THE EPIDEMIOLOGY OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN CHICKENS FROM WEST TIMOR, INDONESIA

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This thesis is submitted for the degree of Doctor of Veterinary Medical Science of Murdoch University

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DECLARATION

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

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ABSTRACT

Highly pathogenic avian influenza (HPAI), caused by subtype H5N1, is a serious viral disease of birds with significant public health concerns due to its zoonotic and pandemic potential. In West Timor (WT), Indonesia the disease resulted in the deaths or slaughter of hundreds of chickens between 2004 and 2006.

The study described in this thesis was designed to provide evidence to support the current HPAI (H5N1) disease freedom status of WT. Data were analyzed to describe the historical spatio-temporal distribution of H5N1 in WT, questionnaire surveys conducted to determine husbandry and biosecurity measures adopted by poultry producers and the market network for poultry and poultry products, and a risk analysis performed to identify the risk of entry of HPAI H5N1 into WT so that suitable preventive programs could be developed.

The retrospective study found that HPAI H5N1 was present at a high prevalence in sampled birds (50.8%, 95%CI: 45.1, 56.5) in 2004 which subsequently reduced in the following years. The highest prevalence was detected in the Kupang City District. Although seropositive chickens were detected in 2012 and 2013 these originated from farms that vaccinated birds against H5N1 and were likely false positive results.

Pharyngeal-cloacal swabs were collected from 300 poultry (292 chickens and 8 Muscovy ducks) from 10 villages and 5 markets between August and October 2013. No positive results were obtained using the Anigen® Rapid H5 AIV antigen test (0%; 95%CI: 0.0-0.8%). A questionnaire was administered to 150 owners of chickens sampled. The results highlighted the adoption of management and husbandry practices, including a lack of biosecurity measures, which had the potential to increase the risk of poultry being exposed to H5N1.

The risk analysis study found that the probabilities of importing infected chickens P(Inf1) and importing asymptomatically infected chickens P(Asym) had significant
impacts on the likelihood of entry of HPAI H5N1, while the probability of infected chickens infecting local chickens $P(\text{Inf}_2)$ had the greatest impact on the probability of having an outbreak of HPAI in WT. The social network analysis conducted demonstrated that there were extensive poultry movements within Kota Kupang and from there to other districts, highlighting that Kota Kupang plays an important role as the potential entry-point for disease into WT. The current management, husbandry and biosecurity practices adopted by poultry farmers/households and the extensive movement of poultry means that there is a sizeable risk of disease spread if it were introduced to the region.

It is concluded that there is a need to: develop suitable educational material to: improve small-holder poultry owners’ knowledge about avian influenza and the adoption of good husbandry and biosecurity measures; encourage disease reporting to the local Department of Agriculture; and to establish farmer groups to disseminate relevant information.
CONFERENCE PROCEEDINGS AND PUBLICATIONS


- The Epidemiology, Control and Zoonotic Potential of Brucellosis under an extensive Husbandry System in The Indonesian Part of The Island Of Timor (Seminar and 2nd Congress/South East Asia Veterinary School Association (SEAVSA)- JW Marriot Hotel Surabaya Indonesia 2011).

- Benefit Cost Analysis of Rabies Control in East Nusa Tenggara Province (Ewaldus Wera, Petrus Malo Bulu, Marieke Opsteegh, and Norhariani Mohd Nor. 2011. Cost-Benefit Analysis of Rabies Control in East Nusa Tenggara

Immunity Level of Broiler chickens against ND virus with different vaccination applications (Ewaldus Wera and Petrus Malo Bulu. 2006. Immunity Level of Broiler chickens against ND virus with different vaccination applications. *Bulletin Partner*. **58**:130-145.)
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<th>Definition</th>
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<tbody>
<tr>
<td>AI</td>
<td>Avian influenza</td>
</tr>
<tr>
<td>AIV</td>
<td>Avian influenza virus</td>
</tr>
<tr>
<td>DOC(s)</td>
<td>Day old chicken(s)</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organizations</td>
</tr>
<tr>
<td>HA/HI</td>
<td>Hemagglutination/Hemagglutination inhibition</td>
</tr>
<tr>
<td>HPAI</td>
<td>Highly pathogenic avian influenza</td>
</tr>
<tr>
<td>H5N1</td>
<td>One of the subtypes of HPAI viruses</td>
</tr>
<tr>
<td>LBMs</td>
<td>Live bird markets</td>
</tr>
<tr>
<td>LPAI</td>
<td>Low pathogenic avian influenza</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organization for Animal Health (Office International des Epizooties)</td>
</tr>
<tr>
<td>SEAVSA</td>
<td>South East Asia Veterinary School Association</td>
</tr>
<tr>
<td>SNA</td>
<td>Social network analysis</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcription Polymerase Chain Reaction</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WT</td>
<td>West Timor</td>
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CHAPTER ONE

Introduction

Highly pathogenic avian influenza caused by H5N1 is an emergent and virulent disease that infects birds, humans and other mammals. The disease is responsible for an ongoing outbreak in Asia, which has now displayed probable human-to-human transmission (Edler, 2006; Ungchusak et al., 2005; WHO, 2012). The virus H5N1 first infected humans in 1997 during a poultry outbreak in Hong Kong SAR, China, then it re-emerged in 2003 and 2004 spreading from Asia to Europe and Africa, resulting in infection of millions of poultry, several hundred human cases, and many human deaths (WHO, 2014a). Outbreaks in poultry have had a significant impact on the livelihood of individual farmers/families, the economy and international trade for affected countries (WHO, 2014a). For instance, Thailand suffered the greatest impact of the disease from January to April 2004, with 75% reduction in exports, followed by China, with 63% reduction, Hong Kong (55%) and 27% in the USA (Taha, 2007). In addition, it has been reported that the virus has caused the death or slaughter of 250 million birds, with an estimated impact of more than US$12 billion, with the disease being responsible for a loss of around 1% of gross domestic product (GDP) in affected countries from 2003 to 2012 (Martins, 2012). Moreover, the disease has caused the death of 449 people in affected countries (WHO, 2015), with 169 of these residing in Indonesia (WHO, 2015).

Chicken is the main meat consumed in Indonesia (Liano and Siagian, 2002) and in 2014 the number of chickens was estimated at almost 2 billion (Statistics Indonesia, 2015). In the Province of Nusa Tenggara Timur (NTT), the total number of chickens was almost 15 million in 2013 (Statistics Indonesia, 2013). In NTT most farmers are small-scale producers (Christie, 2007), and any disease outbreak could have a significant negative impact on food security (Drucker et al., 2006).
This study, conducted from 2013 to 2015, was designed to confirm that West Timor was free from HPAI H5N1. The aim of the study was to provide information (including the spatio-temporal distribution of H5N1, the husbandry and biosecurity measures adopted by poultry owners, the market network for poultry and poultry products in the region, the risk of entry of HPAI H5N1 into West Timor, and the current status of H5N1 in West Timor) to the local government and to the poultry industries to enable the planning of suitable prevention programs for HPAI.

In Chapter 2 the literature on HPAI, with particular relevance to Indonesia, are summarized and in Chapter 3 the methodology adopted for the current project is outlined. In Chapter 4 the spatio-temporal distribution of HPAI H5N1 in West Timor from disease outbreaks reported in 2004 until 2006 are analyzed. The findings from this study are important for a better understanding of the epidemiology of the disease in West Timor.

A targeted survey that was undertaken in order to demonstrate the presence or absence of H5N1 virus in West Timor was outlined in Chapter 5. This study was carried out by testing 300 samples from poultry with a pen-side test.

In Chapter 6 the management of poultry in West Timor is described through the results of interviewing 100 poultry farmers. The routine husbandry and biosecurity practices adopted by these owners provide useful information in the need for further education on disease control in the region.

The movement and trading patterns of poultry in West Timor are described and assessed in Chapter 7. New insights into the trade pattern of live poultry and hence the likely transmission of HPAI through these movements arose from this research.

In Chapter 8 a risk analysis for the entry and transmission of HPAI H5N1 into West Timor is presented. Quantifying the likelihood of the introduction and establishment of the disease through the movement of live poultry was conducted.
Finally in Chapter 9 all the results from this project are discussed and compared with existing literature and recommendations developed to minimize the risk of incursion of HPAI into NTT in the future.
CHAPTER TWO
Literature Review

2.1 Introduction
Avian influenza (AI) is an important viral disease of poultry. Unlike other avian influenza viruses, highly pathogenic avian influenza (HPAI) H5N1 virus causes devastating socio-economic effects around the world. Additionally, the HPAI H5N1 virus has resulted in global public health concerns due to its zoonotic and pandemic potential (Peiris et al., 2007; Perdue and Swayne, 2005) and its impact on the global economy through reduction of farm income through mass losses of poultry (Obayelu, 2007). In this chapter, the history, distribution and epidemiology of HPAI H5N1 are reviewed.

2.1.1 History and Distribution of HPAI H5N1
Historically, avian influenza is not a new disease with the disease being known for centuries. In 1878, Perroncito described the disease as a contagious disease of poultry associated with high mortality (Perroncito, 1878) and subsequently Rivolta and Delprato named the disease ‘Fowl plague’ in 1880 (Stubs, 1943). In 1996 the disease reemerged in Hong Kong commencing a major pandemic (Guan et al., 2002a).

Avian Influenza H5N1 was first isolated from a flock of sick geese in the Guangdong Province of China in 1996 (Xu et al., 1999). In the following year the first case of human infection was documented in Hong Kong, indicating that the H5N1 strain could infect humans directly, without prior adaptation in a mammalian host (Ligon, 2005). The virus is important because of its significant zoonotic potential, the high case-fatality rate in humans and its potential to cause high mortality in poultry, particularly in Southeast Asian countries (WHO, 2005a; de Jong and Hien, 2006).
2.1.2 Emergence and Spread of HPAI Worldwide

The timeline for the reemergence of H5N1 avian influenza virus (AIV) has been reviewed by Webster and Govorkova (2006). This review indicated a hypothetical epicenter for the emergence of H5N1 clades and sub-clades, and discussed the occurrence and number of confirmed human cases and the severity of disease in animals.

In 1996, the HPAI H5N1 strain started to circulate in Asia (Chen et al., 2006b) and, in 1997, 18 humans were affected in the Hong Kong Special Administrative Region, China (Shortridge et al., 1998). Six of these patients died (Bender et al., 1999; Claas et al., 1998b).

In 2003, the HPAI H5N1 strain became endemic in poultry, and subsequently caused outbreaks in nine Asian countries (Webster et al., 2006; Guan et al., 2004; Li et al., 2004; Webster et al., 2005; Sims et al., 2005). In 2006, H5N1 was reported to have spread to four continents (Alexander, 2007b). Whilst it remains a complex problem in Asia, the virus was reported in Turkey, Romania, and Croatia in 2005, and in Africa in 2006 where it was suspected that migratory birds were responsible for introducing the virus (Nicholls, 2006).

From 2003 until 2006, outbreaks and infections of HPAI H5N1 in poultry were reported in China, Japan, South Korea, Laos, Thailand, Cambodia, Vietnam, Indonesia, Malaysia, Mongolia, Iraq, India, Kazakhstan, Azerbaijan, Pakistan, Myanmar, Afghanistan, and Israel in Asia; Albania, Belgium, Croatia, France, Romania, Russia, Turkey, Ukraine, and Sweden in Europe; and Nigeria, Cameroon, and Niger in Africa (Alexander, 2007b). As of 2015, the World Health Organization (WHO) has confirmed HPAI outbreaks in more than 60 countries, with infection reported in 844 humans from 16 countries (Figure 2.1 and Table 2.1). A high case fatality rate in humans (100%) was reported in 2003 with four deaths from four cases (WHO, 2015). The antigenic drift and
genetic shift of the virus are believed to be key contributors to the pandemic nature of the disease (Yang et al., 2010; Horimoto and Kawaoka, 2001; Wan et al., 2005; Webster, 1999; Brown et al., 1998; Capua and Alexander, 2002).

