor behind the higher number of reported outbreaks in Indonesia is the implementation of the Participatory Disease Surveillance and Response (PDSR) approach to disease detection (Buranathai, Amonsin et al. 2007). This involves supporting a community-based response to outbreaks where, by educating and enlisting the help of poultry farmers, traders and community leaders through awareness campaigns, local knowledge as to where and when outbreaks are occurring is adapted and incorporated into the existing outbreak surveillance and prevention framework with rapid tests used to confirm the presence of avian influenza whilst in the field. As of mid-2008, the programme had recruited 2112 officers, operating in 221 of Indonesia's 448 districts. In the first two and a half years of its existence PDSR teams had made nearly 200,000 visits, leading to over 6000 detected outbreaks (Forster 2010).

2.7 Modelling the spread of HPAI

Mathematical models have long been used in infectious disease epidemiology, providing a rigorous framework in which to describe the dynamics of an epidemic and the factors influencing the transmission of disease in a parsimonious manner (Heesterbeek 2002). In 1917 Ronald Ross, the physician responsible for identifying the mosquito as the vector by which malaria is transmitted, and Hilda Hudson described a "critical density of mosquitoes" below which a malaria epidemic could not be sustained (Ross and Hudson 1917). This

concept of a critical population threshold, given a mathematical basis by Kermack and McKendrick in 1927 (Kermack and Mckendrick 1927), was then further developed by George Macdonald in 1952 to provide the first explicit expression for the basic reproductive number (Macdonald 1952), subsequently defined as the average number of secondary infections arising from a typical infective in a previously unexposed population and given the symbol R_0 (Dietz 1993). However the potential for such a threshold, whereby a major outbreak can only occur within a population if $R_0 > 1$, to be estimated directly from epidemiological data and used to assess the effectiveness of control measures was not fully realised until the mid-1980s when brought to prominence, in particular, by the work of Anderson and May (Anderson and May 1982). Since then it has remained arguably the most important quantity in the study of the spread of infectious disease, guiding our understanding of the transmission dynamics of infection (Anderson and May 1991), the effects of herd immunity (Fine 1993) and the role of heterogeneity in driving an epidemic (Yorke, Hethcote et al. 1978; Woolhouse, Dye et al. 1997). Alongside other key measures of spread such as the effective reproductive number (the number of secondary infections arising from a typical infective but taking into account events occurring as a reaction to the spread of a pathogen within the host population such as the depletion of susceptible individuals during an epidemic or the implementation of control measures), the time in which individuals spend in given disease states (e.g susceptible, infectious, immune), the distribution of time between generations of cases, incidence and overall number of cases, calculating the basic reproductive number allows the assessment of the overall effectiveness of control strategies such as vaccination campaigns (Nokes and Anderson 1988), screening programmes (Garnett, Kim et al. 2006) and the implementation of many other treatment strategies for a wide variety of diseases (Woolhouse 1992; Velasco-Hernandez, Gershengorn et al. 2002; Grassly,

Fraser et al. 2006; Hallett, Singh et al. 2008; Ross, Penny et al. 2008), which, due to such non-linear dynamics of infection, would not otherwise be possible.

Moreover, in recent times, spurred on by high-profile event such as major disease outbreaks including Creutzfeldt-Jakob disease (CJD) and severe acute respiratory syndrome (SARS), the spread of resistant bacteria and the 2009 H1N1 influenza pandemic, modelling has increasing become an accepted part of public health decision making (Temime, Hejblum et al. 2008). In each of these examples, as well as numerous others, factors such as the high degree of stochasticity and incomplete observation during the initial stages of infection, the small-population dynamics and environmental contamination associated with nosocomial infection and the evolution of drug-resistance, the apparent high variability in the transmissibility of cases characterising the spread of SARS and the unknown distribution of the incubation period of CJD have all served to highlight the utility of mathematical models in calculating the otherwise unquantifiable uncertainty associated with outcomes of such threats (Austin and Anderson 1999; Ghani, Ferguson et al. 2000; Lipsitch, Cohen et al. 2003; Li, Yu et al. 2004; Fraser, Donnelly et al. 2009).

Whilst mathematical models of the dynamics of disease in animals do not have such a historical pedigree, the notion that they can be also be used to inform disease control policy has become increasingly subscribed to. Models of the spread of diseases such as bovine spongiform encephalopathy, scrapie and classical swine fever have all been developed in order to address pertinent issues regarding ongoing epidemics such as optimal culling strategies, farming restrictions and the impact upon human health (Anderson, Donnelly et al. 1996; Ferguson, Ghani et al. 1998; Stringer, Hunter et al. 1998; Woolhouse, Stringer et al. 1998; Boender, Nodelijk et al. 2008). The 2001 Foot and Mouth Disease (FMD) epidemic in

Great Britain further served to demonstrate that, following advances in computing power and given access to the best available data (Levin, Grenfell et al. 1997), mathematical models can play an active, even decisive, role in providing policy makers with vital information as to the optimal disease control strategy during a rapidly progressing epidemic.

2.7.1 An example of the potential for mathematical models to aid policy making: The 2001 FMD epidemic in Great Britain

Disease tracing suggests that the first outbreak of Foot and Mouth in the UK for over thirty years occurred in Northumberland in February 2001. However, by the time infection was detected, the disease had spread as far as South-west Scotland and Devon, as a result of long-range sheep movements (Gibbens, Sharpe et al. 2001). Thirty-five days into the epidemic, when it became clear the epidemic was out of control and threatening to overwhelm resources, a scientific advisory group was set-up, including mathematical modellers in order to provide policy makers with guidance as to the best control policies to implement (Anderson 2001). Modellers of the 2001 FMD epidemic in the United Kingdom were given full access to databases containing: the location and species-specific livestock numbers of a great majority of all farm holdings in the UK, including all reported infected premises (IP); the date of reporting; the date of slaughter; results of laboratory diagnostic testing; estimates of the date of infection from analysis of lesions and estimates of the date and source of infection for some IPs from contact tracing (Woolhouse 2003). Following the implementation of movement restrictions on the 23rd November, more than 80% of all newly infected IPs arose within 3km of one potential source of infection (Gibbens, Sharpe et al. 2001; Kao 2002).

Before 2001, spatial models of the spread of infectious disease in livestock remained relatively rare with the key exception to this being the "InterSpread" model, a complex stochastic simulation model which requires the specification of a wide array of different parameters or parameter distributions describing the contact structure of farms (including animal and transport contacts) and the implementation of various control measures (Sanson, Struthers et al. 1993; Jalvingh, Nielen et al. 1999; Nielen, Jalvingh et al. 1999). This model was the basis of the analysis of the outbreak conducted by *Morris at al*. (Morris, Wilesmith et al. 2001) The spread of infection in this model was described using a total of 54 parameters. Whilst this is more likely to represent the level of complex heterogeneity which determines a wave of outbreaks in real-life, accurately estimating such values and distributions from data is difficult and unlikely to be possible to validate, leading to problems associated with misspecification and the complexity of this model makes it relatively slow.

In contrast, *Ferguson et al.* used a method whereby only the infection status of spatial clusters of farms less than 3km away from each other were considered in order to determine local spread and a traditional mass action term was used to describe long range transmission (Ferguson, Donnelly et al. 2001). The effects of the local depletion of susceptible farms during the course of the wave, as well as the effect of spatially targeted interventions such as mass culling and ring-vaccination, were then modelled by considering the number of pairs of infectious and susceptible farms within this 3km radius (that is, susceptible-susceptible, susceptible-infectious and infectious-infectious), which is equivalent to the covariance density of infectious and susceptible farms. This second spatial moment of the epidemic, like any moment of a system, in turn depends upon the next

higher moment. This was approximated using a “moment closure” approximation of the number of triples of each possible configuration of infection types which is defined on the basis of the number of pairs, singles and the proportion of triangles (the fraction of the possible pairs of contacts of a given farm which are themselves contacts of one another). Using such an approximation has been termed *moment closure*. This model was intended primarily to provide rapid insight into the spread of infection and the speed and ease of parameterisation allowed an early and detailed analysis of the potential effects of various different interventions. A model by *Keeling et al.* (Keeling, Woolhouse et al. 2001), instead used an individual based stochastic model, based around a spatial kernel function which described how the probability that any infectious IP infects any susceptible IP decays with distance, allowing it to take into account the possibility of more extreme scenarios than the mean represented in the moment-closure model and to capture the long tail at the end of the epidemic.

These models all identified a policy of contiguous culling, whereby animals on farms around infected foci are culled regardless of whether these farms have shown any clinical signs of infection, as being the most effective at reducing the size and overall impact of the epidemic (Ferguson, Donnelly et al. 2001; Keeling, Woolhouse et al. 2001; Morris, Wilesmith et al. 2001; Keeling 2005). The aim of this policy was to shorten the infectious period of infected farms yet to be infected and to remove a significant proportion of the potential contact neighbourhood of these as-yet undetected infected premises (Kao 2002). Although the mass slaughter of apparently healthy animals provoked some opposition from areas within the farming community and veterinary profession, shortly after the measures were implemented the number of infected farms began to decline, the effective reproductive

number fell below unity and the epidemic was brought under control (Ferguson, Donnelly et al. 2001). An inquiry held in the aftermath of the epidemic concluded that the contiguous cull had played a crucial role in controlling the outbreak (Anderson 2001).

2.7.2 Simulation models of the spread of avian influenza

The spread of HPAI in poultry has also led to a surge in interest in the spread of avian influenza within the modelling literature over the last decade. In many cases simulation studies have been carried out in order to obtain a better understanding of the processes likely to affect the spread of infection. These include models exploring how environmental transmission, rather than direct bird-to-bird contact, can facilitate the persistence of avian influenza in poultry populations (Guberti, Scremin et al. 2007; Breban, Drake et al. 2009; Rohani, Breban et al. 2009); evaluation of thresholds for the detection of infection within a flock (Carpenter, Thurmond et al. 2004; Savill, St Rose et al. 2006); the design of methods to estimate the day of disease introduction into a flock (Bos, Van Boven et al. 2007); assessment of the optimum strategies to prevent a pandemic (Jung, Iwami et al. 2009). Further models have been developed to investigate direct and indirect effects of control measures, including the hypothesis that large-scale culling campaigns may impede the evolution of avian host resistance to the virus (Shim and Galvani 2009) and the dynamics of waning immunity (Lesnoff, Peyre et al. 2009), the potential for infection to spread silently, as a result of lower levels of clinical signs and flock mortality (Savill, St Rose et al. 2006), and the evolution and spread of resistance following rounds of vaccination (Iwami, Suzuki et al. 2009; Iwami, Takeuchi et al. 2009). Moreover, in order to explore the potential spread of disease within a particular country more realistically, outbreaks have also been simulated using detailed data on the network of poultry production. This is particularly the case for

poultry production in Great Britain where data on all poultry premises holding more than 50 birds, with details as to the location of the birds and the species and production type these consist of, has been made available to modellers. Models of the spread of infection have then been chiefly characterised by two modes of transmission, local radial spread and infection as a result of long-range movements such as the transport of poultry for slaughter. Simulation using these models have highlighted the areas of the country in which an introduction of infection would be most likely to cause widespread transmission (Dent, Kao et al. 2008), have been used to validate existing control strategies for preventing sustained transmission (Sharkey, Bowers et al. 2008) and have investigated the effectiveness of contingency plans in the event an epidemic takes off, with the finding that according to different measures of cost-effectiveness only nationwide mass vaccination or large radius culls are likely to have a large impact upon the spread of infection (Truscott, Garske et al. 2007). A simulation model has also been used to explore the different pathways of infection from a commercial broiler farm in the USA, using detailed data on the movement of people, livestock and machinery to and from the farm (Dorea, Vieira et al. 2010).

2.7.3 Quantifying avian influenza transmission

As with the models of FMD in 2001, such simulation models, whilst providing useful insight into the infection process, do, however, often require the specification of a range model parameters. In the absence of context-specific outbreak data, sensitivity analyses can, to a certain extent, address issues associated with misspecification of parameter values (Okais, Roche et al. 2010). However, modellers are generally reliant upon existing parameter estimates in the literature and expert opinion in order to obtain a reasonable indication of the ranges of values such inputs may take.

In contrast transmission models fitted to outbreak data are generally a lot simpler, using a smaller number of parameters. This is partially due to data considerations and because they are therefore more straightforward to fit, but also because this provides parsimonious and easily interpretable measures by which to quantify the spread of infection and the incremental effect of interventions.

At the level of transmission between birds, such models are often fitted to data obtained through laboratory experiments. This is because such controlled environments allow relatively detailed data collection, often offer the opportunity to quantify differences in transmissibility between host species, viral strains or subtypes and are straightforward to model, often as a simple SIR or SEIR process. Methods to fit such models to the experimental data then generally fall into two categories, final size methods and the general linear model (GLMs) estimator. The former only requires data on the number of birds infected throughout the course of the experiment along with an assumption as to the overall distribution of the infectious period (discussed further in Chapter 6) whereas the GLM method, first developed by Becker (Becker 1989), fits a rate of transmission to the number of new infections at each time point using GLM formulated with a log-log link function, taking the log of the proportion infected at each time point as an offset variable. This, when multiplied by the mean infectious period, often estimated directly by the time it takes exposed animals to become infected, provides an estimate of the reproductive number. The reproductive number of HPAI H7N7 in chickens, teal, turkey and pheasants (van der Goot, Koch et al. 2005; van der Goot, van Boven et al. 2007), as well as the reproductive number of LPAI H5N2 in chickens (Meijer, van der Goot et al. 2004), have been estimated using these methods (Stegeman, Bouma et al. 2010).

With the exception of a single analysis which used Bayesian methods to fit a survival model including both a parameter determining transmissibility and the distribution of incubation and infectious periods to data on experimentally infected chickens (Bouma, Claassen et al. 2009), all estimates of the reproductive number of H5N1 within a flock have also been obtained in a similar manner. In an experimental context these estimates range from 0.99 to 9 in chickens and an estimate of 20 in Pekin ducks. There have also been experiments in both chickens and ducks where every bird became infected resulting in an unbounded estimate of R_0. Such analyses have also been used to quantify the transmissibility of infection in flocks inoculated using inactivated H5N1 and H5N2 vaccines. Outside of the laboratory it is also possible to estimate the reproductive number within flocks infected in a field context, although this involves making the assumptions that transmission occurs homogeneously within the flock. In Thailand the flock-level reproductive number was calculated using data on daily mortality rates within a flock (Tiensin, Nielen et al. 2007). These numbers were back-calculated according to various assumed fixed infectious periods in order to obtain daily infection rates which were used to produce a GLM estimate of 2.26-2.64 depending on the infectious period chosen. In Vietnam, reproductive numbers were calculated using flock-level final outbreak sizes. Making the assumption that only one bird was infected from a source outside of the flock, it was found that the flock-level reproductive number was significantly lower in outbreaks occurring following the initiation of mass vaccination (see Table 2-1 for full details of existing estimates of the flock level R_0 for H5N1).

Table 2-1| Estimates of within flock transmissibility for HPAI H5N1

Context	Method	Species	Vaccine	R_0 [range[£]]	R_v [range[£]]	Reference
Experiment	Final size	Pekin ducks	Inactivated H5N2	[1.5-∞]	Single dose: [1.5-∞] (after 1 week) 0.6 [0.1-2.2] (after 2 weeks) Double dose: 0.2 [0.005-1.5] (after 2 weeks)	(van der Goot, van Boven et al. 2008)
Experiment	GLM	Pekin ducks	Inactivated H5N2	20[¥]	Single dose: 9.2[¥] (after 1 week) 0.76[¥] (after 2 weeks) Double dose: 0* (after 2 weeks)	(van der Goot, van Boven et al. 2008)
Experiment[#] (High dose)	Final size	Chickens	Inactivated H5N1	E:1.7 [0.4-6.6] F:1.2 [0.37-2.9]	E:0 [0-0.8] F:0 [0-0.67]	(Bouma, Claassen et al. 2009)
Experiment (High dose)	Bayesian	Chickens	Inactivated H5N1	0.99 [0.38-2.1][€]	0*	(Bouma, Claassen et al. 2009)
Experiment[#] (Low dose)	Final size	Chickens	Inactivated H5N1	E:9 [1.9-86] F:3.4 [1.3-7.6]	E:0 [0-0.8] F:0 [0-0.67]	(Bouma, Claassen et al. 2009)
Experiment (Low dose)	Bayesian	Chickens	Inactivated H5N1	2 [0.96-3.6][€]	0 *	(Bouma, Claassen et al. 2009)
Experiment	GLM	Chickens	Inactivated H5N2	12 [4.7-28.7]	0 *	(Poetri, Bouma et al. 2009)
Field	GLM	Chickens	NA	2.26-2.64 [2.01-3.47][&]	NA	(Tiensin, Nielen et al. 2007)
Field[$]	Final size	Poultry	Inactivated H5N2 and H5N1	1.99 [1.87-2.1]	1.17 [1.09-1.25]	(Magalhaes, Pfeiffer et al. 2010)

[3]Range refers to the 95% confidence interval unless otherwise indicated
[€]Range refers to 95% credible intervals.
[¥]Only point estimate given
[*]No birds infected in vaccinated flock providing no information about infectious period required to estimate bounds.
[#]Study where experiments are repeated in chickens inoculated with a low inoculation dose and a high inoculation dose of challenge H5N1 virus. Final size calculations are carried out assuming both a fixed (F) and an exponential (E) infectious period.
[&]Range of values represents different point estimates obtained by assuming different fixed infectious periods.
[$]Study compares final size of outbreaks in flocks before and after the initiation of mass vaccination in Vietnam, R_0 calculated using outbreaks before the campaign and R_v using those occurring after.

Due to the resource-intensive nature of transmission experiments, transmission between flocks is generally only quantifiable in a field setting (Stegeman, Bouma et al. 2010). Using non-spatial SIR models, the reproductive number between flocks has been estimated for the spread of infection between farms in two affected areas during the 2003 wave of H7N7 in the Netherlands, with the effective reproductive number falling significantly following the implementation of control measures such as movement restrictions, and for the 1999-2000 H7N1 epidemic in Italy. Spatial models of the former epidemic have also been fitted using both a continuous spatial kernel (Boender, Hagenaars et al. 2007) and a kernel composed of short, medium and long-range transmission parameters (Le Menach, Vergu et al. 2006), which by calculating local reproductive numbers for each farm, have provided information about areas at risk of future sustained transmission.

However, all of these models make strong assumptions about the underlying infection process. In particular, because the dates on which farms first became infected are not known, the dates of first infection are calculated as occurring a uniform period of time prior to the report of an outbreak (see Table 2-2). *Garske et al.* avoid this problem by fitting the transmissibility and generation time between outbreaks of a non-spatial model to both of these epidemics and an epidemic of H7N3 in British Columbia, Canada (Garske, Clarke et al. 2007). However, this method relies on the assumption of independence of transmission events (Wallinga and Teunis 2004). i.e. that the assignment of the source of infection to any given premises is independent of the assignment of the source of all other outbreaks. Such an assumption is not valid if heterogeneity in the transmissibility of infection, including spatial dependence, is to be incorporated (Lipsitch and Bergstrom 2004; Griffin, Garske et al. 2011). To date the only estimate of between flock transmission for H5N1 comes from a non-

spatial model fitted to a series of outbreaks in Romania in 2006, where, for each farm, the infectious period is assumed to have begun seven days prior to the outbreak's detection.

Table 2-2| Models of the between farm or flock AI transmission

Article	Model Characteristics	Data and farm-level infectious period used	Selected Parameter Estimates and Findings
(Stegeman, Bouma et al. 2004)	SIR Survival model fitted to time series.	HPAI H7N7 Netherlands 2003 farm, infectious for time between detection and culling + 4 days	R =6.5,3.1 in two separate regions, in both R reduced following movement restrictions
(Le Menach, Vergu et al. 2006)	Spatial survival model fitted to data using discretised spatial kernel followed by simulation of control measures.	HPAI H7N7 Netherlands 2003, locations of farms simulated from density data, infectious period as in Stegeman et al.	Reduction in R following movement restrictions, depopulation has greatest impact on number of outbreaks.
(Mannelli, Busani et al. 2007)	SIR model fitted using GLM estimator	HPAI H7N1 1999/2000, infectious from day of detection to date of culling	R=1.8 and 1.5 in two separate regions steadily declining through course of epidemic
(Boender, Hagenaars et al. 2007)	Spatial model fitted to data using continuous spatial kernel.	HPAI H7N7 Netherlands 2003, infectious period as in Stegeman et al.	Identified two regions in Netherlands as high risk regions where R>1.
(Garske, Clarke et al. 2007)	SIR model using branching process estimator to	HPAI H7N1 Italy 1999/2000, H7N7 Netherlands 2003, H7N3 Canada 2004. Generation time	Variation in reproductive numbers ranging from 1.1-2.4 and mean generation time

	simultaneously fit R and generation time.	fitted to data.	ranging from 1.9-8.4 in different settings.
(Ward, Maftei et al. 2009)	SIR model calculating R from daily no. of transmissions, nearest-neighbour and doubling time algorithms.	HPAI H5N1 Romania 2006, infectious for 7 days before outbreak reported.	Estimates of R ranging from 1.9-2.7.

2.7.4 Modelling issues for the spread of H5N1 in Southeast Asia

The transmission dynamics of the waves of H5N1 which spread throughout Southeast Asia have yet to be modelled on a national scale. Instead, macro-level analyses have focussed almost exclusively on identifying risk-factors associated with the observation of an outbreak. These have provided useful insights and hypotheses as to what facilitates transmission, including the findings that H5N1 presence appears to be associated with duck populations chicken numbers, human population, duration of rice harvest, topographical features such as lowlands and areas with high densities of wetland and proximity to roads and slaughterhouses (Gilbert, Chaitaweesub et al. 2006; Gilbert, Xiao et al. 2007; Pfeiffer, Minh et al. 2007; Tiensin, Nielen et al. 2007; Henning, Pfeiffer et al. 2009; Paul, Tavornpanich et al. 2010). These are then used to map the spatial distribution of the relative risks of outbreaks (Gilbert, Xiao et al. 2008; Paul, Tavornpanich et al. 2010). However, such analyses have two primary limitations. Firstly they do not directly estimate and take into account the spatial and temporal dependence of outbreaks. Secondly, they do not provide key quantities, such as the period during which outbreaks remain infectious or the absolute risk

of outbreaks occurring, which are necessary in order to fully evaluate transmissibility and the net impact of control measures.

The main obstacle to quantifying these factors is that the standard of data collected often falls short of that typically required to fit models to the spread of infection. During the initial stages of the epidemic, many countries were slow to recognise the spread of infection within poultry and many outbreaks are thought to have gone unreported (Nguyen 2005; Safman 2010). As a result, it seems unlikely that, using current techniques, modelling can be carried out to estimate the dynamics of this first wave of outbreaks. However, whilst countries such as Cambodia and Laos still really on informal reporting systems, in Vietnam and Thailand attempts at monitoring subsequent waves of H5N1 have proved more successful with the use of specifically developed reporting systems (The National Steering Committee for Avian Flu Prevention and Control 2005; Team 2006), recording dates of detection, locations of outbreaks and, often, other important information such as the species and number of poultry involved. Such surveillance data offer the best opportunity yet to model and quantify the transmission dynamics of waves of H5N1 in Southeast Asia.

However, in order to do so a number of important challenges remain. In particular, data are generally only collected at a sub-district level, which will generally represent multiple farms and neighbourhoods. At this resolution the time it takes for outbreaks to become reported following the introduction of infection into a sub-district is likely to be subject to a high degree of variability, especially when a large proportion of households own poultry. Unfortunately, reliable estimates of this variability do not exist.

2.8 Review of research questions

As stated in the Chapter 1, the overall aim of this thesis is to develop methods in order to quantify key transmission parameters for the spread of H5N1 in Southeast Asia. The first two research questions, concerned with modelling the spread of outbreaks of H5N1 in Vietnam and Thailand, are aimed towards assessing the extent to which transmission models can be fitted to routine outbreak report data in the region in order to assess the transmissibility of infection and effectiveness of control. One of the key challenges to be overcome in order to achieve this is likely to be the uncertainty associated with incomplete observation of the epidemic process.

The quality of data available for outbreak waves in Vietnam and Thailand is likely to be unrepresentative of the rest of the region as other countries rely upon more informal surveillance and reporting infrastructure. In view of this, the remainder of the work for this thesis is focussed upon analysing the spread of infection on a much smaller scale, namely, investigating the extent to which observations of the final size of an outbreak at the flock level can be used to quantify the transmissibility of infection and the effect of individual control measures.

Chapter 3 Quantifying the effects of mass H5N1 vaccination in North Vietnam

3.1 Introduction

In any epidemic setting it is important to evaluate the effectiveness of existing policies designed to mitigate and prevent the spread of outbreaks in comparison to past control strategies and to assess the potential impact of proposed interventions in order to ensure that the optimal level of disease protection is being achieved given the available resources.

This is particularly the case in Vietnam where, in the first year alone, following its implementation in mid 2005, 34.6 Billion VND (≈30.4 million USD) were invested in pursuing a mass vaccination campaign aimed at eliminating outbreaks of HPAI (Dang Ky 2005). During the years prior to the adoption of vaccination, attempts to control the spread of avian influenza through mass "stamping-out" campaigns involved the destruction of millions of poultry and negatively affected the livelihoods of many smallholders without preventing the occurrence of major waves of outbreaks or halting the accumulation of human cases. Between late 2005 and late 2006, following the implementation of mass vaccination alongside more reactive measures such as the culling of infected flocks and the use of ring vaccination (the process of vaccinating uninfected poultry within 5km of a suspected outbreak), Vietnam experienced a year during which no outbreaks in poultry or human cases were reported. However, in 2007, northern Vietnam experienced another prolonged wave of outbreaks and to date sporadic outbreaks and human cases continue to be reported (Ministry of Agriculture and Rural Development 2006; Magalhaes, Pfeiffer et al. 2010).

Both the inactivated H5N2 vaccine used to vaccinate chickens and the recombinant H5N1 vaccine used to vaccinate ducks during this campaign have been found to be efficacious in a laboratory setting (Tian, Zhang et al. 2005; van der Goot, van Boven et al. 2008; Peyre, Fusheng et al. 2009). They have also been shown to be effective within the context of field trials (Ellis, Sims et al. 2006; Peyre, Fusheng et al. 2009) including a trial of both vaccines in two test provinces in Vietnam (To, Bui et al. 2007).

However, whilst field trials do provide a more reliable indication as to whether an intervention is likely to be effective at a population level, there are always factors such as logistic considerations which make the outcome of implementing any intervention on a much larger scale unpredictable. The protective effect of vaccination is determined by the proportion of birds vaccinated and the degree of protection this vaccination then confers. The extensive nature of poultry production in Vietnam and the rapid turnover of poultry (Sims 2007), relative to other livestock such as swine or cows, are the main issues faced when attempting to ensure the former is sufficiently high. The latter can be subject to a degree of heterogeneity due to problems associated with acquiring the technology and resources needed to maintain an adequate "cold chain" of vaccine delivery plus the requirement that each bird has to be vaccinated twice, since one dose is not sufficient to ensure susceptibility and transmissibility are adequately diminished (van der Goot, van Boven et al. 2007; Peyre, Fusheng et al. 2009).

Sero-monitoring of over 576 flocks, with samples taken from three districts in each province, carried out immediately following the first round of two doses of vaccination estimated that the campaign had achieved adequate protection in around 50% of all flocks (Taylor and Dung 2007) which, based upon the disease-free year which occurred soon after,

appeared temporarily sufficient to prevent the spread of infection. In an attempt to maintain control the vaccination campaign was repeated at six month intervals. However the level of overall protection which had been achieved immediately before and during the wave of outbreaks which occurred in 2007 is not known.

The length of time during which a typical outbreak remains infectious is another key determinant as to whether infection can spread. The effect of vaccination on this infectious period at the commune-level (the resolution at which outbreak data is routinely collected in Vietnam) has not been assessed and any resultant impact upon the spread of infection is, therefore, also unknown. On one hand, reducing the number of susceptible birds and flocks within a commune is likely to limit the ability for infection to circulate within the commune, reducing the time the outbreak would remain infectious in the absence of additional control measures. On the other, if infection can spread undetected, either as a result of asymptomatic infection in vaccinated poultry or by virtue of reduced flock or commune mortality, vaccination may result in communes transmitting infection for an extended duration relative to the time it would take to detect infection in immunologically naïve poultry.

Quantifying the parameters which define the spread of infection in both time and space around this change in policy may help to evaluate such key aspects of disease control. Modelling separate waves of infection is likely to provide valuable information about changes in the dynamics of infection and the underlying susceptibility of the population between these waves. From this, an attempt can be made to assess the efficacy of the respective interventions implemented during each wave and the risk posed by similar outbreaks in the future. Though more complicated to formulate, when modelling the

spread of disease within livestock, spatially explicit models are often used in order to incorporate the clustering of cases which occur due to the interdependence of infection events (Keeling 2005; Riley 2007). In the case of H5N1 in Vietnam outbreaks tend to cluster around two main foci: the Red River delta in the North and the Mekong Delta in the South (Pfeiffer, Minh et al. 2007), with smaller clusters of outbreak in the central and northern border region.

A concept featuring heavily in recent disease transmission models in livestock is that of the spatial transmission kernel, a set of parameters which provides an estimate of the risk of disease transmission as a function of distance from a potential infector (Keeling, Woolhouse et al. 2001; Haydon, Chase-Topping et al. 2003; Diggle 2006; Meuwissen, Van Boven et al. 2006; Boender, Nodelijk et al. 2008). Monitoring how this kernel varies between outbreak waves will also provide an indication as to how the potential for infection to spread is changing as a consequence of interventions, as well as other biological and social factors, and allows the identification of areas at high risk of a major outbreak.

Until recently such models relied upon the full observation of all temporal aspects of an outbreak wave, including the times at which infection events occur and the times at which they are removed. However, as is the case in many epidemic settings, such data are not available for commune-level outbreaks occurring in Vietnam, making inference regarding the transmissibility of infection more challenging. Working within a Bayesian framework, which takes into account the uncertainty with regards the missing dates of infection, offers the potential to simultaneously fit the spatial infectivity of infection and the time taken for outbreaks to be officially reported. This provides the opportunity to assess both

interventions aimed at reducing infectiousness and susceptibility and those aimed towards improving the detection of infection.

This chapter introduces a model which uses a spatial kernel to represent the spread of infection between communes in Vietnam and then describes a specially tailored MCMC algorithm designed to fit the model to commune-level outbreak reports in the absence of data on the initial date of infection. This fitting procedure is first validated with simulated data and then fitted to the existing outbreak report data for northern Vietnam between 2004 and 2008, with waves of infection occurring before, during and after the implementation of vaccination in order to obtain parameter estimates of the transmissibility and commune-level infectious period for each wave. Then, by calculating the local reproductive number of each commune, risk maps of areas at high risk of sustaining infection were estimated and compared in order to provide an indication of the relative effects of control measures during each wave and the incremental progress required to prevent transmission. Using the imputed times at which each outbreak became infectious, and the other model parameters, the possible chains of infection (the "epidemic tree") leading to each set of outbreaks reports were also explored. This allows estimation of how the effective reproduction number varied during each wave, providing an indication of how effectively the outbreak was being controlled as each epidemic progressed, and the distances over which infection was transmitted.

Furthermore, by developing an algorithm which prunes branches of the epidemic tree according to a given infection-to-report distribution, it was also possible to investigate the extent to which improved detection of outbreaks could improve the effectiveness of the vaccination campaign.

3.2 Materials and Methods

3.2.1 Description of available outbreak data

Data on the outbreaks of H5N1 (Figure 3-1) in Vietnam between March 2004 and February 2008 were obtained from the Vietnam government's Department of Animal Health's (DAH) disease reporting. The data were processed by the sub-Departments of Animal Health for each of 64 provinces and combined into a single database by the Epidemiology Division of the Department of Animal Health in Hanoi, Vietnam.

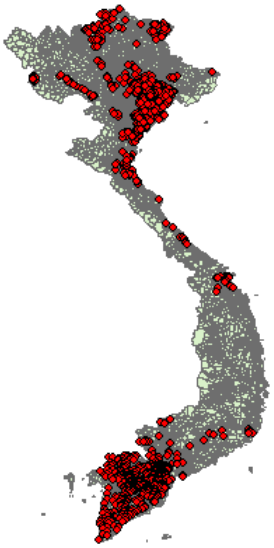


Figure 3-1| Spatial distribution of outbreaks in Viet Nam between November 2004 and February 2007.

The database contains the smallest administrative unit (hereafter referred to as a commune although this can also refer to a town or ward) within which the outbreak occurred, the date on which the outbreak was reported and the species of bird, which include chickens, domestic ducks, Muscovy ducks and quails, involved in each outbreak.

Spatial data were obtained in the form of a shapefile of the geographical boundaries of each of the 10811 communes in Vietnam, as well as data on the population of each commune according to the 1998 LandScan Global Population Database . This database uses spatial data on administrative boundaries, land cover and other factors such as roads and elevation in order to estimate 1998 commune-specific population data using population estimates from the US Bureau of Census, which, in turn, forms annual sub-national estimates based upon the most recent Vietnamese population census. Annual poultry numbers at a provincial level resolution were obtained from the General Statistics Office of Vietnam website (GSO-Vietnamese General Statistics Office 2007).

3.2.3 Model description

Each commune i was assigned a location (x_i, y_i), a date of first infection u_i and a date of removal r_i from the outbreak wave as a result of control measures. Vectors associated with these times $U = \{u_1, \dots, u_N\}$ and $R = \{r_1, \dots, r_N\}$ were also defined, with N the total number of communes in the dataset (including those that never become infected). For ease of notation, communes which did not report an outbreak during any of the wave were assigned values for u_i and r_i arbitrarily larger than the time of the last reported outbreak. Communes were also assigned a poultry population n_i.

It has been shown that infectivity and susceptibility varies between domestic ducks and chickens (Hulse-Post, Sturm-Ramirez et al. 2005). It is also likely that the probability of transmission will also vary when outbreaks involve other species of poultry. However, for a significant proportion of outbreaks, the species in which disease was reported is not available. Moreover, with the data available it is impossible to distinguish between diseased and culled poultry and to estimate the denominator susceptible population of each species

within a commune. In view of this infectivity is parameterised using a single 'black box' per-capita transmission intensity parameter β.