Figure 2.1 Distribution of Avian Influenza H5N1 in the world from 2003 until 2014

Source: (WHO, 2014b)
Table 2.1 Summary of confirmed outbreak in humans of HPAI H5N1

<table>
<thead>
<tr>
<th>Continent</th>
<th>First outbreak</th>
<th>Animals affected</th>
<th>Number of human deaths/cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>March 1997 in Hong Kong</td>
<td>Domestic poultry, wild &amp; zoo birds, pigs, emu, tiger</td>
<td>322/477</td>
</tr>
<tr>
<td>Africa</td>
<td>16 January 2006 in Nigeria</td>
<td>Domestic poultry, wild birds, donkeys</td>
<td>118/353</td>
</tr>
<tr>
<td>Europe</td>
<td>15 July 2005 in Russian Federation</td>
<td>Domestic poultry, wild birds, zoo birds, games birds, cats, dogs, stone martens</td>
<td>5/8</td>
</tr>
<tr>
<td>North America</td>
<td>No outbreak. First case January 2014</td>
<td>None</td>
<td>1/1</td>
</tr>
<tr>
<td>Total worldwide</td>
<td></td>
<td></td>
<td>450/851</td>
</tr>
</tbody>
</table>

Source: (Chan, 2002; Claas et al., 1998b), (FAO, 2012; FAO, 2015), and (WHO, 2016)

2.1.3 Emergence and spread of HPAI in Indonesia

In Indonesia, HPAI H5N1 virus was first detected in domestic poultry in 2003 (Begemann, 2009; Sedyaningsih et al., 2007). By the end of June 2006, the virus had spread with outbreaks reported in 27 provinces (Indonesia Ministry of Agriculture, unpublished data, cited by Sedyaningsih et al. 2007). From 2005 to 2006, sporadic and family clusters of cases occurred in humans throughout Indonesia with a case-fatality proportion of 76% (Kandun et al., 2006; Sedyaningsih et al., 2007). Until January 2009, the virus was reported to be still spreading and was endemic in several provinces, with 141 confirmed human cases, of which 115 died (Yupiana et al., 2010). Most of these human cases in Indonesia (76%) have been associated with contact of poultry or poultry products (Sedyaningsih et al., 2007). HPAI outbreaks have been frequently reported on the islands of Java and Sumatera (Begemann, 2009). Specific information on AI in West Timor will be discussed in Chapter Four.
2.2 Epidemiology of HPAI

2.2.1 Aetiology

Avian Influenza is caused by a type A Avian influenza virus (Chen et al., 2004; Kwon et al., 2005). The infectious agent is a pleomorphic, enveloped RNA virus belonging to the family of Orthomyxoviridae (de Jong and Hien, 2006; Suarez and Schultz-Cherry, 2000), and is divided into subtypes based on differences in its haemagglutinin (HA) and neuraminidase (NA) membrane proteins. The notation HhNn is used to refer to the subtype comprising the h\textsuperscript{th} discovered HA proteins and the n\textsuperscript{th} discovered NA protein (Chen et al., 2004; Kwon et al., 2005). The subtype H5N1 of type A virus is the main cause of avian influenza (Chen et al., 2004; Kwon et al., 2005), and the term highly pathogenic avian influenza (HPAI) has been used for the very virulent forms of avian influenza viruses. It is believed that HPAI viruses emerged from H5 and H7 AIV subtypes of low pathogenicity through mutation (Garcia et al., 1996; Perdue et al., 2003).

2.2.2 Classification of Avian Influenza Viruses

There are two main methods for classification of AI viruses, specifically classification based on their antigenicity or pathogenicity. Understanding the classification of AI viruses helps provide insight into possible strategies for the diagnosis, control and eradication of the disease.

2.2.2.1 Classification of AIV Based on Antigenicity

Influenza viruses are classified as types A, B and C based on antigenic differences in the NP and M proteins of the NA (de Jong and Hien, 2006). All avian influenza viruses are classified as type A. Currently they form 16 HA and 9 NA subtypes based on the antigenicity of the HA and NA surface glycoproteins, designated as H1-H16 and N1-N9, respectively (Fouchier et al., 2005b; Neumann et al., 2009). Influenza B and C viruses are not divided into subtypes.
2.2.2.2 Classification of AIV Based on Pathogenicity

Based on their pathogenicity in chickens, AI viruses are divided into high and low pathogenicity. Highly pathogenic (HP) avian influenza (HPAI) is an extremely contagious, multi-organ systemic disease of poultry leading to high mortality (up to 100%) (Swayne and Suarez, 2000; Alexander, 2000). To date, although only a small percentage of H5 and H7 viruses are highly pathogenic, all HPAI viruses belong only to the H5 or H7 subtypes (Neumann et al., 2009). In contrast, low pathogenic viruses cause only mild respiratory disease, however this may be made more severe through the presence of other infections or adverse environmental conditions (Alexander, 2000; Neumann et al., 2009). Low pathogenic viruses may also cause a reduction in egg production (Neumann et al., 2009), however their importance lies in the fact that they may potentially evolve into HPAI viruses leading to disease outbreaks in poultry (CDC, 2005b).

2.2.3 Antigenic drift and Antigenic Shift

Antigenic drift is the gradual evolution of viral strains at the molecular level due to frequent mutations (Both et al., 1983; García-Sastre, 2009; Lamb, 2008), and is the result of minor antigenic changes in the HA or NA proteins (Lamb, 2008) of previously circulating strains (García-Sastre, 2009). Significant antigenic drift is associated with a more severe, early-onset influenza epidemic, resulting in increased mortality (Treanor, 2004). Antigenic drift results in manipulation of the gene expression (replication) of the virus, particularly the proof reading during RNA transcription, resulting in a high frequency of mutations in any newly replicated virus population (Stephenson and Zambon, 2002). The recent spread of AI is believed to have been driven by antigenic drift (Chen et al., 2005; Liu et al., 2005), as well as the ability of influenza viruses to adapt to different animals (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005) and to cause interpandemic outbreaks (Stephenson and Zambon, 2002).
Antigenic shift or genetic reassortment (García-Sastre, 2009), arises from mixing of genetic material from different viral strains due to co-circulation of different influenza A subtypes (Carrat and Flahault, 2007). It is only seen in influenza A viruses and results from the replacement of HA subtypes with novel ones (Cox and Subbarao, 2000). In addition, it involves major antigenic changes and leads to high infection rates in an immunologically naïve population and is the cause of influenza pandemics (Lamb, 2008; Stephenson and Zambon, 2002). Moreover, genetic reassortment of the virus during replication may cause new or even more dangerous subtypes (Edler, 2006), and could result in a novel avian influenza virus that can be efficiently transmitted between humans (Yuen and Wong, 2005).

2.2.4 Incubation Period

The incubation period for AI ranges from 2 to 5 days, however it can be extended up to 17 days (Swayne, 2012; WHO, 2005a). An incubation period of seven days has been recommended to be used for trace-back during field investigations and when monitoring contacts (WHO, 2011). In humans the clinical signs typically last from 5 to 24 days (Edler, 2006). In Sumatera research by Yang et al. (2007) showed that the incubation period had a uniform distribution of 3 to 7 days with an infectious period of 5 to 13 days.

2.2.5 Factors contributing to the presence and survival of avian influenza virus

A range of factors affects the survival of AI viruses. Understanding the source of the virus and its survival are important in understanding the nature of the virus and how to control it. Physical, chemical and biological factors contribute to the presence and the survival of the virus in the environment, in faeces and manure, eggs, raw meat and poultry products and in water.


2.2.5.1 Survival in the Environment

In the environment, the primary factors affecting the transport, survival and fate of viruses in liquid environmental media are temperature, ionic strength, chemical constituents, microbial antagonism, the adsorption status of the virus and the type of virus (Sobsey and Meschke, 2003). Of these, temperature is the most important factor influencing the survival and persistence of the viruses in different media. At a temperature between 4 and 37°C, H5N1 can survive for up to 200 days (Benedictis et al., 2007). On nonporous surfaces, the virus has been shown to survive for up to 6 days post contamination (Tiwari et al., 2006).

Benedictis et al. (2007) suggested that the strain of virus and the structural characteristics (pellets or loose) influenced the survival of AIV in faeces. Infected birds can shed AIV in their faeces, saliva and nasal secretion for the first 2 weeks after infection (WHO, 2006c; Swayne, 2008b; de Jong and Hien, 2006). In addition, in wet faeces, the infectivity of the virus is maintained for at least 4 days at 25°C (Shortridge et al., 1998). In allantoic fluid or fresh faeces at 33 to 35°C, the virus can survive for only 30 minutes (Songserm et al., 2006b). In chicken faeces, the virus has been found to die within one week at 15-20°C (Lu et al., 2003), however in wet manure it can survive for up to 105 days (Fitchner, 1987).

2.2.5.2 Survival in Eggs

The HPAI virus can be found within and on the surface of eggs laid by infected birds (WHO-INFOSAN, 2005), or on the surface as a result of faecal contamination (Swayne and Beck, 2004). Infective virus has been detected in albumen and yolk samples, as well as on the surface of the egg shells, in up to 57% of eggs laid within 18 days after the appearance of clinical signs during an HPAI outbreak (Swayne and Beck, 2004). One study revealed that H5N1 was also present within the eggs and oviducts of naturally infected quail, with titres of $10^{4.6} - 10^{6.2}$ EID50/mL (Promkuntod et al., 2006).
2.2.5.3 Survival in Raw Meat and Meat Products

Raw meat can play an important role in the transmission of H5N1. The virus can survive in contaminated raw poultry meat and therefore can be spread through the marketing and distribution of contaminated food products, such as fresh or frozen meat (WHO-INFOSAN, 2005). Das et al. (2008) detected virus in meat and the tracheas of clinically normal birds within 6 hours of infection and all samples were positive 24 hours post-infection (PI).

2.2.5.4 Survival in Water

The LPAI viruses have been observed to remain infective in water for up to 102 and 207 days at 28°C and 17°C, respectively (Stallknecht et al., 1990). In lake water, the viruses can remain infective for up to 30 days at 0°C and 4 days at 22°C (Fouchier et al., 2007; Olsen et al., 2005). In relation to HPAI viruses, H5N1 has also been reported to be persistent in water of different temperatures and salinities. Domanska-Blicharz et al. (2010) demonstrated the persistence of H5N1 isolated during the epidemic in wild birds in Poland in 2006 in three water samples derived from sources known to host wild water birds. These authors found that the persistence of the virus varied considerably depending on the temperature and salinity of the water. Virus survival was the highest at 4°C and lowest at 20°C in all water samples. Brown et al. (2007) revealed that two Asian HPAI H5N1 viruses survived in water with salinities of 0, 15 and 30 ppt (parts per thousand) at 17°C for up to 26, 30 and 19 days respectively and at 28°C for up to 5, 5 and 3 days respectively.

2.2.5.5 Inactivation of AIV

Avian influenza virus (H5N1) can be inactivated or killed by different treatments or methods, specifically by thermal, physical and chemical means. H5N1 can be inactivated at 56°C within 3 hours and at 60°C within 30 minutes (WHO, 2005b). Swayne (2006) investigated the effect of heat treatment on HPAI virus
(A/chicken/Korea/ES/2003, H5N1 subtype) in chicken meat. He found that the virus was completely inactivated after exposure at 70°C for 1 second and at 70°C for 5 seconds in the breast and thigh meat. Normal cooking (temperatures at or above 70°C in all parts of the product) will inactivate the virus (WHO-INFOSAN, 2005).

Studies in Thailand demonstrated that H5N1 (initial dose of $10^{6.3}$ EID$_{50}$/ml) mixed with chicken faeces under different environmental conditions was completely inactivated within 30 minutes after direct sunlight exposure at a temperature of 32–35°C. In contrast infectivity was retained for 4 days when the mixture was kept in the shade at 25–32°C (Songserm et al., 2006b). Another study conducted in Thailand showed that the virus lost its infectivity in chicken manure within 24 hours when maintained at 25°C, and within 15 minutes when kept at 40°C (Chumpolbanchoom et al., 2006). At room temperature (28°C), the virus lost its infectivity after 24 hours, but at 56°C the virus was found to be inactivated within 30 minutes (Shahid et al., 2009). Drying of faeces at ambient temperatures also has been shown to inactivate the virus (Shortridge et al., 1998).