Spatial heterogeneity in the transmissibility of infection was incorporated into the model using a spatial transmission kernel which describes how the risk of transmission between an infectious and susceptible commune scales with distance. This had the functional form:

$$k(d_{ij}) = (1 + \frac{d_{ij}}{\rho})^{-\alpha} \quad 3.1$$

where d_{ij} is the distance between commune i and commune j and the power α and offset ρ are parameters to be estimated. Normalising this function by all potential transmission distances transforms it into a probability distribution whereby changes in either of the kernel parameters only affect the relative probability of transmission over varying distances and not the overall rate of transmission. Moreover, disentangling the baseline transmission rate and the effect of distance on transmission in this way is likely to improve the identifiability of these parameters when the model is fitted to the data. Ideally, such normalisation should be carried out thus:

$$k^*(d_{ij}) = \frac{k(d_{ij})}{\int_0^\infty rk(r)\,dr}. \quad 3.2$$

However, this integral is only proper when $\alpha > 2$. Otherwise, it is necessary to approximate the normalisation constant using a finite upper bound which is larger than the maximum distance between any two points in the dataset.

From this the instantaneous risk of that commune i is infected at time t can be defined:

$$\lambda_i(t) = \sum_{\substack{i=0,\\ i\neq j}}^{N} \beta n_i n_j k\big(d_{ij}\big)\mathbb{1}(i \text{ susceptible and } j \text{ infectious at } t) \qquad 3.3$$

where N *is* the number of communes in the dataset and $\mathbb{1}(.)$ is the indicator function.

If the time of infection and the time of removal from the outbreak wave of each commune are known, and assuming constant infectivity following an incubation period of a day (Shortridge, Zhou et al. 1998) until the day of removal, the wave of outbreaks can then be modelled as a space-time survival point process, where communes avoid infection until they are infected at a rate which depends upon the temporal and spatial distribution of other outbreaks. Such models have applications in ecology and geography as well as epidemiology (Cressie 1991; Rathbun and Cressie 1994).

Modelling the outbreak wave this way, using the instantaneous risk of transmission above, given the history of the wave and the associated accumulated exposure to infection $\Lambda(t) = \int_{\tau=0}^{\tau=t} \lambda_i(\tau)\, d\tau$, the probability that a commune escapes infection until time t is:

$$q_i(t) = \exp\big(-\Lambda(t)\big) \qquad 3.4$$

Although the above formulation assumes continuous time, in reality the reporting of outbreaks occur on a daily basis. Therefore, in practice, each wave was fitted as a discrete approximation of the transmission process with a time-step size of a day. As a result, transmissibility was assumed to remain constant throughout each day, with communes becoming infectious on the day following infection. As a result, if the commune remains susceptible to infection by day t, the probability it becomes infected on this day is

$$p_i(t) = 1 - \exp\big(-\lambda_i(t)\big). \qquad 3.5$$

3.2.2 Data assumptions

Based upon experimental data on the pathology of H5N1 infection in birds (Shortridge, Zhou et al. 1998; Bouma, Claassen et al. 2009), communes were assumed to become infectious on the day following infection and, based upon official advice distributed to Vietnamese paraveterinary professionals, an infected commune was assumed to be removed from the outbreak on the day following its reporting (FAO 2005).

In an attempt to capture backyard transmission, and in the absence of more precise data, this population is calculated as the province-level poultry population multiplied by the proportion of the province-level human population residing within the commune. This then represents the assumption that, whilst the concentration of poultry with respect to human population differs between provinces (this is especially likely when comparing the predominantly rural provinces in the North with city provinces such as Ha Noi province), this concentration is uniform between communes within the province.

The analysis of spread of outbreaks was restricted to those occurring in the north of Vietnam, with the 18° latitude line defined as a cut-off point. This was chosen in an attempt to capture the spread of infection around the Red River Delta outbreak focus whilst easing the computational demands of the model and reducing the problems associated with a estimating the kernel between communes close to the east coast of Vietnam where travelling between communes in a straight line would involve a journey by sea. Moreover, this cut-off ensured that all the virus isolates used in phylogenetic analyses of the outbreaks during 2007 (Nguyen, Nguyen et al. 2008), where the virus in the North was identified as a different clade to that in the South, were assigned to the correct foci.

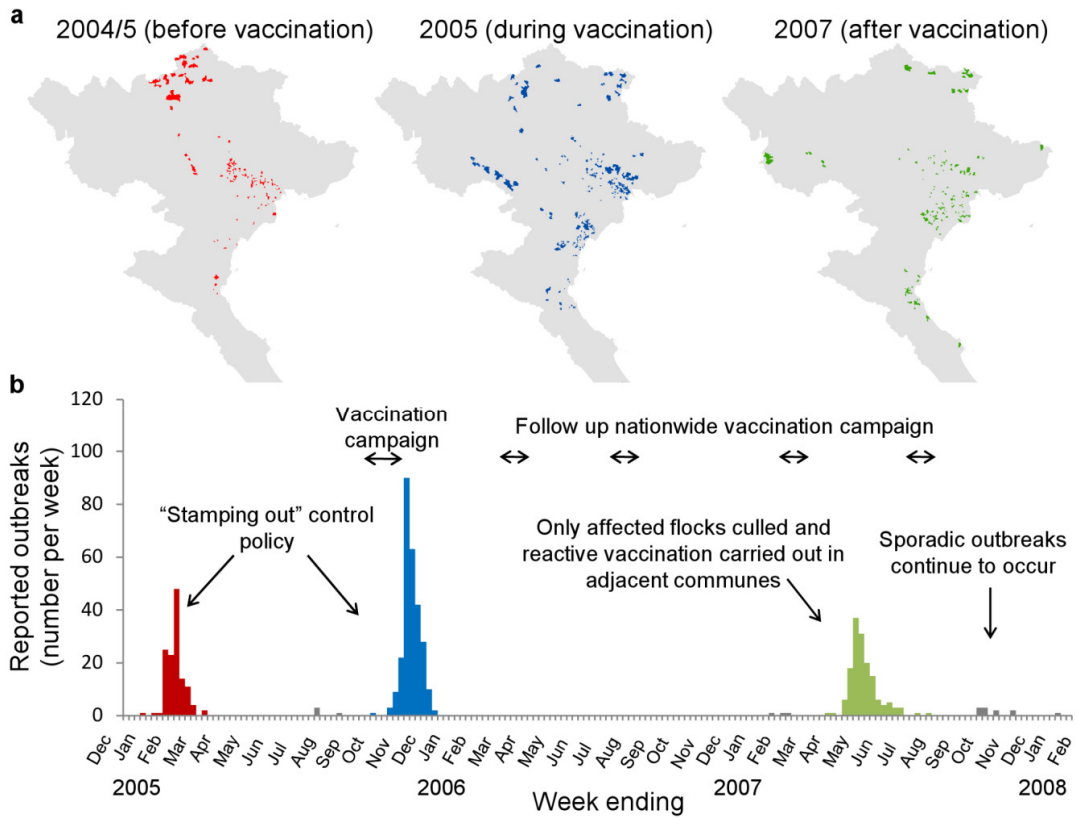


Figure 3-2| Spatial and temporal distribution of outbreaks of H5N1 in poultry in northern Viet Nam Dec 2004-Feb 2008.

Figure shows, **(a)**, Commune-level spatial distribution of reported outbreaks, **(b)**, Weekly incidence of outbreak reports in northern Viet Nam. (As sufficiently accurate data on the timing and location of outbreaks during the first wave is not available, figure shows data from the second wave onwards).

Three outbreak waves in Northern Vietnam were defined (Figure 3-2). The first, which involved 129 communes, began in December 2004 and lasted for 84 days, this was followed by the second taking place in late 2005, lasting 59 days and affecting 271 communes and a third wave taking place in mid-2007, lasting for 126 days and affecting 152 communes (from this point onwards these will be referred to, respectively, as 2004/5, 2005 and 2007 waves). The beginning and end of each wave were defined, respectively, as the closest period of at least 21 days where no outbreaks were reported before the first and after the last reported outbreak.

Commune locations were calculated as the centroid of the polygon representing the area of the commune. A small proportion of infected communes reported more than one outbreak

during the course of a single wave. A threshold period of 14 days was defined, loosely based upon the first wave of outbreaks during which a maximum time an outbreak could go undetected of 21 days was identified (Nguyen 2005). Any two outbreaks which occurred within this period of time within the same commune were classed as the same event and the commune was assumed to have remained infectious throughout. If a commune was reported as having two outbreaks more than 14 days apart, and no other outbreaks were reported in the interim, then the possibility that the latter outbreak had an external source was allowed and the locations of both the earlier and latter outbreak, rather than being calculated as the centroid, were randomly simulated within the commune affected.

3.2.4 Bayesian framework for inference of model parameters

If the times of infection and removal were observed then conducting likelihood-based inference about the transmission kernel parameters would be relatively straightforward as it would be possible to compute the overall likelihood of the epidemic process. This would consist of the product of the likelihood that all of the communes which never become infected avoid infection on any of the T days during which the outbreak wave takes place, the likelihood that infected communes escape infection until the day they are infected and the likelihood that they become infected on that day:

$$P(U,R|\theta) = \prod_{i=0}^{N} \left\{ q_i(T)^{\left(1-I(u_i<T)\right)} \left(q_i(u_i)\, p_i(u_i) f(r_i - u_i; \omega) \right)^{I(u_i<T)} \right\} \quad 3.6$$

here $I(x)$ is an indicator function, $f(t;\omega)$ is an assumed parametric distribution of the duration of time between a commune first becoming infected and the date an outbreak is reported (the contribution of which to the likelihood, if infection and removal times were

observed, would be a constant multiplier) and θ denotes the set of kernel and infection time distribution parameters. From this maximum likelihood estimates of the kernel parameters can be obtained (Boender, Hagenaars et al. 2007) or the posterior distribution of the parameters can be sampled using various methods, including standard MCMC techniques (O'Neill 2002; Chis Ster and Ferguson 2007).

In Vietnam, it is possible to make rough estimates of the date an outbreak is removed from the day of report and other information such as official control policies and guidelines (FAO 2006). If the infection time of a commune was observed it would also be possible to make an estimate of the time a commune becomes infectious from experimental data (Shortridge, Zhou et al. 1998; Bouma, Claassen et al. 2009). However, the time between the infection of a commune and the report of an outbreak is likely to vary widely between each outbreak and depend upon a multitude of factors including the type of farm involved, the size of the flock, the species of bird involved, the background mortality previously experienced, the willingness to report disease and the level of infection in the vicinity of the infected premises.

Without observing these infection times, in order to write down the fully conditional likelihood it would be necessary to integrate the above likelihood with respect to the vector of times of infection:

$$P(R|\theta) = \int_U P(U, R|\theta) dU. \qquad 3.7$$

This likelihood has very high dimensionality and would be very computationally intensive to work with.

To overcome this problem a data augmentation approach was used, with unobserved infection times treated as extra model parameters (O'Neill and Roberts 1999; Streftaris and Gibson 2004; Chis Ster, Singh et al. 2008). Conditioning on the existence of a unique first infection U_0 and using Bayes theorem (noting that $P(U,R,\theta|U_0) = P(U,R|\theta,U_0)P(\theta) = P(\theta,U|R,U_0)P(R)$), assuming that all outbreaks are eventually reported, the appropriate joint posterior distribution of the model and infection time parameters is:

$$P(\theta,U|R,U_0) = \frac{P(U,R|\theta,U_0)P(\theta)}{P(R)} \propto P(U,R|\theta,U_0)P(\theta) \qquad 3.8$$

Here $P(R)$ does not depend upon the unknown parameters and so can be treated as a constant.

Table 3-1| Prior distributions of the model parameters

Parameter	Description	Prior distribution
$\boldsymbol{\beta}$	Transmission intensity	$U(0,10000)$
$\boldsymbol{\rho}$	Spatial kernel offset	$U(0,10000)$
$\boldsymbol{\alpha}$	Spatial kernel power	$U(0,10000)$
$\boldsymbol{\varphi}$	Shape of infection to report distribution	$U(0,10000)$
$\boldsymbol{\gamma}$	Shape of infection to report distribution	$U(0,10000)$
$\boldsymbol{U_0}$	Timing of first infection	$U[r_0 - 30, r_0 - 1]$

The duration between communes becoming infected and the report of an outbreak within a commune were assumed to follow a Gamma distribution and the parameters of this distribution, as well as the infectivity and spatial kernel and model parameters, were given uniform bounded prior distributions (Table 3-1), whilst the timing of the first infection U_0

was given a uniform prior bounded in the interval $[r_0 - 30, r_0 - 1]$, where r_0 is the day this outbreak was reported.

3.2.5 An MCMC algorithm to fit the model to the data

One way of integrating over such high dimensional probability distributions is then to use MCMC methods. This involves designing a Markov chain which converges to the required distribution, which can then be approximated through Monte Carlo integration (Gilks, Richardson S et al. 1996). The following describes one such algorithm which provides samples of the posterior distribution of model parameters described above:

For ease of notation U will be used to denote the set of infection times at any given stage of the Markov chain and $U' \subset U$ to denote the set of imputed infection times for the communes in which outbreaks were reported, initial values for all $u_i \in U'$, were calculated according to the duration between infection to report which was drawn from a uniform proposal distribution bounded between 1 and 30 days. Setting $U_0 = \min(u_i \in U')$, these were repeatedly sampled until a set of infection times was obtained which satisfied the criteria of a unique time of first infection $\left(\sum_{u_i \in U'}\{\mathbb{1}(u_i = U_0)\} = 1\right)$ and that, on the day each other outbreak was infected, there was at least one ongoing, infectious outbreak:

$$\sum_{j=0}^{N} \mathbb{1}(u_j < u_i).\mathbb{1}(r_j \geq u_i) > 0, \text{for all } u_i \in U'/U_0, \qquad 3.9$$

Beginning with the obtained starting set of imputed infection times, the posterior distributions of the model parameters were then sampled by proceeding according to the following two-step algorithm:

(1). For each of the kernel and time to report parameters, $\theta_i \epsilon\, \theta$, a new parameter value ${\theta_i}^*$ is drawn on a log-scale from a Normal distribution centred on the logarithm of the existing parameter:

$$\log {\theta_i}^* \sim N(\log \theta_i\,, {\sigma^2}_i). \qquad 3.10$$

Denoting the set of current values of all the remaining model parameters θ_{-i}, the proposed value is then accepted with probability

$$\min\left(\frac{P(U,R|{\theta_i}^*,\theta_{-i})}{P(U,R|\theta)}.\frac{{\theta_i}^*}{\theta_i},1\right). \qquad 3.11$$

The variance of the proposal distribution of each parameter, ${\sigma^2}_i$, was calibrated by assessing the ratio of proposed moves of the parameter which are accepted so as to achieve good MCMC mixing (ideally as close to the 'golden' acceptance ratio of 23% (Roberts, Gelman et al. 1997) but a ratio of 10-35% was judged to represent adequate mixing).

(2). The duration between infection and report, and thus the infection time itself, of individual outbreaks are updated using a Metropolis-Hastings independence sampler (Tierney 1994) with the same proposal distribution of the time between infection and report as those used to obtain the starting set of infection times. Using U now to denote the set of current infection times, the current infection time u_j of a randomly selected commune j is replaced with the proposed infection time u_j^* with probability

$$\min\left(\frac{P\left(U-\{u_j\}+\{u_j^*\},R\middle|\theta\right)}{P(U,R|\theta)},1\right) \qquad 3.12$$

where $U-\{u_j\}+\{u_j^*\}$ denotes the set of infection times with the proposed replacing the current infection time. In order to achieve faster convergence it is

desirable to update more than one infection time during this step (updating 10% of infection times appears to be a reasonably good rule of thumb). Evaluating the entire likelihood for each of these proposed times results in the algorithm becoming computationally intensive. In view of this, the sampler was designed to calculate the acceptance probability by comparing the existing and proposed likelihood in the interval$\left[\min\left(u_j, u_j^*\right), \max\left(u_j, u_j^*\right)\right]$, the only section of the likelihood affected by the proposed move.

Both steps are repeated for a 'burn in' period of 20000 moves, determined by examining parameter traces from different starting parameter values, at which point the MCMC is deemed to have converged to its stationary distribution and subsequent moves are treated as samples of the posterior distribution of each of the parameters. The chain is then run until its stopping time 250000 moves later, which was assessed to be suitable by checking that the Monte Carlo standard error of each of the five main parameters was less than 5% of its posterior standard deviation and by determining that the posterior distribution was invariant to different starting values (see Figure3-3).

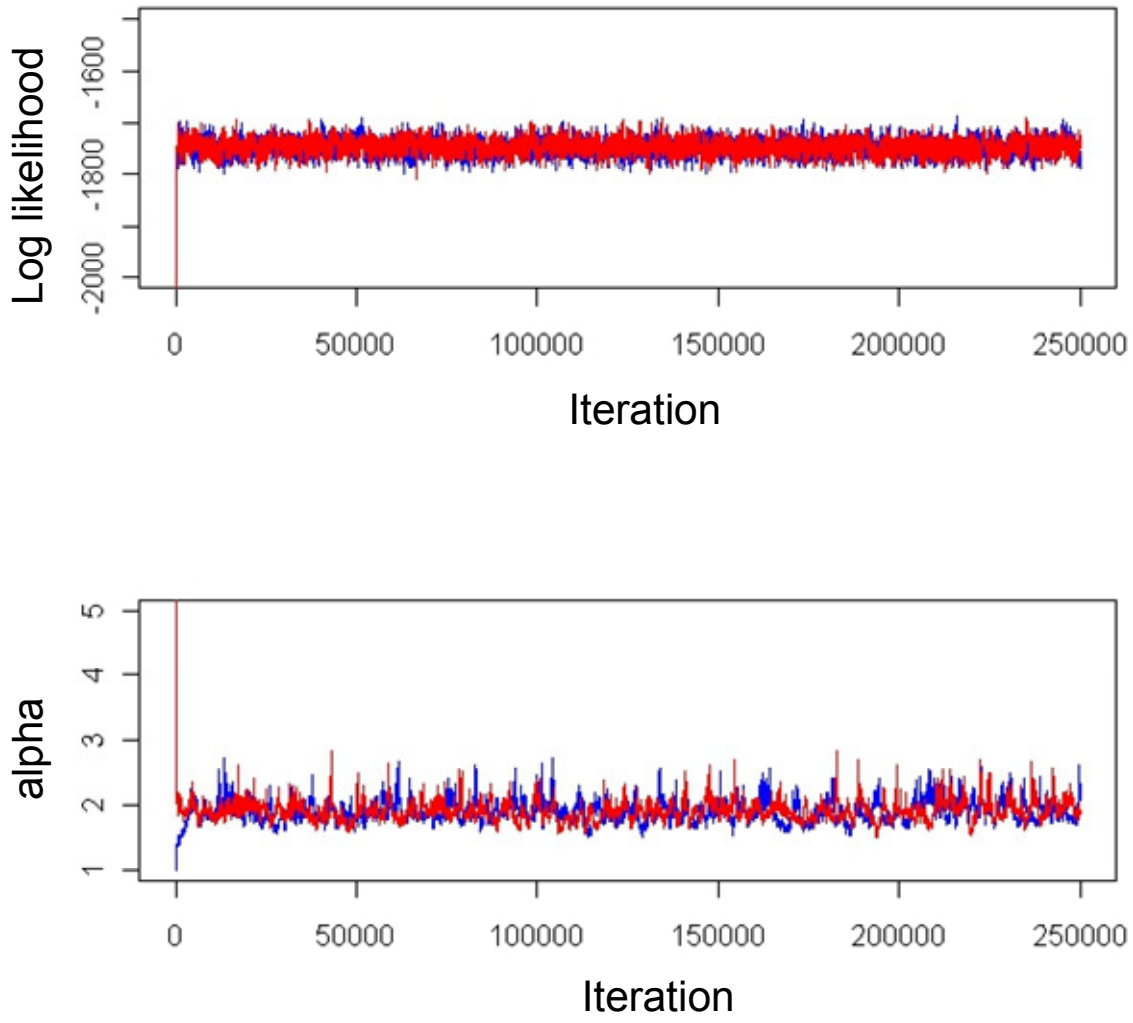


Figure 3-3| Sample MCMC output.
Plots show the log likelihood and power kernel parameter $\boldsymbol{\alpha}$ traces of the 2007 outbreak data under the baseline model assumption for two sets of initial parameter values: $\boldsymbol{\alpha = 1,\ \gamma = 1,\ \beta = 10^{-7}, R - U \sim \Gamma(3,3)}$ (blue lines) and $\boldsymbol{\alpha = 10,\ \gamma = 100,\ \beta = 10^{-10}, R - U \sim \Gamma(6,1)}$.

3.2.5 Fitting the model to simulated datasets

To assess the ability of the fitting procedure to estimate transmission parameters accurately, outbreaks of HPAI were simulated over the spatial distribution of communes in Vietnam. This was done by seeding an outbreak within a commune in the high-risk Red River Delta region with an infectious period drawn from a predefined Gamma distribution (communes were assumed to become infectious the day following infection and to have

been reported the day before removal thus the infectious period and the duration between infection and report are of equal length). Secondary infections were then selected with an infection probability as described in the model, calculated using a preselected set of parameter values and commune level poultry populations calculated using annual province-level poultry data from 2005. Infectious periods for these communes were drawn from the same gamma distribution as before. This procedure was repeated at each time-step, representing a day of the outbreak wave, and infected communes were removed from the dataset at the end of their infectious periods. The simulation was continued until no infectious communes remained. Once simulated datasets had been obtained the fitting procedure was applied, using only the spatial location and date of report of each outbreak (entered as the day before the outbreak was removed).

3.2.6 Quantifying the change in infectivity between waves

In order to obtain insight as to the adequacy of the different control measures implemented during each of the three waves it is important to obtain estimates of the relative ease with which infection spread between communes. To begin with, this was assessed by calculating the difference between the posterior distributions of the transmissibility of infection at varying distances between each wave, according to the joint posterior distributions of the infectivity and kernel parameters obtained from fitting each wave independently. Then, in order to take into the fact that, in terms of replicating the spatial pattern of outbreaks during each wave, estimates of transmissibility at some distances may be more important than others, an overall estimate of the relative transmissibility of infection between two waves was calculated by fitting both waves simultaneously and constraining the changes in the spatial kernel for the latter wave to match the changes occurring as a result of fitting the

spatial kernel to the former wave. Denoting the respective estimates of β in each likelihood as $\beta_i$and β_j, the protective effect of the control policies applied during wave j compared to those during wave i can then be calculated using the proportional change in infectivity occurring during the wave:

$$\gamma_{ij} = \frac{\beta_j}{\beta_i}. \quad 3.13$$

It should, however, be noted that this estimate does not directly take into account differences between the individual fitted kernels of the two waves in the same way as fitting an unconstrained kernel, therefore the two methods were compared to assess the extent to which the two methods agree about the change in transmissibility over distances at which transmission was estimated to occur, as calculated by reconstructing the epidemic tree (subsection 3.2.8).

3.2.7 Calculating risk maps

The local reproduction number of a commune estimated from an outbreak wave is defined as the number of secondary infections which could have been expected to occur had the first outbreak of the wave occurred in that particular commune, assuming that all other communes are susceptible to infection. Within a large population, where it can be assumed there are no local saturation effects caused by tertiary infection, this can be calculated by summing the probabilities that a given commune infects all other communes to produce the expected number of secondary infections. It should be noted that in this analysis, due to the spatial dependence between infection events, such an assumption is not valid. However, summing such probabilities has become an accepted measure of assessing regions most at risk of sustained transmission (Ferguson, Donnelly et al. 2001; Boender, Hagenaars et al.

2007; Truscott, Garske et al. 2007) and, for the sake of simplicity, values calculated in this manner will still be referred to as local reproduction numbers. When the infectivity kernel and distribution of the infectious period T are available, such estimates can be obtained analytically (Boender, Hagenaars et al. 2007) using the expression:

$$\begin{aligned} R_i &= E\left[\sum_{j\neq i} p(d_{ij})\right] = \sum_{j\neq i}\left\{1 - E\left[e^{-k(d_{ij})T}\right]\right\} \\ &= \sum_{j\neq i}\left\{1 - M_T\left(-k(d_{ij})\right)\right\} \\ &= \sum_{j\neq i}\left\{1 - \left[\frac{a}{a + abk(d_{ij})}\right]^a\right\}. \end{aligned} \qquad 3.14$$

Here $p(d_{ij})$ is the probability that transmission occurs between an infectious and susceptible commune d_{ij}km apart and the infectious period is assumed to take the form of a Gamma distribution, $T \sim \Gamma(a, b)$, with $M_T(x)$ the moment generating function of this distribution.

Thus, using the posterior distribution of the model parameters it is possible to obtain the posterior distribution of each commune-level local reproduction number. Then, plotting these on a map, it is possible to highlight geographical areas which are most at risk of experiencing sustained inter-commune transmission in the event of an outbreak.

Another easily obtainable statistic which can illustrate how changes in the spatial transmission kernel translate to the spatial distribution of risk within communes is the expected number of infections which would occur on the first day of an outbreak, denoted Λ_i:

$$\Lambda_i = \sum_{j=0, j\neq i}^{N} \left[1 - \exp\left(-\beta n_i n_j k(d_{ij})\right)\right]. \quad 3.15$$

Although these maps only provide information about the second generation of outbreaks and not the eventual size or spread of the outbreak wave, which is determined by the extent to which an outbreak accesses these spatial pockets of high risk communes, comparing risk maps may provide an indication as to how likely it was that sustained onward spread of any size occurred. Where risk maps are being used to estimate the effects of control policies it is necessary to standardise the risk maps by using the same denominator poultry population for the each set of parameter posterior distributions.

3.2.8 Reconstructing the epidemic tree

If the times at which each commune became infected and the duration they remained infectious were observed the effective local reproduction number and the expected distance over which infection was transmitted could by estimated by reconstructing the epidemic tree (Haydon, Chase-Topping et al. 2003). Conditional on such data, the probability that commune i is infected by j is then

$$\mu_{ij} = \frac{\beta n_i n_j k(d_{ij}) \mathbb{1}(j \text{ infectious on day } i \text{ infected})}{\sum_{k=0}^{N} \beta n_i n_k k(d_{ik}) \, \mathbb{1}(k \text{ infectious on day } i \text{ infected})}. \quad 3.16$$

However, when the times of infection are not available, a sufficiently large number of samples of μ_{ij} have to be drawn at regular intervals during the MCMC. The probability that i infects j is then estimated by:

$$\mu_{ij}^{*} = \overline{\mu_{ij}} \quad 3.17$$

where $\overline{\mu_{ij}}$ is the posterior mean of μ_{ij} estimated by calculating the mean of the obtained samples.

From this the expected distance D_i of an infected commune i from its parent can then be defined as

$$D_i = \sum_{j \in N} \mu_{ij}^* \, d_{ij}. \qquad 3.18$$

The effective local reproduction number, R_i^*, of any outbreak commune i (here defined as the number of secondary infections arising from a given commune when it is infected during the wave) can also be calculated by summing the infection probabilities between the commune and all other outbreak communes which were infected during its infectious period:

$$R_i^* = \sum_{j \in N} \mu_{ji}^*. \qquad 3.19$$

Monitoring how the average of these local effective reproductive numbers varies during a wave of outbreaks provides an indication of the extent to which transmission is being effectively contained due to factors such as changes in the application of control measures, the depletion of susceptible flocks and the extent to which a wave stochastically infects areas at high risk of sustained transmission (Lipsitch, Cohen et al. 2003; Wallinga and Teunis 2004; Cauchemez, Bhattarai et al. 2011). During any wave, if the average local effective reproduction number of outbreak communes remains below unity for a sufficiently long period of time the wave will come to an end.

3.2.9 Evaluating the effects of more rapid detection and culling

Once the epidemic process has been reconstructed, the effect of detecting and removing an outbreak at an earlier time-point can be assessed. First a sample epidemic tree is drawn by assigning a source of infection for each outbreak probabilistically from the list of communes which were infectious on the day the outbreak commune was first infected. From this, secondary infections which arose between the "real-life" removal time of an outbreak and a putative earlier removal time, occurring as a result of improved surveillance, are identified, according to the assumed impact of this surveillance. Then, by pruning clades of outbreaks attributed to these secondary infections, where a clade is defined to consist of all future generations of outbreaks arising from an individual infection, the epidemic tree which would have occurred as a result of the earlier removal time is obtained, although it should be noted that this makes the assumption that pruned outbreaks would not have been infected from alternative sources at a latter stage of the wave. Sampling epidemic trees repeatedly (100000 draws in this analysis) provides an estimate of the effects of the earlier detection for a given set of parameters and infection times and, in turn, repeating this for the sets of infection times and model parameters at regular intervals during the MCMC provides an estimate of the posterior distribution of the impact of this earlier detection.

This approach was also used to estimate the size and duration of the wave which would have occurred in 2007 had the rate of detection been maintained to that estimated for either of the earlier waves in my analysis. At regular intervals during the MCMC the cumulative distribution function of the time to report distribution fitted to the 2007 wave, the parameters of which are here denoted ω, was evaluated at $r-u$, the individual

imputed time to report, for each outbreak commune. The time the outbreak would have been detected during a previous wave was then calculated as the r^* which satisfies

$$P(T < r - u|\omega) = P(T < r^* - u|\omega^*), \quad 3.20$$

where ω^* is a randomly drawn sample from the posterior distribution of the infection-to-report distribution of the earlier wave. The epidemic tree was then pruned according to this new set of removal times. This was repeated 100000 times at each interval during the MCMC, randomly sampling a different ω^* each time.

3.2.10 Hypothesis testing using the Bayes Factor

In this chapter (and at various points throughout the thesis), Bayes Factor (BFs) are used to judge the evidence for or against a given hypothesis H_1, relative to that of an alternative hypothesis H_2, given the data D. This is defined as

$$\mathrm{BF} = \frac{P(H_1|D)P(H_2)}{P(H_2|D)P(H_1)}, \quad 3.21$$

which is equivalent to the ratio of the posterior odds of H_1 to its prior odds. The extent to which this provides evidence in favour of H_1 is then judged according to the subjective scale developed by Jeffreys, namely, if BF varies between 1 and 3, the evidence for a given hypothesis is poor; if it is between 3 and 10, this evidence is substantial; if it is between 10 and 30, it is strong; if it is between 30 and 100, it is very strong; and if it is above 100 it is decisive (Jeffreys 1961). When such an approach is used to test for the level of evidence supporting the hypothesis that the value of a parameter for a given model fitted to one dataset is greater than the equivalent parameter from the same model fitted to another dataset, provided the prior distributions of the two sets of model parameters are specified

to be equal, the BF will be equivalent to the posterior odds as the prior odds for this hypothesis are even.

3.3.1 Simulation Study

50 datasets were generated using the transmission kernel and infectious period represented by the black lines in Figure 3-4 and 13 simulations where the size of the outbreak exceeded 20 communes were selected. For all the simulations the simulated parameter values fell within the fitted 95% credible interval and the overall mean values from all the fits recaptured the simulated kernel and infection-to-report distribution well (Figure 3-4).

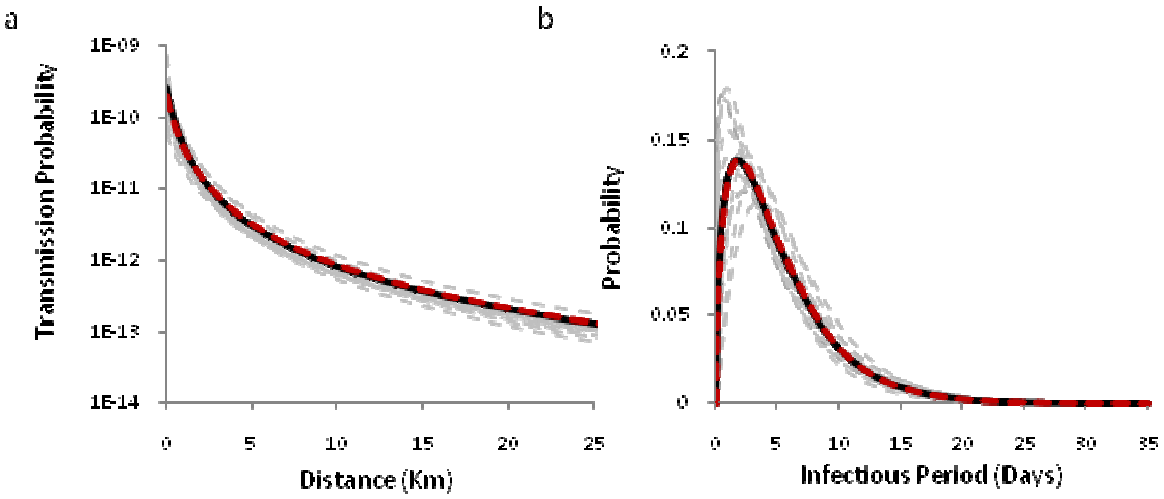


Figure 3-4| Simulation study results.
Parameters used during simulation of outbreaks waves (black line), estimates from the posterior mean of the model parameters estimated from fitting the model to each simulated wave (dashed grey lines) and the overall mean of each of these estimates (dashed red line) for **(a)**, infectiousness over space as described by the estimated spatial kernel multiplied by the fitted value of and **(b)**, the commune-level infectious period.

3.3.2 Fitting the model to outbreaks in northern Vietnam

The Markov chains used in fitting the model converged adequately for all three outbreak waves, providing posterior distributions of both the spatial transmissibility and infectious period parameters (Table 3-1, Figure 3-5 and Figure 3-6).

Table 3-1| Posterior mean and 95% credible intervals for the baseline model fitted to each of the three outbreak waves.

Model Parameters		**Posterior mean (95% C.I.)**		
Parameter	**Description**	**2004/5**	**2005**	**2007**
		Spatial Transmissibility		
$\boldsymbol{\beta * 10^8}$	Intensity	3.96 (0.45-14.5)	1.85 (0.75-4.88)	2.06 (0.57-6.02)
$\boldsymbol{\rho}$	Offset	4.49 (0.36-13.68)	1.45 (0.22-3.42)	0.77 (0.04-2.21)
$\boldsymbol{\alpha}$	Power	2.14 (1.57-3.07)	2.02 (1.75-2.35)	1.81 (1.60-2.08)
		Duration between infection and report		
ϕ	Shape	1.81 (1.24-2.75)	2.33 (1.71-3.21)	2.45 (1.49-4.54)
γ	Scale	3.37 (2.04-5.01)	2.09 (1.33-3.12)	3.98 (2.11-6.15)
$\phi\gamma$	Mean	5.87 (4.59-7.40)	4.74 (3.84-5.91)	9.10 (7.29-11.21)
$\phi\gamma^2$	Variance	20.04 (10.25-34.82)	10.09 (5.29-17.80)	36.29 (18.69-60.14)

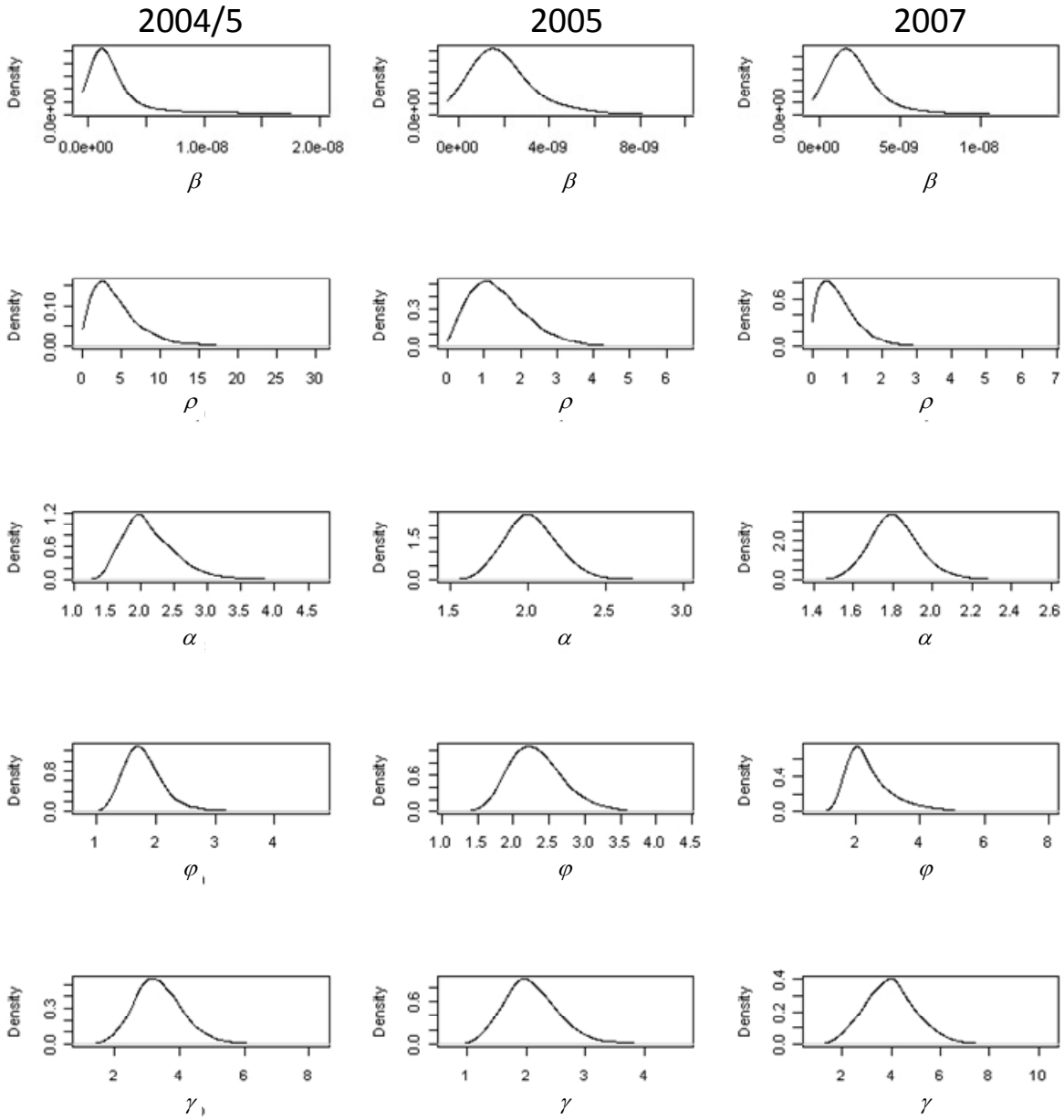


Figure 3-5| Posterior probability density plots of the fitted infectivity, spatial kernel and infection–to-report distribution model parameters for each of the three waves.

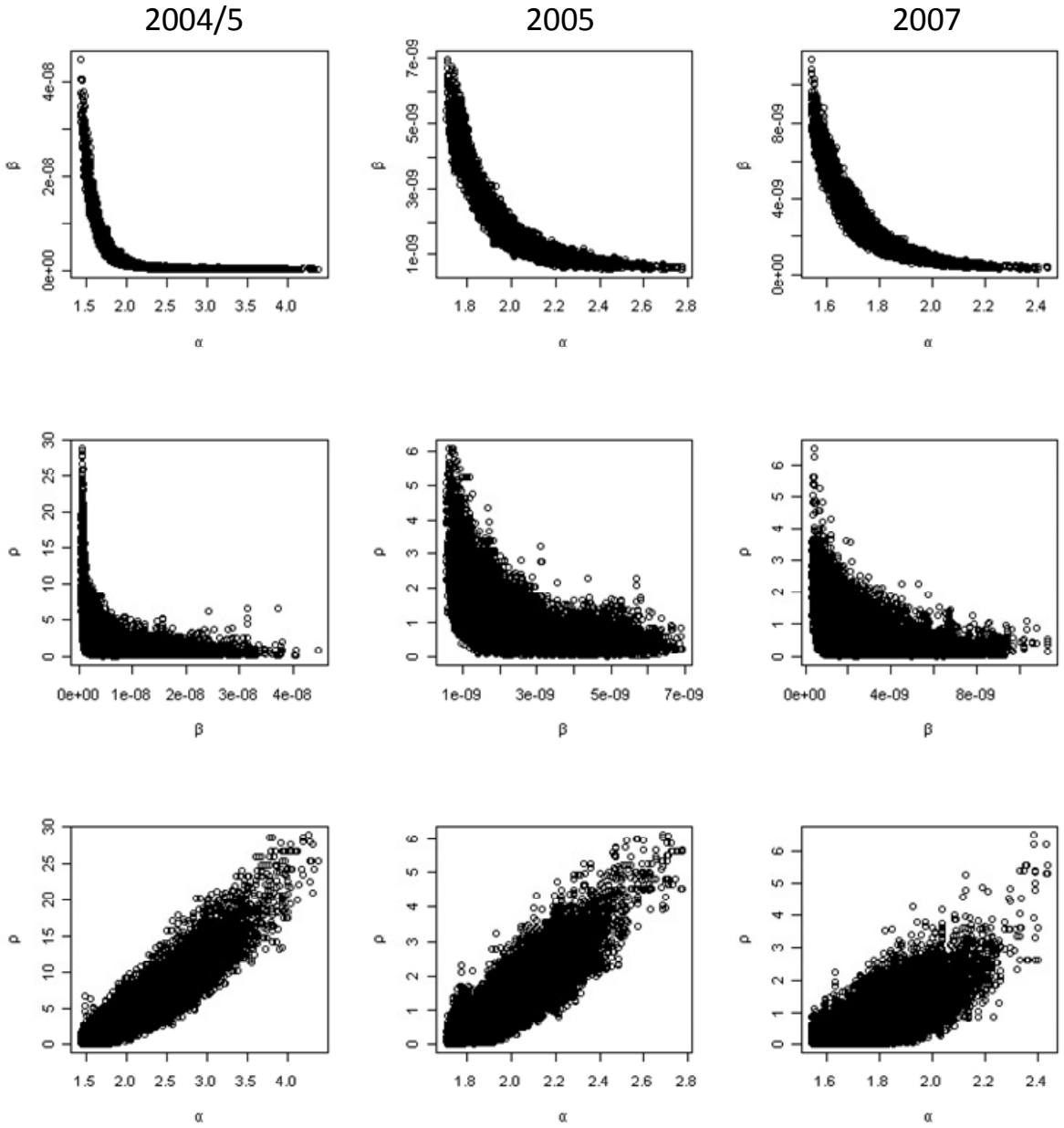


Figure 3-6| Bivariate plots of the joint posterior distributions of the fitted spatial kernel and infectivity parameters for each of the three waves.

The fitted spatial kernel varied little between the waves occurring before and during the first round of the vaccination campaign. However, the posterior mean per-bird probability of transmission during the 2007 wave appears to have lowered, especially over long-range distances (Figure 3-7(b)). As a result the expected daily number of secondary outbreaks

generated by one infected commune was, in general, substantially reduced (Figure 3-7(a) and (c)), suggesting that, whilst a commune remained infectious, transmission would take place at much slower rate compared to the previous two waves.

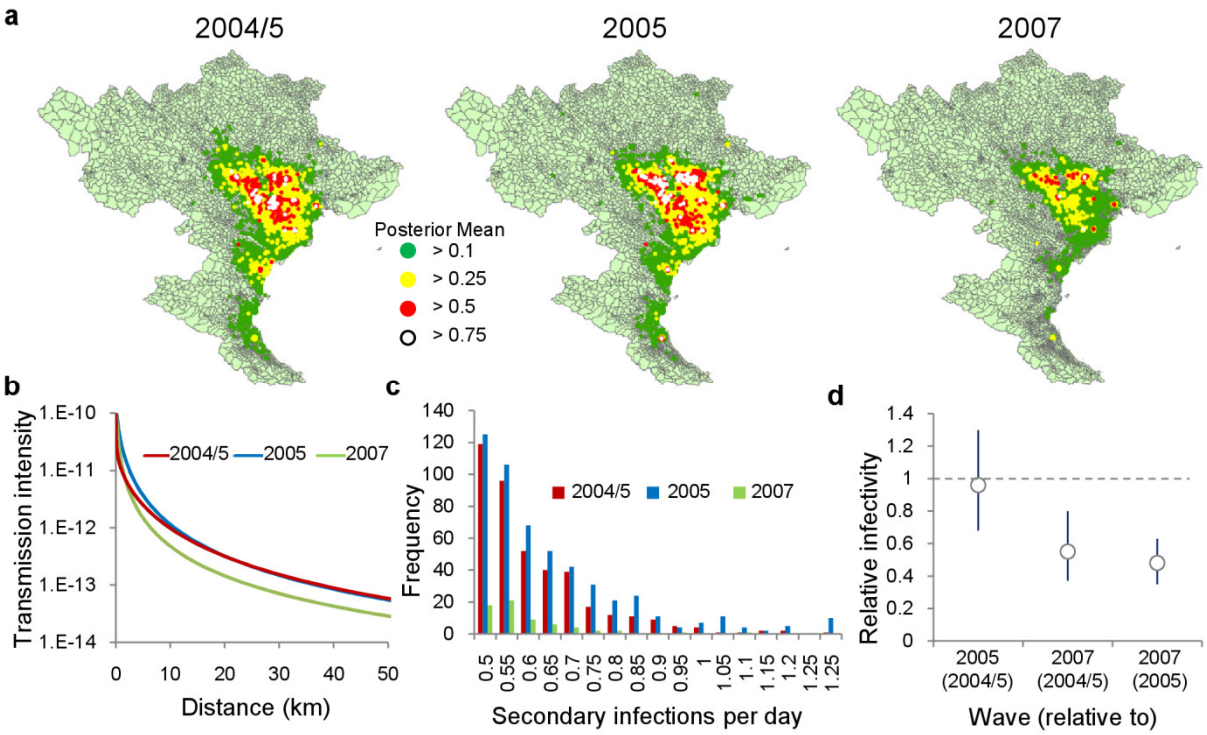


Figure 3-7| Between-commune infectivity.
(a), Risk map of the expected daily number of secondary infections arising from infected commune during each of the three waves, under the assumption all other communes remain susceptible. **(b)**, Plot of how the daily probability of between-commune transmission scales with distance, **(c)**, Histogram of commune-specific daily rate of infection (only those over 0.5 plotted), **(d)**, Relative changes in per-capita infectivity parameter between waves with associated 95% credible intervals.

Estimates of the change in infectivity occurring between each wave appear to suggest, when either unconstrained or constrained spatial kernels are used, that infectivity did not change significantly between the 2004/5 and 2005 wave. However, relative to both these waves, over distances where the vast majority of transmission was estimated to occur, both estimates suggest that infectivity was substantially lower in 2007. When this change was fitted as a proportional reduction, there was decisive evidence (BF>100) for such a reduction

with a posterior mean reduction of 55% relative to that during the 2004/5 wave and of 48% relative to that during the 2005 wave (Figure 3-6(d)). Making the assumption that changes in infectivity are caused by vaccination and not influenced by other factors including changes in the effectiveness of other control measures such as bio-security or movement restriction, this can then be compared to the effects of a vaccination campaign achieving, respectively, 45% and 52% uniform effective coverage (where effective coverage refers to the overall vaccination coverage multiplied by the protective efficacy of the vaccine).

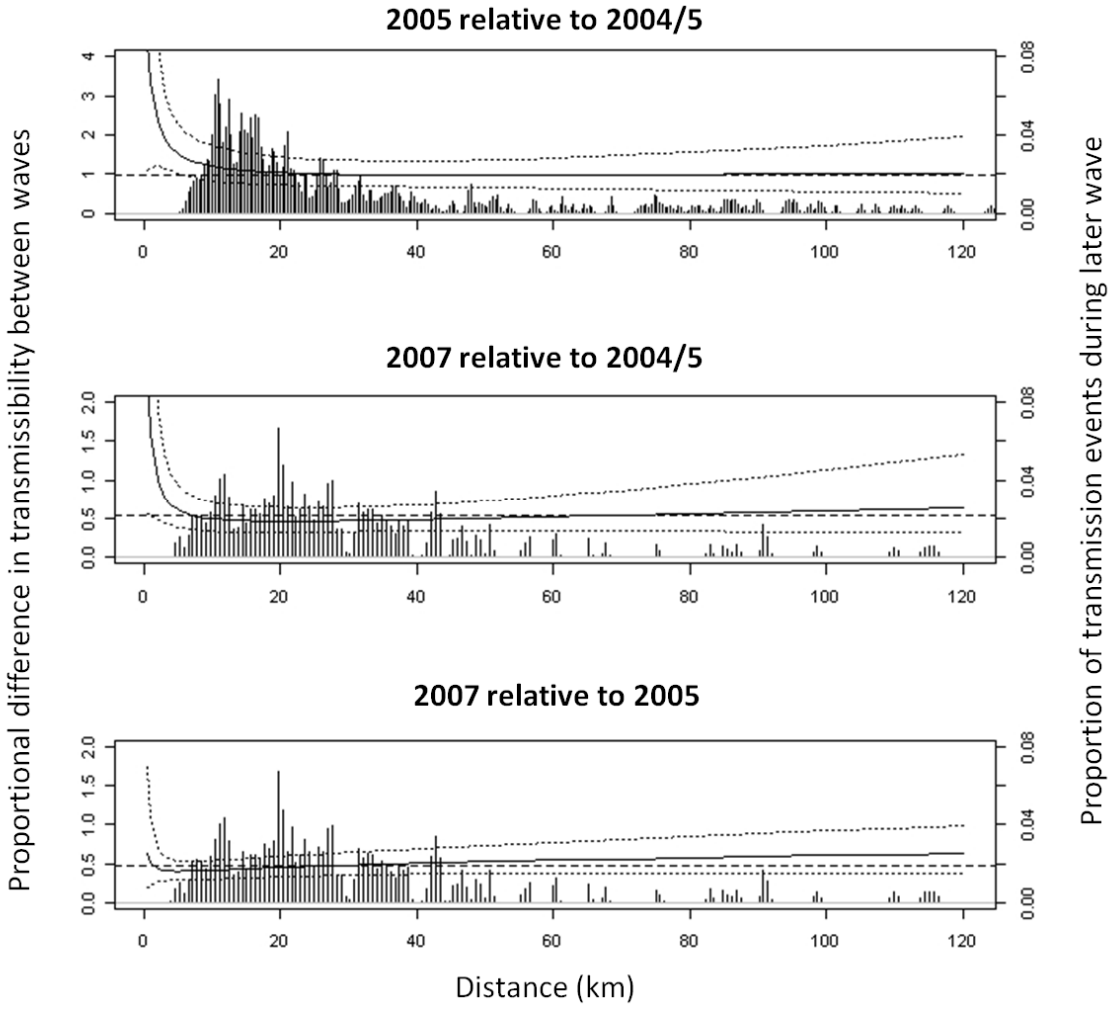


Figure 3-8| Estimates of the difference in spatial transmissibility between waves, both when the kernel of the latter wave is unconstrained and when it is constrained to match that of the earlier wave.

Solid black line shows how the posterior mean proportional difference in transmissibility varies with distance when the shape of the kernel is allowed to differ between waves, dotted lines show the associated 95% credible intervals, dashed line shows the posterior mean proportional difference when the kernel of the latter wave is constrained to match that of the earlier wave. Bars show the proportion of transmission events occurring over these distances as estimated by the reconstructed expected mean distance $\boldsymbol{D_i}$.

The posterior distribution of the infection-to-report parameters of the 2004/5 and 2005 outbreaks provide substantial evidence for the hypothesis that there was a contraction in the mean infectious period between these two waves (Bayes factor (BF)=7.88), with a point estimate of a reduction from around 6 days to 5 days (Table 3-1). As a result despite the fact infection spread more rapidly in the initial stages, the wave was brought under control more rapidly than during the 2004/5 and 2007 waves (Figure 3-11(b)). In contrast, relative to both previous waves, the average infectious period of an outbreak commune increased significantly during the 2007 wave (Table 3-1) to around 9 days, with some outbreak communes estimated to remain infectious for over 20 days (Figure 3-9(b)). This pattern can also be seen when examining the posterior mean imputed infection-to-report of individual outbreaks, with an overall imputed mean of 5.8 days in 2004/5, 4.3 days in 2005 and 8.6 days in 2007 (Figure 3-11(a)).

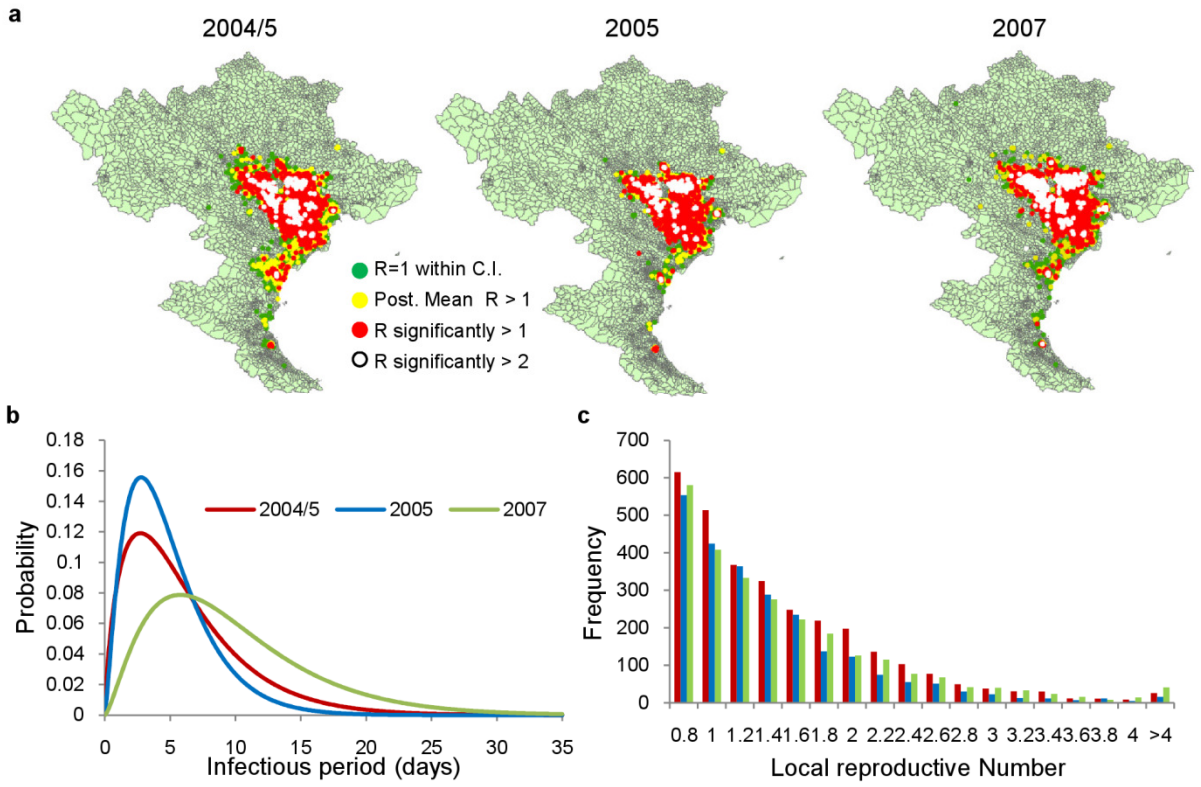


Figure 3-9| The infectious period and local reproductive numbers.
Figure shows**, (a)**, Spatial distribution of local reproductive numbers calculated analytically using the infectivity and infectious period estimates, **(b)**, Estimate of probability (assumed gamma) distribution of the infectious period during each of the three waves,**(c)**, Histogram of local reproductive numbers for the three waves (only those with values greater than 0.8 plotted).

This reduction in the rate at which infection was reported during the 2007 wave relative to the previous two waves, when used to translate the daily rate of transmission into local reproduction numbers, highlights why, despite the coincident reduction in the daily transmission probability, a wave of outbreaks could be sustained, with the increased opportunity to spread infection making transmission likely in a similar number of communes as in the previous waves (Figure 3-9(c)). Once again, the majority of transmissibility focussed around the RRD region (Figure 3-9(a)). This also may explain why, despite the relatively low intensity of transmission during the early stages of the wave, the 2007 wave was more prolonged than the preceding waves.

Overall, accounting for changes in the distribution of poultry between the 2004/5 and 2007 waves, there was an 11% reduction in the number of communes with estimated local reproduction numbers above unity (Figure 3-10)

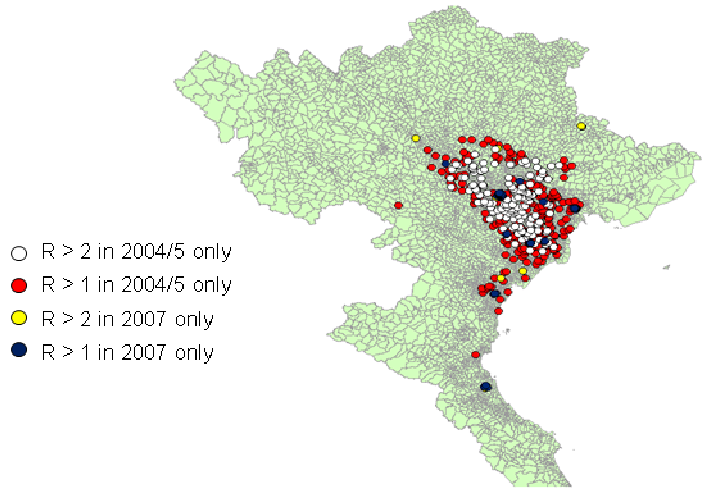


Figure 3-10| Comparing local reproductive numbers before and after vaccination.
Map highlighting communes where the mean posterior estimate of R exceeded 1 or 2 either only 2004/5 or only in 2007, with poultry populations standardised to those caluclated for 2007. Map shows an 11% (191 communes) reduction in number of communes with R>1 and a 14.1% (79 communes) reduction in those with R>2.

Transmission was more localised during the wave which took place concurrently with the vaccination campaign, with the mean transmission distance contracting by 2.5km relative to the 36.8kms estimated for the 2004/5 wave and a much higher proportion of transmission occurring over less than 20km than in either other wave. Outbreaks were most spatially dispersed during the 2007 wave where the estimated mean transmission distance was 40km with the around 5% of outbreaks estimated to have occurred more than 150km from the source of infection (Figure 3-11(c)).

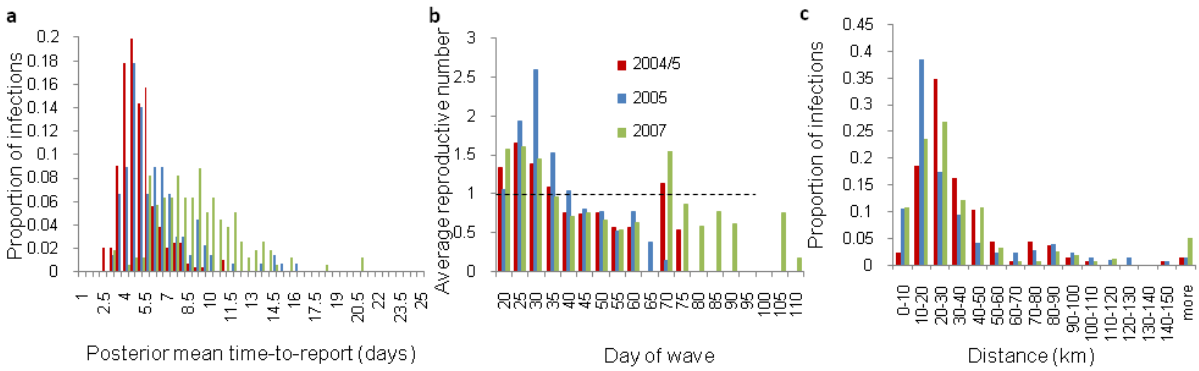


Figure 3-11| Reconstructing the epidemic tree.
(a), Density of posterior mean imputed infection to report durations, **(b)** Temporal pattern of average local reproductive numbers (see equation (3.19)) throughout the three waves, **(c)**, Proportion of transmissions occurring at different distances for each of the three waves.

Treating each outbreak as the root of a separate sub-epidemic it was found that, had all outbreaks been identified and successfully removed within two weeks of the initial infection, and had all subsequent outbreaks within the clade avoided infection for the remainder of the wave, the expected eventual size of the wave would have been reduced by 39% and the wave would have been eliminated 41% more quickly, with a 14% probability that the wave would have become extinct within the first 10 outbreaks. Moreover, if the rate of detection had been maintained to that estimated for the wave preceding mass vaccination, this probability rises to around 23%, with a 61% reduction in the size of the wave, and 42%, with an 76% reduction in the size of the wave, based upon the infection-to-report distribution of the 2005 wave (Table 3-2).

Table 3-2| Impact upon the 2007 wave of truncating the infectious period according to a shorter distribution of the time between infection and report.

Modified infectious period	Mean number of outbreaks averted (% of wave)	Mean reduction in length of wave (% of wave)	Probability of extinction within first 10 outbreaks
Truncated at 21 days	12.05 (7.6%)	17.72 days (14.0%)	0.8%
Truncated at 14 days	60.62 (38.6%)	51.71 days (40.7%)	13.8%
Truncated at 10 days	122.95 (78.3%)	86.02 days (67.7%)	49.9%
2004/5 infectious period	104.88 (60.8%)	65.83 days (51.8%)	23.4%
2005 infectious period	120.02 (76.4%)	83.24 days(65.5%)	42.6%

3.4 Sensitivity analysis of the changes in dynamics post-vaccination

The underlying assumptions made by any model about an epidemic are likely to influence the quantitative estimates of the transmission dynamics of an epidemic. As a result it is always worth assessing the sensitivity of such estimates to as wide a range of assumptions as possible. Moreover, when these estimates suggest a particular qualitative conclusion it is important to ensure that such a conclusion is not simply an artefact of the modelled assumptions. Therefore, an assessment of the sensitivity of the finding that, following vaccination, infectivity had been reduced but that outbreaks during the 2007 wave were reported less quickly, to a number of the basic underlying modelling assumptions was carried out.

3.4.1 24 hour duration between report and removal

Thus far the modelled assumption was that outbreaks in all three of the waves are removed according to the guidelines distributed to veterinary paraprofessionals in the field (FAO 2006). Thus, using a combination of movement restrictions, quarantine measures and the

immediate culling of flocks within which infection has been detected, an outbreak is removed from the wave successfully within 24 hours of the outbreak being reported. In reality, it is possible that it takes a longer time to achieve effective control in some, or even all, cases. The sensitivity of the model to this uncertainty was investigated by repeating the analysis with different report to cull durations. It was found that the estimated infection-to-report duration was negatively correlated with time between report and removal. However, as Figure 3-12 illustrates, if the duration between report and removal remains constant across all three waves, our estimates still suggest that outbreaks were not detected as rapidly during the 2007 wave.

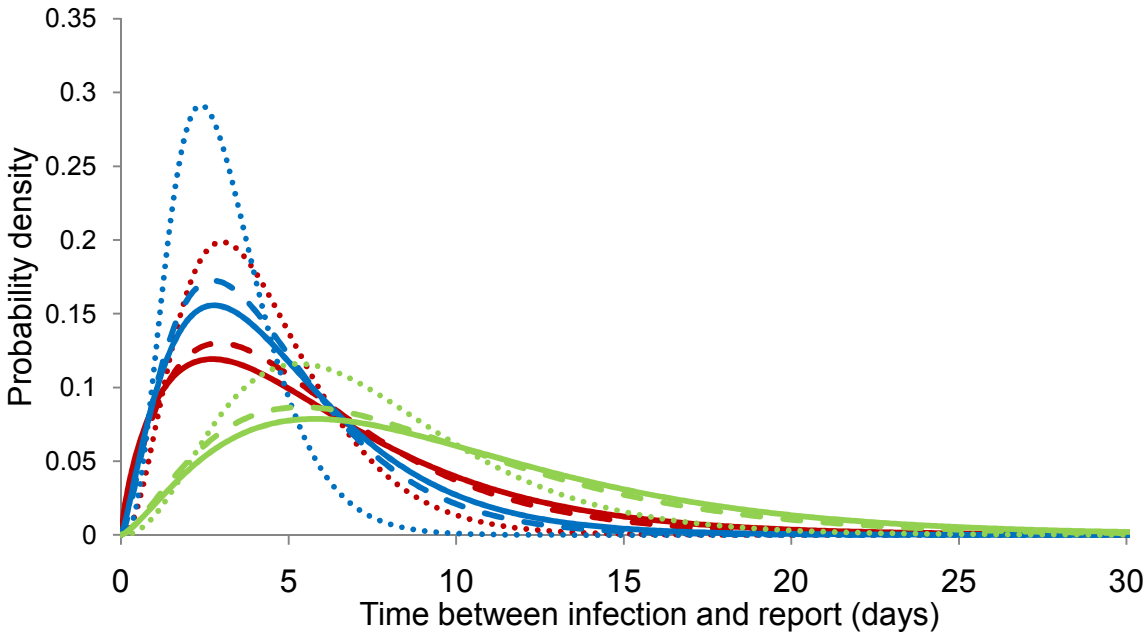


Figure 3-12| Sensitivity analysis of the time between the report of an outbreak within a commune and the removal time of the outbreak.
Estimated probability distribution of the duration between the beginning of an outbreak and the date it is reported (plotted values are the posterior mean parameter estimates) for the 2004/5 wave (red lines), 2005 wave (blue lines) and 2007 wave (green lines) under the assumption that communes remain infectious for at a constant level for 1 day (solid lines), 3 days (dashed lines) and 8 days (dotted lines).

Moreover, whilst the estimate of the reduction in per-capita infectivity following vaccination, obtained by once again fixing the kernel slope across all waves, is incrementally

muted as the duration between reporting and removal is increased across the three waves (arising from the disproportionate increase in the estimated infectious period of the two earlier waves relative to that in 2007 (Figure 3-12)), these estimates are still consistent with a decisive reduction in infectivity until the report-to-removal delay reaches approximately ten days (Table 3-3).

Another plausible scenario tested was that, as a possible consequence of the intervention being more time-consuming to implement or due to the time needed for vaccine-induced immunity to be acquired (Peyre, Fusheng et al. 2009), outbreaks in 2007 took a longer time to be effectively controlled by ring vaccination in comparison to the mass culling campaigns during the 2004/5 and 2005 waves. However, it was found that even if outbreaks took over a week longer to control in 2007, the estimated mean detection time remained greater than during the previous two waves (Figure 3-12), whereas the reduction in infectivity, calculated similarly as before by holding the kernel slope fixed across each of the waves, became more pronounced (Table 3-3).

Table 3-3| Sensitivity analysis of the assumed duration between the report and the removal of an outbreak.

Time between report and removal during 2004/5 wave	Time between report and removal during 2007 wave	Relative infectivity (95% C.I.s)
1 day	1 day	0.55 (0.37-0.8)
1 day	6 days	0.41 (0.28-0.58)
3 days	3 days	0.65 (0.45-0.91)
3 days	6 days	0.58 (0.38-7.5)
6 days	6 days	0.7 (0.53-0.96)
11 days	11 days	0.82 (0.63-1.01)

3.4.2 Constant infectivity profile

As an alternative to the assumption that the infectivity of a commune remained constant throughout the duration of the infectious period, a second scenario was explored whereby infectiousness increases monotonically until the outbreak is detected, growing rapidly during the early stages of infection but saturating quickly. For this the cumulative distribution function of an exponential distribution was used, with parameter $\delta = 2.5$ calibrated so as to level off by the 10th day of the outbreak:

$$\beta(t-u) \propto 1 - \exp\left(-\frac{(t-u)}{\delta}\right) \qquad 3.22$$

Following the reporting of an outbreak the infectivity of the commune was then assumed to decay exponentially for the following week, with a 24 hour 'half-life'.

Fitting this infectivity curve to the outbreak data it was once again found that the model estimated that, for the average duration of infection of the earlier waves, infectivity was lower following vaccination but that this was offset by a longer infectious period (Figure 3-13).