H5N1 is sensitive to some chemical agents or disinfectants such as glutaraldehyde, quaternary ammonium compounds, chlorine and phenol, as well as detergents. This is believed to be due to the presence of lipid in the virus envelope (AUSVETPLAN, 2011). In order for H5N1 to be inactivated, disinfectants must be applied for the recommended exposure time and at the recommended concentrations (Shahid et al., 2009). Most disinfectants have optimum efficacy at temperatures above 20°C (Samberg and Meroz, 1995). One study, conducted by Ito et al. (2006), demonstrated that H5N1 and other HPAI viruses could be destroyed by using povidone-iodine (PVP-I) products.
2.2.6 Host Range/Species and Reservoirs

2.2.6.1 Host Range

Influenza A viruses infect a variety of animals including humans, pigs, horses, marine mammals and birds (Alexander, 2000; Hinshaw et al., 1979; Lvov, 1978). Mammalian species naturally or experimentally susceptible to AIV include *Macaca fascicularis* (*Cynomolgus monkey / Crab-eating macaque*), mice (*Mus musculus*), hamsters (*Mesocricetus auratus*), pigs (*Sus scrofa*), ferrets (*Mustela putorius furo*), stone marten (*Martes foina*), dogs (*Canis lupus familiaris*), domestic cats (*Felis catus*), tigers (*Panthera tigris*), leopards (*Panthera pardus*) and civets (*Paradoxurus hermaphrodites*) (Brunei and Petter, 2010). In addition, dogs can shed virus from the nose without showing any apparent signs of disease. Receptors for avian (H5N1) virus are present, not only in the lower part of the respiratory tract of dogs, but also in the trachea and nose, which are potential ports for the entry of virus (Chen et al., 2010; Maas et al., 2007).

All currently known influenza A subtypes have been isolated from birds (Webster, 1997), indicating that subtypes, other than those routinely found in humans (H1 to H3), have the potential to cross the species barrier and infect people (Riedel, 2006; Peiris et al., 2007).

2.2.6.2 Reservoirs for AIV

Wild aquatic birds (Anseriformes), including ducks, are the natural reservoir of influenza type A viruses. These animals play an important role in the mutation and replication of the viruses without displaying any clinical signs of disease (Guan et al., 2002b; Sturm-Ramirez et al., 2005; Alexander, 2000; Webster et al., 1992), and the viruses appear to have achieved an optimal level of host adaptation in these species (Webster et al., 1992). In relation to H5N1, Songserm et al. (2006a) indicated that free-ranging ducks are a potential reservoir of the virus. As such, ducks are considered to
play an important role in transmitting the virus, with a potential consequence of disease in domestic poultry (Gilbert et al., 2006; Gilbert et al., 2008).

2.2.7 Transmission

Transmission is a key process in host–pathogen interactions of a disease (McCallum, 2000). There are different means for AIV to be introduced into a new flock of poultry, to people, or to other mammalian species. The transmission of infection from one continent to another is believed to be due to either the movement of poultry and/or migratory birds (Kilpatrick et al., 2006).

2.2.7.1 Transmission of Avian influenza H5N1 amongst Waterfowl

All AIV, including H5N1, circulate in wild waterfowl. In waterfowl viral reassortment occurs, resulting in a huge pool of constantly changing viruses. The H5N1 is known to cause occasional sporadic deaths in migratory waterfowl, but generally it does not kill them in large numbers and consequently can be carried by them over long distances (WHO, 2006a). Additionally, in aquatic birds, influenza viruses replicate in the epithelial cells of the intestinal tract and consequently these birds shed viruses in high concentration in their faeces (Lamb, 2008). The transmission of H5N1 between wild waterfowl is by the faecal-oral route and this is facilitated by contaminated water (Hinshaw et al., 1979; Hinshaw et al., 1980; Webster et al., 1978). In this medium the virus can survive for a long period of time (Brown et al., 2009a; Brown et al., 2007; Hinshaw et al., 1979; Hinshaw et al., 1980), up to 60 days if the water is between 4 and 10°C (Domanska-Blicharz et al., 2010).

2.2.7.2 Transmission of Avian Influenza H5N1 amongst Poultry

In a poultry flock, AI can be transmitted by five means: direct exposure to infected birds; exposure to AIV contaminated equipment or materials; movement of people with contaminated shoes or clothing; contaminated water; or by air (Swayne, 2008b). The
WHO (2006c) highlighted that direct contact with infected birds, which have the potential to shed large amounts of virus, is the most efficient way of introducing the virus into a naïve population.

In relation to H5N1, transmission between individual birds is by ingestion or inhalation (The Merck Veterinary Manual, 2011). Transmission experiments conducted in chickens in Hong Kong revealed that H5N1 is spread by the faecal–oral route, rather than by the aerosol route (Shortridge et al., 1998). It can also be transmitted directly or indirectly through contact with contaminated fomites (de Jong and Hien, 2006; Gambotto et al., 2008). An experimental study using a/chicken/Korea/ES/2003 (H5N1) found that HPAI virus produced high titres of virus in the breast meat and feeding this meat to other chickens resulted in infection and subsequent deaths (Swayne and Beck, 2004).

Transmission between farms is the result of breaches in biosecurity practices, basically through the movement of infected poultry or contaminated fomites (Capua and Marangon, 2006; Swayne, 2012). Airborne transmission over long distances between farms has not yet been demonstrated. For these reasons, if biosecurity measures are implemented at the farm level, AI infections should be preventable.

2.2.7.3 Transmission of H5N1 from Chickens to Pigs

It was thought that pigs act only as a reservoir for H5N1 and were a dead-end host of the disease because they only experienced subclinical infection (Brunei and Petter, 2010). However, the results of Kida et al. (1994) demonstrated that AIV could be transmitted from pigs with or without non-human-type HAs (H4 to H13). Similarly, Lipatov et al. (2008) found that pigs fed breast and thigh meat from chickens that had died from infection with WS/Mong/05 H5N1 virus became infected, indicating that transmission of H5N1 from chickens to pigs was possible.
Pigs have an important role in the ecology of AIV, and are recognized as efficient replicating hosts or mixing vessels for reassortment of avian and human influenza viruses with the potential for a pandemic virus to emerge (Ma et al., 2009). This is due to the presence of GAL receptor cells for both avian and human viruses in the pig’s trachea (Ito et al., 1998). Supportive evidence for the “mixing vessel” theory has been provided from the double (avian/human; human/swine) and triple (human/avian/swine) reassortant influenza A viruses isolated from pigs in the USA and China (Ma et al., 2009). These authors further described that novel reassortant H2N3 influenza viruses isolated from pigs in the USA most likely originated from AIV in surface drinking water. These viruses had similar genetic make-ups to earlier (1957) isolates of the H2N2 human pandemic virus where these novel H2N3 swine viruses were able to cause disease in pigs and mice and were infectious and highly transmissible in pigs and ferrets without prior adaptation.

2.2.7.4 Transmission of H5N1 from Chickens to Humans

Humans can be infected through close contact with sick poultry (Suarez et al., 1998; Subbarao et al., 1998; Pappaioanou, 2009) and direct physical contact with sick or dead poultry has been identified as the primary risk factor for the transmission of H5N1 from poultry to humans in Indonesia (Kandun et al., 2007). Additionally, plucking and preparing diseased birds; handling fighting cocks; playing with poultry, particularly asymptomatic infected ducks; and consumption of duck’s blood or possibly undercooked poultry products have also been implicated in infection of humans (Beigel et al., 2005). In an outbreak of H5N1 in Hong Kong in 1997, disease in humans was associated with exposure to live poultry a week prior to the onset of illness (Mounts et al., 1999).


\[ \text{2.2.7.5 Transmission of H5N1 between Humans} \]

Until 2000, there was no human-to-human transmission of H5N1 reported through social contact (Bridges et al., 2000; Katz et al., 1999); and it was thought that the virus was transmitted to humans only through direct contact with infected birds. However, recent findings have suggested that the virus can potentially be transmitted between humans (Normile, 2007; MacKenzie, 2008). Research conducted in Thailand by Ungchusak et al. (2005) indicated probable person to person transmission of H5N1, due to clustering of cases within a family. In this situation it was hypothesized that the virus was transmitted from the index patient to her mother and aunt. Similar findings were found in Sumatera in 2006, and in that study the statistical evidence of human-to-human transmission was very strong \((p=0.009)\) (Yang et al., 2007). Bridges et al. (2000) suggested that human-to-human transmission of AI might increase the chances for the emergence of a novel influenza virus with pandemic potential. These authors described that having H5-specific antibody was the factor that enabled the person to person transmission of H5N1 from the patients to health care workers (HCWs), and the seroconversion of 2 exposed HCWs strongly suggested the occurrence of person to person transmission.

\[ \text{2.2.7.6 The Role of Ducks in the Transmission of AIV} \]

Ducks have been found to play an important role in the propagation of H5N1 and wild ducks act as long-distance vectors of HPAI H5N1 (Keawcharoen et al., 2008). Hulse-Post et al. (2005) conducted a study and found that ducks did not show any signs of infection, however they continued to shed virus. Consequently they represent a potential pandemic threat, and play a key role in the propagation and biological evolution of HPAI H5N1 in Asia. Another study conducted in Egypt on wild ducks revealed the genetic diversification of HPAI AIV with different amino acid substitutions in ducks,
suggesting that ducks can generate novel variants that might serve as aetiological agents for new outbreaks and epidemics (Watanabe et al., 2011).

2.2.8 Risk Factors

There are many factors which facilitate the transmission of H5N1 into animal and human populations. These factors include trade and movements of live birds and fighting cocks, and live-bird markets (Gilbert et al., 2006; Sims, 2007; Begemann, 2009). Many previous studies have shown that live bird markets (LBMs) serve as a ‘fertile ground’ for mutation and emergence of new influenza viruses with increased virulence to infect other species and are closely related to the occurrence of AI in commercial poultry (Senne et al., 2005). For example, emergence of H5N1 in Hong Kong in 1997, which killed 6 people, occurred in LBMs (Claas et al., 1998a). Paul et al. (2011) reported that trade of poultry between farms, farming practices and environmental characteristics were the risk factors for the transmission of HPAI H5N1 in Thailand. In Bangladesh, human population density, commercial poultry population (number of commercial poultry per sub-district), and the number of roads per sub-district were risk factors for HPAI transmission (Loth et al., 2010). In Thailand and Vietnam, cropping intensity was found to be consistently associated with the presence of HPAI H5N1 (Gilbert et al., 2008). In north-eastern Italy, poultry species, type of production and farm size were identified as risk factors for HPAI infection (Mannelli et al., 2006). In Indonesia, research conducted in the province of West Java, highlighted that two risk factors (specifically poultry density and road density) were significantly associated with HPAI H5N1 outbreaks in poultry (Yupiana et al., 2010). In addition, recent outbreaks in South Sulawesi and Bali were shown to be associated with poultry movements from Java and Sumatera (Begemann, 2009). However, pre-emptive culling, greater distance from infected farms and higher altitudes were protective to infection (Busani et al., 2009).
2.2.8.1 Identification of the HPAI H5N1 Risks

The H5N1 virus is classified in the family Orthomyxoviridae, which at present consists of five genera including Influenza viruses A, B and C, Thogoto virus, tick-borne viruses, and Isa virus, however only viruses of the Influenza virus A genus are known to infect birds (Alexander, 2007a). This author further explained that this virus has segmented, negative sense, single strand RNA genomes. The HPAI viruses infect all types of domestic birds with the isolation rate of 15% for ducks and geese, and 2% for other species, but are rarely isolated from wild birds (Alexander, 2000). In Thailand, the disease has affected various types of poultry with most infected flocks reported to be either backyard chickens (56%), ducks (27%), broilers (6%), layers (5%), quails (2%), or other birds (3%) (Tiensin et al., 2005). HPAI H5N1 is particularly important because it is able to cross the species barrier and cause high mortality in mammals, as well as humans (Gauthier-Clerc et al., 2007). In addition, it was listed by the OIE in 2014 as a disease of great importance for both animal and human health (OIE, 2014b). The transmission of the virus in Indonesia has been reported to be attributed to the movement of poultry within the country, and it is considered unlikely by some authors that the virus entered through bird migration (Smith et al., 2006).

2.3 Disease

Avian influenza viruses have the potential to cause serious impacts on both animals and humans around the world. The disease has different rates of morbidity and mortality depending upon the pathogenicity and virulence of the infecting viral strain.

2.3.1 Disease in Animals and Humans

Highly pathogenic avian influenza H5N1 has caused disease in a wide range of animals and humans. Since 2003, more than 589 people in 15 countries in parts of Asia, Africa, Eastern Europe and the Middle East have become ill from H5N1, and approximately 60% of these have died. Indonesia, Vietnam and Egypt have reported the highest
number of human cases (CDC, 2012). Infants and young children are the highest affected age groups in humans (Lahariya et al., 2006). Deaths occur an average 9 to 10 days after the onset of illness (range 6 to 30 days) (Chotpitayasunondh et al., 2005; Hien et al., 2004), and most patients die from progressive respiratory failure (Chotpitayasunondh et al., 2005; Hien et al., 2004; Yuen et al., 1998).