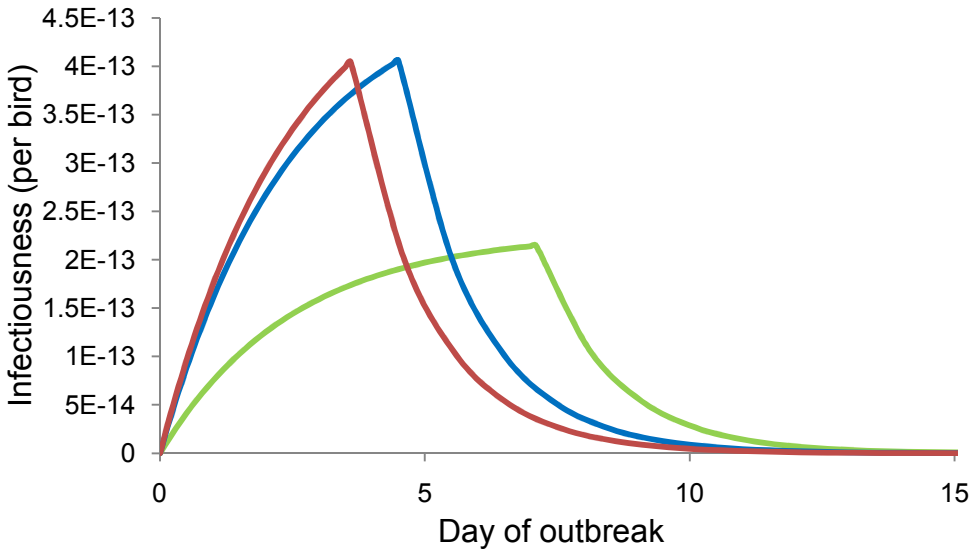


Figure 3-13| Modelling non-constant infectivity throughout the infectious period.
Estimates of infectivity for the 2004/5 (red line), 2005 (blue line) and 2007 (green line) waves. Values shown correspond to an outbreak with wave specific mean infectious period and show the per-bird instantaneous probability of transmission to a susceptible commune 25km away (corresponding to the estimated median transmission distance calculated for the 2007 wave).

3.4.3 Impact of unobserved outbreaks

Applying the fitting procedure to a wave where some outbreaks remain unobserved and unreported throughout the duration of infection would result in connecting outbreaks which are further apart in both time and space. As a result, especially at a high level of unreported outbreaks, the fitting procedure is likely to overestimate the commune-level infectious period. As this factor is of particular concern following a vaccination campaign where 'silent spread' may be an issue (Savill, St Rose et al. 2006; Webster, Peiris et al. 2006), the effect this has upon the estimate of the spatial kernel was investigated.

To do this the model was fitted to simulated data where a proportion of outbreaks were selected at random and relabelled as susceptible for the duration of the wave. As expected, it was found that the estimate of infectious period increases as the proportion of detected outbreaks decreases (Figure 3-14(a)). However, as the wave is increasingly 'thinned out' in

this way, the fitting procedure increasingly estimates transmission over longer distances. This produces a kernel which underestimates short range and overestimates long range transmissibility (Figure 3-14(b)). These results suggests that it is unlikely that an increased proportion of unobserved outbreaks during the 2007 wave could fully explain the kernel estimate for that wave, where the proportional reduction in inter-commune transmissibility increased with distance (Figure 3-6(b)). Moreover, any increase in the level of unobserved outbreaks obviously supports the conclusion that there has been a decrease in overall detection capacity. It should be noted, however, that this assumes that the ability to detect outbreaks is uniform throughout Vietnam and throughout the wave of outbreaks and also that the infectious period of an outbreak would not affect, or be affected by, whether or not the outbreak was reported. In reality it is highly probable that this will not be the case. For example, short-lived outbreaks may be less likely to be detected and unreported outbreaks may remain infectious for a longer length of time than would have been the case had they been detected.

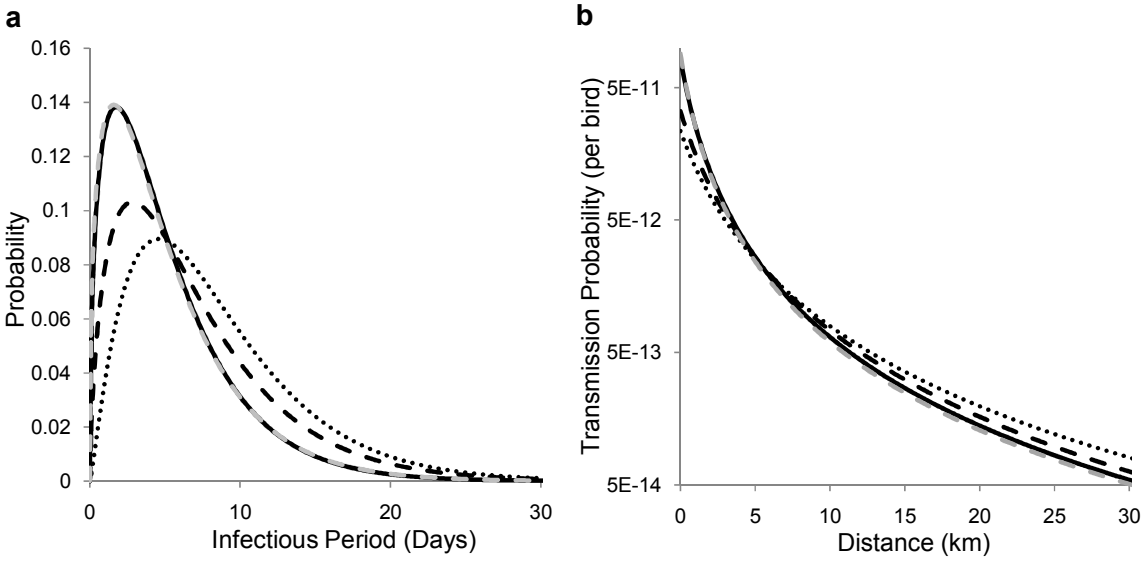


Figure 3-14| Simulation study of the effect upon model estimates of underreporting.
(a), fitted commune level infectious period, **(b)**, fitted spatial kernel. Plotted values are those used to generate the simulated outbreak (dashed grey lines), those calculated using the posterior mean model parameter estimates obtained from the complete simulated dataset (solid black lines), the overall mean posterior parameter estimates from fitting the model to five datasets where 20% of outbreak are randomly not observed and therefore treated as susceptible throughout the wave (dashed black lines) and five datasets where 50% of outbreaks are randomly not observed.

3.5 Discussion

This analysis demonstrates the application of data augmentation techniques to existing livestock disease modelling methodologies in order to quantify the spatial and temporal spread of disease in a setting where the times of infection are unobserved. It was found that, following the implementation of vaccination, the day-to-day probability of infection spreading between communes was significantly reduced, with the estimate of effect comparable to 45% effective vaccination coverage suggesting that population immunity may not have fallen too far below the level estimated from post-campaign sero-surveillance (Taylor and Dung 2007). However, the duration of time taken to report outbreaks had also increased significantly, allowing infection to spread. This result appears to support the

hypothesis that mass vaccination can make outbreaks harder to detect. One way in which it has been suggested this could occur is if a small proportion of birds in a flock go unvaccinated as the lower level of mortality may make outbreaks harder to detect or if vaccination leads to the asymptomatic transmission of infection between birds (Savill, St Rose et al. 2006). In Vietnam, where backyard poultry already experience high degree of background mortality, any reduction in mortality due to infection with H5N1 within an infectious flock is likely to have strong implications for the ability to detect signs of circulating infection, especially if farmers believe their birds are protected. Moreover, at a commune-level scale, it may also be the case that outbreaks are only likely to be reported when a high number of flocks have been affected by the disease. As a result, even if vaccination is completely effective at providing protection from infection within a flock, if a sufficient proportion of flocks remain unvaccinated in order to sustain transmission within the commune with a lower number of affected flocks, infection may become more difficult to detect. Such "silent spread" has been demonstrated in experiments where, as a result of a single inoculation, birds were only partially protected and infection could transmit asymptomatically (van der Goot, van Boven et al. 2007). However, whilst it was suggested by outbreak investigators that infection was harder to detect in 2007 (To, Bui et al. 2007), this is the first quantitative evidence of a reduction in detection capacity on a macroscopic scale.

Although the finding of a reduction in infectivity coupled with a decrease in the rate at which outbreaks were reported following the introduction of infection into a commune proved robust to different assumptions about the infectivity profile of a commune and the rate at which outbreaks where controlled following detection, there are other potential

factors which could not be controlled for in the analysis. In particular, it wasn't possible to quantify the extent to which the model estimates may have been affected by unobserved outbreaks occurring outside the dataset. It was found that, if uniformly distributed in space and time, unobserved outbreaks have the affect of inflating the estimated infectious period and underestimating short range transmission whilst overestimating long range transmission. However, it was not possible to assess the extent to which clusters of unreported outbreaks may have affected the obtained result. Future work could potentially address the issue of unreported outbreaks within the study region by allowing the data augmentation algorithm to also impute entire outbreaks (O'Neill and Roberts 1999; Chis Ster, Singh et al. 2008; Jewell, Keeling et al. 2008). However, this would not address the issues associated with the potential for infection to be imported from outside the study region, in particular in neighbouring countries such as Laos, Cambodia and China where data on both the prevalence of infection and the population of poultry at risk were not available.

Similarly, whilst these qualitative findings were also robust to an increase in the time between the report of an outbreak and its removal from a wave, the assumption that this duration is constant across all outbreaks is unlikely to be realistic. Future work could focus upon estimating this distribution by including the time between the report and the removal of each outbreak as additional model parameters. This may also provide insight as to whether the speed and effectiveness of the response to reported outbreaks varied between the three waves. Such an analysis would, however, add to the already high computational demands of the model fitting procedure.

It may also be the case that the assumption that transmissibility between communes is a function of Euclidean distance may not realistically represent the spread of infection in

Vietnam, particularly over long ranges where the risk of infection may also depend upon measures of accessibility such as the provision of roads or transport. If these data were available it may have been possible to find a better fitting model which incorporates a more realistic measure of the shortest effective distance between communes, although the reliability of such a measure based upon distance by road could equally be affected by factors such as the distribution of live bird markets or slaughterhouse facilities which are likely to act as key nodes in the spread of infection (Fournie, Guitian et al.).

Inferring the exact mechanism by which outbreaks remained unreported for longer is not straightforward from the existing data. For example, there does appear to have been a shift in the distribution of host species involved in reported outbreaks. During the 2004/5 wave, of outbreaks were there were data on the species involved, only 30% were identified as ducks whereas in 2007 this figure rose to 77% (Minh, Morris et al. 2009). This change has previously been attributed to the lifting of a duck-hatching ban in February 2007 with outbreaks also coinciding with the end of rice-harvest season when ducks are allowed to graze freely on rice paddies and are therefore harder to vaccinate (Peyre, Fusheng et al. 2009). This, combined with the fact the wave did not follow the seasonal pattern associated with previous epidemics and the lower level of pathogenicity associated with H5N1 infection in waterfowl, makes it tempting to conclude that ducks may be acting as a reservoir of infection which is difficult to detect, thus contributing to a longer commune-level infectious period. However, the longer ranges over which transmission was estimated to take place during 2007 wave may suggest that some outbreaks went completely reported and, even within communes where outbreaks are reported there is no way to confirm that the flock in which disease is reported was responsible for introducing infection into the commune. In

fact, if silent spread was occurring, it seems plausible that flocks reported in outbreaks, many of which had not been vaccinated, may have been subject to an ascertainment bias based upon higher pathogenicity of infection relative to vaccinated flocks within which infection was circulating. Changes in the transmissibility or reporting rates of outbreaks may also be linked with other factors such as differential vaccine efficacy or observed changes in the genotype of the virus (Nguyen, Nguyen et al. 2008).

It was estimated that a large proportion of transmission during the wave occurring concurrently with the first round of vaccination took place over relatively short distances. Such an localised infection could be due to the movement of vaccinators who, it has been suggested, may have acted as carriers of infection from one commune to another (Pfeiffer, Minh et al. 2007). It is also interesting that the obtained estimates didn't suggest a reduction in the daily per-capita poultry transmissibility during the 2005 wave, despite the concurrent vaccination campaign which was taking place. This may suggest that outbreaks predominantly occurred within large regions yet to be vaccinated or in areas where vaccinated poultry had yet to develop effective immunity.

It was also demonstrated how imputed infection times can be used to explore all possible chains of infection and to assess the range of possible effects which can be achieved by 'pruning' an outbreak from the epidemic tree. Applying this to outbreaks occurring during the 2007 wave, it was found that if the reporting rates had been maintained to those achieved during the previous waves, the size and the duration of wave would have been considerably diminished. It should, however, be noted that this analysis made the strong assumption that, having avoided the transmission event which led to it becoming initially infected, the "pruned" outbreak commune remained uninfected for the remainder of the

wave. As a result, this is likely to represent an over-estimate of the impact such improved surveillance would constitute. One method which avoids this issue which could be used in future analyses would be to adopt the approach of *Cook et al* which uses the Sellke construction whereby individuals are assigned a threshold which, when passed by the infectious pressure accumulated during the wave, results in infection. It is then possible to use this concept of infectious pressure to infer both the infectious status of such a pruned outbreak throughout the remainder of the wave and the resultant effect this has upon the trajectory of the epidemic (Sellke 1983; Cook, Gibson et al. 2008).

The finding that, despite a sizeable reduction in infectivity, infection was still able to spread following a decrease in the rate at which outbreaks were reported does, however, highlight the fact that, in order to ensure that such reductions in infectivity achieved through vaccination are translated into a noticeable impact on outbreak size, it is essential that rates of reporting are maintained through adequate surveillance. Measures towards this have already been attempted in Vietnam with the trial of a pro-active surveillance programme whereby resources are allocated according to known risk factors such as the presence of live bird markets and high semi-commercial farm density and a prior history of outbreaks (Desvaux, Do et al. 2007). The programme also included an awareness campaign informing stakeholders about how outbreak detection criteria should change following the vaccination of a flock. An alternative approach is to use unvaccinated birds, or those vaccinated with an inactivated heterologous strain of avian influenza, as sentinels for the detection of the spread of infection. This has been implemented under the Differentiating Infected from Vaccinated Animals (DIVA) guidelines in industrial production systems such as those in Italy

and the U.S. and has been successful in helping to control outbreaks of low pathogenic avian influenza (LPAI) (Capua, Cattoli et al. 2004; Suarez 2005).

The current GETS project in Vietnam, which includes a trial of the use of sentinel birds in vaccinated flocks, age-based vaccination of ducks and active surveillance of mobile duck flocks in five high and low risk provinces(FAO 2010), may provide valuable insight as to how increasing detection capacity can be achieved and, as Vietnam enters a period of transition towards targeted vaccination in the ongoing attempts to control the spread of H5N1, how this may be harnessed in order to manage the spread of infection in a sustainable way.

Chapter 4 Outbreaks of H5N1 in poultry in Thailand: The relative role of poultry production types in sustaining transmission and the impact of active surveillance in control.

4.1 Introduction

Outbreaks of H5N1 spread widely throughout Thailand, with two large waves occurring between late 2003 and mid-2005 (Tiensin, Nielen et al. 2007). However, in terms of the level of control currently achieved relative to the scale of the epidemic during these years and the measures employed to obtain this, the Thai experience of H5N1 is unique to the region. Unlike other countries and territories including China, Hong Kong, Vietnam and Indonesia where large-scale waves of outbreaks resulted in the adoption of mass vaccination strategies, the use of vaccination has remained prohibited in Thailand, a decision which has been strongly supported by the large industrial exporters (Safman 2010). Instead Thailand pursued a policy of stringent movement restrictions, including 90-day bans on movement from or through affected areas unless a permit had been obtained from local authorities, and a campaign to restrict chicken production to closed farms. These policies were aimed towards enhancing bio-security and preventing the circulation of infection in small-holder flocks (NaRanong 2007), the type of flock reported as infected in the majority of recorded outbreaks (Tiensin, Chaitaweesub et al. 2005).

The implementation of these measures also coincided with a concerted effort by industrial producers to bring aspects of the supply chain such as hatching and slaughtering 'in-house',

with this trend towards such vertically integrated production fuelled by a desire to reduce the risk of infection and improve access to overseas markets (NaRanong 2007; Safman 2010). Since the second wave of outbreaks came to a conclusion in mid-2005, the spread of infection has successfully been reduced to low levels. However, the extent to which the measures aimed towards improving the bio-security of small-holders were responsible for achieving this level of control can only be assessed accurately if the extent to which small-holders played a role in driving the transmission of infection is known.

Poultry production in Thailand differs markedly from that in neighbouring countries. Rather than a system where the majority of poultry are raised extensively by smallholders and consumed locally (both in terms of the number of poultry produced and income generated) production is dominated by relatively few large-scale industrial integrators, many of whom play a role in Thailand's large poultry export market (Rushton, Viscarra et al. 2005).

It has been estimated that the overall odds of an outbreak being reported in backyard flocks were significantly lower than within commercial layer flocks in Thailand, apart from southern provinces where there was no significant difference, and lower than within commercial broiler flocks in northern and central provinces (Otte, Pfeiffer et al. 2006). This challenges the general assumption that the epidemic had been primarily spread by small-holders. However, to date, no attempt has been made to quantify the respective roles of these two sectors in propagating the spread of infection directly.

As part of the 'X-ray' surveillance campaigns deployed by the Thai authorities in response to the developing second wave of infection, detailed data were collected at a sub-district (otherwise known as "tambon") level. As a result the composition of poultry production in both tambons which experienced infection and those which remained uninfected at the

time of the wave are available, not only in terms of the number of poultry or flock, but also the composition of these poultry in terms of production type and species (Tiensin, Chaitaweesub et al. 2005).

In this chapter data from outbreak reports obtained from these surveillance campaigns and those from the existing surveillance infrastructure are used to fit a model of the spatial spread of the second wave of H5N1 in 2004/5 similar to that of the previous chapter but incorporating large-scale industrial production. From this the relative roles of commercial and backyard chickens, as well as those of ducks and geese, in the transmission of infection are investigated to assess whether the control policies implemented, with the severe implications for smallholders they entailed, are likely to have been effective. Particular focus is also paid as to whether 'X-ray' surveillance was successful at increasing the extent to which outbreaks were rapidly detected.

4.2 Materials and Methods

4.2.1 Description of data

Spatial information in the form of a shape-file with the administrative boundaries at the tambon level was obtained from the Geo-informatics and Space Technology Development Agency and the Department of Land Development, Thailand. Data on the outbreaks and poultry population were obtained from the Department of Livestock Development.

Poultry census data were collected as part of the first 'X-ray' survey between October and mid-November 2004. The data included the number of backyard chickens, broilers, layers, quails, turkeys, geese, meat and free-grazing ducks and the number of flocks of each type within each tambon.

Data on H5N1 outbreaks reported between January 2004 and November 2005 were compiled by the Department of Livestock Development and comprise the date at which the outbreak was reported, the tambon within which the outbreak was detected and the species and production type of the flock in which disease was detected (Figure 4-1a).

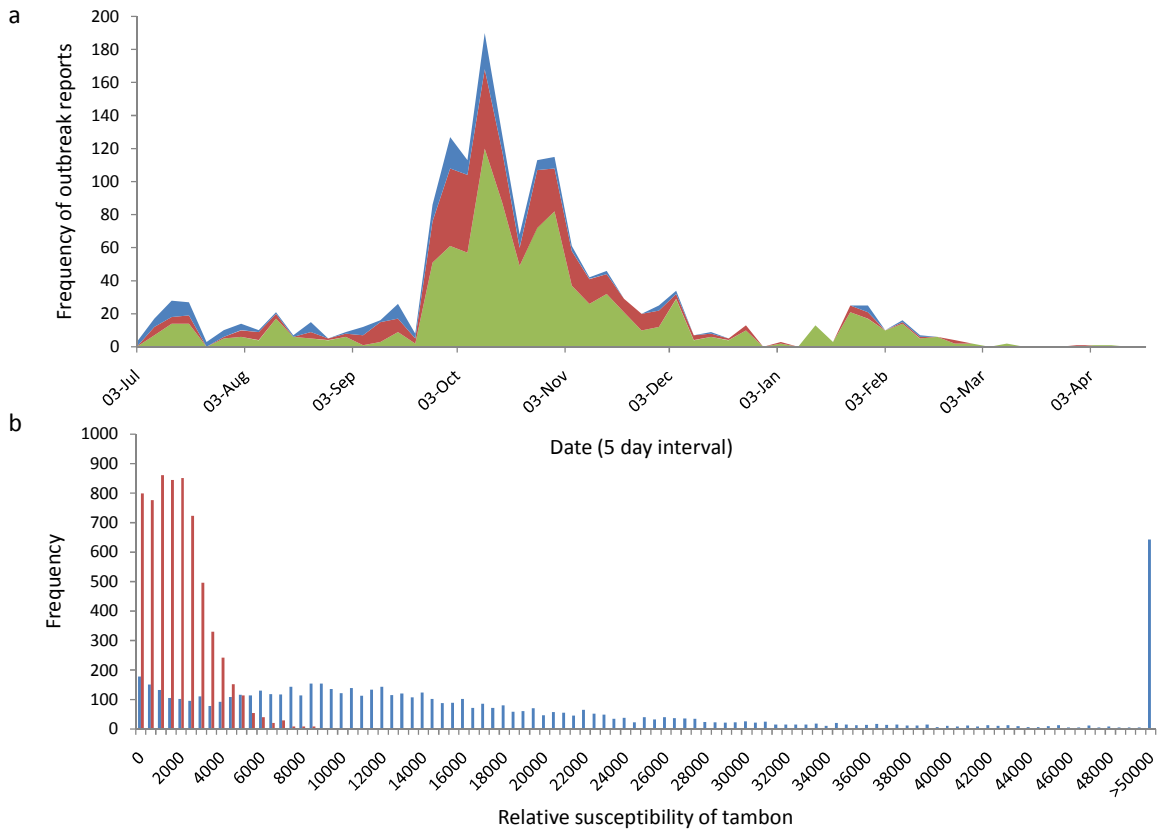


Figure 4-1| Outbreak and poultry population data.
(a), Outbreak report data by date of report and production type: Commercial chicken (blue), ducks and geese (red), backyard chicken and other (green), **(b)**, tambon-level susceptibility histogram: Tambon poultry population corresponding to linear assumption (blue columns) and logarithm of mean flock size within tambon multiplied by number of flocks in tambon corresponding to logarithmic assumption (red columns).

4.2.2 Data assumptions

Outbreak locations were calculated as the tambon centroid. Whilst uninfected, all poultry within the tambon were assumed to be susceptible to infection. In the absence of data on the size of flocks in which infection occurred, infection was assumed to occur independent of flock size with the number of infectious birds during an outbreak calculated as the

number of flocks within which infection was detected multiplied by the average size of a flock within the tambon. In three instances an outbreak was reported within a tambon which, according to the census, contained no poultry. In this case the number of poultry was calculated as the average number of backyard birds in all adjacent tambons, making the assumption that the presence of commercial premises would have been recorded.

Fitting of a model using a Euclidean spatial kernel to all outbreaks in Thailand would involve measuring transmissibility between country borders and over long stretches of ocean. The study region was therefore confined to the 6403 northernmost tambons in order to produce a more convex area. As a result, outbreaks and poultry data in Southern Thailand were not included in the analysis.

The lengthy duration of the wave resulted in a high number of tambons experiencing outbreaks during multiple distinct time periods. As in the previous chapter, outbreak reports within 14 days of each other in the same tambon were treated as being part of the same outbreak, leaving a total of 136 tambons where there was a period of more than 14 days between outbreaks. In these instances outbreaks were classified according to the Thai control policies prohibiting movement and restocking of birds in infected areas for 90 days following the confirmation of an outbreak (NaRanong 2007; Safman 2010). Tambons with outbreaks re-occurring later than 14 and less than 90 days following the previous report were assumed to have remained susceptible throughout, with the number of susceptible birds halved following the controlling of the earlier outbreak. Outbreaks occurring more than 90 days following a previous outbreak were assumed to be the result of restocking and the tambon was assumed not to be susceptible to infection during those 90 days. All tambons were assumed to become re-susceptible to infection 90 days following an outbreak

with the susceptibility to infection equal to that estimated before the tambon was first infected.

4.2.3 Modelling homogenous infectivity and susceptibility

To begin with, all heterogeneity in the type of poultry production was ignored and two assumptions as to how infectivity and susceptibility scale with the number of poultry within a tambon were explored. The first is that they both scale linearly, i.e. the instantaneous force of infection exerted upon a susceptible tambon i by an infectious tambon j is:

$$\lambda_{ij} = \beta x_i y_j k^*(d_{ij}) \quad 4.1$$

where β is the transmission intensity parameter and $k^*(d_{ij})$ is the normalised spatial kernel, parameterised in the same way as in the previous chapters, evaluated at distance d_{ij}, $x_i = n_i$, the number of birds in tambon i, and y_j is the estimated number of infectious birds in tambon j. For the linear scaling assumption this is calculated by multiplying the average flock size, $\overline{x_j} = n_j / f_j^n$ (where f_j^n is the number of flocks within the tambon) by the number of flocks in which an outbreak was reported, f_j^y. The second assumption explored was that the infectivity and susceptibility of each individual bird within a flock saturates as the flock becomes large and, as a result, the flock-level risk of infection scales logarithmically with the size of a flock. With this assumption the natural logarithm was taken of the average flock size within the tambon and y_i and x_i were calculated by multiplying this by, respectively, the number of infectious and susceptible flocks within the tambon (see Table 4-1).

The outbreak wave was modelled at a sub-district (tambon) level resolution as in the previous chapter for Vietnam. However, here the likelihood of an infection event occurring

between tambons at time at u_i (equation (3.8)) was altered to additionally utilise data on number of flocks within the tambon in which infection was observed. Whilst the dynamics of infection within the tambon were not explicitly modelled, the modification takes into account the fact that flocks in which infection was not reported would have remained susceptible to infection from other infectious tambons until the date the outbreak was removed. Thus the likelihood that tambon i is infected at time u_i and removed (culled) at time r_i is given by:

$$p_i(u_i, r_i) = \left[1 - \exp\left(-\sum_{j=0}^{N} \beta x_i y_j k^*(d_{ij}) I(j \text{ infectious on } u_i)\right)\right] \times \exp\left(-\int_{t=u_i}^{r_i} \sum_{j=0}^{N} \beta(x_i - y_i) y_j k^*(d_{ij}) I(j \text{ infectious on } t)\, dt\right) \quad (4.2)$$

Here the term in square brackets represents the likelihood that the tambon became infected at time u_i and the second exponential term represents the likelihood that the non-infected flocks avoided infection from any infectious source outside of the tambon until the tambon was culled at time r_i.

As a result, the likelihood of the overall epidemic becomes

$$P(U, R|\theta) = \prod_{i=0}^{N} \left\{ q_i(T)^{\left(1 - I(u_i < T)\right)} \left(q_i(u_i) p_i(u_i, r_i) f(r_i - u_i; \alpha) \right)^{I(u_i < T)} \right\} \quad (4.3)$$

Where $q_i(u_i)$ and $f(r_i - u_i; \alpha)$ are, respectively, the likelihood of tambon i avoiding infection until the time of infection u_i (as defined in equation 3.4) and that the outbreak is subsequently reported $r_i - u_i$ days later according to a Gamma-distributed infection-to-report distribution (as described in chapter 3).

4.2.4 Introducing heterogeneity in production type

Outbreak reports and poultry population data were disaggregated into three separate production types (Figure 4-1(a)):

1. commercial chicken
2. ducks and geese (classed together due to their similar methods of production (Otte, Pfeiffer et al. 2006))
3. backyard chickens and any other species including wild birds.

Heterogeneous between-tambon transmissibility with regards to these types of poultry production was then incorporated into the model using type-specific infectivity and susceptibility parameters (Chis Ster and Ferguson 2007). This involved modelling the force of infection with five additional infectivity parameters:

$$\lambda_{ij} = \left(\varphi^1 {x_i}^1 + \varphi^2 {x_i}^2 + {x_i}^3\right)\left(\psi^1 {y_j}^1 + \psi^2 {y_j}^2 + \psi^3 {y_j}^3\right)k^*\left(d_{ij}\right). \qquad (4.4)$$

Here the three ψ are infectivity parameters for commercial chicken, duck and geese and backyard flocks respectively and the two φ are the susceptibility of commercial chicken and duck and geese flocks relative to backyard flocks. The x_i and y_j terms represent the number of infectious and susceptible birds of each type within the tambon which was estimated in the same way as for the basic model but using the type-specific number of poultry, poultry flocks and outbreak reports (${n_i}^k$, f_i^{nk} and f_i^{yk} respectively for $k = 1,2,3$).

Table 4-1| Characteristics of the four transmission models fitted to the data

Model	Production-type parameters	Calculation of susceptible and infectious poultry
Model 1	No	$y_i = f_i^{\,y}\overline{x_\iota}$ $x_i = n_i$
Model 2	No	$y_i = f_i^{\,y}\ln(1+\overline{x_\iota})$ $x_i = f_i^{n}\ln(1+\overline{x_\iota})$
Model 3	Yes	${y_i}^k = f_i^{\,yk}\overline{{x_\iota}^k}$ ${x_i}^k = {n_i}^k$
Model 4	Yes	${y_i}^k = f_i^{\,yk}\ln(1+\overline{{x_\iota}^k})$ ${x_i}^k = f_i^{\,nk}\ln(1+\overline{{x_\iota}^k})$

Once these parameters have been fitted to the outbreak data the relative roles of each production type in driving transmission during the wave can be investigated by reconstructing the epidemic tree. From a given set of infection times, the probability (conditional on these infection times, the observed outbreak data and values of the other model parameters) that tambon i is infected by infectious poultry of type m in tambon j is

$$\mu_{ij}^{m} = \frac{\psi^m {y_j}^m s_{ij}}{\sum_{k=0}^{N}(\psi^1 {y_k}^1 + \psi^2 {y_k}^2 + \psi^3 {y_k}^3)s_{ik}}, \tag{4.5}$$

where $m = 1,2,3$ refers to, respectively, commercial chicken, backyard chicken and ducks and geese and the total susceptibility to infection from tambon j of the flocks eventually infected in tambon i on the day they were infected

$$s_{ij} = (\varphi^1 {y_i}^1 + \varphi^2 {y_i}^2 + {y_i}^3)k^*(d_{ij})\mathbb{1}(j \text{ infectious on day } i \text{ infected}). \tag{4.6}$$

These type-specific infection probabilities can then be summed to obtain the number of tambons infected by tambon i which are the result of infected flocks of production type m:

$$T_i^m = \sum_{\mathrm{j}=0}^{\mathrm{N}} \mu_{ji}^m. \quad (4.7)$$

4.2.5 Model fitting procedure and assessment of model adequacy

A similar MCMC algorithm to that used in the previous chapters was implemented with the model parameters sampled on a logarithmic scale and updated according to a classical random-walk Metropolis-Hastings algorithm and infection-to-report durations updated according to an independence sampler with a uniform proposal distribution between 1 and 30 days. Convergence was determined visually by running the chain from different initial values. For the basic model a burn-in period of 10000 steps followed by 100000 steps was deemed adequate. Acceptance probabilities of 10-35% were deemed adequate.

The two possible assumptions about the way in which infectivity and susceptibility scale (linearly or logarithmically) and the choice as to whether production-type-specific infectivity and susceptibility parameters were included in the model led to a total of four separate models being fitted to the data. These were labelled Models 1-4 according to their characteristics as described in Table 4-1.

Poultry type-specific model parameters were given the prior distributions listed in Table 4-2 and all other model parameters were given the same priors as those used in Chapter 3. The relative susceptibility φ^1 and φ^2 were given log-normally-distributed priors in order to ensure adequate prior probability was assigned to the possibility that the values of these are between 0 and 1. An analysis of the sensitivity of these parameters to the chosen standard deviation of their prior distributions was also conducted (Table 4-2).

Table 4-2| Prior distributions of the model parameters

Parameter	Description	Prior distribution
$\boldsymbol{\psi^1}$	Infectivity of commercial chicken	$U(0,10000)$
$\boldsymbol{\psi^2}$	Infectivity of ducks and geese	$U(0,10000)$
$\boldsymbol{\psi^3}$	Infectivity of backyard chicken	$U(0,10000)$
$\boldsymbol{\varphi^1}$	Susceptibility of commercial chicken relative to backyard	$LN(0,3)$ (Baseline) $LN(0,1)$ $LN(0,5)$
$\boldsymbol{\varphi^2}$	Susceptibility of ducks and geese relative to backyard	$LN(0,3)$ (Baseline) $LN(0,1)$ $LN(0,5)$

For both φ parameters, all prior distributions tested have been listed and the chosen baseline prior has been specified.

The data augmentation techniques used to fit these models to the data, which avoid directly integrating the entire likelihood with respect to the uncertainty in the date at which infection was introduced into a tambon, make it difficult to calculate established measures of model fit such as the deviance information criterion. Instead, using an approach formulated by *Cauchemez et al* (Cauchemez, Bhattarai et al. 2011), an exploratory procedure was carried out, comparing the predictive distribution of the times of infection on each day of the wave with next-step-ahead predictions based upon the predictive distribution of infection times prior to that day, in an attempt to assess whether, for any of the models, there are any stages of the wave where the fitted parameters predict an abnormal level of transmission.

Denoting $U_{t-1}(U)$ the dates of infection of tambons infected before day t and

$$n_t(U) = \sum_{j=0}^{N} \mathbb{1}(u_i = t) \quad (4.8)$$

the number of tambons infected on day t, the predictive distribution $n_t|R$ can be sampled directly using the imputed infection times at regular stages of the MCMC. Samples of the joint posterior distribution of the model parameters and imputed infection times can then be used to simulate a prediction of the number of newly infected tambons on each day given the imputed history of the infection process up to that day, providing a sample of the next-step-ahead distribution $n^{+1}{}_t|U_{t-1}, \theta$. The probability that the "reconstructed" predictive value of n_t is less than or equal to the "expected" next-step ahead prediction is then

$$P^{\leq} = \int \mathbb{1}(n_t(U) \leq n^{+1}{}_t) P(n^{+1}{}_t|U_{t-1}(U), \theta) P(U, \theta|R) dn^{+1}{}_t dU d\theta. \quad (4.9)$$

The probability that the reconstructed value of n_t is greater than or equal to the next-step ahead prediction, $P^{\geq}$ can be similarly obtained. The assumption of adequacy for the model fit on this day was then rejected if $P^{\leq}$ or $P^{\geq}$ was less than 10% (Cauchemez, Bhattarai et al. 2011).

4.2.6 Investigating the effects of 'X-ray' surveillance

The stated aim of 'X-ray' surveillance is to provide a cross-sectional view of the H5N1 status of poultry throughout Thailand. This involved implementing measures such as sampling all sick poultry and assessing every household for poultry die-offs aimed at providing additional detection capacity during that period. As a result, one measure of whether this policy was effective is likely to be the speed at which outbreaks were reported following the introduction of infection. In order to investigate this a supplementary analysis was

implemented where two separate Gamma-distributed infection-to-report distributions were fitted, one to outbreaks reported during periods where 'X-ray' surveillance was taking place (1-31 October 2004 and 1-28 February 2005) and a second to outbreaks reported outside these periods.

4.3 Results

Each model was successfully fitted to the data, with estimates of the mean infectious period varying between 7.8 and 8.8 days (Table 4-2). The estimates of production-type-specific relative susceptibility parameters, in particular the susceptibility parameter for commercial flocks φ^1**,** proved to be quite sensitive to the choice of prior distribution. However, the choice of prior had no effect upon the overall estimate of the respective role of each production type in propagating the epidemic, estimated using the reconstructed epidemic tree (Table 4-3).

Table 4-2| Posterior mean and 95% credible intervals for each of the 4 fitted models

Parameter	Posterior Mean (95% Credible Intervals)			
	Model 1 (Basic linear)	**Model 2 (Basic logarithmic)**	**Model 3 (Type-specific linear)**	**Model 4 (Type-specific log)**
Kernel power	1.88 (1.69-2.08)	2.80 (2.53-3.15)	2.88 (2.55-3.32)	3.11 (2.76-3.58)
Kernel offset	6.34 (3.28-10.34)	11.07 (6.93-17.10)	13.25 (7.69-21.49)	14.87 (9.07-23.13)
Infectivity	0.044 (0.040-0.048)	48.79 (44.60-53.65)	see Table 4-3	see Table 4-3
Mean infectious period	8.77 (7.78-9.81)	8.37 (7.15-9.70)	8.43 (7.38-9.61)	7.79 (6.85-8.66)
Infectious period variance	33.45 (25.84-40.80)	26.07 (17.06-33.78)	27.25 (20.91-34.03)	23.66 (17.53-30.62)

For Model 3 and Model 4 values shown are from the model fitted using baseline priors.

Table 4-3| Analysis of the sensitivity of the production-type-specific transmission parameters and role in transmission to the choice of relative susceptibility prior distributions.

	Model 3			Model 4		
Prior	LN(0,1)	LN(0,3) (baseline)	LN(0,5)	LN(0,1)	LN(0,3) (baseline)	LN(0,5)
$\boldsymbol{\psi^1}$	0.018[0.012-0.24]	0.018[0.013-0.25]	0.019[0.013-0.26]	65.5[44.6-90.3]	65.3[44.6-90.3]	64.5 [42.7-89.1]
$\boldsymbol{\psi^2}$	0.009[0.001-0.02]	0.009[0.001-0.02]	0.009[0.002-0.02]	6.1[1.6-12.05]	6.80[2.0-12.5]	6.11[1.73-11.94]
$\boldsymbol{\psi^3}$	2.89[2.53-3.28]	2.9[2.53-2.30]	2.92[2.53-3.34]	74.2 [59.7-90.2]	75.71[61.7-90.5]	73.09[58.79-89.41]
$\boldsymbol{\varphi^1}$	0.006[0.002-0.011]	0.003[0.0006-0.007]	0.002[0.0001-0.0016]	0.84[0.12-2.06]	0.63[0.06-1.81]	0.53 [0.01-1.7]
$\boldsymbol{\varphi^2}$	0.18[0.13-0.23]	0.17[0.12-0.22]	0.17[0.12-0.22]	3.55[2.28-5.18]	3.63[2.38-5.32]	3.72[2.40-5.4]
$\overline{\boldsymbol{T_i^1}}$	0.22	0.22	0.22	0.28	0.28	0.28
$\overline{\boldsymbol{T_i^2}}$	0.04	0.04	0.04	0.07	0.07	0.07
$\overline{\boldsymbol{T_i^3}}$	0.74	0.74	0.74	0.65	0.65	0.65

Table displays posterior means of each parameter with 95% credible intervals in brackets. $\overline{\boldsymbol{T_i^1}}$, $\overline{\boldsymbol{T_i^2}}$ and $\overline{\boldsymbol{T_i^3}}$ denote the number of outbreaks caused by infectious poultry of each production type averaged over the total number of outbreak tambons.

4.3.1 Comparing model fit

Making the assumption that the infectivity and susceptibility of a tambon scale linearly with the poultry population (and assuming these birds mix homogeneously within the tambon itself) necessitates, due to the high concentrations of poultry associated with areas with industrial production, fitting the model to a highly dispersed distribution of poultry populations (Figure 4-1(b)). This provides little space within which to find a set of parameter values which allows a realistic spatial distribution of outbreaks whilst ensuring that the risks of infection and transmission in those areas with high densities of poultry are not overly represented. As a result, the parameter estimates obtained from the model suggest a scenario where only a relatively small number of tambons contained a sufficient number of birds to transmit infection (Figure 4-2) with the wave being driven by a few isolated, extremely high risk, tambons (including local reproduction numbers in excess of 40). There

were also 5 days when based upon the next-step-ahead distribution, the predictive distribution of Model 1 estimated abnormal levels of transmission. Whilst the addition of poultry-type-specific parameters (Model 3) reduced the number of instances where this occurred, there were still days during the early stages of the wave where the model predicted a lower number of outbreaks than would be expected from the next-step ahead predictions (Figure 4-3).

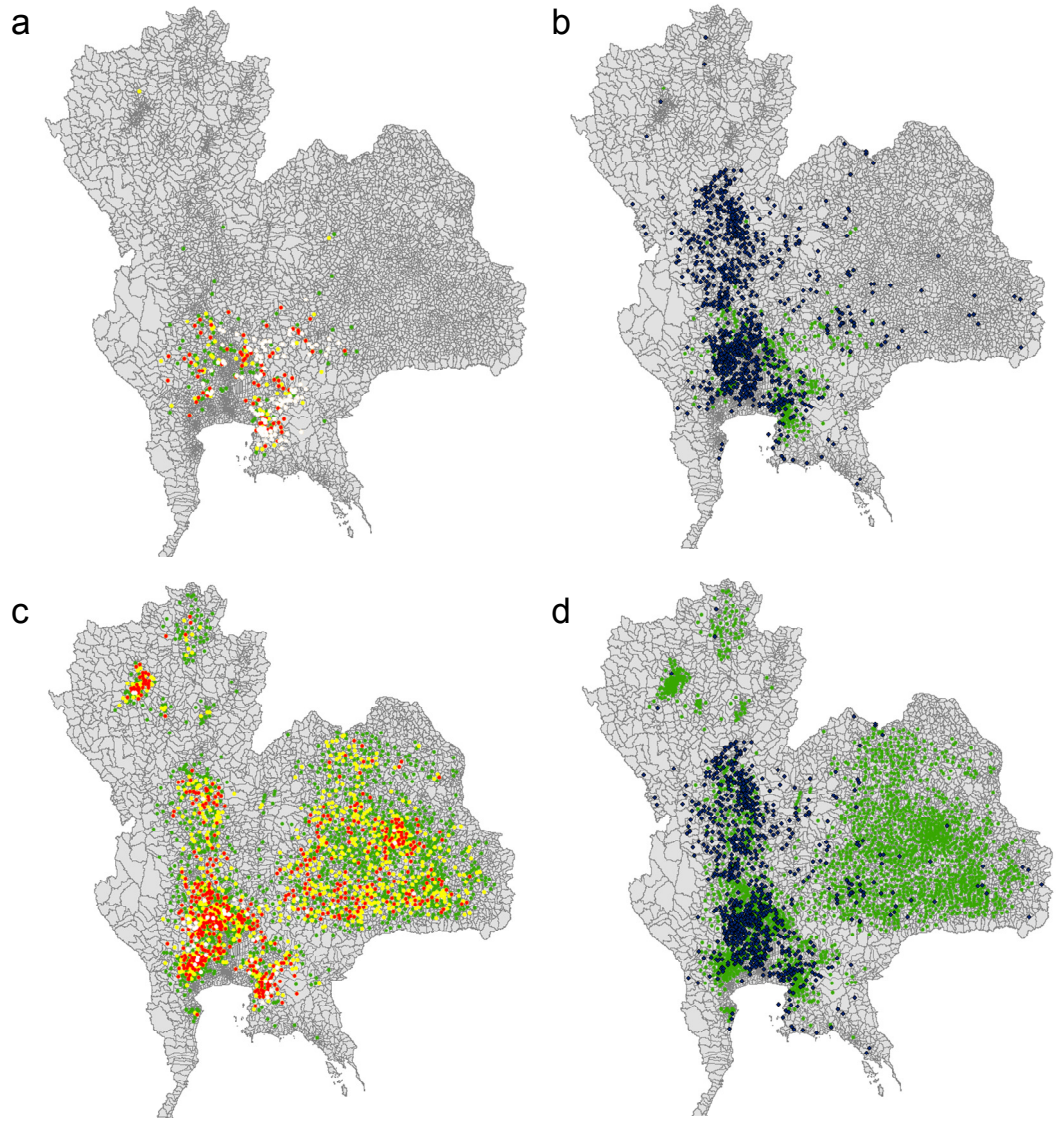


Figure 4-2| Risk maps of local reproductive numbers.
Estimates are based an outbreak involving 1.66 infected flocks (the average for outbreak communes in the dataset), with each infected flock size calculated as the average flock size within the tambon. Figure shows both linear (**(a)** and **(b)**) and logarithmic assumptions (**(c)** and **(d)**). Figures **(a)** and **(c)** shows tambons where R>2 (white dots), R>1 (red dots), R>0.75 (yellow dots) and R>0.5 (green dots). Figures **(b)** and **(d)** compare spatial locations of outbreak reports (blue circles) with the distribution of tambons where R>0.5 (green dots).

In contrast if, as in Model 2, the infectivity and susceptibility of a flock is assumed to scale logarithmically, the fitted parameters provide a scenario where a much larger number of tambons are capable of transmitting infection. As a result, the burden of transmission is aggregated over a much larger amount of space, producing a risk map which more closely resembles the observed distribution of outbreaks (sustained transmission throughout central Thailand with sporadic transmission in the north-eastern Khorat Plateau) and the inputted epidemic curve does not feature any days with abnormal levels of transmission.

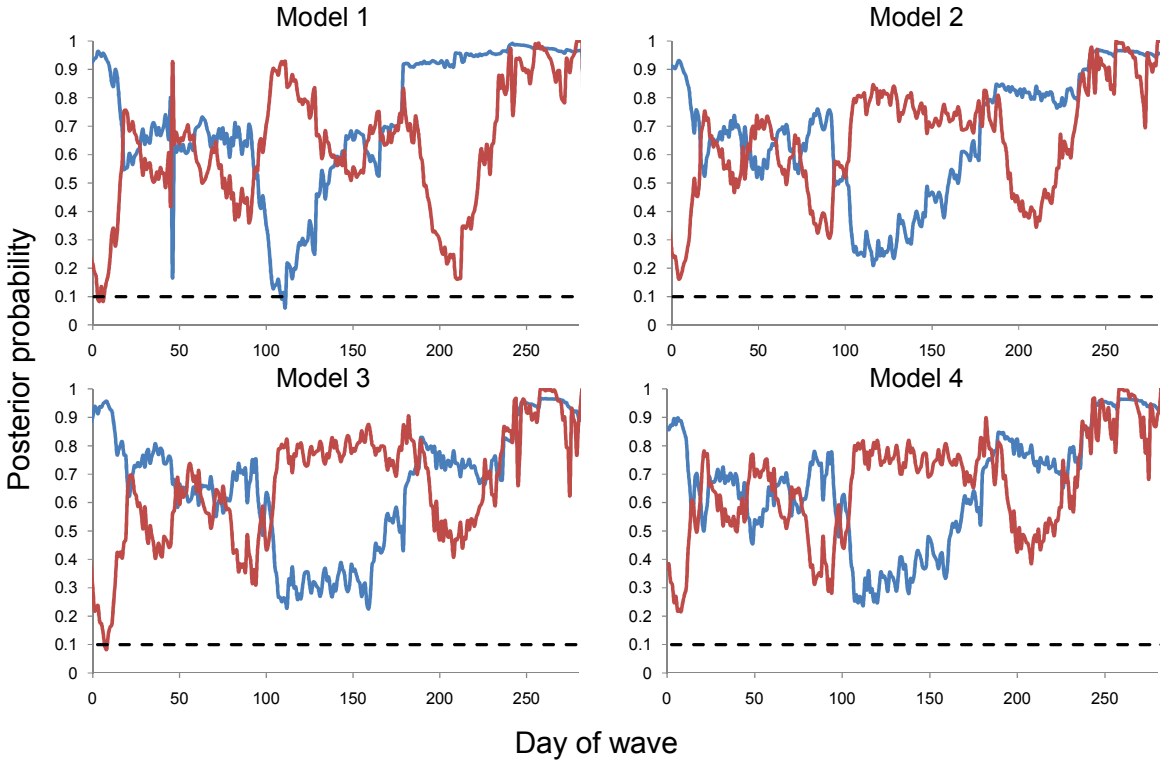


Figure 4-3|Model consistency throughout the wave.
Red lines show $P^{\leq}$ the posterior probability that the "reconstructed" number of infections per day are less than or equal to the number of infections predicted by the next-step ahead distribution. Blue lines show $P^{\geq}$, the posterior probability the equivalent values are greater than or equal to the next-step ahead prediction and the dashed black lines show the 10% rejection threshold.

4.3.3 Respective roles of commercial and non-commercial poultry

The results suggest that, overall, the majority of transmission occurred as a result of infectious backyard poultry. However, backyard poultry constitutes by far the largest category both in terms of the number of flocks infected and the number of flocks in total. As a result, for both Models 3 and 4, when averaged over the total number of flocks in the region, commercial flocks were over ten times more likely to transmit infection. Moreover, when only considering flocks which were reported infected during the wave, of which commercial chicken flocks constitute 11% of the total number, the fact that infected commercial chickens were the source of an estimated 22% of between tambon transmission according to Model 3 and 28% according to Model 4 also suggests that they played a disproportionate role in spreading infection during the wave. In contrast, infectious ducks and geese were estimated to contribute least to the spread of infection and, per flock, were the least likely to transmit (Table 4-4).

Table 4-4| Number of transmissions and transmissibility by production type according to the reconstructed epidemic tree.

Source of infection	Linear model			Logarithmic model		
	Commercial chicken	Ducks and Geese	Backyard	Commercial chicken	Ducks and Geese	Backyard
Total number of transmissions	207.54	38.40	702.06	263.37	63.89	620.74
Transmissions per infectious flock	1.19	0.088	0.72	1.51	0.15	0.64
Transmissions per flock ($\times \mathbf{10^3}$)	2.41	0.060	0.19	2.83	0.13	0.17

4.3.2 Effect of enhanced 'X-ray' surveillance

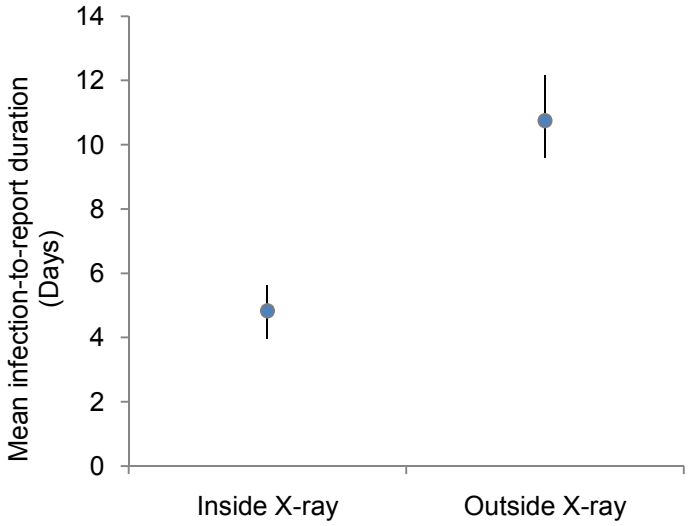


Figure 4-4| Distribution of infection-to-report inside and outside of 'X-ray' periods.
Figure shows posterior distribution of the mean infection-to-report inside and outside of X-ray surveillance periods according to the time to infection-to-report distributions fitted to Model 4. Points represent posterior mean estimates and bars represent 95% credible intervals.

The results from fitting a separate infection-to-report distribution to outbreaks reported during periods of 'X-ray' surveillance suggest that such measures were successful at enhancing the reporting of outbreaks. When carried out for Model 4 this resulted in an estimated infection-to-report duration of 4.8 days for outbreaks reported during an 'X-ray' period and 10.7 days for outbreaks reported outside this (Figure 4-4). This would suggest that the reporting of outbreaks was improved as a result of such active surveillance, probably as a result of a combination of detecting outbreaks more rapidly than those reported as a result of the existing surveillance structure and by combating any underreporting of outbreaks. The latter factor would mean, however, that it isn't possible to make the simple conclusion that 'X-ray' surveillance reduced the mean time between infection and report by 6 days as the underreporting occurring outside 'X-ray' is likely to lead to an overestimation of the time to report during these periods. This is because the

fitting algorithm is forced to join outbreaks occurring further apart in time (as demonstrated by the simulation study in subsection 4.3.4).

4.4 Discussion

The nature of poultry production and differences in control and surveillance infrastructure and policy can be important determinants of the spread of H5N1. The commercial poultry sector in Thailand creates localised areas of extremely high concentrations of poultry. As a result, according to an analysis of the predictive distribution of outbreaks, a model which assumes the risk of infection scales linearly with the population of poultry within a tambon, when fitted to the outbreak data, is forced to impute abnormal levels of transmission at different stages of the wave. In order to avoid such inconsistencies, it was necessary to introduce some form of heterogeneity in per-bird transmissibility, either in the form of a model which makes the assumption that the risk of infection saturates as flock size increases or that the susceptibility and infectivity of flocks varies by production type. Whilst the number of days tested and the relatively conservative rejection threshold of 10% make it feasible that the finding that there were some days where abnormal transmission may have arisen by chance, the suggestion that additional heterogeneity in the transmissibility of infection needs to be incorporated into any tambon-level transmission model is supported by the risk map produced when Model 1 was fitted to the data. In particular, this model could not replicate the levels of sustained transmission observed in the central region of Thailand.

Considering the challenges associated with the assessment of models fitted using augmented data (Cauchemez, Bhattarai et al. 2011), an analysis comparing the overall predictive distribution of the model to next-step ahead predictions provides a valuable

indicator as to whether, for each day of the wave, the fitted model is able to adequately replicate the observed data. Moreover, as the 10% rejection threshold is likely to be conservative given the number of days of the wave tested, the finding that this threshold wasn't reached for models 2 and 4 suggests there is good agreement between the next-step-ahead and predictive distributions of these models. It does not, however provide a direct measure of the extent to which simulations of the model would replicate the dataset in the same way as a deviance statistic would, nor does it provide any measure of the complexity of the model in order to assess the extent to which the model may be over-fitted, making inference about which was the best of the fitted models difficult.

The results obtained from fitting production-type-specific transmissibility parameters appear to support the use of a multi-sectoral approach such as that used to control the spread of infection in Thailand. Due to their large numbers, it was estimated that the majority of transmission occurred as a result of infectious backyard poultry flocks, suggesting that any measures which successfully reduced the susceptibility of these flocks to infection are likely to have had a large impact on the ability of the disease to spread. However, the finding that, per-flock, backyard flocks had a low relative risk of transmission compared to commercial flocks supplements previous analyses which found evidence they were also at a low relative risk of infection (Otte, Pfeiffer et al. 2006; Tiensin, Ahmed et al. 2009; Paul, Tavornpanich et al. 2010). Moreover, the estimated disproportionate role played by commercial poultry in spreading infection between tambons appears to support the assertion that once infected, large-scale commercial operations can spread infection more effectively over longer distances (Otte, Pfeiffer et al. 2006; Tiensin, Ahmed et al. 2009). This suggests that measures to reduce the risks of this occurring, such as the drive

towards vertical integration (NaRanong 2007), are also likely to have had a significant effect on transmission.

In contrast, based upon those outbreaks that were reported, these results would appear to suggest that ducks and geese play a relatively minor role in spreading infection. However, field studies have shown that ducks can act as silent carriers of infection (Chen, Deng et al. 2004; Hulse-Post, Sturm-Ramirez et al. 2005). It has also been shown that the relative risk of an outbreak occurring within a tambon increases with both density of free-grazing and commercial duck production (Gilbert, Chaitaweesub et al. 2006; Gilbert, Xiao et al. 2008; Paul, Tavornpanich et al. 2010), suggesting that this analysis may be underestimating the contribution of ducks in the spread of infection.

The estimates of the relative roles of each production type are also likely to be sensitive to differences in the proportion of outbreaks reported between production types and the nature and extent of any such ascertainment bias is difficult to assess. For example, the shorter infection-to-report fitted during 'X-ray' surveillance, where officials go door-to-door looking for signs of infection, may suggest that a proportion of outbreaks in backyard flocks went undetected outside of 'X-ray', whereas the presence of HPAI may be more easily detected in large-scale commercial operations (Otte, Pfeiffer et al. 2006). It may also be the case that the relative reporting rates in backyard and commercial operations change during such periods of active surveillance, causing the extent of such a bias to vary as the wave progresses. The nature of this kind of surveillance may also result in clusters of outbreak reports, correlated in both space and time, which will distort both the estimated infectious period of outbreaks during this period and their contribution to the estimate of the other spatial infectivity parameters. On the other hand, from the perspective of evaluating control

policies, the finding of a shorter infection-to-report distribution for outbreaks reported during “X-ray” surveillance does at least suggest that such campaigns are effective at providing surveillance capacity in excess of that achieved by relying upon the existing passive surveillance infrastructure alone. It should also be noted that, as the poultry census data were collected as part of the first of these campaigns, it is possible that the number of poultry involved in outbreaks occurring before this time was underestimated due to depopulation via culling which may have had an impact upon this result. Ensuring such data are collected regularly in the absence of outbreaks could prevent this being an issue for the analysis of future outbreaks. Data on the distribution of infected flock sizes would also allow assessment of the validity of the assumption that infection was independent of flock size and, if this is not the case, provide added insight into the relationship between the size of a flock and the risk of infection.

The formulation of the production-type-specific transmissibility parameters used in this analysis also makes the assumption that poultry mix randomly according to production type. In reality there may be interactions between the type of poultry within a susceptible flock and the type of poultry within an infectious flock which affects the transmissibility between these flocks. Such interactions could be parameterised using a 3x3 matrix with each element determining the transmissibility of infection between a different poultry-type-specific configuration of infectious and susceptible flocks (Chis Ster, Singh et al. 2008). Such a model would, however, require the fitting of additional transmission parameters, making the fitting procedure more computationally intensive to run, and in the absence of a more precise measure of model fit, it would not be possible to determine the extent to which this model was an improvement upon the fitting models used in this analysis. The results would

also still be subject to the same caveats with regards to ascertainment bias and the differences in surveillance capacity at different stages of the wave.

Despite the limitations discussed above, this analysis, through the identification of the different roles of the poultry sectors and species composition in the transmission of infection throughout Thailand, does highlight the need to ensure that the response to, and the analysis of, the transmission of avian influenza within a country is tailored to the specific poultry production systems within which disease is spreading. This is especially true when the respective consequences of both infection and the application of stringent control measures can be severe. Moreover, the finding that the rate of reporting increased during "X-ray" surveillance, also suggests that, in addition to providing a more accurate snapshot of the disease status of a country at the time when such information is most important, resources aimed towards actively detecting outbreaks are also likely to contribute strongly to the level of control achieved.

Chapter 5 Estimating heterogeneous vaccine efficacy from animal transmission experiments using final size distributions

5.1 Introduction

The work presented in the previous chapters concerned the dynamics of infection in Vietnam and Thailand with administrative areas equivalent to a group of a few rural communities or an urban centre treated as the epidemiologic unit of interest. This involved making the assumption that, at such low-level resolution, transmissibility could be assumed to scale with distance. Quantifying the dynamics of infection at a higher resolution such as the transmission between or within flocks is likely to provide more nuanced insight into the factors which may drive an epidemic or influence the effectiveness of control measures.

Animal transmission experiments have been proven to be an important tool by which to assess the impact of individual factors such as vaccination, strain type or host species within a controlled environment. When assessing the impact of control measures aimed at reducing transmission, such as vaccination or anti-viral therapy, these experiments generally follow a well-established template (De Jong and Kimman 1994; Velthuis, Bouma et al. 2007). Animals are first partitioned into control and intervention groups. For diseases in poultry, resource constraints often mean that the sizes of these groups are rarely greater than 10-40 birds. Within each of these groups a set number of birds (half the total in each group has been suggested as good rule of thumb (Velthuis, Bouma et al. 2007)) are randomly selected, separated and infected with the disease in question at a set titre, whilst the remaining birds

in the intervention group receive the appropriate treatment. Both groups may be further divided in order to carry out multiple experiments and, at a set time post-inoculation, the infected birds are introduced into the uninfected flocks and the progression of disease is monitored.

Methods to fit the reproduction number to the final outbreak sizes (calculated from the number of birds susceptible at the start of the outbreak and the number left once it reaches its conclusion) from such experiments have been used to quantify aspects of the transmission of various diseases in animals (De Jong and Kimman 1994; De Jong and Bouma 2001). This includes, for avian influenza, the relative transmissibility of low and high pathogenic strains (Van der Goot, De Jong et al. 2003), the relative effects of different anti-viral drugs (Meijer, van der Goot et al. 2004) and the effects of vaccination for a range of disease and vaccine strains and host species (van der Goot, Koch et al. 2005; van der Goot, van Boven et al. 2007). In particular, they have been used to explore the transmissibility of H5N1 between chickens (Bouma, Claassen et al. 2009) and the effects of an inactivated H5N2 vaccine upon its spread within Peking ducks (van der Goot, van Boven et al. 2007).

In an initially wholly susceptible population, the relationship between the reproduction number, R_0, and the final number of individuals who ever become infected was first explored by Kermack and McKendrick (Kermack and Mckendrick 1927) who derived a relation for a homogeneously mixing deterministic SIR model with an exponentially distributed infectious period. However, when an experiment only involves small flocks of poultry, or when only a small number of poultry are initially infected, stochastic effects are likely to play a large role in the number of birds who ever become infected especially as, when assessing the effects of interventions aimed at reducing flock-level transmission, it is

desirable to allow for the possibility that, despite the presence of limited transmission, R_0 has actually been brought below unity. Derivation of estimators of the reproduction number from the final size of an outbreak using stochastic models of transmission have principally been based on the *general* stochastic epidemic*,* a model which fulfils the Markovian property that the future state of the epidemic depends only on its current state, requiring that the infectious period is exponentially distributed (Bartlett 1949). Many useful results can be obtained from this model construction, including approximations of the entire trajectory and duration of an outbreak (Andersson and Britton 2000) and, in particular, using martingale methods, an estimator of the reproduction number from the final size of an outbreak which is straightforward to implement (Becker 1989) and has previously been applied to animal transmission experiments (De Jong and Kimman 1994; Bouma, De Smit et al. 2000; Orsel, Dekker et al. 2005). However, the use of an exponentially distributed infectious period, whilst analytically convenient, is not always a realistic representation of how long hosts remain infectious before they are removed from an epidemic. In the case of poultry infected with H5N1, results from recent transmission experiments which estimated very low variability in the length of time chickens remain infectious (Bouma, Claassen et al. 2009) suggest that this assumption is very unlikely to be appropriate. As a result the methods used in this analysis are based on a construction proposed by Ball (Ball 1986) which allows calculation of the final size distribution of the *generalised* stochastic epidemic (GSE), in which the infectious period can take any given form.

Generally, in such analyses of the effects of vaccination, all the non-infected poultry within both the intervention and the control flocks are assumed to have homogenous susceptibility and the respective estimates of β in each of the flocks are compared with particular focus as

to whether the reproduction number in the intervention flocks has been successfully brought below unity. This approach is then equivalent to the assumption that the intervention reduces the susceptibility of each bird by a factor ϵ, as a result

$$\epsilon = \frac{\beta_v}{\beta} = \frac{R_v}{R_0} \qquad 5.1$$

where β_v and R_v are the transmission intensity and associated reproduction number in the intervention flock. Often, this is then used to determine whether the herd immunity induced by the vaccine is sufficient to prevent sustained transmission (when $R_v < 1$) (Van der Goot, De Jong et al. 2003; van der Goot, Koch et al. 2005).

This assumption of a homogenous immune response to vaccination is often described as a "leaky vaccine" model and is only one of many other possible vaccine actions whereby heterogeneous individual immune responses can combine to induce herd immunity (Halloran, Haber et al. 1992). The simplest and most widely used alternative to this is an "all or nothing" model of vaccine action where individuals either acquire full or no protection. In this model the same efficacy and reduction in the reproduction number is achieved when individuals who receive the vaccine acquire full immunity to infection with probability ϵ and remain fully susceptible otherwise.

This chapter extends existing final size methods for modes of vaccine action and for intermediate models. Using simulation, the extent to which the choice of model of vaccine action is likely to influence estimates of the impact of vaccination during transmission experiments and the consequences this may have upon any assessment of vaccine efficacy are then investigated. The possibility that there are scenarios in which the correct model of vaccine action can be distinguished from final size outbreak data is also explored.

5.2 Methods

5.2.1 Model comparison using the deviance information criterion (DIC).

DIC is a method of model comparison which can be calculated as the posterior mean deviance of a fitted model penalised by the addition of a measure of the effective number of model parameters (calculated as the difference between the posterior mean deviance and deviance calculated at the mean of the parameters). As such, it is intended as a measurement of how well the model would reproduce the observed data, balanced by the desire to ensure the model is not over-fitted (Spiegelhalter, Best et al. 2002). Exact rules by which to judge whether a difference in DIC is important do not exist, however, a reasonable rule of thumb is to view a difference greater than 5 as evidence that the model with the lowest DIC is a substantially better fit (Spiegelhalter, Best et al. 2002; Bolker 2008).

5.2.2 The final outbreak probabilities of the GSE

The general stochastic epidemic is a standard S-I-R model in a closed population of n individuals, with y initially infectious individuals and s initially susceptible. Once infected, infectives remain infectious for a period of time according to a given distribution I, which can take any form within reasonable limits. These individuals then transmit infection according to the points of a homogeneous Poisson process with intensity β/n. Once an individual reaches the end of their infectious period they are removed and play no further role in the epidemic. This is then a mass-action transmission model whereby the rate of transmission is independent of population size and, therefore, an infectious individual infects a susceptible individual at a rate inversely proportional to the size of the population. The validity of this assumption has not been tested for the spread of H5N1 in poultry. However it was shown to be a relatively realistic approximation of the transmission of

pseudorabies virus in swine (Bouma, Dejong et al. 1995). The assumption also results in an R_0 independent of population size which provides an easily interpretable measure of the spread of infection when analysing flocks of different sizes. Under this mass-action assumption the basic reproduction number is then

$$R_0 = \beta E[I]. \tag{5.2}$$

Using this construction, Ball (Ball 1986) derived the following relation between the final size outbreak probabilities $p_{y,s}^n(k)$, the transmission parameter β and the infectious period distribution I:

$$\sum_{k=0}^{l} \frac{\binom{s-k}{l-k} p_{y,s}^n(k)}{\left[\phi\left(\frac{\beta(s-l)}{n}\right)\right]^{k+y}} = \binom{s}{l}, \quad 0 \le l \le k \le n. \tag{5.3}$$

Here, $\phi(x) = E[\exp(-xI)]$, is the Laplace transformation of the assumed probability distribution of the infectious period I and $p_{y,s}^n(x) = P(Z = x|s, y, n, \beta, I)$ is the probability that $Z = x$ initially uninfected individuals are infected during the course of the outbreak given an initial susceptible population s, infectious population y, population size n, transmission parameter β and infectious period distribution I . These probabilities can then be rewritten as a triangular set of equations which can be solved recursively

$$p_{y,s}^n(k) = \frac{1 - s_k}{a_{kk}}, \quad s_k = \sum_{l=0}^{k-1} a_{kl} p_{y,s}^n(l), \quad k = 0, \dots, N, \tag{5.4}$$

where

$$a_{lk} = \frac{\binom{s-k}{l-k}}{\binom{s}{l}\left[\phi\left(\frac{\beta(s-l)}{n}\right)\right]^{k+y}}. \quad 5.5$$

5.2.3 Quantifying the impact of vaccination

To assess how well different models of vaccine action can explain observed outbreak sizes, two different scenarios, with the following assumptions about available data, were first explored:

(i) A "leaky" vaccine where all individuals of the population are assumed to be equally susceptible and thus R_v can be calculated independently of R_0.

(ii) An "all or nothing" vaccine where R_0 is known (perhaps through analysis of outbreaks in a control group) but the fraction of individuals who have been protected as a result of vaccination, f_v, is not.

Whilst the two models of vaccine action described above have the same impact upon the reproduction number within a flock, they affect the final outbreak size probabilities quite differently. For a "leaky" vaccine, the outbreak probabilities can be calculated in the same way as for an unvaccinated flock but with transmission intensity $\epsilon\beta/n$. However, for an "all-or-nothing" vaccine, the outbreak can be seen as a multitype epidemic (Ball 1986; Andersson and Britton 2000), in a large population this would result in a situation where $\epsilon(n-y)$ individuals have no chance of acquiring infection, thus the outbreak probabilities of the remaining $(1-\epsilon)(n-y)$ become:

$$p^{n}_{y,(1-\epsilon)(n-y)}(x), \quad 5.6$$

These two models constitute only two of many possible assumptions about the distribution of vaccine response within a flock. In order to explore a wider range of assumptions, a beta

distribution was used to model the proportional reduction in the susceptibility of each individual bird, ε_i, with the following parameterisation:

$$P(\mathrm{E} = \varepsilon_i; \mu, \rho) = \frac{1}{\mathrm{B}(\mu\rho, (1-\mu)\rho)} {\varepsilon_i}^{\mu\rho-1}(1-\varepsilon_i)^{(1-\mu)\rho-1}. \qquad 5.7$$

Here μ can be interpreted as the overall efficacy of the vaccine (i.e. the proportional reduction in the reproduction number) and ρ as a measure of the extent to which the reduction in overall susceptibility is distributed evenly between individuals. "All or nothing" and "leaky" distributions can be obtained by, respectively, letting $\rho \to 0$ and $\rho \to \infty$ (Figure 5-1) (Maire, Aponte et al. 2006).