2.3.2 Clinical Signs of HPAI

2.3.2.1 Clinical Signs in Humans

Infection of humans with H5N1 results in typical flu-like symptoms which include: cough (dry or productive); diarrhoea; difficulty breathing; fever (greater than 38°C); and a runny nose (Medical Encyclopedia, 2011; Kandun et al., 2006; Oner et al., 2006; WHO, 2005a). In the early course of illness, patients present symptoms such as diarrhoea, vomiting, abdominal pain, pleuritic pain and bleeding from the nose and gums (Chotpitayasunondh et al., 2005; Hien et al., 2004; Tam, 2002; WHO, 2005a). Dyspnoea often occurs five days after the onset of illness (range 1 to 16 days) (Chotpitayasunondh et al., 2005). Some clinical presentations include extensive, often bilateral infiltration, lobar collapse, focal consolidation and air bronchograms (Gambotto et al., 2008). During the 1997 epidemic in Hong Kong patients presented with fever, headache, malaise, myalgia, sore throat, cough and rhinitis (Medical Encyclopedia, 2011; Chen et al., 2004; Yuen et al., 1998). Analysis of data from 12 patients in Thailand, found that fever was often the first symptom seen (Chotpitayasunondh et al., 2005).

2.3.2.2 Clinical Signs in Animals

In animals, clinical signs of H5N1 are variable and are dependent upon the onset of the disease and the species infected. In cats and tigers, the clinical signs are fever, decreased activity, protrusion of the nictitating membrane, conjunctivitis and laboured breathing (Kuiken et al., 2004). Nervous signs, including convulsions and ataxia, are consistent
with brain lesions. In severely affected cats, sudden death may occur as early as two
days after the onset of illness (Songserm et al., 2006a).

In experimentally infected dogs, signs include anorexia, fever, conjunctivitis, laboured
breathing and coughing (Chen et al., 2010), however they only developed mild disease
(Maas et al., 2007).

Unlike the low pathogenic form that usually causes only mild signs in poultry (such as
ruffled feathers), HPAI spreads rapidly through flocks, and may cause disease that
affects multiple internal organs with a mortality level up to 100% (Alexander, 2000;
Cox and Subbarao, 2000), often within 48 hours of infection (Cox and Subbarao, 2000).
In peracute cases, most birds are found dead before clinical signs develop (The Merck
Veterinary Manual, 2011; CIDRAP, 2012). However, in acute cases, a range of clinical
signs can be observed including inappetence, lack of energy, drastic decline in egg
production, oedema of the head and neck, swollen and cyanotic combs and wattles,
petechial haemorrhages on internal membranes, excessive thirst, watery diarrhoea,
swollen and congested conjunctiva with occasional haemorrhages, diffuse
haemorrhages between the hocks and feet, coughing/sneezing, incoordination, nasal and
ocular discharge, mucus accumulation and nervous signs (paralysis) (de Jong and Hien,
2006; Swayne, 2008a; The Merck Veterinary Manual, 2011). In severely affected birds,
greenish diarrhoea is commonly observed (CIDRAP, 2012; Swayne, 2008b; The Merck
Veterinary Manual, 2011). However, birds that survive the acute form can develop
torticollis, opisthotonus, or incoordination (The Merck Veterinary Manual, 2011). In
waterfowl, clinical findings include inappetence, reluctance to move, inactivity,
dullness, quietness, weakness and staggering (Ellis et al., 2004a).
2.3.2.3 Pathological and Histopathological Signs

2.3.2.3.1 Pathological Findings

A number of pathological signs can be observed in animals infected with AIV. In tigers and leopards on necropsy gross lesions of severe pulmonary consolidation and multifocal haemorrhages in the lungs, heart, thymus, stomach, intestine, liver and lymph nodes have been observed (Keawcharoen et al., 2004). Serosanguinous nasal discharge and icterus in cases with diffuse haemorrhagic lesions, multifocal mottled necrosis in the pancreas with splenomegaly, hepatomegaly and swollen kidneys can also be observed (Kwon et al., 2005; Keawcharoen et al., 2004).

In bar-headed geese (Anser indicus), great black-headed gulls (Chroicocephalus ridibundus), brown-headed gulls (Chroicocephalus brunnicephalus), and great cormorants (Phalacrocorax carbo), pathological signs including severe hyperaemia and oedema of the brain, haemorrhage and necrosis of the pancreas, and severe cloudy swelling of the kidneys have been observed (Abdel-Moneim et al., 2010). In infected chickens, haemorrhages in the conjunctiva, oedematous swelling of the face and wattles, hydropericardium, haemorrhages of the proventriculus and bursa of Fabricius, increased secretion of tracheal mucus, and congestion and oedema of lungs can be observed (Nakamura et al., 2008). During the outbreak in Hong Kong in 2002, congestion of the intestines, trachea, brain, liver, and kidneys; congestion and oedema of the lungs; swollen, mottled or congested spleen; increased pericardial fluid; petechial haemorrhages on cardiac fat; red-brown mottled pancreas; haemorrhagic duodenal contents; focal liver necrosis; friable liver; thickened air sacs; and minimal contents in the gizzard and proventriculus were observed in dead waterfowl (Ellis et al., 2004a).

2.3.2.3.2 Histopathological Lesions

Abdel-Moneim et al. (2010) reported histopathological evidence of coagulative necrosis with non-suppurative inflammation in the pancreas, as well as degenerative foci of
cardiomyocytes with nonsuppurative inflammation in a moribund bar-headed goose (A. indicus). Tanimura et al. (2006) similarly observed histopathological changes in large-billed crows (Corvus macrorhynchos) during the 2004 outbreak in Japan. The lesions included severe multifocal necrotising pancreatitis, focal degeneration and necrosis of neuronal and glial cells of the central nervous system, and focal degeneration of cardiac myocytes. Perkins and Swayne (2001) also reported lesions in multiple organs which were characterized by exudate, haemorrhage, necrosis and inflammation. The lung, heart, brain, spleen and adrenal glands were the most consistently affected, and viral antigen was most often detected by immunohistochemistry in the parenchyma of these organs. Kwon et al. (2005) diagnosed HPAI H5N1 in three dead black-billed magpies (Pica pica sericea) in South Korea. At necropsy, the authors found the prominent lesions of multifocal or coalescing necrosis of the pancreas with enlargement of the livers and spleens. Microscopically, necrotising pancreatitis and lymphocytic meningoencephalitis were observed.

2.4 Diagnosis

2.4.1 Clinical Diagnosis

Diagnosis based on presenting clinical signs alone is usually not possible because there are several diseases (Section 2.4.2) that present with similar clinical signs to that of AI.

2.4.2 Differential Diagnosis of HPAI H5N1

Highly pathogenic avian influenza due to H5N1 must be differentiated from other causes of high mortality such as virulent Newcastle disease, peracute septicaemic fowl cholera, heat exhaustion and severe water deprivation (Swayne, 2012), as well as infections with reovirus, infectious bursal disease virus, infectious laryngotracheitis virus, Mycoplasma gallinarum and Mycoplasma gallinaceum (Gall et al., 2009).
2.4.3 Laboratory Diagnosis

A number of laboratory diagnostic techniques have been developed to confirm the disease from a wide variety of samples and situations (OIE, 2012). These include identification of the virus, assessment of its pathogenicity, serological tests, antigen capture and molecular techniques. Nevertheless, virus isolation has been recognised as the gold standard for diagnosis and is essential for characterization of the virus (de Jong and Hien, 2006).

2.4.4 Virus Isolation

2.4.4.1 In Humans

Samples for virus isolation from suspect human cases include rectal swabs, serum, cerebrospinal fluid (de Jong et al., 2005; Schultsz et al., 2005), conjunctival swabs and respiratory specimens (nasal or throat secretions) (Fouchier et al., 2005b; Yuen et al., 1998; Hien et al., 2004). Sera can be tested with enzyme linked immunosorbent assays (ELISAs) (Schultsz et al., 2005) and further tested with H5-specific microneutralization assays (Rowe et al., 1999), while nasal swabs can be tested by using reverse transcription polymerase chain reaction (RT-PCR) (Hien et al., 2004).

2.4.4.2 In Animals

Avian influenza viruses can be isolated in embryonated eggs or in cell culture, using permissive cells such as Madin Darby canine kidney (MDCK) cells or rhesus monkey kidney (LLC-MK2) cells. The HPAI viruses from animals do not require the addition of exogenous trypsin for efficient replication in cell cultures, unlike human strains (de Jong and Hien, 2006). The immunofluorescent test (IF) can be used to detect virus-infected cells directly in specimens taken from animals (Madeley and Peiris, 2002). Initial identification of influenza A virus can be performed by IF staining with monoclonal antibodies against the nucleoprotein, and then can be tested further with a PCR or haemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays.
using a panel of reference antisera against various subtypes (de Jong and Hien, 2006).

2.4.5 Antigen Detection

2.4.5.1 In Humans

Rapid immunochromatographic assays or direct immunofluorescence (de Jong and Hien, 2006; Peiris et al., 2007) and enzyme immunoassays (EIA) have been widely used to allow the rapid detection of influenza A viral antigens in humans (Peiris et al., 2007). However, additional subtype-specific diagnostic methods (e.g., RTPCR or culture) are required to differentiate avian from human influenza virus (Peiris et al., 2007).

2.4.5.2 In Animals

Several tests have been used to detect AIV antigen. These include antigen capture Enzyme Immunoassay (IAC-EIA) (Davison et al., 1998), influenza A antigen detection ELISA (Sims et al., 2003; Chua et al., 2007), H5 avian influenza-specific antigen detection ELISA, H5 rapid immunoblot assays, chromatographic immunoassays (Chua et al., 2007), HI tests (Maines et al., 2005), nucleic acid sequence based assay (NASBA) (Sims et al., 2003), and microarray technology Flu Chip (Liu et al., 2006). The ELISA test can be performed in 20 minutes and the NASBA in 6 hours (Sims et al., 2003). A double antibody sandwich ELISA assay (DAS-ELISA), employing a monoclonal antibody directed against type-specific influenza A nucleoprotein (McAB anti-NP), has also been used to detect antigen of influenza A viruses (Siebinga and Boer, 1988). These authors concluded that this test was sensitive, rapid and easy to use.

Recently, virus biosensors with label-free electrical detection have been developed, including Si nanowire field-effect transistor (FET) sensors for influenza A detection (Patolsky et al., 2004) and Indium-tin-oxide thin film transistor biosensors for label-free detection of H5N1 (Guo et al., 2013). These biosensors have been described
as promising tools for on-site use in field investigations of H5N1 due to their high sensitivity, rapidity, selectiveness and low cost (Guo et al., 2013; Patolsky et al., 2004).

2.4.6 Reverse Transcription Polymerase Chain Reaction (RT-PCR)
Reverse transcription polymerase chain reaction offers a cheaper and more rapid alternative than virus isolation for the diagnosis of AI, with confirmation of clinical samples within 3 hours (OIE, 2012). de Jong and Hien (2006) described that the RT PCR allows for the sensitive and specific detection of viral nucleic acids compared to culture or antigen detection methods. Respiratory specimens are usually assessed by an RT-PCR assay specific for the haemagglutinin gene of influenza A H5N1. The RT-PCR methods were the diagnostic tools of choice in H5N1 outbreaks in Hong Kong and other Southeast Asian countries (Chotpitayasunondh et al., 2005; Yuen et al., 1998; Hien et al., 2004).

2.4.7 Serology
Agar gel immunodiffusion (AGID), haemagglutination and haemagglutination inhibition (HI) tests, and ELISAs have been used to detect antibodies against AIV (de Jong and Hien, 2006; Gan, 2004). de Jong and Hien (2006) described that the detection of subtype-specific antibody was important for epidemiological investigation purposes, particularly during outbreaks. For human influenza viruses, HI assays are the gold standard for detecting antibodies to H5N1 and are the preferred serological assay to detect antibodies to AIV in some countries such as China (Huang et al., 1990). For avian infection, a latex agglutination test (LAT), based on polystyrene beads sensitized with inactivated avian influenza virus H5N1 particles, has been developed (Xu et al., 2005). Another test, a colloid gold strip (CGS) test for detecting antibodies to H5 subtype AIV has also been developed. The test is based on membrane chromatography and uses colloidal gold conjugated with inactivated AIV-H5N1 as the tracer. This test is described as suitable for the on-site detection of antibodies or antigens because of its
simplicity, speed of operation and production of results, low cost, the fact it can be performed by non-skilled technicians and does not require expensive equipment (Peng et al., 2007). In addition, the latex and CGS tests have similar sensitivity and specificity of 88.8 and 97.6%, respectively (Cui et al., 2008; Xu et al., 2005). Unfortunately, however, these tests are not available throughout the world.