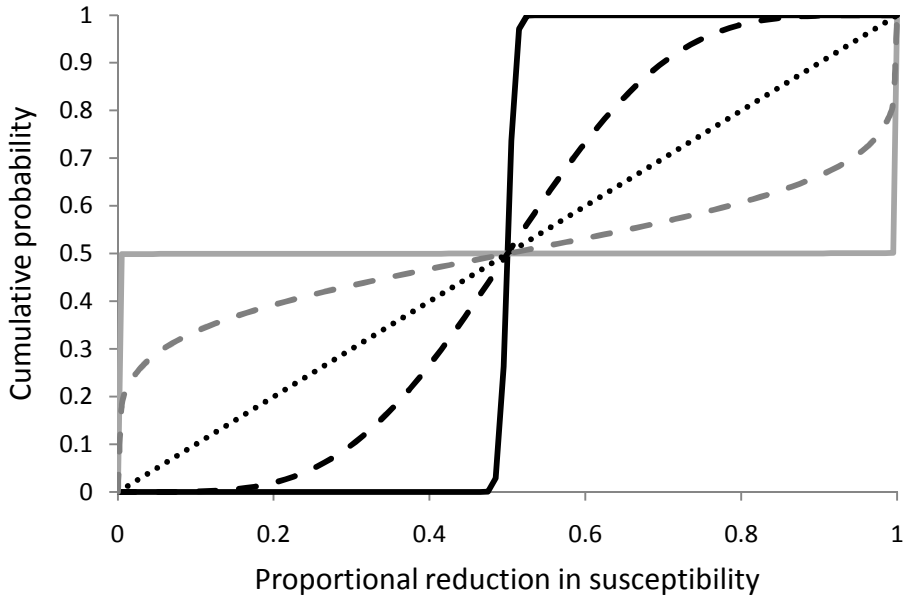


Figure 5-1| Cumulative probability distribution of a 50% reduction in susceptibility according to different values of $\boldsymbol{\rho}$.
In all cases a 50% reduction in susceptibility is achieved by setting $\boldsymbol{\mu = 0.5}$. Examples shown are $\boldsymbol{\rho}$ =0.001 (solid grey line), 0.4 (dashed grey line), 2 (dotted black line), 10 (dashed black line), 4000 (solid black line).

If the reduction in susceptibility of each individual is drawn from such a distribution, the final size distribution of the outbreak then has the form of a multi-type epidemic with n types, each with population size 1:

$$\sum_{\underline{\boldsymbol{l}}=0}^{\underline{\boldsymbol{k}}} \frac{p_{y,n-y}^{n}(\underline{\boldsymbol{l}})}{\prod_{i=0}^{n-y}\phi_i\left(\sum_{j=0}^{n-y}\frac{\beta\varepsilon_j(1-k_j)}{n}\right)^{y+l_i}} = 1, \quad \underline{\mathbf{0}} \leq \underline{\boldsymbol{k}} \leq \underline{\boldsymbol{n}}. \qquad 5.8$$

Here $\underline{\boldsymbol{k}}$ and $\underline{\boldsymbol{l}}$ are vectors representing a possible outbreak configuration (k_i=1 if the ith individual is infected in the course of the outbreak and 0 otherwise) and $\underline{\boldsymbol{a}} \leq \underline{\boldsymbol{b}}$ means $a_i \leq b_i,\ i = 1, \ldots, k$ and $\sum_{\underline{\boldsymbol{b}}=0}^{\underline{\boldsymbol{a}}} = \sum_{b_1=0}^{a_1} \ldots \sum_{b_n=0}^{a_n}$. With this formulation the infectious period distribution can be allowed to vary for each bird. However, for the scenario considered throughout this chapter, when only the total number of birds infected within the flock, rather than the final infection status of the bird in each specific category, is known, allowing such variation is difficult to justify. Therefore, where this formulation is used in this analysis the infectious period distribution is assumed to be equal for all birds.

The probability of an outbreak of size k occurring is then:

$$p_{y,n-y}^{n}(k) = \sum_{\underline{\boldsymbol{l}}=0}^{\underline{\boldsymbol{n}}} p_{y,n-y}^{n}(\underline{\boldsymbol{l}})\mathbb{1}(||\underline{\boldsymbol{l}}|| = k), \qquad 5.9$$

where $\mathbb{1}(.)$ is the indicator function.

Simulated data for any flock size, number of initial infectives, value of R_0 and overall vaccine efficacy can then be obtained for any of the above models of vaccination simply by randomly drawing outbreak sizes according to the outbreak size probability distribution these generate**.**

5.2.4 Bayesian estimation of the reproduction number

Given the probability distribution of I, the infectious period distribution (although, for simplicity, this is assumed to be a constant throughout the analysis), it is possible to obtain the likelihood of any set of N final outbreak sizes, number of initial infectives and population sizes $(\underline{x}, \underline{y}, \underline{n}) = \{(x_0, y_0, n_0), \dots, (x_N, y_N, n_N)\}$ for any chosen value of β in an unvaccinated population:

$$\pi\left(\beta \middle| \left(\underline{x}, \underline{y}, \underline{n}\right), I\right) \propto \pi\left(\left(\underline{x}, \underline{y}, \underline{n}\right) \middle| \beta, I\right) \pi(\beta)$$

$$\text{with} \quad \pi\left((\underline{x}, \underline{y}, \underline{n}) \middle| \beta, I\right) = \prod_{i=0}^{N} p_{y_i, n_i - y_i}^{n_i}(x_i) \qquad 5.10$$

This likelihood can then be explored using a standard random-walk Metropolis-Hastings algorithm to reach and draw from the posterior distribution of β and thus R_0. This method can also be applied to estimate R_v in a vaccinated population under the assumption of a "leaky" vaccine. Unless otherwise specified, in each analysis β was assigned a uniform prior distribution bounded between 0 and 10000.

In order to quantify the protection achieved by an "all or nothing" vaccine when R_0, and therefore β, is known, it is necessary to calculate $\pi\left(\epsilon \middle| \left(\underline{x}, \underline{y}, \underline{n}\right), \beta, I\right)$, where ϵ is the proportion of individuals who are successfully protected by vaccination. For a single experiment this can be estimated by obtaining the posterior distribution of the number of initially susceptible individuals, s, and calculating the efficacy as $\frac{n-y-s}{n-y}$. However, when the data consists of multiple outbreaks, the likelihood has to be integrated over the distribution

of the number of individuals who achieve full immunity for a given "all or nothing" coverage and efficacy (Ball and Lyne 2002; Becker, Britton et al. 2003):

$$\pi\left(\epsilon|\left(\underline{x},\underline{y},\underline{n}\right),\beta\right) \propto \prod_{i=0}^{N} \int_{s_i=0}^{n_i-y_i} P(x_i|s_i,y_i,n_i,\beta,I)\, P(s_i|\epsilon) ds_i$$

$$= \prod_{i=0}^{N} \sum_{f=0}^{n_i-y_i} \binom{n_i-y_i}{s_i} {s_i}^{\epsilon} (n_i-y_i-s_i)^{1-\epsilon} p_{s_i,y_i}^{n_i}(x_i). \qquad 5.11$$

When vaccine response is assumed to follow a beta distribution with given heterogeneity ρ, as described in equation (5.7), the likelihood of any overall vaccine efficacy $\epsilon = \mu$, for any set of outbreak sizes can be written as:

$$\pi\left(\mu|\left(\underline{x},\underline{y},\underline{n}\right),\beta,\rho\right) \propto \prod_{i=0}^{N} \int_{\underline{\mathbf{E_i}}} P\left(x_i|\underline{\mathbf{E_i}},y_i,n_i,\beta,I\right) P\left(\underline{\mathbf{E_i}}|\mu,\rho\right) d\underline{\mathbf{E_i}}, \qquad 5.12$$

where $\underline{\mathbf{E_i}}$ is an ordered vector of vaccine responses in the ith population. Integrating this likelihood directly would be computationally intensive. Instead the likelihood was integrated using stochastic methods, with each individual response treated as a nuisance parameter to be estimated. An uniform prior bounded between 0 and 10000 was assumed for μ and each unobserved vaccine response was assigned a uniform prior bounded between 0 and 1. The set of vaccine responses $(\epsilon_j)_i \in \underline{\mathbf{E}} = \{\underline{\mathbf{E_1}}, \ldots, \underline{\mathbf{E_N}}\}$ and the overall mean vaccine efficacy μ, initially set to values randomly drawn from a uniform distribution between 0 and 1 were then updated according to the following two step MCMC algorithm:

(1) Proposed values of the mean overall efficacy, μ^* were drawn from a normal distribution truncated and centred at the current value of μ with variance σ

calibrated to ensure adequate acceptance and updated according to a standard Metropolis-Hastings algorithm, with proposed values not within the interval [0,1] assigned zero likelihood.

(2) An experiment i and individual within this flock j was chosen randomly and a new value for this individual's response to the vaccine, $(\widehat{\epsilon_j})_i \sim U[0,1]$, was proposed. This process was repeated γ times. Denoting $\{\underline{\hat{\mathbf{E}}}_1, \ldots \underline{\hat{\mathbf{E}}}_N\}$ the full set of vaccine responses this generates, these proposed responses are then accepted with probability:

$$\alpha = \min\left(1, \prod_{i=0}^{N} \frac{P\left(x_i|\underline{\hat{\mathbf{E}}}_{\mathbf{i}}, y_i, n_i, \beta, I\right) P\left(\underline{\hat{\mathbf{E}}}_{\mathbf{i}}|\mu, \rho\right)}{P\left(x_i|\underline{\mathbf{E}}_{\boldsymbol{i}}, y_i, n_i, \beta, I\right) P\left(\underline{\mathbf{E}}_{\mathbf{i}}|\mu, \rho\right)}\right), \qquad 5.13$$

with γ calibrated so as to ensure adequate acceptance. This process was then repeated δ times, with δ calibrated to ensure the chain mixed adequately.

These steps were repeated until the algorithm had converged and a sufficient sample of the posterior distribution of μ has been obtained.

5.2.5 Calculating the minimum flock size needed to reject $R_v > 1$ for an "all-or-nothing" vaccine

The best case scenario, in terms of the vaccine efficacy achieved during a transmission experiment, is that all vaccinated birds become effectively protected. However, with the all-or-nothing" vaccine action model described above, there will always be a possibility that such an event can occur despite an underlying failure rate of the vaccine which is not sufficiently low to bring the effective reproductive number below unity. However, if the basic reproductive number is known, it is possible to calculate the upper bound of the confidence interval, with associated significance level τ, of this failure rate, f. This would be

the largest value of $\hat{f}$ which satisfies $P(X = 0) < \tau$ where $X \sim Bin(n, \hat{f})$, with n the total number of vaccinated birds used in the experiment.

Similarly, if R_0 is known, it is possible, even before the experiment has been conducted, to calculate the minimum number of vaccinated birds required in order to have any prospect of demonstrating that, under the assumption of an "all-or-nothing" vaccine, $R_v < 1$. This number would be the smallest value of n_{min} which satisfies

$$P(X = 0) < \tau, \qquad X \sim Bin\left(n_{min}, \frac{1}{R_0}\right). \tag{5.14}$$

5.2.6 The final size distribution and reproduction number when both challenge and non-challenged birds are vaccinated.

One of the assumptions implicit in the types of experiment and analyses described above is that, once infected, vaccinated birds are as infectious as non-vaccinated birds. However, an effective vaccine may inhibit transmission within a flock by reducing the infectivity of any infected vaccinated bird. In an attempt to account for this possibility and thus more accurately capture transmission within a flock of vaccinated birds, final size estimates of the effects of HPAI vaccines upon the reproduction number are often applied to experiments where both the birds challenged with infection and the flock they are subsequently introduced to have received vaccination (van der Goot, van Boven et al. 2007; van der Goot, van Boven et al. 2008; Bouma, Claassen et al. 2009). In such analyses all challenged birds are assumed to have become infected and be equally infectious, regardless of the results of any cloacal or tracheal swabs taken, and all non-challenge birds are assumed to be equally susceptible, with positive swabs assumed to indicate the presence of infection. When a vaccine is leaky and, as a result, all challenged vaccinated birds are equally infectious, the average number of infections arising from a single challenged bird is equivalent to the

reproduction number and such an analysis would provide a valid estimate of the reproduction number within a wholly vaccinated flock. However, when vaccine response is not homogenous, these two values are no longer equivalent. As a result, the average number of infections arising from a challenged bird no longer provides a threshold indicator as to whether a major outbreak can occur and the estimate of the reproduction potential of infection within a flock is subject to further potential bias. Instead, when vaccine responses are distributed into a finite number of compartments, the reproduction number can be calculated as the dominant eigenvalue of the next generation matrix this creates (Diekmann, Heesterbeek et al. 2010). In the case of an all-or-nothing vaccine (or indeed an "all-or-something" vaccine where the vaccine fully protects a proportion of birds and reduces the transmissibility and susceptibility of the remainder by uniform amounts), as they are the only birds which become infected, the overall effective reproduction number (remembering that this is the average number of secondary cases arising from a single *infectious* bird) is simply the number of cases arising from a bird in which vaccination has not been fully effective. The final size probabilities in this scenario then become

$$p^{n}_{s-s_v,y-y_v}(x_i) \qquad 5.15$$

where y_v is the number of challenged and s_v the number of non-challenged birds protected by the vaccine.

5.3 Results

As shown by others, holding the overall efficacy of the vaccine fixed, the expected final size of an outbreak following vaccination with an "all or nothing" vaccine is lower than that of a "leaky" vaccine (Ball, Britton et al. 2004). Essentially this is because, whilst both vaccines increase the probability that the outbreak will die out within the first few infections, the "all

or nothing" vaccine also reduces the eventual size of any major outbreak that may occur (Figure 5-2).

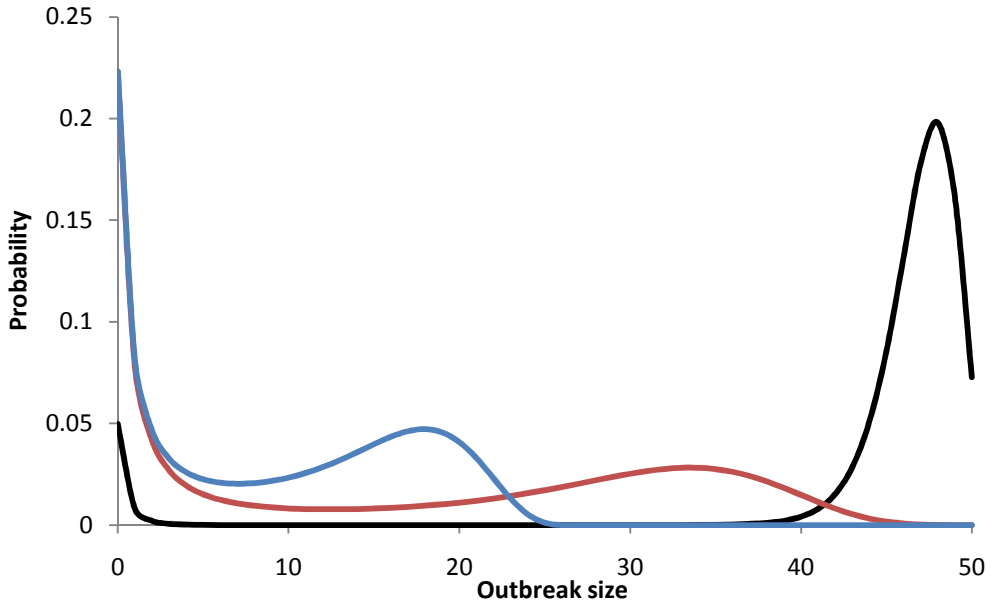


Figure 5-2| Different final outbreak distribution for $R_0 = 4$ for different models of vaccine action in a population size 50.
No vaccination (black line), leaky vaccination with efficacy 0.5 (red line), all or nothing vaccination with efficacy 0.5 (blue line).

5.3.1 Fitting vaccine models to data from one experiment

In order to assess the extent to which data from a single experiment within a vaccinated flock can provide information about the likely action of a vaccine, the relative fit of the "leaky" and "all-or-nothing" assumptions of vaccine action to all possible outbreak sizes in a flock of 50 birds were calculated, with the exception of the scenario where all birds become infected which, as Figure 5-3 shows, results in a posterior distribution of the leaky vaccine estimate which is highly sensitive to changes in the prior distribution (the posterior distribution of all other outbreak sizes were invariant to these choices of prior).

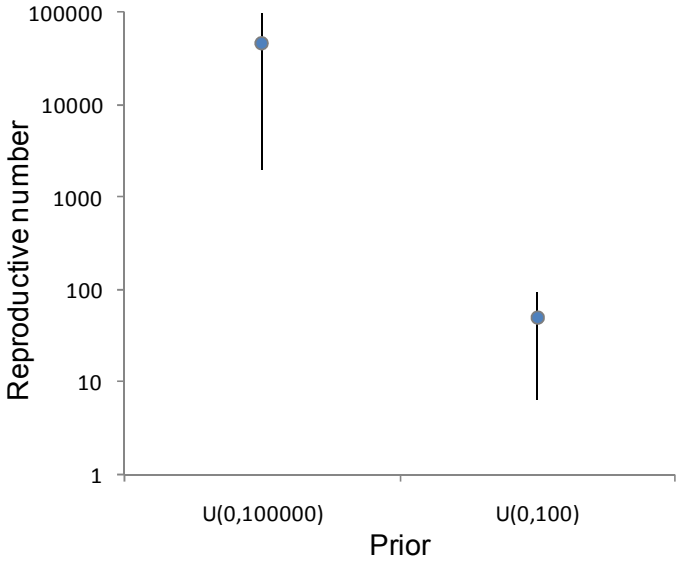


Figure 5-3| Sensitivity of the leaky vaccine model to the choice of prior in the scenario where all birds become infected.
Results shown are for a single flock of 50 birds where all 50 become infected during the experiment, circle represent posterior means and bars represent 95% credible intervals.

With the exception of scenarios where the vaccine proves counter-productive (i.e. the fitted effective number within the vaccinated flock is greater than the known basic reproduction number), such data would always favour an all-or-nothing vaccine, with the difference in DICs between the models increasing rapidly as the size of the outbreak passes $x/(nR_0)$, the susceptible population size at which, under the all-or-nothing assumption, R_v reaches unity (Figure 5-4).

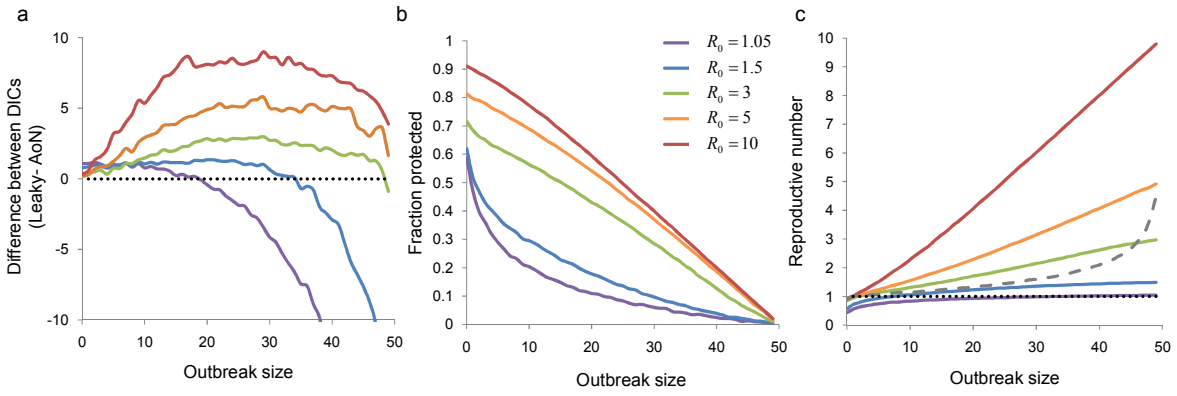


Figure 5-4| Fitting different models of vaccine action to all possible outbreak sizes in a population of 50 birds.
(a) Difference between DICs of leaky and all-or-nothing models for different $\boldsymbol{R_0}$ and outbreak sizes.**(b)** Estimated fraction vaccinated for all-or-nothing models with different $\boldsymbol{R_0}$ **(c)** Estimated $\boldsymbol{R_v}$ for all- or-nothing models with different $\boldsymbol{R_0}$ and "leaky" vaccine model (dashed grey line).

This is because, as illustrated in Figure 5-5, it becomes possible to fit a fraction completely immune sufficient to prevent larger outbreaks whilst allowing the effective reproduction number to remain above unity, readily infecting the remaining susceptible individuals. This results in much higher values of the likelihood of the particular outbreak size than for the equivalent fitted leaky vaccine estimator.

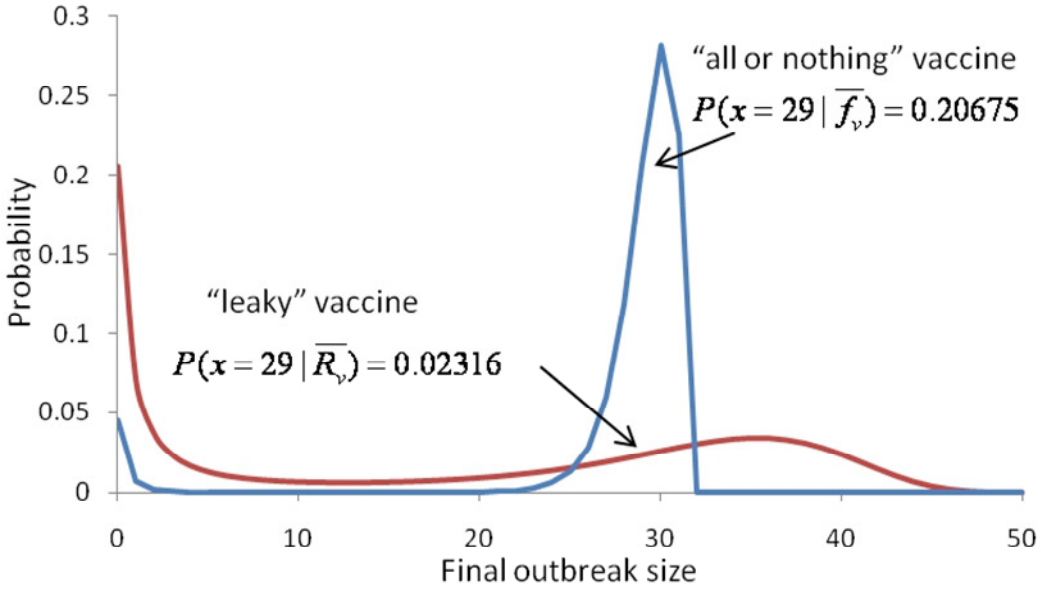


Figure 5-5| Posterior mean final size distribution fitted to an outbreak involving 29 birds in a flock of 50.

When an outbreak infects either a majority or a minority of the flock, it becomes more difficult to differentiate between the two models as the bimodal outbreak probabilities which characterise the “leaky” vaccine model are more easily fitted. At smaller flock sizes, such as those used during vaccine experiments, this bimodality becomes compressed to the extent that it proves impossible to differentiate between vaccine actions with any confidence based upon a single experiment. However, when an all-or-nothing vaccine reduces the mean size of an outbreak it also results in a lower variance in the size of an outbreak than a leaky vaccine of the same overall efficacy (Figure 5-6). This suggests that, if multiple experiments are carried out, the variation in the size of outbreaks, rather than the mean outbreak size, may, using a measure of the ability of a fitted model to reproduce the observed data such as DIC, make it possible to differentiate between the fit of the two models.

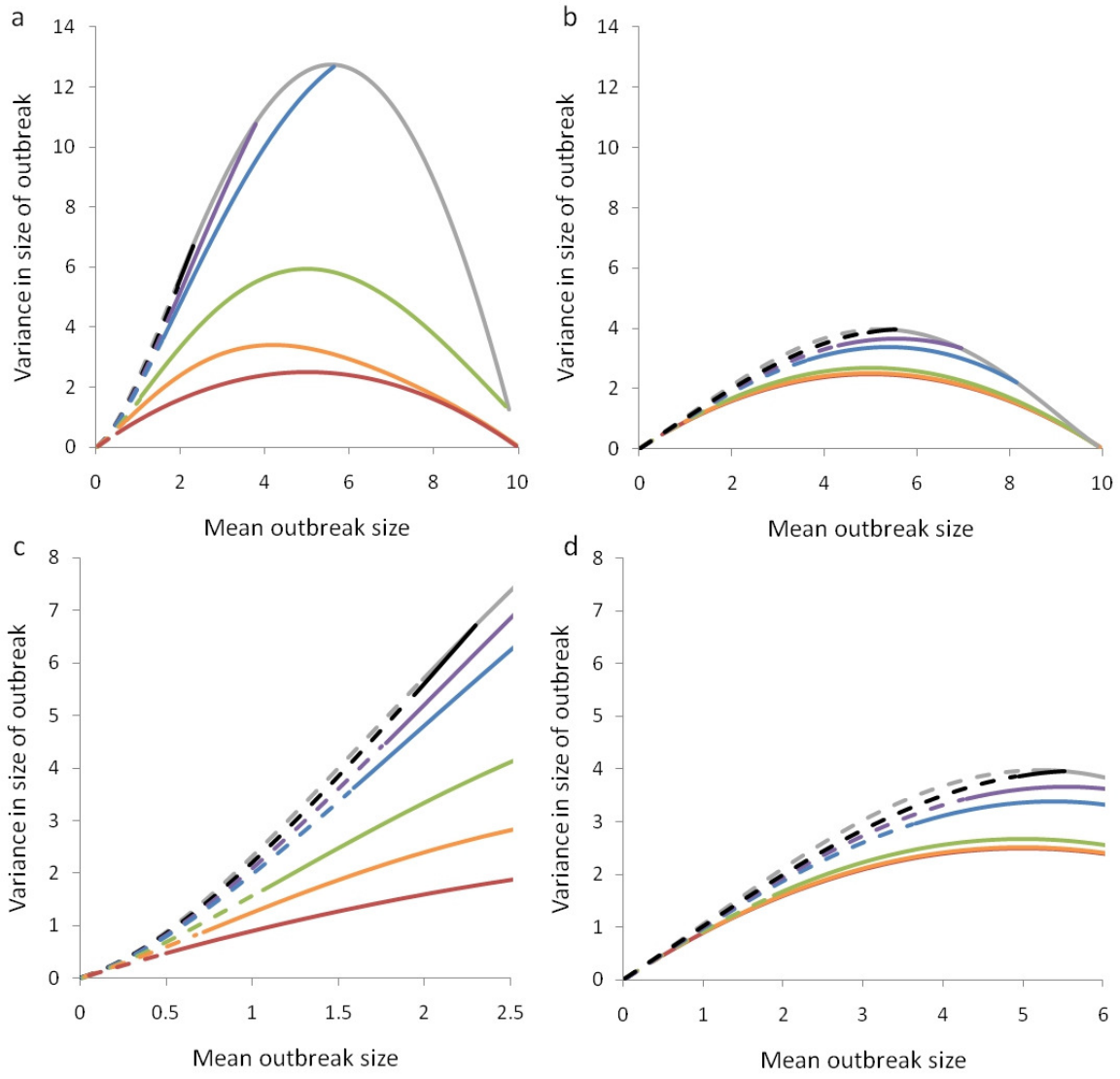


Figure 5-6| Plot of variance in the size of outbreaks against average outbreak size in flocks of ten vaccinated birds for "leaky" and "all-or-nothing" vaccination scenarios.
Grey line shows the mean and variance for the distribution of final outbreak sizes as the basic reproductive number (or equivalently the effective reproductive number under a leaky vaccine) increases from 0 to 5, the remaining lines then show the mean and variance for the distribution of final outbreak sizes for an all-or-nothing vaccine as vaccine efficacy decreases (moving from left to right on the figure) from 1 to 0.05 for the situations where $\boldsymbol{R_0}$=1.1 (black line), 1.5 (purple line), 2 (blue line), 5 (green line), 10 (orange line) and 20 (red line). The dashed segment of each line highlights scenarios where $\boldsymbol{R_v} < 1$.**(a)** shows the possible combinations of mean and variances when a single infected bird is introduced to a vaccinated flock of ten birds and **(b)** shows the same figure when 10 infected birds are introduced to the a vaccinated flock of ten birds. **(c)** and **(d)** show the same two figures but where the mean outbreak size axis has been truncated to show the most important scenarios (where $\boldsymbol{R_v}$ is near 1) more clearly.

5.3.2 Fitting vaccine models to data from multiple experiments

With higher values of R_0, the difference between the variance in outbreak sizes resulting from the use of an all-or-nothing vaccine and that of a leaky vaccine increases (Figure 5-6), thus the number of outbreaks needed to differentiate between the two models decreases. As a result, if R_0 is known and is sufficiently high, it may be possible to identify which model fits the observed final outbreak sizes from a relatively low number of transmission experiments. However, this is likely to hold only when a vaccine fails to reduce the reproduction number below unity (Figure 5-7). In such a scenario, if experiments are repeated a sufficient number of times, an all-or-nothing vaccine is likely to generate a high number of mid-sized outbreaks which would be difficult to reproduce using a model of leaky efficacy (Figure 5-7(a)). In contrast, only scenarios where a leaky vaccine reduces susceptibility substantially, but not to the extent that large scale outbreaks can never occur, give rise to sets of outbreaks sizes which are not possible to reproduce using an all-or-nothing vaccine model of any efficacy. As a result, the range of efficacies where a leaky vaccine can be correctly identified within a plausible number of experiments is relatively narrow (Figure 5-6(b)).

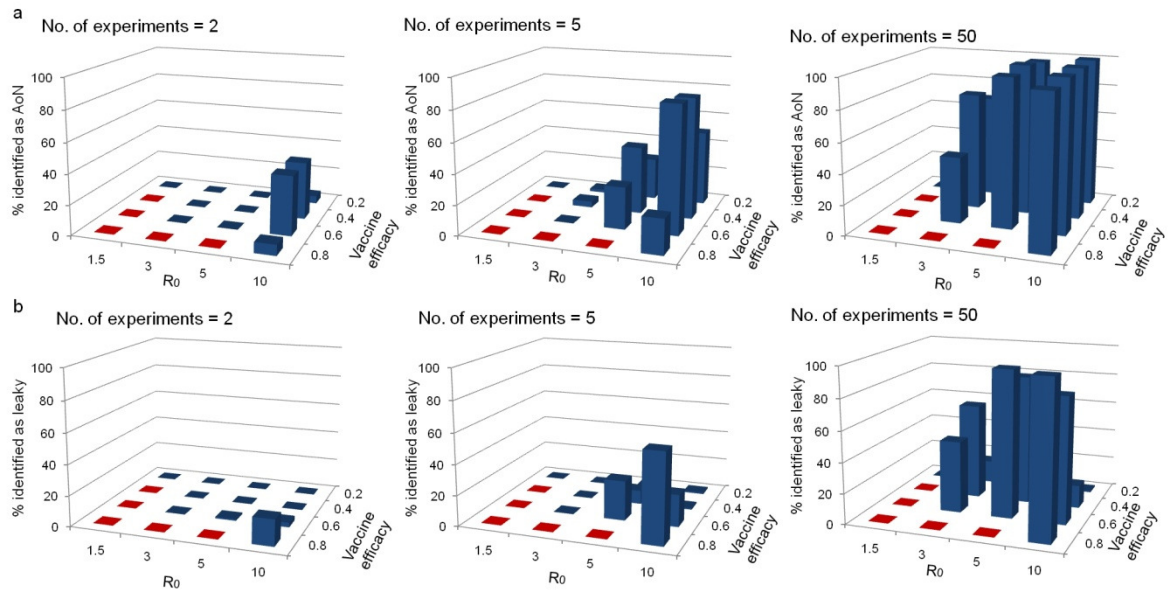


Figure 5-7| Scenario analysis of the ability to correctly favour a leaky or all-or-nothing vaccine model.

For both the leaky and all-or-nothing vaccine models, 100 datasets were simulated for each combination of $\boldsymbol{R_0}$, vaccine efficacy and number of experiments. Experiments involved a single infectious bird introduced into a vaccinated flock of ten birds. Scenarios where $\boldsymbol{R_V} < 1$ are highlighted in red. The DICs of the leaky vaccine model and the all-or-nothing model were fitted to each of these simulations were then recorded. **(a)** shows the percentage of simulated all-or-nothing datasets which were correctly identified as being generated by an all-or-nothing model (when the fitted model had a DIC value at least 5 lower than the fitted leaky model), similarly **(b)** shows the percentage of simulated leaky datasets which were correcty identified as being generated by a leaky vaccine model.

5.3.3 The effect of misspecification of vaccine action upon estimates of vaccine efficacy.

Figure 5-8(a) illustrates that, of all possible assumptions regarding the distribution of vaccine response within a flock, the all-or-nothing and leaky models produce extreme estimates of R_v. The all-or-nothing vaccine assumption is the most conservative assumption regarding the level of control achieved whilst the leaky vaccine assumption is the most optimistic. The extent to which the leaky model will underestimate the reproduction number depends upon the degree to which it overestimates the level of homogeneity in vaccine response (Figure 5-8(a)). Importantly, this may lead to scenarios where, as result of

assuming a leaky mode of action of the vaccine and thus underestimating the level of heterogeneity in vaccine response, a vaccine may be wrongly estimated to have brought the effective reproduction number below unity (Figure 5-8(a) and (b)).