2.4.8 Enzyme Linked Immunosorbent Assays (ELISAs)

Commercial ELISA kits have been widely used to detect antibodies against the nucleocapsid protein. Kits with an indirect and competitive format have been developed and are now being used to detect AIV-specific antibodies (OIE, 2012). The competitive ELISA (C-ELISA) has been shown to be more sensitive and specific than the AGID test, and as sensitive and specific as the HI test (Zhou et al., 1998). However, this method takes several hours to perform and requires trained personnel and specialised equipment (Oh et al., 2006).

2.4.9 Type of Samples Required for the Diagnosis of AI

In order to get a correct diagnosis or to get the best results from a test, suitable samples need to be collected. The WHO (2006b) described the preferred specimens to be collected to diagnose the presence of AIV. These preferred samples include upper respiratory tract, posterior-pharyngeal (throat) (which have the highest yield for detecting influenza type A H5N1), naso-pharyngeal and nasal swabs. From the lower respiratory tract, a tracheal aspirate can be collected. Serum can also be collected from both acute and convalescent patients; however in acute and peracute cases a negative antibody response may be obtained due to insufficient time to develop antibodies. Secondary specimens may include plasma in EDTA (for detection of viral RNA), rectal swabs (especially if the patient has diarrhoea), and cerebrospinal fluid.

Avian Influenza viruses are identified by demonstrating the presence of: influenza A matrix or nucleoprotein antigens using the AGID or other suitable immunoassays; or
viral RNA using an influenza A-specific reverse transcriptase-PCR test (Swayne, 2012). For a rapid test, a real-time multiplex polymerase chain reaction (PCR) has been used to detect influenza A and B in clinical specimens. The commercial PCR assays can be completed in less than 30 minutes and have a sensitivity of 93.3% and a specificity of 100% (Zou et al., 2007).

2.5 Impacts of HPAI H5N1

The virus HPAI H5N1 has been described to have impacts on animal health and production (Brahmbhatt, 2005), as well as human health (Capua and Alexander, 2004b; Katz, 2003; Katz et al., 2009; Perdue and Swayne, 2005; WHO, 2013). The WHO (2013) described two main risks for human health as a result of the widespread persistence of H5N1 in poultry populations: sporadic human infections; and the potential for emergence of a pandemic influenza strain. Direct impacts on livestock producers include livestock deaths and reduced production along with disruption to the supply and distribution networks of poultry traders, feed mills and breeding farms. It is likely that short-term losses are followed by longer-term impacts, which results in increased direct costs and foregone income (Otte et al., 2008; Brahmbhatt, 2005). McLeod et al. (2005) described four factors that contributed to the economic and social impact of HPAI H5N1. These include the zoonotic potential of the disease resulting in human deaths, loss of production and livelihoods of vulnerable people, endemic status of the disease that leads to a prolonged financial drain because of the control costs, and disruption to the global poultry population and trade in poultry and poultry products through widespread outbreaks caused by the movement of migratory birds.

2.5.1 Impact of H5N1 in Asian Countries

It has been recorded that Asian countries have experienced the biggest impact of H5N1 since its reemergence in 2003. With respect to animal production, the entire chicken population of Hong Kong of over one million birds was slaughtered (Alexander, 2000)
as a result of the apparent spread of this virus to humans (Class et al. 1998). In the outbreaks in Thailand and Vietnam in 2003, the agricultural costs alone were estimated to be US$880 million and US$120 million, respectively, excluding the costs of human sickness and death, or the damage done to other areas of the economy, such as tourism (Fouchier et al., 2005a). Additionally, direct cost to the livestock sector in Cambodia, Thailand and Vietnam has been estimated at US$560 million (Bloom et al., 2005). These authors further explained that annual GDP could potentially reduce by USD14.2 to 99.2 billion (Bloom et al., 2005). From the onset of HPAI outbreaks in 2004 until 2006, 63.8 and 50 million birds were culled in Thailand and Vietnam, respectively (NaRanong, 2007). As well, 294 people have died from infection with H5N1 in Asian countries since 2012 with Indonesia, Vietnam and China being the three most affected countries (WHO, 2017).

2.5.2 Impact of H5N1 in European Countries

In Europe, H5N1 was first detected during the autumn of 2005 (Williams et al., 2011), specifically in Croatia and Romania, and subsequently the virus gained entry to other countries including Denmark (Bragstad et al., 2007), Germany, France, Greece, Italy, Sweden, Czech Republic (Barral et al., 2008), Slovenia and Austria (Enserink, 2006; Fink et al., 2010). In Europe, between December 1999 and April 2003, over 50 million birds died or were depopulated following HPAI infection (Capua and Alexander, 2004a).

2.5.3 Impact of H5N1 in African Countries

The disease was reported on poultry farms in at least three states in northern Nigeria in early 2006 (Enserink, 2006; Ducatez et al., 2007). Since then, other African countries have reported H5N1 infections including Niger, Egypt, Djibouti, Burkina Faso, Sudan and Ivory Coast (Ducatez et al., 2007). By 2007, the disease had caused infection in 40 African people with a 40% fatality rate with most infected individuals (80%) being
female (Fasina et al., 2007). Until 2012, the WHO had recorded the deaths of 121 people from H5N1 in the continent of Africa (WHO, 2017).

2.5.4 Impact of H5N1 in Indonesia

In Indonesia, the disease has been reported to affect all of the four poultry sectors and in all bird species including chickens, ducks, quails and songbirds (Samaan, 2007). Hartono (2004) reported that around 17.1 million poultry (15 million layers, 2 million parent stock and 0.1 million broilers) died or were culled between July 2003 and January 2004 because of HPAI H5N1. As well the disease has caused a significant number of deaths in people in some provinces. As of 2015, the (WHO, 2016) recorded that 167 people had died across the country since the first case was reported in humans. Moreover, the disease has caused some poultry producers to go bankrupt and has had a significant financial burden on people involved in poultry enterprises (Poultry Indonesia, 2012).

2.5.5 Impact of H5N1 on Trade and Consumption of Poultry Meat and Eggs

Although the WHO has suggested that the consumption of meat and eggs during an outbreak is safe (WHO, 2013), the trade and consumption of poultry meat and eggs has fluctuated dramatically during outbreaks. Taha (2007) reported that the “import demand for both uncooked and cooked poultry declined substantially in 2003, due to consumers’ fear of contracting avian influenza by eating poultry meat” in Europe. The author noted that the market demand for poultry products increased in 2005, but again decreased in 2006 because of further outbreaks. In Indonesia, until 2011, outbreaks of H5N1 have affected the consumption level of chicken, especially in Bogor, and consequently has resulted in a reduced price for chickens (Poultry Indonesia, 2012).
2.6 Control Strategies

The major tools of primary prevention are preventing the disease from occurring in the first place and these include surveillance, threat identification, reporting and containment (Edler, 2006). Traditionally, public health preparedness has included basic hygiene and special procedures designed to prevent exposure and contain infection in health-care settings (Edler, 2006). Other researchers have sought alternative control strategies for HPAI H5N1 in poultry. Control strategies based on surveillance, stamping out, movement restriction and enforcement of biosecurity measures have not prevented the virus from spreading, particularly in developing countries. Instead the use of antiviral chemotherapy and natural compounds, avian-cytokines, RNA interference, genetic breeding and/or development of transgenic poultry are being evaluated as integrated intervention strategies for control of HPAI H5N1 in poultry (Abdelwhab and Hafez, 2012). In Indonesia, stamping out has been used to control the disease in outbreaks. However, it was limited to individual infected flocks in villages, but is hampered by a lack of an appropriate compensation scheme (Begemann, 2009).

There are three main goals or outcomes for controlling AI: prevention; management; and eradication (Swayne, 2008a). These can be achieved by combining components including education, biosecurity, diagnostics and surveillance, elimination of infected poultry, and decreasing host susceptibility. However, the success of any strategy is dependent on industry-government trust, co-operation and interaction (Swayne and Akey, 2005). For instance, during the outbreak of H5N2 in Mexico in 1994, the policies undertaken by the government included immediate stamping out of affected flocks, disinfection of affected premises, quarantine measures in the region, strict movement controls on poultry and their products and vaccination. With these policies, the disease was eradicated in a relatively short time (Villarreal, 2006). This eradication strategy has
also been successfully applied in Europe and North America in response to HPAI outbreaks (Swayne and Akey, 2005; Alexander, 2007b).

Recently, in order to prevent the disease transmitting to people, travelers have been advised by the WHO to avoid visiting live-bird markets in areas with AI outbreaks. It has also been suggested that people who work with birds should use protective clothing and special breathing masks and all people should avoid eating undercooked or uncooked poultry meat to reduce the risk of exposure to the virus (Medical Encyclopedia, 2011).

As a part of prevention, vaccination is a means that has been used to increase the immunity of susceptible poultry. Many experimental studies have shown that AI vaccines provide good protection against the development of clinical signs, including death, and reduced viral shedding (Swayne et al., 2001; Tian et al., 2005; van der Goot et al., 2008). The use of extensive vaccination programs in poultry to control the H5N1 epidemic has been increasingly used in developing countries (Peyre et al., 2009). These programs have primarily used killed, whole virus-adjuvanted vaccines (Suarez, 2005). However, vaccination alone does not prevent infection entering a population. Vaccination can be a powerful tool to support eradication programs if it is used in conjunction with other control methods, and proper vaccination can reduce or prevent clinical signs, reduce virus shedding in infected birds and vaccinated birds, and increase the resistance to infection, as well as reduce transmission (Suarez, 2005; van der Goot et al., 2005b). Therefore, researchers have recommended that if vaccination is adopted for the control of AI, vaccine banks, including companion diagnostic tests, must be established and made available for immediate use (Capua and Marangon, 2003). In line with the use of vaccines for AI control, Swayne (2006) and Capua and Alexander (2008) suggested that vaccines should only be used as part of a comprehensive control strategy that also includes biosecurity, quarantine, surveillance and diagnostics,
education, and elimination of infected poultry. In certain conditions, however, when there is risk of major spread and depopulation is not feasible or desirable, the FAO recommended vaccination using the differentiating infected from vaccinated animals (“DIVA”) strategy (FAO/OIE/WHO, 2004). This is believed to be the only way to enable detection of field exposure in a vaccinated population and has been successfully implemented in the field resulting in disease eradication (Capua and Marangon, 2006). However in developed countries vaccination is not considered to be a control option, instead stamping out is usually recommended for H5 and H7 AIV (Capua and Marangon, 2006). In the USA, for instance, immediate stamping out has been the preferred option for HPAI outbreaks (Swayne and Akey, 2005). This method is preferred because the virus is still able to replicate in clinically healthy vaccinated birds, and this is probably why vaccination alone has been unsuccessful in achieving eradication (EU Commission, n.d). In response to outbreaks of HPAI H7N7 in the Netherlands, a set of measures was successfully implemented to reduce viral transmission. These measures included depopulation of infected flocks, culling of flocks within a 1 km radius of an outbreak, establishment of culling within 48 hours after notification of an outbreak, performance of forward and back tracing by official veterinarians, implementation of a transport ban, establishment of a protection and surveillance zone around infected flocks, and establishment of a buffer zone (Stagemen et al., 2005).

Biosecurity, is regarded as the first and most important means of prevention, as it can create a barrier against the entry of infection (Capua and Marangon, 2006). However, in developing countries, application of biosecurity measures has always been a challenge. This is because most poultry in these countries (around 80%) are village poultry (Conan et al., 2012). These represent the largest number of domestic animals in the world
(FAO:FAOSTAT, 2012), and these village poultry units are characterized by small flocks with generally low biosecurity measures (Pym et al., 2006).

2.7 Monitoring and Surveillance

Disease monitoring and surveillance systems (MOSSs) have become one of the major components of veterinary activity. These are used to assess the existing levels of prevalence, the effectiveness of control programmes and, after disease eradication, to document the continued absence of disease from a given region or zone (Doherr and Audige, 2001). In addition, continued growth in international trade and the developing concepts of zoning and risk assessment demand effective assessment of national surveillance and monitoring systems for animal health (Hueston, 1993). Surveillance studies undertaken in wild animals have provided information on the prevalence of AIV in the environment, and to enable banking of reference reagents and putative vaccine strains to be used in times of outbreaks in humans, as well as in animals (Munster et al., 2005). In Indonesia, Participatory Disease Surveillance and Response (PDSR) programmes were introduced in 2006. These have provided information on the incidence of disease in the village poultry sector and have been facilitated by the introduction of pen-side rapid antigen testing (Begemann, 2009; Azhar et al., 2010a).

2.8 Demonstrating Disease Freedom

Disease freedom is defined as the absence of clinical, epidemiological or other evidence of an infectious agent (Welte et al., 1998). For the purpose of international trade of animals and animal products, the documentation of freedom from disease has significant importance, and needs to be undertaken through annual surveys at a farm or animal level (Schwermer et al., 2009). In order to obtain official recognition of disease freedom, countries have to provide substantial evidence to the OIE and/or their trade partners (FAO, 2013). Surveillance is widely used to assist in making these decisions and supporting claims of freedom from disease (Cameron, 2007). Current methods to
demonstrate zone or country freedom from disease are based on either quantitative analysis of the results of structured representative surveys, or qualitative assessments of multiple sources of evidence (including complex non-representative sources) (Martin et al., 2007). The qualitative assessment approach has been applied by the OIE to assess claims of freedom at the completion of disease eradication programs. In the structured representative surveys, data are used to estimate the probability that the negative results of the survey would be achieved if the disease were present at a specified level (the design prevalence). If the probability is less than an agreed level, the population is considered free (Martin et al., 2007). When demonstrating a zone is free from infection, there is considerable flexibility in relation to convenience or cost, in setting the number of herds tested and the number of tests per herd that will meet the requirements of the survey (Cannon, 2002). A computer program, ‘FreeCalc’, has been developed to enable the accurate calculation of survey sample-size requirements and the precise analysis of survey results to minimise costs and to provide reliable survey results (Cameron and Baldock, 1998). Confirming disease freedom from AI has significant benefits for the international trade of poultry and poultry products and these benefits are obtained by producers, the industry and the nation.

Central to controlling a disease in a region is understanding the epidemiology of the disease in question. In the next chapter the general materials and methods used for the current study on HPAI in West Timor are outlined.
CHAPTER THREE

Materials and Methods

3.1 Location of Data Collection.

East Nusa Tenggara (Indonesian: Nusa Tenggara Timur - NTT) is a province of Indonesia, located in the eastern part of the Lesser Sunda Islands. The province consists of 566 islands, but is dominated by the three main islands of Flores, Sumba and Timor (Provincial Government of NTT 2010). West Timor is situated on the island of Timor and shares this island with the Republic of Democratic Timor Leste (RDTL). West Timor is split into five districts; from west to east the five regencies are: Kupang District; Kupang City; Timor-Tengah Selatan (South Central Timor); Timor-Tengah Utara (North Central Timor); and Belu (See Figure 4.1). The economy in West Timor is mainly agricultural, with the livestock sector playing an important role in the economy of families (Provincial Government of NTT 2010).

3.2 Existing Data Collection

Existing data (the number of cases in the last 10 years, control measures applied, and other related data) were collected from the Provincial Livestock Services of NTT. Informal meetings were conducted with the heads of villages of selected districts to provide an update on the available data for that village. At these meetings the purpose of the study was also explained.

3.3 Study Design

3.3.1 Study type

A standard cross-sectional study was conducted in selected districts of West Timor. This type of study is commonly used in epidemiology to obtain information about the baseline characteristics of a population (Pfeiffer, 2010), and is usually designed to select a random sample of the target population (Thrusfield, 2005). The external population
was the total poultry population in West Timor, with the poultry population in selected districts being the target population. The unit of interest was individual poultry.

3.3.2 Sampling Method

A purposive sampling approach was used to select the districts, sub-districts, villages, poultry owners and poultry in this study. Inclusion and exclusion criteria to be used for the sampling are outlined in the Table 3.1.

Table 3.1 Inclusion and Exclusion Criteria for Selection of Animals

<table>
<thead>
<tr>
<th>Site</th>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>District</td>
<td>Expected HPAI status known – based on reporting of clinical disease to Dinas (Livestock Services).</td>
<td>Unknown HPAI status - based on no or unreliable reporting of clinical disease to Dinas (Livestock Services)</td>
</tr>
<tr>
<td>Sub-district</td>
<td>Has a history of HPAI infection</td>
<td>Does not have a history of HPAI infection</td>
</tr>
<tr>
<td>Village</td>
<td>Has a history of HPAI infection</td>
<td>Does not have a history of HPAI infection</td>
</tr>
<tr>
<td>Poultry owner</td>
<td>Present on day of visit&lt;br&gt;Given consent to participate&lt;br&gt;Currently is raising poultry</td>
<td>Not present on the day of visit</td>
</tr>
<tr>
<td>Individual Poultry</td>
<td>Known to belong to the interviewee</td>
<td>Unknown ownership.</td>
</tr>
</tbody>
</table>

3.3.2.1 Questionnaire Design

Questionnaires were designed which contained a range of closed, open and ranking questions. The questionnaires (Appendices 1 to 5) were developed in English and then translated into Bahasa Indonesia prior to implementation. The questionnaires were approved by the Human Ethics Committee, Murdoch University and the survey was approved by local authorities.
3.3.2.2 Questionnaire Pilot

The questionnaires were pretrialed in Indonesian Language with 10 poultry farmers (6 men and 4 women) in Kupang to ensure that sufficient time was allocated for the interviews and to ensure there were no ambiguities with questions. The educational backgrounds of the respondents ranged from Primary School to University graduated. Minor adjustments to the questionnaires were made to avoid uncertainty over the meaning and wording of some questions.

3.4 DATA COLLECTION

3.4.1 Questionnaire Administration

The questionnaires were delivered to selected poultry owners using a personal (face-to-face) interview method. Prior to commencement of the interview, the interviewer explained the purpose of the survey and obtained the consent from the participant. The interview was completed in full before any blood samples from poultry were collected.

3.4.2 Recruitment of Interviewees

Interviewees (farmers, sellers, buyers) were selected based on the criteria of: being present on the day of visit; giving their consent to participate; and currently rearing, selling and/or buying poultry, respectively. For the expert opinion survey, experts were selected from the local livestock services (Provincial Dinas Peternakan East Nusa Tenggara, Dinas Peternakan of Kota Kupang and Belu Districts) and Agricultural quarantine officers of Class 1 Tenau Kupang who had a good knowledge on the diseases of poultry and/or were working with or in the poultry industry.

3.4.3 Husbandry, Biosecurity Practices and Market Chain Survey

These studies were undertaken in selected areas of West Timor which were considered to have a high risk of HPAI. Questionnaires were distributed to farmers in order to: identify the husbandry and biosecurity practices being adopted by poultry owning
households; determine the movement of poultry in order to obtain information about poultry trading practices; and determine the knowledge on poultry health and avian influenza of workers from selected markets in West Timor. All questionnaires had been approved by the Murdoch University Animal and Human Ethics Committee (approval number Human Ethics Committee: 2013/106 and Animal Ethics: R2589 13), and local authorities (Governor of Nusa Tenggara Timur Province-approval number:070/2617/KPPTSP/2013). The questionnaires included closed, open and ranking questions (Village Farmer Questionnaire-Appendix 1, Poultry Buyer Questionnaire-Appendix 2 and Poultry Seller Questionnaire- Appendix 3). They were initially developed in English and then translated into Bahasa Indonesia prior to implementation. After development the questionnaires were discussed with local veterinary staff from the Provincial Dinas Peternakan (Livestock Services) NTT and then modified slightly to improve the meaning and clarity of specific questions. The questionnaires were then pretrialled on a group of 10 poultry farmers from Kupang. Based on the responses from these farmers slight modifications were further made to the questionnaires. The final questionnaires were administered to 100 selected farm owners through face-to-face interviews. Participation consent was read out loud to the respondents at the beginning of the interviews. The interview teams included Dinas Staff from Kota Kupang and Belu. These teams were trained on the questionnaire administration and were familiarized with the questionnaires prior to the surveys being conducted. The questionnaire interviews were conducted with the assistance of local staff from the local Dinas (Livestock Services) who could provide information in the range of local languages (Dawan and Tetun), as well as Bahasa Indonesia. Prior to conducting an interview the purpose of the survey was explained to the owners.
3.4.4 Demonstration of freedom from HPAI H5N1

3.4.4.1 Selection of Sites

A targeted sampling approach was used to select the sites, where high-risk districts were specifically targeted for sampling. These high-risk districts were selected based upon previous reports, discussions and findings documented by the Provincial Government Livestock Services of NTT. The sites selected for sampling included bird markets and farms. The city of Kupang was considered to be a high-risk district due to the previous documented reports and findings and it also is the main poultry production region in East Nusa Tenggara.

3.4.4.2 Sample Size Calculation

The total number of birds’ blood sampled per village was 20. This sample size was based on the formula to estimate the prevalence of infection with adjustment for test sensitivity (84.3%) and specificity (97.7%) (Meseko et al., 2010) and for the estimated poultry population per village and with level of confidence set at 95%. The design prevalence in the selected districts of West Timor was set at 20%.

3.4.4.3 Sample Type and Test Methods

In order to demonstrate the free status of West Timor from H5N1 virus, pen-side tests were conducted in the selected sites. At first, a screening test was undertaken on samples collected from birds located at the selected sites. Cloacal and tracheal swabs were taken from live birds. These samples were tested immediately using the Anigen® Rapid H5 AIV Antigen. Test Kit as described by Meijer (2006). The Anigen Rapid® H5 AIV Antigen Test Kit is a chromatographic immunoassay for the qualitative detection of avian influenza type A virus antigen in avian cloacal or tracheal swabs, or faeces (BioNote. Inc, Seoul, Republic of Korea). The test has a reported sensitivity of 100% at the farm-level and 82% at the individual level compared with virus isolation during an outbreak of H5N1 in Korea in 2004 (Meijer, 2006). During the outbreak in Nigeria
between 2006 and 2007, the sensitivity of the Anigen® Rapid H5 AIV antigen test was reported to be 84.3% (95% confidence interval (CI), 78.1-88.9%), whereas the relative diagnostic specificity was 97.7% (95% CI, 94.2–99.1%) (Meseko et al., 2010). In Indonesia, Anigen® has been the preferred test because it is easy to use, and has adequate sensitivity and specificity (Boland et al., 2010). Sera was collected from any positive chickens for further testing with a c-ELISA or HI test in the Type B Veterinary Laboratory of the Provincial Livestock Services of Kupang.

3.5 Data Management

A purpose built database was designed in Excel 2007 (Microsoft) for the entry and storage of data from the farmer questionnaire and the poultry-recording sheet. Data were recorded as either categorical or continuous variables depending on the type of data collected. Initial cleaning of the data involved checking record numbers, ensuring there were no duplicates, checking for missing or unusual values and ensuring consistency between variables. The data were then exported to SPSS for subsequent statistical analysis. To avoid potential loss of data, data were backed up onto hard disks.

3.6 Data Analyses

A qualitative and stochastic analysis was used to analyze the risk of introduction of HPAI into West Timor. This involved the use of the Excel add-in PopTools (Version 3.5) (http://www.poptools.org/functions/). Social Network analysis was used to analyze the market chain of poultry movement in West Timor using Ucinet® (Borgatti et al., 2002).

The quantitative data sets were analyzed using a range of statistical tests in SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). After tabulating the data in SPSS, normality tests were performed in SPSS by plotting the data and conducting homogeneity tests for variances. For discrete data a chi-square test ($\chi^2$) for independence or Fisher’s exact test was used. The prevalence and their 95% confidence
intervals (CI) were calculated using the Exact Binomial method (Daly, 1992). Odds ratios and their 95% CI were also calculated.

3.7 Control of Bias

3.7.1 Selection Bias

Purposive sampling is a non-random selection process. With subdistricts and villages being selected for questionnaires in this manner, there is the potential for selection bias. The selection criteria for both the subdistricts and villages were biased towards those villages that were accessible and considered safe for the researcher. This type of sampling influences the external validity of the study as it is not representative of the reference population.