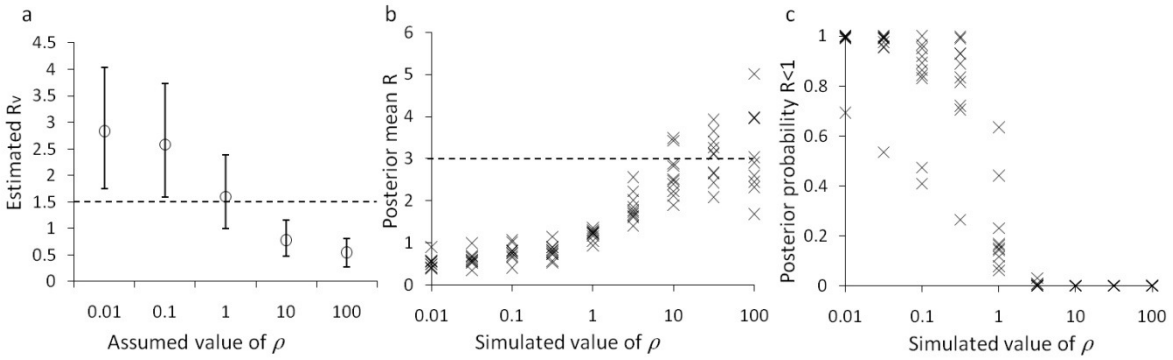


Figure 5-8| The effect of misspecification of the level of heterogeneity in vaccine response.
Figure shows **(a)** the posterior mean and 95% credible intervals of $\boldsymbol{R_v}$ when different values of $\boldsymbol{\rho}$ are fitted to a set of 10 experiments where 5 infected birds are introduced to 5 vaccinated birds, $\boldsymbol{R_0}$=10, $\boldsymbol{\mu = 0.15, \rho = 1}$. **(b)** and **(c)** show results obtained from fitting a leaky vaccine model using different values of $\boldsymbol{\rho}$. For each choice of $\boldsymbol{\rho}$, 10 sets of 10 experiments with 5 infected birds introduced to 5 vaccinated birds where simulated with the parameters $\boldsymbol{R_0}$=10 and $\boldsymbol{\mu = 0.3}$. In **(a)** and **(b)** the dotted line represents the true value of $\boldsymbol{R_v}$ used to simulate outbreaks.

As an example, the likelihood of making such an erroneous conclusion for an all-or-nothing vaccine which only reduces the effective reproduction number within a flock to 1.25 was assessed for different values of R_0 and study sizes (Figure 5-9). When a leaky vaccine model was fitted to simulated experiments which involved introducing a single initially infected bird into a flock, the reproduction number was only wrongly estimated to be below unity when the basic reproduction number was relatively high, with the evidence for such a conclusion increasing with the number of experiments performed. However, in such scenarios, if the basic reproduction number had previously been estimated, it would often be possible to ascertain that an all-or-nothing estimate produced a better fit to the data (Figure 5-9).

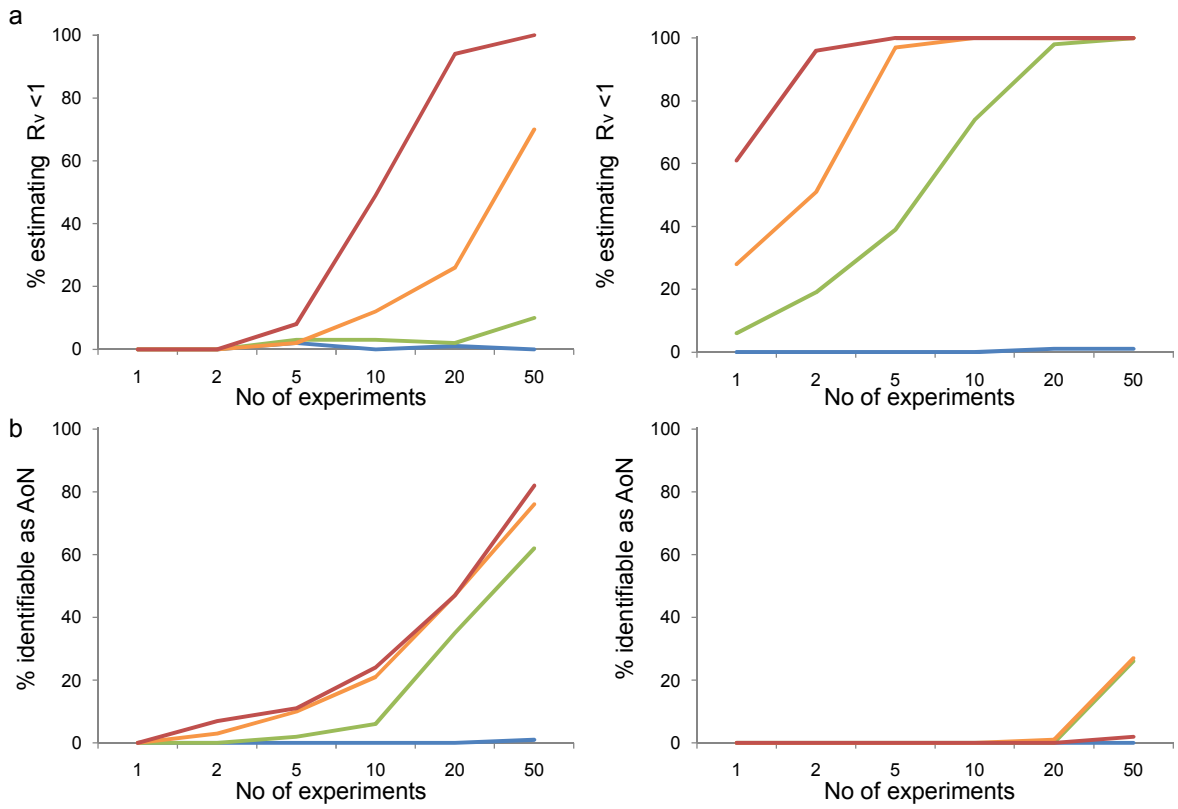


Figure 5-9| Effect of assuming a leaky vaccine for experiments where an all-or-nothing vaccine results in $R_v = 1.25$.

100 sets of experiments involving ten vaccinated birds are simulated for each combination of study size and reproductive number using the all-or-nothing vaccine outbreak probabilities with efficacy adjusted to ensure $R_v = 1.25$. Both the leaky and all-or-nothing vaccine models were then fitted to each of these sets. **(a)** shows the percentage of simulations where the one-tailed 95% credible interval of the leaky vaccine estimator did not include 1**, (b)** shows the percentage of simulations where the difference in DIC was greater than five in favour of the all-or-nothing model. For both **(a)** and **(b)** the left hand column shows the results for scenarios where 1 infected bird is introduced to a flock of 10 vaccinated birds and the right hand column shows the results for the equivalent scenarios but with 10 infected birds introduced to 10 vaccinated birds. Blue, green, orange and red lines correspond to scenarios where R_0=1.5, 3, 5 and 10 respectively.

In contrast, when, as has been recommended, multiple infected birds are introduced into the vaccinated flock, the likelihood of mistakenly concluding that the critical vaccine threshold has been reached increases dramatically and there is little chance of detecting any difference between the fit of the different actions of vaccine (Figure 5-9). Using $R_0 = 5$, a value well within the distribution of estimates for the R_0 of H5N1 (Table 2-1), as an example, a leaky vaccine model to pairs of experiments produced an estimate of $R_v < 1$ in 43% of the scenarios tested. For the tested scenarios involving a larger number of experiments or a

higher R_0 this occurred more often than not and, with a sufficient number of experiments, became inevitable for all scenarios except for those where, if $R_v > 1$, the vaccine had not actually had much effect (e.g. $R_0 = 1.5$). Moreover, with multiple initial infected birds, it is possible to choose a reproduction number which generates final size distributions which are more similar to those of any given all-or-nothing vaccine (Figure 5-6). This makes it extremely difficult to distinguish between the final outbreak size distribution of a leaky vaccine reducing the reproduction number below unity and that occurring as a result of infection spreading readily between birds not protected by an all-or-nothing vaccine (Figure 5-9(d)).

If a high R_0 is estimated, smaller transmission experiments may not have the power to establish that $R_v < 1$, under the conservative assumption of an "all or nothing" vaccine. For example, a study involving 20 vaccinated birds, all of which avoid infection, would only be sufficient, making the assumption that all birds avoided infection as a result of being fully protected, to conclude, at a 5% significance level, that the critical vaccine threshold had been reached for $R_0 < 7.2$ (Figure 5-10).

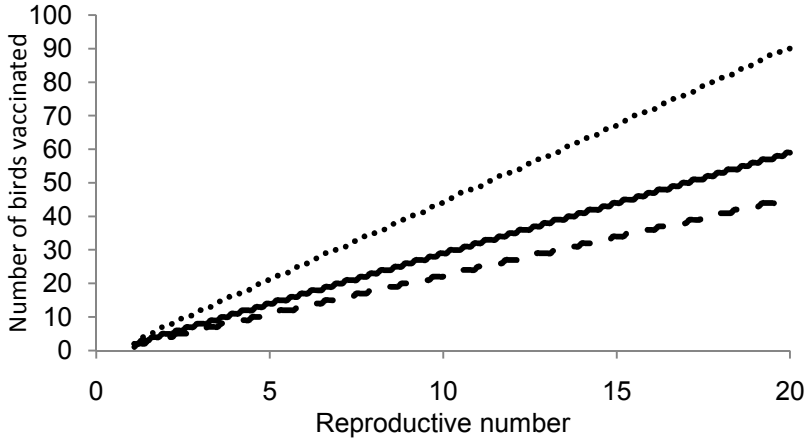


Figure 5-10| Minimum number of vaccinated birds required to reject $R_v > 1$ under the assumption of an all-or-nothing vaccine.
Significance level: 5% (solid line), 1% (dotted line) and 10% (dashed line).

5.3.4 Effect of the misspecification of vaccine action when both challenge and non-challenged birds are vaccinated.

Vaccinating both challenge and non-challenge birds further exacerbates the extent to which a leaky vaccine model will underestimate the reproduction number from experimental data using an all-or-nothing (or all-or-something) vaccine. This is because the leaky model overestimates the number of infected birds, as well as the number of susceptible birds, within the flock. As a result, for many scenarios where an all-or-nothing vaccine is not sufficient to bring the reproduction number below unity, there would be the distinct possibility of mistakenly inferring that $R_v < 1$. This probability decreases, for a fixed R_v, with both a lower R_0 and when fewer experiments are conducted. However, even at levels of R_0 within the lower range of those estimated for H5N1 (Table 2-1) and at study sizes equivalent to those conducted to estimate the efficacy of H5N1 vaccines (van der Goot, van Boven et al. 2008; Bouma, Claassen et al. 2009), there exist scenarios where wrongly inferring $R_v < 1$ would be the most likely outcome when using a leaky vaccine estimator (Figure 5-10).

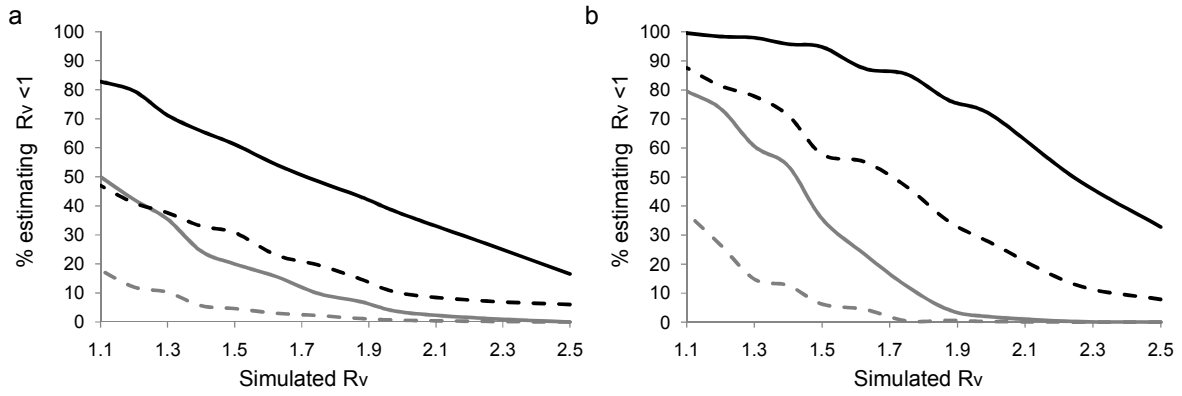


Figure 5-11| Probability of wrongly inferring $R_v < 1$ based upon the assumption of a leaky vaccine when both challenged and unchallenged birds are vaccinated with an all-or-nothing vaccine.
All-or-nothing vaccine efficacy was adjusted for each value of R_0 to ensure the correct R_v. For each combination the result shown is percentage of 500 simulations where the estimated one-sided 95% credible interval of the leaky vaccine estimate was below unity. (a) shows the results when fitted to pairs of experiments and (b) for sets of five experiments. Black lines represent $R_0 = 6$ and grey lines $R_0 = 3$, dashed lines show the equivalent values when only unchallenged birds are vaccinated.

For example, in an analysis by *Van der Goot et al* (van der Goot, van Boven et al. 2008), virus was isolated from only four out of nine Pekin ducks inoculated with H5N1 virus following a double dose of an inactivated H5N2 vaccine, suggesting the existence of some heterogeneity in vaccine response. These birds were then separated into a group of four and a group of five and introduced into two unchallenged flocks of five birds which had previously received the same vaccine regimen. Making the assumption that this heterogeneity can be characterised by an all-or-nothing or all-or-something vaccine and using the maximum likelihood estimate of the efficacy of this vaccine (4/9=0.44), it was found that for a basic reproduction number of 2.3 (so that R_v would be slightly above unity), the probability that a leaky vaccine estimator would wrongly find strong evidence that the reproduction number had been brought below unity was around 41%. This probability then decays relatively slowly with increasing R_0, saturating at around 5% for values of $R_0 > 10$ (Figure 5-12). The real-life result of this experiment resulted in virus being isolated from one unchallenged duck, resulting in a leaky vaccine estimate of $R_v = 0.2$ which was below unity at the 10% significance level.

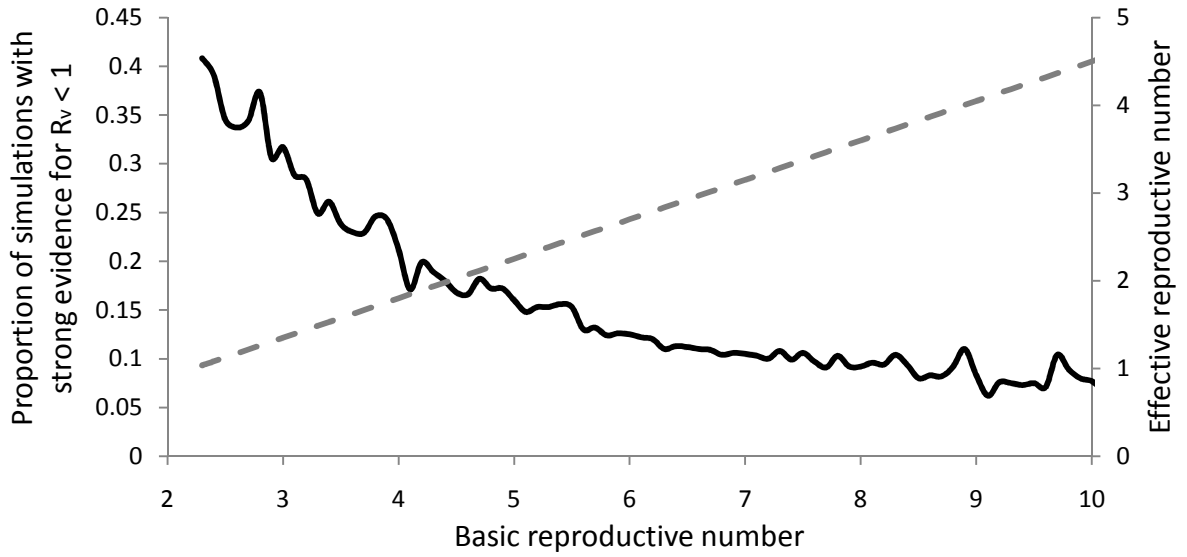


Figure 5-12| Probability of wrongly inferring $R_v < 1$ from two experiments when assuming an all-or-nothing vaccine with 44% efficacy which is leaky.
A leaky vaccine model was fitted to 1000 simulated datasets for experiments with 4 and 5 challenged vaccinated birds introduced to two sets of 5 unchallenged vaccinated birds for reproductive numbers between 2.3 and 10 and the proportion which resulted in an estimate of strong evidence for $R_v < 1$ was calculated.

5.4 Discussion

Final outbreak size distributions provide a framework for inference about the spread of infection within a flock which have the advantage of requiring data which is very often readily available, especially within an experimental context. However, implementing these methods generally require assumptions are made about the infection process, with regards to the infectious period distribution and the nature of mixing within a flock. When using these methods to compare the spread of infection within a vaccinated and control flock the action by which vaccination achieves protection becomes an additional unknown.

As this analysis shows, the assumption made regarding this factor can have a large effect upon the estimate of reproduction number within the vaccinated flock. In the existing literature, estimates of this from the final outbreak size within the context of transmission experiments tend to make the implicit assumption of a "leaky" vaccine. This has the advantage of not requiring any knowledge about the basic reproduction number. However,

in terms of the estimate of the level of control achieved by the vaccine, it represents the most optimistic assumption possible. Moreover, various scenarios were found where, if flock sizes or the number of repeat experiments are sufficiently large, alternative models of vaccine action may provide a significantly better fit. In particular, when R_0 is sufficiently high, medium-sized outbreaks within relatively large vaccinated flocks and a lower level of variance in the size of outbreaks than that expected from a "leaky" vaccine may indicate that observed outbreak sizes are more easily explained by a model which incorporates heterogeneity in individual response to the vaccine and, therefore, a higher R_v.

The benefits and drawbacks of introducing different numbers of infected birds at the beginning of an experiment need to be more carefully evaluated when establishing the protocol of any transmission experiment and are likely to depend upon the size of the study. Vaccine experiments often introduce multiple infected birds into a vaccinated flock in order to challenge the level of herd immunity that has been achieved robustly and reduce the chances that a minor outbreak would occur stochastically despite an ineffective vaccine (Velthuis, Bouma et al. 2007). However, this will further bias the estimate of R_v as a result of any misspecification of the level of heterogeneity in individual response to vaccination and may directly lead to the mistaken conclusion that the level of immunity within the flock has passed the critical vaccination threshold. Thus simply fitting an effective reproduction number to final size data from such experiments, in the absence of information with regards to the action of a given vaccine, is not an adequate measure of vaccine efficacy. Reducing the number of initially infected birds is also likely to make it easier to choose between competing models of vaccine action and, as it also reduces the probability that the entire

flock becomes infected during the experiment, may make estimation of R_0 from the control group using final size methods more straightforward.

As well as having an effect on susceptibility to infection, vaccines may also reduce the onwards transmission of disease when a vaccinated bird becomes infected. Attempts to quantify the transmission of HPAI in vaccinated flocks have involved analysing the final size of outbreaks within flocks where challenged birds also receive vaccination. However, this compounds the extent to which the assumption of a leaky vaccine will underestimate the true reproduction number within the flock. Within the current literature there are examples of transmission experiments involving H5N1 vaccines where not all vaccinated birds challenged with infection became infected, suggesting that the level of heterogeneity in vaccine response was under-represented by the leaky vaccine final size estimator which was applied (van der Goot, van Boven et al. 2008; Bouma, Claassen et al. 2009). This may suggest that some vaccines are less effective at reducing the transmission potential of HPAI within a flock than these studies would appear to suggest. This highlights the fact that in order estimate transmission and the adequacy of any vaccine accurately, the distribution of vaccine response, both in terms of the susceptibility to infection and infectiousness of vaccinated birds, need to be better understood. In the absence of such data, assessing the transmissibility and susceptibility of vaccinated birds in separate experiments may produce a more reliable estimate of the effects of a vaccine and provide greater insight as to the action by which the vaccine confers protection. The resources necessary to conduct such analyses may, however, be prohibitive.

An alternative, more robust way to ensure the efficacy of a vaccine is adequate would be to establish a range of plausible basic reproduction numbers, perhaps based upon the

experiments conducted on the control group, use this to estimate the minimum number of birds required, using the method described in this chapter, and then challenge this number of vaccinated birds to infection, either directly or through a transmission experiment with a sufficiently high number of initially infected birds. This method would also, provided it is safe to assume that vaccination does not enhance transmissibility once infected, be conservative with respect to assumptions regarding the extent to which vaccination reduces infectivity. However, in the context of H5N1, very high reproduction numbers have been estimated from transmission experiments involving flocks of both chickens and ducks (van der Goot, van Boven et al. 2007; Poetri, Bouma et al. 2009). Taking the most recent as an example, an R_0 of 12 was estimated for the transmission of H5N1 between chickens with a lower 95% confidence interval of 4.7. This estimate is well within the region where misspecification of the action of the vaccine can lead to an important underestimation of R_v. To reject the hypothesis R_v>1 using the method described above would require that the experiment involves a minimum of 35 vaccinated birds, suggesting that typical vaccine experiment sizes may not be sufficient.

Wrongly inferring that R_v has been brought below unity as a result of a leaky vaccine would be of little consequence if all outbreaks occurred within the context of a small closed population, with the high coverage achieved by an all-or-nothing vaccine having a similar effect in reducing transmission as the reduction in susceptibility induced by the leaky vaccine. However, in larger flocks an all-or-nothing vaccine which fails to induce sufficient herd immunity may still lead to a sizeable outbreak within the remaining susceptible sub-population. This is likely to have consequences during real life epidemics with larger flock sizes and where transmission routes exist between flocks and could feasibly lead to a

situation where infection is sustained within a flock but mortality is reduced to a level which makes outbreaks difficult to detect. Such “silent spread” may have serious implications for the overall effectiveness of any large-scale vaccination campaign (Savill, St Rose et al. 2006), where any prolonging of the time it takes to detect outbreaks may offset the reduction in infectivity and susceptibility induced by the vaccine. This suggests that for H5N1, and other pathogens with similar transmissibility, further trials may be necessary to establish that, even in the controlled context of a transmission experiment, vaccines are sufficiently efficacious to prevent sustained transmission within a flock.

Chapter 6 An estimate of the effect of H5N1 vaccination upon within and between flock transmission in Indonesia

6.1 Introduction

In the previous chapter the use of final size methods to estimate the reproduction number within the context of animal transmission experiments were explored. In this chapter the same methods are applied to data collected from field investigations into outbreaks of H5N1 in Indonesia. These investigations were carried out in areas where the Operational Research in Indonesia for more effective control of highly pathogenic avian influenza (ORIHPAI) study was taking place. This study was undertaken within 16 districts on the island of Java where the Participatory Disease Surveillance and Response (PDSR) programme (a reporting network which covers much of Indonesia and combines active and reactive surveillance as well as rapid testing for the presence of Influenza A and the collection of samples for laboratory confirmation is well established (Forster 2010). ORIHPAI was a year long study designed to assess whether mass vaccination can feasibly reduce the incidence and intensity of outbreaks within populations of small scale commercial and backyard poultry which characterise the majority of poultry production in Indonesia. It involves randomly selected sub-districts which are then randomly allocated either treatment or control status. Those sub-districts allocated treatment status are then further separated into two categories: 1) PDSR with mass vaccination with an inactivated Indonesian H5N1 avian influenza (AI) strain (A/Ck/Legok/2003) and 2) PDSR with mass vaccination with both

AI and Newcastle Disease vaccine. In both treatment arms AI vaccination was then offered to small-scale commercial and backyard farms free of charge and on a voluntary basis, with the aim of vaccinating each flock with an initial inoculation and 3 week booster 4 times over the study period. Sero-monitoring following the first round of vaccination found 11% of poultry in the control sub-districts with antibody titre >0, 55% in zones which received AI vaccination alone and 47% in areas which received AI and ND vaccination, suggesting that the programme had achieved partial coverage. Vaccination was re-administered every six months with three rounds occurring between July 2008 and July 2009.

Here the data obtained from these field investigation are used to estimate the reproduction number for outbreaks which arose during the study period, both at a within and between flock level. The aim of this analysis was to assess whether outbreaks in vaccinated areas differed substantially from those within control measures and, if so, attempt to infer to what extent this can be attributable to the impact of vaccination.

6.2 Methods

6.2.1 Data collection

Data were collected in the form of field outbreak investigations within each of the 16 ORIHPAI districts between February and November 2009. In each of these districts a list of confirmed outbreaks was generated, with only those verified by either a positive PDSR rapid antigen test, laboratory PCR and/or virus typing included. Depending on available time and the number of outbreaks which had occurred within the district, 1-4 outbreaks were then randomly selected and investigated with the constraint that at least one of the outbreaks was from a vaccination (either AI or AI and ND) zone and at least one from an area where only standard PDSR control was implemented, provided such outbreaks existed. In each of

these districts data on the number of birds affected in individual flocks and the size of these flocks, as well as data on the timing of first and last observed signs of infection where possible, were obtained via in-depth interviews with poultry-holders within the *rukun tetangga* (RT- the smallest administrative unit in Indonesia and roughly translated as "neighbourhood") in which the outbreak occurred. The total number of households within the RT, confirmed by a transept walk through the study RT, and the number of households which had experienced infected poultry was also recorded.

In total, within the 16 selected sub-districts, field investigations were carried out in 41 RTs where outbreaks had been reported, with 16 of these within sub-districts which had been allocated treatment status (either AI or AI and ND vaccination) within ORIHPAI. The remaining 25 were in sub-districts which only received PDSR as an intervention. During these field investigations, data on the flock size and total number of birds infected was collected from a total of 109 farmers, 40 in vaccination zones and 69 in control zones, as well as data on whether any birds were the sold and slaughtered during the course of the outbreak. In total 43 of the farmers reported they had sold or slaughtered poultry in the face of an outbreak. As the infection status of these birds, and thus the likely impact selling or slaughtering had upon the final outbreak size, could not be established, the outbreaks within these flocks were excluded from the analysis. This left 25 flock-level outbreaks in vaccination zones and 41 in the control zone (Figure 6-1).

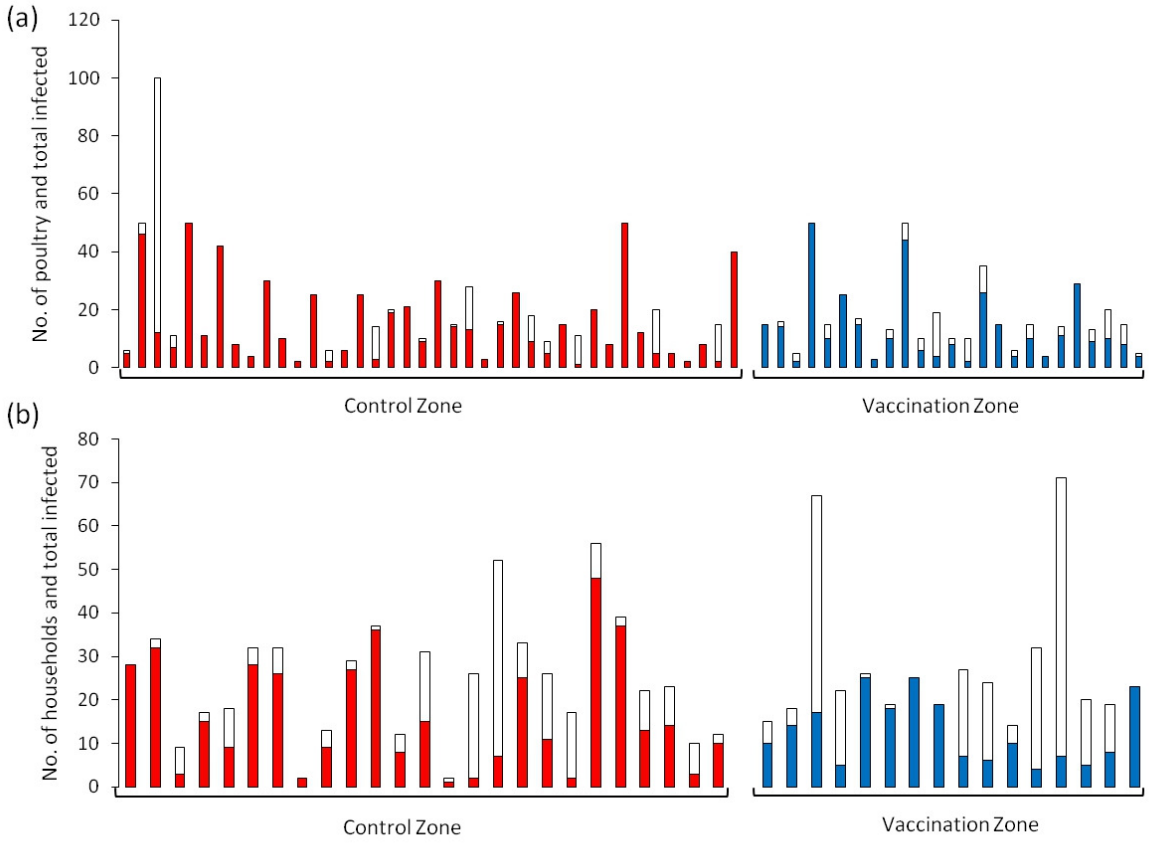


Figure 6-1| Flock and RT sizes and proportion infected.
Figure shows **(a)** Flock level outbreaks, **(b)** RT level outbreaks, height of bars represent, respectively, the total number of birds within each flock and the total number of flocks within the RT, with the proportion infected represented by the coloured proportion of each bar.

6.2.2 Reproduction number estimation

Analysis was conducted at two different resolutions, that of within-flock transmission where each flock was treated as a closed population and transmission takes place between individual birds, and between-flock transmission where each RT was treated as a closed population and transmission takes place between flocks. At both of these levels outbreaks were modelled as GSEs (see previous Chapter), with a single initial infective, and the posterior distribution of the reproduction numbers was obtained using the methods as described in the previous chapter, with the likelihood of each final outbreak size calculated using the construction proposed by *Ball* (Ball 1986). For each estimate, uniform priors

bounded between 0 and 10000 were used for the parameter β, a burn-in period of 3000 steps of the MCMC was used and the posterior distribution was sampled every 5 steps for a total of 30000.

Estimation of the reproductive number from the data required a separate estimate of the infectious period distribution. within- and between-flock transmission. For within-flock transmission, an estimate from a recent analysis within the existing literature (Bouma, Claassen et al. 2009) of a mean infectious period of 2.5 days with a variance of 0.16 was used (Figure 6-3(b)). For between-flock transmission the distribution was fitted to the observed outbreak durations (estimated from the dates at which infection was first and last observed within a flock and assuming there was a day of infectivity before infection was first observed).

6.2.3 Dealing with larger population sizes

For values of $R_0 < 1$, outbreaks can be expected to die out within the first few infections, whilst for $R_0 > 1$ outbreak probabilities are bimodal in nature, with outbreaks either dying out stochastically within the first few infections or taking off and infecting a large proportion of the population (Figure 6-2(b)). As a result, extreme scenarios such as large outbreaks when $R_0 < 1$ and, more importantly due to the recursive way of calculating the outbreaks probabilities, the intermediate outbreak sizes between the plausible "major" and "minor" outbreak scenarios for $R_0 > 1$ can become numerically unstable (Demiris and O'Neill 2006). This is because the recursive calculation of small outbreak probabilities leads to rounding errors and negative outbreak probabilities when the required accuracy exceeds the precision available for the standard data types used in many programming languages (Figure 6-2(a)). For double precision arithmetic these errors tend to occur in populations of around

50 or more. In order to avoid such problems calculations for larger outbreaks were carried out using the arbitrary precision floating point classes available from the Number Theory Library (NTL) package (Shoup 2003).

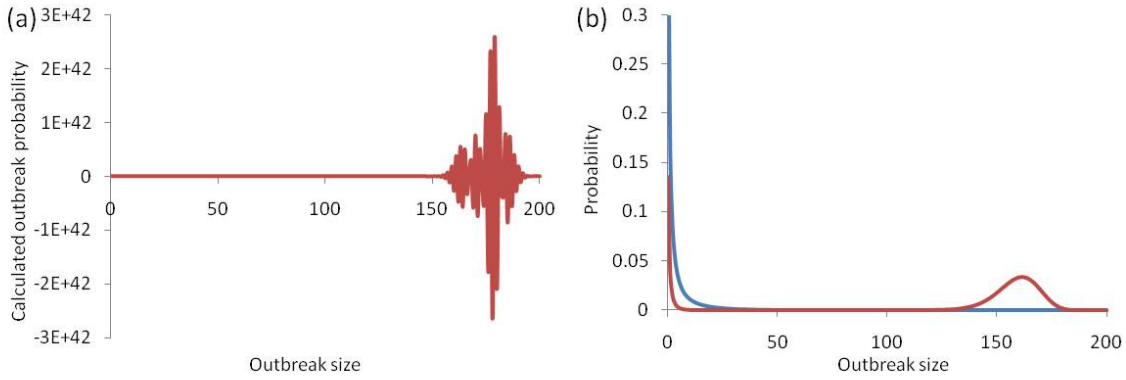


Figure 6-2| Dealing with numerical instabilities.
Figure shows **(a)** Outbreak probabilities for $\boldsymbol{R_0}$=2 in a population size 200 as calculated using standard double precision. **(b)** Outbreak probabilities for $\boldsymbol{R_0}$=2 (red line) and $\boldsymbol{R_0}$=0.5 (blue line) as calculated using 150 bit precision.

6.2.4 Estimating the protective effect of vaccination

As discussed in the previous chapter, any estimate of the effective reproduction number in birds which have received vaccination depends upon the assumption made about the distribution of vaccine response within individual birds. However, when vaccination coverage is unknown, directly calculating the likelihood of an outbreak size for any given assumption about the action of the vaccine involves both integrating over all possible vaccine coverages and, as the vaccination status of the birds which were infected is also unknown, all possible combinations of infections within vaccinated and unvaccinated birds which could produce the observed outbreak size, making the likelihood overly computationally intensive. When attempting to estimate the coverage of the vaccine, the only assumption about the action of the vaccine which eliminates the latter uncertainty is that of an "all-or-nothing" vaccine where vaccinated birds are either totally immune (and

therefore don't feature within the number of infections) or totally susceptible. With regards to the action of the vaccine, this assumption constitutes the least optimistic estimate of the proportion of birds protected by vaccination. This is because, for any other assumption, larger outbreaks would be expected for the same coverage. Therefore, as shown in Chapter 5, for any given R_0, a higher estimated protective coverage would be required to fit the observed outbreak sizes.