3.7.2 Measurement of Bias

Using data gathered from questionnaires, there is the potential for measurement bias. To limit this potential bias, interview teams were trained to allow them to become familiar with the questionnaires and to provide instructions as the most appropriate manner in which to conduct the interviews.
CHAPTER FOUR

Retrospective Study of the History of HPAI H5N1 in West Timor

4.1 Introduction

A highly pathogenic avian influenza virus subtype H5N1 was first detected in Indonesia in August 2003 (Sumiarto and Arifin, 2008), although it was not officially reported until 2004 (Loth et al., 2011). Sims et al. (2005) hypothesised that the virus was introduced into Indonesia either through wild water birds or the illegal importation of infected poultry or poultry-associated products from neighbouring countries. In West Timor, the disease was first diagnosed in 2004 when five samples of chickens from two different farms tested positive on the haemoglobin inhibition (HI) test (unpublished data from Provincial Dinas Peternakan, Nusa Tenggara Timur). Smith et al. (2006) suggested that the virus originated from West Indonesia (Java) through two separate introductions from the movement of poultry and/or poultry products.

Retrospective studies are often used as the first step in the investigation and subsequent control of a disease, and involve the analysis of existing data to understand the distribution and patterns of spread of a disease (Ayebazibwe et al., 2010). Although data on testing for HPAI through active surveillance are available in West Timor, to date no analysis has been undertaken on these data to understand the epidemiology of this disease. Therefore the study described in this chapter was carried out to analyze this existing epidemiological data on HPAI in West Timor, including describing the spatio-temporal distribution of reported HPAI H5N1 outbreaks in West Timor since the original disease incursion.
4.2 Materials and Methods

4.2.1 Study Area and Data Collection
The study area is outlined in Chapter 3. Data on reported outbreaks of HPAI H5N1 and surveillance for the virus on West Timor between 2004 and 2013 were obtained from the Provincial Dinas Peternakan (Department of Agriculture) of NTT. No samples had been collected between 2007 and 2011, and some samples collected in 2012 and all samples in 2013 were from commercial farms that obtained day old chickens (DOC) from Java. As the parent stock of these DOC had a history of vaccination against HPAI, these samples/data were excluded from the analysis due to the potential for false positive serological reactions (Wang et al., 2011; Capua et al., 2003b). The samples which generated the data used in this study were collected through the national active surveillance system aimed at detecting avian influenza viruses. For this surveillance, swabs, sera and samples of organs from diseased and apparently healthy chickens were collected. The swabs were tested using the Anigen® Rapid H5 AIV antigen test kit (Bionote, Republic of Korea-Anigen Rapid AIV Ag Test Kit), sera samples were tested with HA/HI tests, and organ samples were examined by ELISA. Sera and organ samples were tested at the Center for Veterinary Investigation, Denpasar, Bali.

4.2.2 Data Analysis

4.2.2.1 Descriptive analysis
Descriptive analyses were conducted in Excel (Microsoft® Office Excel 2010 for Mac) and statistical tests carried out in SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). The exact binomial method was used to calculate the 95% confidence intervals for the test prevalence as described by Ross (2003). Odd ratios and their 95% confidence interval were also calculated. The $\chi^2$ test was used to compare the differences between years, sample types and between species of poultry.
4.2.2.2 Calculation of Real Prevalence

The real prevalence (true prevalence) of HPAI in 2004 was calculated based on the data of the outbreak in that year by using the following formula as described (Pfeiffer, 2010):

\[
\text{Real Prevalence} = \frac{\text{Apparent prevalence} + (\text{Specificity} - 1)}{\text{Specificity} + (\text{sensitivity} - 1)}.
\]

In this formula the apparent prevalence was the prevalence attained after testing (test prevalence), the sensitivity of the HA/HI Test was set at 80.6%, and the specificity at 100% (Zarkov, 2007), the Se and Sp for the Anigen® Rapid H5 AIV antigen test were 84.3% and 97.7%, respectively (Meseko et al., 2010).

4.3 Results

A total of 1838 samples originating from the five districts of West Timor were tested from 2004 to 2013 and after excluding the samples from potentially vaccinated animals (177), analysis was conducted on the remaining 1661 samples (Table 4.1). The samples were taken from chickens, pet birds and Muscovy ducks (Cairina moschata); and from both clinically healthy and dead birds, and it was possible that multiple samples were taken from the same birds for diagnosis with the different tests used (personal communication with Cahyo Sunarno - Coordinator of Participatory Disease Surveillance and Response (PDSR) of NTT staff at Provincial Dinas Peternakan of NTT).
Table 4.1 Samples examined in the different years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Poultry type</th>
<th>Type of sample</th>
<th>Number of positive/total tested</th>
<th>Overall percent positive (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Organs</td>
<td>Sera</td>
<td>Swabs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chicken Muscovy ducks</td>
<td>Chicken Muscovy ducks</td>
<td>Chicken Song Birds Muscovy ducks</td>
</tr>
<tr>
<td>2004</td>
<td>Commercial</td>
<td>38 0</td>
<td>251 0</td>
<td>20 0 0</td>
</tr>
<tr>
<td>2005</td>
<td>Backyard</td>
<td>8 1</td>
<td>319 3</td>
<td>115 0 3</td>
</tr>
<tr>
<td>2006</td>
<td>Backyard</td>
<td>8 1</td>
<td>74 2</td>
<td>57 3 3</td>
</tr>
<tr>
<td>2012</td>
<td>Commercial*</td>
<td>0 0</td>
<td>397 0</td>
<td>232 0 0</td>
</tr>
<tr>
<td></td>
<td>Backyard</td>
<td>0 0</td>
<td>79 28</td>
<td>13 0 6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>54 2</td>
<td>1120 33</td>
<td>437 3 12</td>
</tr>
</tbody>
</table>

* Only birds sourced from non-vaccinated adult stock included in the results

Source: Data from Provincial Dinas Peternakan of Nusa Tenggara Timur
Of these samples, 1153 were sera, 452 were swabs and 56 were from organs/tissues. The overall test prevalence from the samples was 12.2% (95%CI: 10.6, 13.8); however data on prevalence in individual animals were not available as multiple samples were potentially collected from individual animals, although their identity was not recorded. Eleven of the 56 organ/tissue samples exhibited histopathological lesions consistent with HPAI (19.6%, 95%CI: 10.2, 32.4), 191 of the 1153 sera were positive (16.6%, 95%CI: 14.5, 18.8) while all 452 swabs were test negative on the Anigen® Rapid H5 AIV antigen test (0.0%; 95% CI: 0.0, 0.8). All samples from song birds (caged pet birds) (n = 3) and Muscovy ducks (n = 47) were negative. The prevalence in commercial chickens (16.7%) was significantly higher than that in village chickens (6.7%) ($\chi^2 = 36.1; \text{df} 1,1; P < 0.0001; \text{OR} 2.8, 95\% \text{CI} 2.0, 4.0$).

4.3.1 Temporal Distribution

In Table 4.2 the prevalence of HPAI H5N1 in West Timor from 2004 to 2006 and in 2012 is outlined for all samples. Overall there was a significant difference in the prevalence between years ($\chi^2 = 548.5; \text{df} 3, 1; P < 0.0001$), with the prevalence in 2004 (50.8%, 95%CI: 45.1, 56.5) significantly higher than that in 2005 (OR 12.9, 95% CI: 6.7, 24.7). The prevalence in 2005 (7.6%, 95%CI: 5.3, 10.5) was similar to that of 2006 (7.4%, 95%CI: 3.8, 12.9). No positive results were found in 2012 (0.0%, 95%CI: 0.0, 0.5). The prevalence of HPAI based on the type of samples is summarized in Table 4.3. The proportion of positive results was significantly different between sera, organ and swab samples in 2004, 2005 and 2006 (Table 4.3).

Table 4.2 Prevalence of HPAI in West Timor in different years (results of all samples)

<table>
<thead>
<tr>
<th>Year</th>
<th># tested</th>
<th>Percent positive (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>309</td>
<td>50.8 (45.1, 56.5)</td>
<td>12.9 (6.7, 24.7)</td>
</tr>
<tr>
<td>2005</td>
<td>449</td>
<td>7.6 (5.3, 10.5)</td>
<td>1.02 (0.5, 2.1)</td>
</tr>
<tr>
<td>2006</td>
<td>148</td>
<td>7.4 (3.8, 12.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>2012</td>
<td>755</td>
<td>0.0 (0.0, 0.5)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4.3 Comparison of the HPAI prevalence between samples collected in different years from West Timor.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sample type</th>
<th># tested</th>
<th>Percent positive (95%CI)</th>
<th>OR (95%CI)</th>
<th>P value</th>
<th>df</th>
<th>χ² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Sera</td>
<td>251</td>
<td>58.2 (51.8, 64.3)</td>
<td>3.4 (1.6, 7.2)</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>33.36</td>
</tr>
<tr>
<td></td>
<td>Organ</td>
<td>38</td>
<td>28.9 (15.4, 45.9)</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swab</td>
<td>20</td>
<td>0 (0.0, 16.8)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Sera</td>
<td>322</td>
<td>10.6 (7.5, 14.5)</td>
<td>-</td>
<td>0.0007</td>
<td>2</td>
<td>14.51</td>
</tr>
<tr>
<td></td>
<td>Organ</td>
<td>118</td>
<td>0 (0.0, 3.1)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swab</td>
<td>9</td>
<td>0 (0.0, 33.6)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Sera</td>
<td>76</td>
<td>14.5 (7.5, 24.4)</td>
<td>-</td>
<td>0.0036</td>
<td>2</td>
<td>11.26</td>
</tr>
<tr>
<td></td>
<td>Organ</td>
<td>63</td>
<td>0 (0.0, 5.7)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swab</td>
<td>9</td>
<td>0 (0.0, 33.6)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Sera</td>
<td>504</td>
<td>0 (0.0, 0.7)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swab</td>
<td>251</td>
<td>0 (0.0, 1.5)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The estimated true prevalence of HPAI in the different sample types and years of sampling is outlined in Table 4.4. The true prevalence varied from 0% (all samples in 2012) to 72.2% in sera from 2004.

Table 4.4 True prevalence of HPAI in West Timor based on the sample type

<table>
<thead>
<tr>
<th>Year</th>
<th>Sample type</th>
<th>True Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Sera</td>
<td>72.2% (66.2, 77.6)</td>
</tr>
<tr>
<td></td>
<td>Organs</td>
<td>31.1% (17.1, 48.1)</td>
</tr>
<tr>
<td></td>
<td>Swabs</td>
<td>0.0% (0.0, 16.8)</td>
</tr>
<tr>
<td>2005</td>
<td>Sera</td>
<td>13.2% (9.7, 17.4)</td>
</tr>
<tr>
<td></td>
<td>Organs</td>
<td>0.0% (0.0, 33.6)</td>
</tr>
<tr>
<td></td>
<td>Swabs</td>
<td>0.0% (0.0, 3.1)</td>
</tr>
<tr>
<td>2006</td>
<td>Sera</td>
<td>18.0% (10.1, 28.5)</td>
</tr>
<tr>
<td></td>
<td>Organs</td>
<td>0.0% (0.0, 33.6)</td>
</tr>
<tr>
<td></td>
<td>Swabs</td>
<td>0.0% (0.0, 5.7)</td>
</tr>
<tr>
<td>2012</td>
<td>Sera</td>
<td>0.0% (0.0, 0.7)</td>
</tr>
<tr>
<td></td>
<td>Swabs</td>
<td>0.0% (0.0, 1.5)</td>
</tr>
</tbody>
</table>
4.3.2 Spatial Distribution

4.3.2.1 Districts Affected

In Table 4.5 the prevalence of HPAI H5N1 in the different districts of West Timor are summarized. Kota Kupang had the highest prevalence of 21.4% (95%CI: 18.6, 24.5) and South Central Timor the lowest (0.0%; 95%CI: 0.0, 1.5). The prevalences in the five districts were significantly different ($\chi^2 = 123.3; \text{df} 4, 1; P < 0.0001$). The distribution of the disease in West Timor is displayed in Figure 4.1, with four of the five districts having some test-positive birds during this outbreak.