If R_0 is known then, using the notation introduced in the previous chapter for final size probabilities, the number of individuals (birds for within flock transmission, whole flocks for between flock transmission) vaccinated within the population, v_i, can be modelled by a beta distribution with shape parameters θ and γ. This provides the likelihood:

$$\pi\left(\theta,\gamma|\left(\underline{x},\underline{n}\right),R_0,I\right) \propto \prod_{i=0}^{N} \int_{v_i=0}^{n_i-1} P(x_i|v_i,n_i,\beta,I)\,P(v_i|\theta,\gamma)dv_i \qquad 6.1$$

Integrating over all possible levels of vaccine coverage can then also be avoided by treating each v_i as a nuisance parameter to be augmented. Using uniform priors, bounded between 0 and 10000 for the θ and γ parameters of the beta distribution and between 0 and 1 for each unobserved vaccine coverage, a Metropolis-Hastings algorithm similar to that used to update individual susceptibilities in the previous chapter was implemented in order to estimate the posterior distribution of θ and γ.

Whilst this will only provide an estimate of the protective coverage, and not the overall number of birds actually vaccinated, from the perspective of assessing the effectiveness of a vaccination programme, a lower-bound of the overall coverage achieved with regards to

assumptions about the action of the vaccine can be obtained by assuming a perfect or wholly effective vaccine.

6.3 Results

When all outbreaks were analysed together at the within-flock level (Figure 6-3(a)), using the infectious period based upon the results of a transmission experiment (Figure 6-3(b)), R_0 was estimated as 2.54 with 95% credible interval (95% CrI) (2.35-2.74). Disaggregating outbreaks in control and vaccination zones produced reproduction numbers of 2.79, 95% CrI (2.52-3.10) within flocks in control zones and of 2.23, 95% CrI (1.96-2.51) in vaccination zones (Fig 7-3(a)). This difference is decisive according to Jeffreys' scale of evidence (Jeffreys 1961) (a Bayes factor (BF)> 100 see subsection 3.2.10).

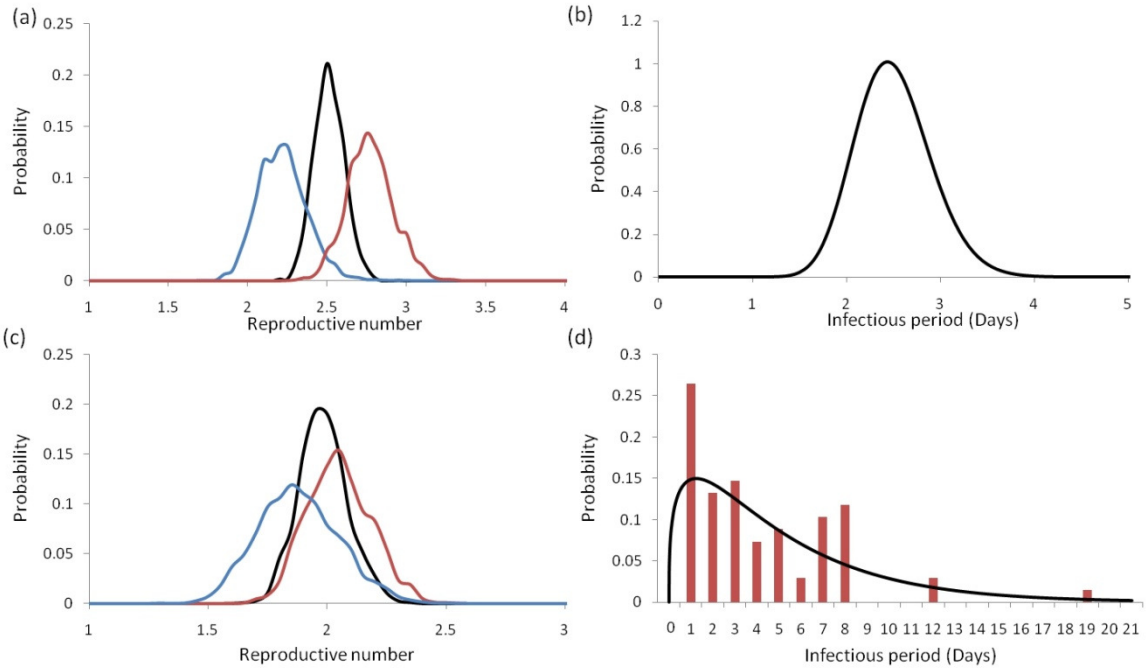


Figure 6-3| Reproductive numbers for a variable infectious period.
Figure shows **(a)** Posterior distribution of within-flock reproductive number outbreaks in vaccination zones (blue lines), control zones (red lines) and the aggregated data (black lines). **(b)** Within-flock infectious period distribution used in analysis. **(c)** Posterior distribution of between-flock reproductive number for outbreaks in vaccination zones (blue lines) and control zones (red lines) and the aggregated data (black lines). **(d)** Histogram of observed outbreak durations (red bars) and between-flock infectious period distribution fitted to those outbreaks (black line).

Carrying out the same analysis for the final size data at the RT level produced a lower between-flock reproduction number of 2.01, 95% CrI (1.81-2.23). The reproduction number of outbreaks in the vaccinated zone (1.9, 95 % CrI(1.59,2.26)) were, again, estimated to be lower than that in the control zone (2.07, 95% CrI(1.82,2.35)), with the posterior distributions providing substantial evidence of a lower level of transmissibility in the outbreaks within the vaccination zone (BF=7.69) (Fig 3(c)).

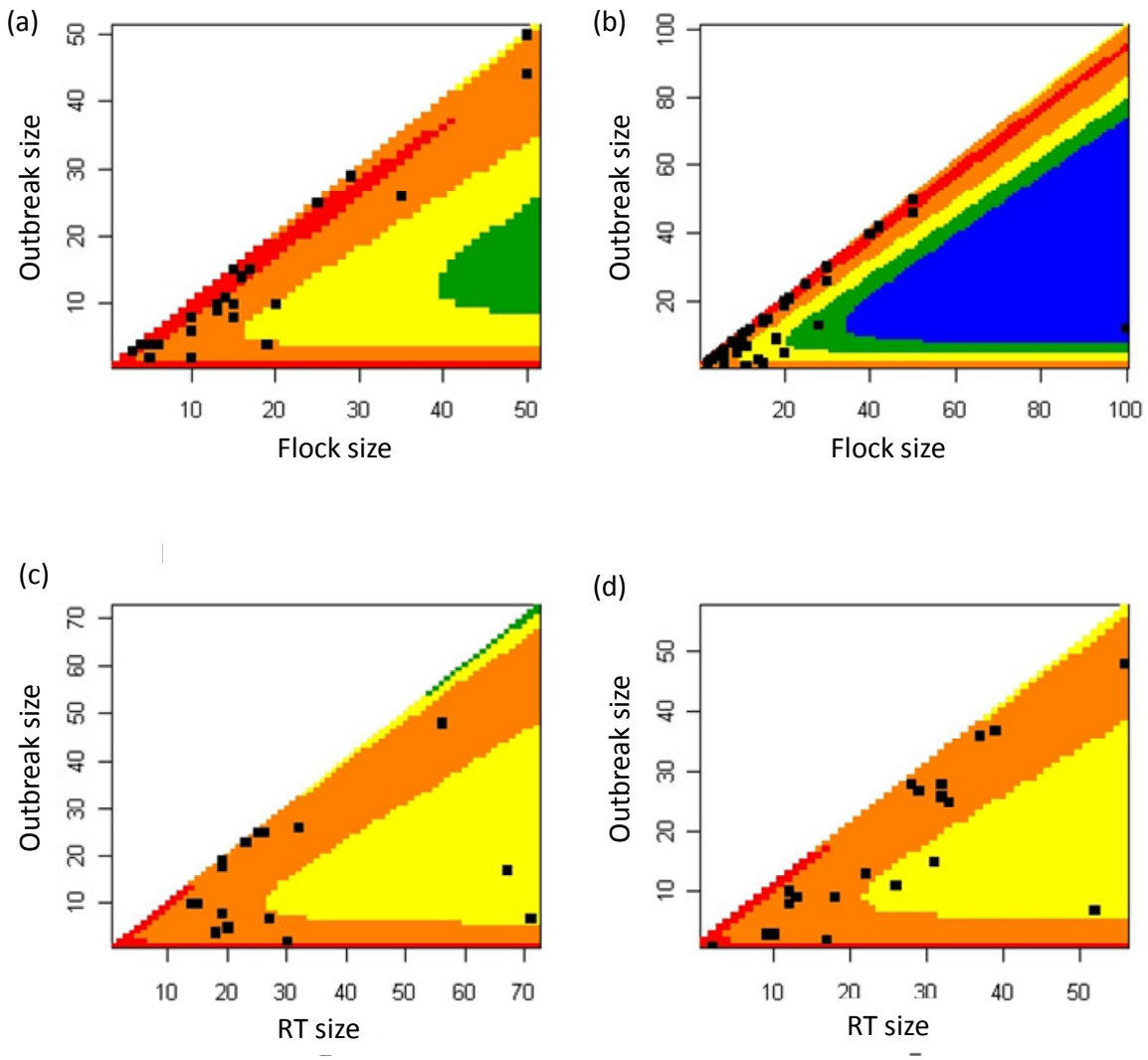


Figure 6-4| Comparison of the posterior predictive probability of outbreak sizes and the data. Posterior predictive probabilities are calculated as the mean outbreak size probability generated by sampling the posterior 1000 times. Outbreak sizes are then coloured according to this probability: red >0.1, orange >0.01, yellow >0.001, green > 0.0001, blue >0. Figure shows **(a)** within-flock transmission for outbreaks in vaccination zones, **(b)** within-flock transmission for outbreaks in control zones, **(c)** between-flock transmission for outbreaks in vaccination zones, **(d)** between-flock transmission for outbreaks in control zones. In each figure observed outbreak sizes are displayed using black squares.

Figure 6-4 shows that, whilst the model was able to fit a lot of outbreaks reasonably well, there are a few noticeable outliers, with the two worst fitting within-flock level outbreaks (an outbreak of 12 in a flock of 100 and an outbreak of 13 in a flock of 28) occurring within non-vaccination areas. Moreover, both the fitted within-flock and between-flock

transmission outbreak probabilities suggest a greater number of low-level outbreaks than were observed within the dataset. In particular, whilst the dataset contained no flocks where only a single bird was infected and only a single RT where only one flock reported infected birds, from the fitted outbreak probabilities, it would be expected that this would be the case in 11% of flocks and 17% of outbreak RTs in vaccination zones and 6% of flocks and 14% of outbreak RTs in control zones.

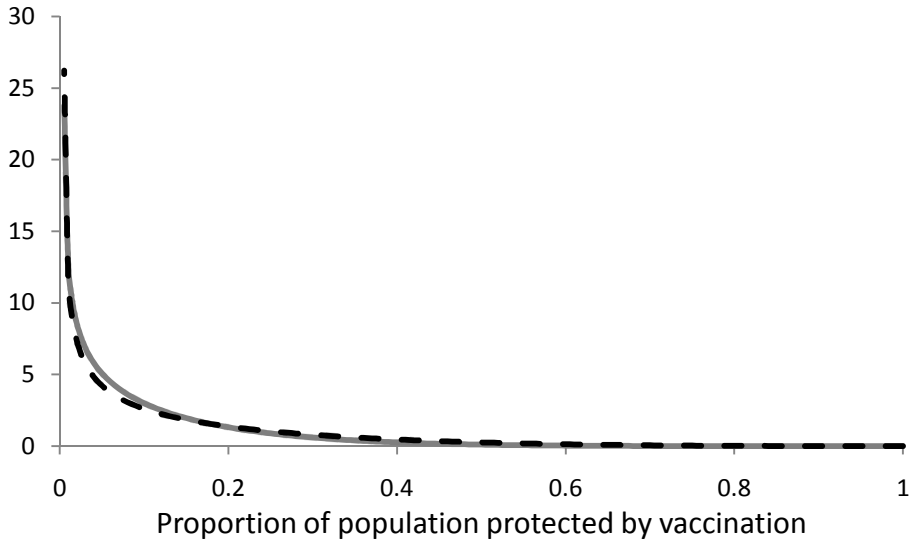


Figure 6-5| Probability density function of fitted vaccination coverage using posterior mean R_0.
Within-flock coverage (grey line) and between-flock coverage (black dashed line).

When fitted with the posterior mean estimate of R_0 from the outbreaks which occurred in control zones, similar distributions of vaccine coverage were estimated for between and within flock transmission (Figure 6-5). In both cases the estimated mean vaccination coverage was significantly lower than the approximately 50% coverage estimated from the post-vaccination sero-monitoring (Table 6-1).

Table 6-1| Posterior distribution of vaccination coverage using posterior mean R_0

Measure of coverage	Within Flock posterior mean (95% CrI)	Between Flock posterior mean (95% CrI)
Shape θ	0.61 (0.15-1.54)	0.53 (0.19-1.24)
Shape γ	5.42 (1.49-17.41)	3.63 (1.12-10.63)
Mean coverage	11% (5%-19%)	14% (5%-27%)
R_v	2.45 (2.22-2.63)	1.71 (1.47-1.89)

6.4 Discussion

This analysis provides the first quantitative estimates of the transmissibility of H5N1 between and within small-holdings of poultry in Indonesia and one of only a handful of estimates of the reproduction number of H5N1 in poultry from outbreak data (Tiensin, Nielen et al. 2007; Ward, Maftei et al. 2009; Magalhaes, Pfeiffer et al. 2010). The estimate of a within flock reproduction number of 2.52 agrees closely with that estimated for outbreaks in Thailand (Tiensin, Nielen et al. 2007) and the finding of a significant reduction in the reproduction number following vaccination found for outbreaks in Vietnam (although that analysis found a lower reproduction number than that estimated in Thailand (Magalhaes, Pfeiffer et al. 2010)). The between-flock reproduction number obtained in this analysis is also well within the distribution of the only existing estimate, that of outbreaks of H5N1 in Romania (Ward, Maftei et al. 2009) and the finding that this was significantly lower than that for within-flock transmission is important because it highlights the potential impact that measures which reduce between flock transmission such as heightened biosecurity can have upon the spread of infection. The estimate from this analysis suggests

that if half the flocks in an RT can be fully protected, the level of spread within an RT may be significantly diminished.

The fact that it was not possible to fit all outbreaks using a generalised stochastic epidemic, even within areas not receiving vaccination, may suggest that either one of the modelling assumptions, such as that of a closed population or a single initial infective, may be inadequate or that heterogeneity in transmissibility needs to be incorporated into the model. Potential factors causing this may include species of poultry, the method by which poultry are kept or housing density. Modelling such heterogeneity would require more detailed data. In particular, data would be needed on those households within the RT which avoid infection.

Assuming surveillance effort is equally distributed, the design of this study, where outbreaks are investigated retrospectively, is always likely to result in investigations taking place in areas where control measures are having the least success in preventing transmission, as these are the areas where outbreaks are most likely to occur and be detected. In fact, out of the sixteen RTs investigated in vaccination zones only four had officially received vaccination sufficiently early to ensure immunity could develop whilst, of the remaining twelve RTs, three received vaccination within a week of an outbreak occurring and the remaining RTs had yet to be vaccinated as part of the most recent round of the vaccination campaign The estimate in this analysis of vaccine coverage was also significantly below the coverage estimate obtained from sero-surveillance which was carried out within the entire vaccine zone, appearing to support the suggestion that poorly vaccinated areas are over-represented in the data. It should, however, be noted that sero-conversion is not necessarily an accurate representation of protective coverage if some birds sero-convert but are not

fully protected or of the overall coverage of the vaccination campaign if there are instances where vaccination fails to induce sero-conversion. Moreover, the coverage estimate obtained from the model was calculated assuming a fully effective vaccine. This was because, as neither the vaccination status of infected or uninfected birds was available, this was the only assumption which was computationally feasible to fit. If such data were available it would have been possible to assess the sensitivity of this estimated coverage to the assumption of vaccine action by fitting different vaccine response profiles to the data as discussed in Chapter 5. However, given that outbreaks were studied using retrospective field investigations, data on the vaccination status of any birds, independent of whether they were infected, at the time of the outbreak is likely to be impossible to collect.

For similar reasons regarding the design of the study, these results also provide no information as to the number of RTs which have been protected from infection within vaccinated areas, especially as the estimates of the between-flock reproduction number suggest that a reasonable proportion of unprotected RTs could be expected to avoid any outbreaks following the introduction of infection. As a result, the estimated reproduction numbers of investigated outbreaks cannot be viewed as an estimate of the overall reproduction number within ORIHPAI vaccination zones.

The estimate of outbreaks occurring in some areas despite a partial reduction in the reproduction number of infection agrees with recent modelling work which found that, four months following vaccination, in a typical tropical free range poultry population, that the level of population immunity would fall below the critical vaccination threshold required to prevent sustained transmission due to population turnover (Lesnoff, Peyre et al. 2009). Therefore, in light of the dynamism of small-scale poultry production in Indonesia where

approximately 80% of traded poultry are sold alive (Sumiarto and Arifin 2008), it is perhaps not surprising that there were reported outbreaks in the vaccination and control zones where despite some residual level of immunity, the reproduction number had once again risen above unity.

Another factor contributing to the lower estimate of the reproduction number in outbreaks in the vaccination zones may be that vaccination has been successful in preventing outbreaks in areas within which infection was most intense and R_0 is highest. As a result, this study may be sampling traditionally lower-risk areas where, on average, a lower level of between and within flock transmission takes place. It could also be the case that, as the observed level was higher, but not significantly so, within the vaccination zone, the selling and slaughtering of infected flocks also had some impact on the results of the analysis, especially if this occurs to a greater extent in flocks in which the burden of infection is heaviest.

Whilst it would be tempting to view the smaller number of outbreaks investigated in vaccination zones as evidence of a lower incidence of outbreaks than in non-vaccinated areas, the stated aim to investigate at least one outbreak in a vaccination zone and one outbreak in a control zone would seem to suggest that surveillance effort was not in fact equally distributed across the different treatment groups. The extent to which this may have biased the distribution of observed outbreaks sizes across treatment groups in the data is also not clear. More detailed data on the overall and treatment group and district specific incidence of outbreaks, as well as the respective resources allocated to investigate outbreaks, would help to assess this factor.

These results suggest that there are still areas within designated ORIHPAI vaccination zones where infection is able to spread and vaccination coverage hasn't been maintained sufficiently to keep the reproduction number below unity. However, our estimates may imply that, even within these areas, incremental progress has been made towards controlling the spread of infection or that the programme has been effective at curtailing the most intense outbreaks which had been previously occurring. In order to achieve full control within areas where poultry turnover occurs so rapidly, a programme where the time between repeated campaigns of vaccination is reduced may be the only way to ensure that sufficient population level immunity is maintained.

Chapter 7 Conclusions and limitations

7.1 Key Findings

The research conducted towards my PhD focussed upon evaluating and applying methodologies for calculating key transmission parameters from the forms of outbreak data likely to be collected for the H5N1 subtypes currently circulating in Southeast Asia and determining the extent to which these can be used as measures of the effectiveness of different control measures.

In Vietnam and Thailand nationwide surveillance data has been collected and collated at a sub-district resolution for waves of outbreaks occurring since 2004. As a result, using Bayesian techniques to deal with the uncertainty inherent in partially observed infection processes, it was possible, using MCMC, to fit spatial models which provided the opportunity to quantify, compare and contrast the estimated dynamics of infection for these waves and during different phases of control strategies.

However, in many settings such systematic data collection is not carried out and it is not possible to model the spread of infection at such a macroscopic level. Instead analysis is carried out on a smaller scale but at a higher resolution, either within an experimental setting or where data is collected in the field in an attempt to obtain a snapshot of different aspects of the overall transmission process. In such contexts, final size of outbreak methods, due to the relatively low data requirements they impose, provide a useful means by which to quantify transmissibility within or between a flock of birds. In recent years such techniques have been applied to assess the efficacy of many of the vaccines currently used in various countries in Southeast Asia and beyond.

In light of this, the second half of this thesis concerned the use of final size methods to quantify the effects of vaccination at the flock level with an investigation as to the possible effects of different vaccine actions upon estimates of vaccine efficacy fitted to experimental data and assessing the effects of a vaccination field trial in Indonesia.

7.1.1 The development of techniques to assess the impact of interventions when data are limited.

Bayesian methods constitute valuable tools for obtaining the maximum amount of information from an epidemic (Streftaris and Gibson 2004; Cauchemez, Temime et al. 2006). In particular, working within a Bayesian framework makes it possible to treat missing data in the form of nuisance parameters. For partially observed epidemics, this substantially simplifies what would otherwise be a prohibitively cumbersome likelihood which can then be fitted to the observed data using relatively straightforward to implement methods for simulating from such distributions such as MCMC (O'Neill and Roberts 1999; Streftaris and Gibson 2004; Chis Ster, Singh et al. 2008; Jewell, Keeling et al. 2008).

Using these methods during my PhD I was able to simultaneously estimate the spatial transmissibility and infection-to-report distribution for waves of outbreaks in Vietnam and Thailand based solely on routine outbreak report and poultry population data, to use the reconstructed epidemic tree from this to assess how infectivity varies at different stages of these waves and how model fit varies with surveillance effort and to explore the potential effects of more rapid detection of outbreaks. Also, although the way the data was collected is likely to mean it cannot be used to interpret the overall effect of vaccination in the region, I was able to estimate the effect of vaccination upon outbreaks investigated during a field trial in Indonesia based only upon final outbreak sizes in the absence of explicit data on

vaccination coverage. This serves to highlight that, by reducing the data requirements necessary to allow the fitting of transmission models to reports of outbreaks and outbreak investigations, such techniques have the potential to enhance the role that modelling can play in quantifying the spread of infection and the effects of control measures in a wide range of settings. In particular, such models could plausibly be fitted to waves of outbreaks of many different animal diseases including HPAI, ND, FMD, CSF, sheep and goat pox and any future outbreaks of rinderpest where information gathering and outbreak reporting follows standard FAO guidelines (FAO 1999) and data on the denominator population are also available.

7.1.2 The impact of vaccination in Vietnam

My results suggest that, a year following the implementation of mass nationwide vaccination in Vietnam, outbreaks occurring during the 2007 wave of outbreaks in northern Vietnam were less readily reported than during waves which occurred both before vaccination and around the time the campaign was first implemented. As a result, although the rate at which infection could spread between infectious communes had been substantially reduced, sustained transmission could still occur in the form of a less intense but more prolonged wave of outbreaks. Such an increase in the time between a commune becoming infected and the presence of infection being reported supports the notion that vaccination can lead to a scenario where, either through increasing the prevalence of asymptomatic infection or by reducing flock mortality below established thresholds, outbreaks are harder to detect.

An exploration of the possible chains of transmission which led to the wave found that, had such a reduction in detection capacity been avoided, the overall impact of vaccination, in

terms of limiting the size and duration of the wave and reducing the likelihood of a wave occurring, would have been improved markedly. Whilst these findings were based upon strong assumption regarding the future of the epidemic, they do highlight the importance of supplementary strategies aimed towards improving outbreak surveillance such as the use of sentinel birds or small-holder awareness campaigns to ensure that the potential for vaccination to reduce transmission is realised, both for future mass vaccination campaigns in different contexts and as Vietnam moves towards a transitional strategy of more focussed vaccination.

Comparing the waves occurring in late 2004 and mid-2005 I found that, whilst transmissibility of infection did not vary much between the two waves, there was substantial evidence that the rate at which outbreaks were reported had increased following a rise in the compensation offered by the Vietnamese government to farmers with infected flocks, suggesting that measures aimed towards ensuring farmers are less negatively affected as a result of reporting outbreaks may substantially improve disease surveillance.

7.1.3 Active surveillance and the role of different poultry sectors in Thailand

The large industrial production sector in Thailand has created areas with extremely high poultry concentrations. As a consequence, unlike in Vietnam, it was not possible to generate a model with infectivity increasing linearly with the poultry population within a tambon which produces a spatial distribution of risk of transmission resembling the observed distribution of outbreaks. As when modelling these outbreaks, some form of heterogeneity in per-bird infectivity and susceptibility with increased intensity of production should be

incorporated into any model, this can be done by either modelling saturating infectivity as flock size increases, producing a more plausible spatial distribution of risk or by stratifying the poultry population by production type.

Reconstructing the epidemic tree from this model it was found that, whilst the majority of infection was transmitted from backyard flocks, both in terms of those infected and the total number of flocks, the per-flock probability of transmitting infection was highest in commercial flocks, suggesting that commercial flocks played a disproportional role in propagating the wave of outbreaks. This result was, however, based upon the assumption that outbreaks of each production type were equally likely to be reported which may not be the case. The finding that the estimated time between infection to report contracted during periods of active 'X-ray' surveillance also suggests that the extent to which different production types were likely to have been detected also varied throughout the wave, although this would at least suggest that, when applied, these measures improve the overall level of control achieved.

7.1.4 Using final size methods to estimate the effects of vaccination

When experimental vaccination studies are conducted, inference is often carried out to determine whether the vaccine has successfully brought the reproduction number within a flock below unity. The simplest, and most often used, way of doing this, based upon data on the number of birds within a flock infected at the end of the experiment is to make the assumption that the vaccine affects the transmissibility of all birds equally. This does, however, produce the most optimistic estimate of vaccine efficacy. When such a model is fitted to data where unvaccinated infectious birds are introduced into a flock of vaccinated birds and, if R_0 has already been estimated to be relatively high, it may be possible to fit

more conservative assumptions about the action of a vaccine which explain the experimental data more readily. Moreover, when, as is often the case, multiple birds are infectious at the beginning of an experiment, such an assumption can easily lead to scenarios where a vaccine which resembles an all-or-nothing model of vaccine action but fails to reduce the reproduction below unity is wrongly assessed to be sufficiently efficacious to prevent sustained transmission in the context of a larger flock size. If these challenged birds have also already received vaccination, the effect of any misspecification of vaccine action and, as a result, the extent to which the leaky vaccine estimator is likely to underestimate the true reproduction number will be exacerbated.

Such methods were also used to calculate the between and within flocks transmissibility of H5N1 from outbreak investigations in Indonesia. These contribute to, and agree quite closely with, the limited number of existing estimates in the literature. When outbreaks were stratified according to treatment group, the final size estimate of the within-flock reproduction number in the vaccination zone was lower than in those which had not been assigned vaccination and there was also substantial evidence of a lower between-flock reproduction number. Using the estimates of the reproduction number in the non-vaccination areas, and treating the unobserved protective effect of vaccination in each flock as extra model parameters, it was found that this effect was equivalent to a mean protective effect of 11% of the flock for the outbreak flocks and 14% of the number of households within the outbreak neighbourhoods investigated in vaccinated areas. This may suggest that, even in a field trial context, biannual vaccination campaigns may not be sufficient to ensure that vaccination coverage is maintained at sufficiently high levels throughout all designated areas to prevent onward transmission, although the inherent

biases resulting from data collection based upon such retrospective outbreak investigations do make it difficult to assess the extent to which these results can be generalised.

7.2 Limitations and potential avenues for future research

There are a number of aspects of the modelling of waves of outbreaks in both Vietnam and Thailand which could have been improved had additional or more specific outbreak data been available. For Vietnam, higher resolution poultry data may have improved the accuracy of model, particularly in urban areas or for outbreaks in provinces with relatively low overall poultry populations. For similar reasons, it would have also been interesting to see the extent to which outbreaks occurring in the border regions corresponded to the intensity of infection in the equivalent border regions in Laos and China. Species-specific poultry data may also have offered the opportunity to elucidate the role of waterfowl in the dissemination of infection, an issue which has been the subject of much debate (Hulse-Post, Sturm-Ramirez et al. 2005; Gilbert, Chaitaweesub et al. 2006; Webster, Hulse-Post et al. 2007). The results of such an analysis would, however, come with similar caveats to that of the Thai data as to the problems associated with asymptomatic infection, especially during the second half of the 2005 wave and in 2007 when vaccination protocols varied between species.

It would also be interesting to assess the extent to which the obtained results are sensitive to some of the remaining modelling assumptions. In particular the assumption that all outbreaks were eventually reported and that the time taken to control an outbreak following the report of infection within a commune was constant. The former would involve imputing both the time of infection and the time of report for unreported outbreaks whilst the latter would involve treating all removal dates as nuisance parameters. Both of these

analyses would, however, substantially increase the number of model parameters necessary to fit the outbreak wave and would, therefore, either require the development of a more efficient model-fitting algorithm or additional computing power.

As it constituted the main change in control strategy which occurred before the 2007 wave, it is justifiable to highlight ways in which the changes in estimated dynamics of infection for this wave relative to previous waves may be attributable to the move towards mass vaccination. However, it cannot be shown conclusively that this is, directly or indirectly, the case and other factors such as mutations in the virus, changes in the production patterns of ducks or changes in the willingness of farmers to report outbreaks may explain these differences. Data on the timing, location, and coverage of vaccination may have helped to assess whether vaccination could be linked to changes in infectivity and infectious period and provided more nuanced understanding as to mechanism of any such effects and the potential role of vaccinators in the spread of infection. It would also be interesting to explore the extent to which other suggested risk factors such as the presence of live bird markets, altitude or vegetation factors play a role in determining the infectiousness and susceptibility of a commune (Gilbert, Xiao et al. 2008; Henning, Pfeiffer et al. 2009).

The method used to assess the impact of improved surveillance following vaccination used in this analysis also involved making strong assumptions about the epidemic which are likely to result in the overestimation of the potential benefits of such control measures. Future analyses could avoid this problem by instead modelling the spread of infection using the concept of thresholds of infectious pressure (Cook, Gibson et al. 2008), allowing a fully retrospective analysis of the effect of different durations between the infection entering a commune and the report of an outbreak.

For both Thailand and Vietnam the use of a Euclidean kernel made it necessary to limit the areas of each country over which transmission was estimated, resulting in outbreaks in each dataset being excluded from the analysis. One way to overcome this limitation, if such data became available, would be to use some other measure of spatial connectedness such as the shortest distance by road or by incorporating some estimate of the extent to which communes share the same live bird market. Moreover, as there were distinct genotypes of the virus circulating in Vietnam during the time period studied (Nguyen, Nguyen et al. 2008), if there had been specific data on the genotype of each outbreak, it may have been possible to reduce the level of uncertainty in the reconstructed epidemic tree and assess whether changes in the genotype of the virus affect the transmissibility of infection.

The poultry population data available were more detailed in Thailand than for Vietnam. However the fact that they were collected during the ongoing wave means the accuracy of the tambon-level data for outbreaks occurring before the poultry census in October 2004 is questionable, especially as Thailand had implemented stringent stamping out measures. This is highlighted by the existence of a handful of outbreaks occurring in tambons where the census had found no poultry. The estimate of an extended infection-to-report distribution for outbreaks reported outside of “X-ray” active surveillance also highlights the relative shortcomings of the existing passive surveillance infrastructure and the effects of these upon the results obtained is not easy to predict. Whilst some degree of heterogeneity in transmission risks for different types of poultry production was explored, there is likely to be further important heterogeneity within the three categories into which poultry production was stratified in these models. Other poultry farming factors such as whether

ducks are free-grazing, the presence of fighting cocks, and different categories of industrially produced have all been shown to have significant effects upon the relative risk of outbreaks occurring within a tambon (Gilbert, Chaitaweesub et al. 2006; Paul, Tavornpanich et al. 2010). Further developing the model to incorporate such detailed heterogeneity would, to a certain extent, be possible with the available data. However, adding additional complexity to the model would make fitting the model even more computationally intensive and any further estimates would be subject to the same data limitations as those currently obtained.

The chapter exploring the impact of different modes of vaccine action upon observed estimates of vaccine efficacy highlighted some of the limitations associated with the final size methods currently used to assess the adequacy of vaccines from experimental data. However, whilst it is important to understand the extent to which estimates may mistakenly overemphasize the effective level of control achieved, the only alternative I found constitutes a conservative assumption as to the action of the vaccine, both in terms of the reduction in susceptibility and the reduction in infectivity achieved, and relies upon knowledge of the basic reproduction number. Whilst this method may be useful in establishing that an estimate of effectiveness is robust to such assumptions it would fail to identify some vaccines which, despite leading to limited amounts of transmission, successfully reduce the reproduction number below zero (i.e. those where the method overestimates the level of heterogeneity in vaccine response or underestimates the effect of the vaccine upon reducing secondary infections when vaccinated birds become infected). Instead it would be useful to investigate further the types of experiments which are likely to provide information about determinants of the outcome of these transmission experiments such as the effect of vaccination upon infectivity and susceptibility and the inherent level of

heterogeneity this creates. It may then be possible to find an estimator of within-flock transmissibility which provides the most sensitive test of vaccine effectiveness, for a set experiment size in terms of the number of birds used, whilst ensuring that estimates of the control achieved are not overly-optimistic.

The data collected during outbreak investigations of H5N1 at the flock level in Indonesia included details on various possible risk factors including farming practices such as whether birds were caged or allowed to range freely, whether birds bought and sold at market, the actions of farmer in response to an outbreak such as quarantine, slaughter and selling and the presence of different species of birds within an RT. However, more detailed analysis as to the influence of these factors wasn't possible due to the relatively small sample size and the fact that, for many variables, data weren't available for any of the flocks which avoided infection. The relatively simplistic model used in this analysis, which makes the assumption of outbreaks involving single introduction of infection into a closed population, is also unlikely to reflect reality. Furthermore, as there were no data on the vaccination status of individual birds, the measure of the effect of vaccination was made using the strong assumption of a perfectly effective vaccine. More rigorous outbreak investigation protocols and a larger study size may have avoided these problems however the additional resources necessary to make this worthwhile may be restrictive.

7.3 Conclusion

In conclusion this thesis demonstrates that fitting of epidemic models using data augmentation techniques to account for missing data can be beneficial in better evaluating the effectiveness of past, future or current interventions for animal diseases even when data are limited. This is especially important in the case of H5N1 in Southeast Asia where

significant resources continue to be invested in an attempt to reduce the spread of infection. In countries such as Thailand, where the large industrial sector and export market provides a strong incentive to prevent infection, the control of infection appears to be achievable, although the equity of the consequences of achieving this control has been debated. In contrast, in countries such as Indonesia and Vietnam, the failure of stamping out measures has led to the adoption of mass vaccination as a key control policy. Whilst this has, especially in Vietnam, resulted in reductions in the burden of disease and the number of human cases in the short term, the findings in this thesis suggest that maintaining a level of coverage and detection capacity sufficient to have a long-term impact on the circulation of disease remains extremely challenging. Such obstacles need to be considered carefully as control strategies enter an inevitable phase of transition as it increasingly becomes accepted that the threat H5N1 poses to livestock, livelihoods and human health will not be eliminated in the near future.

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