Table 4.5 Prevalence of HPAI in different districts in West Timor (2004-2006, 2012)

<table>
<thead>
<tr>
<th>District</th>
<th># tested</th>
<th>Percent positive (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kota Kupang</td>
<td>775</td>
<td>21.4 (18.6, 24.5)</td>
<td>19.6 (2.7, 142.3)</td>
</tr>
<tr>
<td>Kupang District</td>
<td>526</td>
<td>6.3 (4.4, 8.7)</td>
<td>4.8 (0.7, 35.8)</td>
</tr>
<tr>
<td>North Central Timor</td>
<td>40</td>
<td>5.0 (0.6, 16.9)</td>
<td>3.8 (0.3, 43.2)</td>
</tr>
<tr>
<td>Belu</td>
<td>73</td>
<td>1.4 (0.0, 7.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>South Central Timor</td>
<td>247</td>
<td>0.0 (0.0, 1.5)</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 4.1 Subdistricts in West Timor which had positive diagnoses of HPAI
4.3.2.2 Species Affected

In Table 4.6 the prevalence of HPAI H5N1 in the different bird species tested are listed. The prevalence was higher in chickens 12.5% (95% CI: 11.0, 14.3) than in pet (song) birds and Muscovy ducks (both 0%) and overall there was a significant difference between the species ($\chi^2 = 7.1; \text{df} 2,1; P = 0.03$).

<table>
<thead>
<tr>
<th>Species</th>
<th># tested</th>
<th>Percent positive (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td>1611</td>
<td>12.5 (11.0, 14.3)</td>
</tr>
<tr>
<td>Song Birds</td>
<td>3</td>
<td>0.0 (0.0, 70.8)</td>
</tr>
<tr>
<td>Muscovy Ducks</td>
<td>47</td>
<td>0.0 (0.0, 7.5)</td>
</tr>
</tbody>
</table>

4.4 Discussion

This is the first study examining historical data covering the outbreaks of HPAI H5N1 in West Timor from 2004 to 2006 and subsequent surveillance data (2012). In this study no pet birds or Muscovy ducks were positive, with disease and positive test results only detected in chickens. Village chickens, commercial chickens (broilers and layers), and ducks are the most common types of poultry raised by households in West Timor. In Timor most ducks reared originate from the Mallard duck (Anas platyrhynchos) as opposed to Muscovy ducks (Dean and Sandhu, 2008), however no Mallard type ducks were sampled in this study because the owners were not present on the sampling days (personal communication with Cahyo Sunarno - Coordinator of Participatory Disease Surveillance and Response (PDSR) of NTT/ staff at Provincial Dinas Peternakan of NTT). This study found that disease was most prevalent in 2004, the first year it had
been detected in the Province. The prevalence in 2004 was also significantly higher than subsequent years. Although seropositive poultry were detected in 2012 and 2013 (data not shown), the samples were all obtained from clinically normal poultry from commercial farms that imported DOC from Java. The parent stock of these DOC had been vaccinated against HPAI (Personal communication with Lola Allung, staff of Provincial Dinas Peternakan of NTT) and consequently these data were excluded from the analysis because of the likelihood of false positive reactions arising from maternally derived immunity (Funkhouser and Robinson, 2003).

Poultry play an important role in providing households with income and are a valuable source of protein and micronutrients (Alders and Pym, 2009; Guèye, 2000a; Randolph et al., 2007; de Bruyn et al., 2015). In West Timor most villagers keep small flocks of poultry under backyard settings (categorized as Sector 4) (FAO, 2004). Based on the animal census in 2013, 98% of the livestock population in Indonesia were poultry (Central Bureau of Statistics, 2013), while in West Timor poultry represent 90% of the total livestock population (Central Bureau of Statistics of Nusa Tenggara Timur, 2013). In addition, based on the data from the National Research Council of Indonesia, poultry, especially chicken meat, provided 67% of the total animal protein consumed by humans in 2013 (National Research Council of Indonesia-Dewan Riset Nasional, 2015). Consequently it is important that the status of AI in West Timor in particular, and in Indonesia in general, is understood to minimize potential losses to poultry owning households and the industry from this disease.

The prevalence of H5N1 was highest in Kota Kupang (21.4%). This could be due to this district being the center of poultry trade in West Timor. All poultry and poultry products originating from Java and Bali (DOC and eggs) are unloaded in Kupang and are then distributed to their final destination on West Timor. This practice would account for the higher prevalence reported in this district than in other districts as opportunities for
diseased birds being detected is higher here, plus potentially a greater probability of contact with local susceptible birds. Studies in other Asian countries have shown that trading patterns have contributed to the spread of the disease (Sims, 2007; Sims et al., 2009; Barennes et al., 2007) and results of studies on trading and movement patterns of chickens in West Timor are outlined later in this thesis (Chapter 7).

In the current study evidence of infection/positivity was only detected in chickens. No data were available on the poultry population in West Timor from 2004 to 2007, however in 2012 the population of chickens was 39 times larger than that of ducks and Muscovy ducks in the five districts (BPS NTT, 2014). Chickens were reported to be the poultry species most affected during the outbreak of H5N1 in Hong Kong in 1997 when high mortalities (70 to 100%) were reported on affected farms (Claas et al., 1998a) and over one million chickens were also slaughtered (Alexander, 2000). In addition chickens have been shown to be the most susceptible species of poultry to HPAI H5N1 virus (Perkins and Swayne, 2003). Chen and Chen (2014) reported that environmental factors play an important role in the spread and distribution of the disease, particularly in relation to poor management and biosecurity applied to chickens, especially those raised under village chicken systems. It is likely that the poor biosecurity adopted by poultry owners in Timor (discussed in detail in Chapter 6) facilitated some spread of infection within and between flocks.

The real prevalence of HPAI H5N1 in West Timor in 2004 was higher than in other years. This could be due to several factors including the lack of immunity of the chicken population as none had been vaccinated prior to the outbreak, along with the movement of birds through routine trade practices. The host ecology, immunity and phylogeny have important impacts on the prevalence of HPAI (Garamszegi and Møller, 2007). Chickens without immunity are vulnerable to the disease, whilst chickens with immunity have minimal to no virus shedding, hence result in a reduction in the
transmission of virus in field settings (Ellis et al., 2004a). In addition the progeny (DOC) of vaccinated hens have high levels of maternally derived antibody providing clinical protection against HPAI H5N1 (Maas et al., 2011). In Indonesia, vaccination against HPAI was initiated in 2004 (Swayne et al., 2015) in certain areas as an emergency measure (Begemann, 2009). However, if biosecurity is strong there is less need to vaccinate chickens (Conan et al., 2012). In response to the H5N1 outbreak in West Timor in 2004 to 2006 vaccination was not adopted, instead slaughter/stamping out was implemented based on the Government’s control strategic plan and regulation in 2004 (Decision of Directorate General of Livestock No:17/Kpts/PD.640/F/02.04 Guidelines of prevention, control and eradication of zoonotic disease of AI) (Directorate of Animal Health Indonesia, 2012). In addition, no clinical cases have been reported since 2006, hence vaccination was not subsequently adopted as a control measure for the disease. Besides slaughter, the Government implemented movement control of poultry (importation of chickens from infected areas was prohibited with importation of DOC only permitted from high biosecurity level test-negative farms approved by the Government).

Although retrospective studies can be useful in understanding a disease and providing direction for the development of control programs, they are based on historical data which may change with time and may not be representative of the total population or the current situation. Consequently there is a need for up-to-date data collected in a sound epidemiological manner to understand the current situation of infection in West Timor. The results of a serological study in West Timor are outlined in the next chapter (Chapter Five).

4.5 Conclusions

This study established that HPAI H5N1 was present in West Timor between 2004 and 2006. On the basis of available data the disease occurred at a high prevalence in 2004
and then reduced. This study found that poultry from Kota Kupang had the highest prevalence and there was a significant difference in the prevalence between districts. Also, significant differences were found between the bird species tested and between years. The findings of this study can be used as baseline epidemiological data for further research to understand the epidemiology of avian influenza H5N1 in West Timor.
CHAPTER FIVE

Targeted Surveillance to Demonstrate the Freedom of West Timor from HPAI H5N1

5.1 Introduction

The first outbreak of disease caused by H5N1 occurred in humans in Hong Kong in 1997, with six of 18 infected individuals dying (case fatality rate of 33%) (Chan, 2002). Subsequently early in 2004 the virus caused major outbreaks of disease in poultry in Indonesia (Smith et al., 2006) and other outbreaks were also reported in the same year in Vietnam (Hien et al., 2004), Thailand (Grose, 2004; Chotpitayasunondh et al., 2005), Republic of Korea, Japan, Cambodia, and Lao People’s Democratic Republic (WHO, 2005b). The outbreaks were associated with high levels of mortality, up to 100%, in poultry (WHO, 2014a), as well as high levels of mortality in cases affecting humans (more than 50%) (WHO, 2005a). Highly pathogenic avian influenza not only resulted in the restriction of international trade of live birds and poultry meat products but also affected tourism (Rushton et al., 2005) and public health with 167 people dying of 199 reportedly infected in Indonesia (case fatality rate of 83.9%) from 2003 to 2015 (WHO, 2016).

In West Timor, HPAI (H5N1) was reported to have caused the death of a number of chickens between 2004 and 2006 as described in Chapter 4. However, since then no evidence of clinical disease has been reported (unpublished data of Provincial Dinas Peternakan of East Nusa Tenggara and Chapter 4). According to the Terrestrial Animal Health Code, OIE (2015) a country, zone or compartment can be considered free from infection with HPAI viruses in poultry when: it has been shown that infection with HPAI viruses in poultry has not been present in the country, zone or compartment for the preceding 12 months, although its LPAI virus status may be unknown; or based on surveillance, any virus detected has not been identified as HPAI virus; or if infection
has occurred in poultry in a previously free country, zone or compartment, highly pathogenic notifiable avian influenza free status can be regained three months after a stamping-out policy (including disinfection of all affected establishments) is applied, providing that surveillance has been carried out during that three-month period.

Previously either representative surveys of the studied populations or qualitative assessments by a panel of experts of multiple complex sources of evidence were used to provide evidence of freedom from infectious diseases, including HPAI (Martin et al., 2007). However quantitative analysis of the results of structured representative surveys can also be used to demonstrate a zone or country is free from disease (Martin et al., 2007). To support freedom from HPAI, a surveillance programme targeting all susceptible species is required in a country/zone/region. This surveillance programme can be carried out using appropriately designed randomised sampling techniques or by using targeted surveillance of high risk species in specific locations or birds/farms undertaking high-risk practices (OIE, 2009). In order to provide evidence that HPAI H5N1 was absent from West Timor targeted surveillance was undertaken in high-risk areas composing of markets (100 samples) and farms (200 samples). The results of this surveillance are reported and discussed in this chapter.

5.2 Materials and Methods

5.2.1 Study Area

This study was undertaken in two (City of Kupang and Belu which borders Timor Leste) of the five districts in West Timor and involved sampling in a total of five subdistricts, 10 villages and five markets (Figure 5.1). Alak, Maulafa and Kelapa Lima were the subdistricts selected from the City of Kupang, while the subdistricts of Tasifeto Barat and Kota Atambua were selected from the district of Belu. Four villages were selected from the district of Belu (Naitimu, Bakustulama, Tenukiik, and Manumutin), while from the City of Kupang six villages were selected (Alak,
Namosain, Sikumana, Naikolan, Lasiana, and Oesapa) (Figure 5.1). The selection of the districts, subdistricts, villages and markets included in this study was made using a targeted sampling approach, where high-risk locations and live-bird wet markets were specifically targeted for sampling. These districts and locations were classified as high-risk based upon previous reports, and through discussions and findings documented by the Provincial Government Livestock Services of NTT, as outlined in Chapter 3. Five markets (one market in the district of Belu and four markets in the district of Kota Kupang) were also selected. The markets (pasar in Indonesian) selected were Pasar Baru in Belu, and Pasar impres Naikoten, Pasar Oebobo, Pasar Oeba, and Pasar Oesapa in Kota Kupang. These markets were selected because they were the largest markets in these districts and hence were considered to have the highest risk of having infected birds, if infection was present.

Figure 5.1 Map of West Timor showing the location of villages and markets of Kota Kupang and Belu where sample collection was undertaken.
5.2.2 Study Design

The study design was outlined in Chapter Three.

5.2.3 Sampling and Collection of Samples

A total of 300 swabs were collected from poultry (292 chickens and 8 Muscovy ducks) from the 10 villages and 5 markets between August and October 2013. The samples were collected from both village and commercial poultry farms, as well as from markets. Twenty birds were sampled from each of the 10 villages. This number was based on a design prevalence of 20% (if disease is present at least 20% of birds would be expected to be affected). Using a test with a sensitivity of 84.3% and a specificity of 97.7% (Meseko et al., 2010), an estimated poultry population of one thousand and a level of accuracy of 95%, 20 birds were required to be sampled per village. Only 8 (2.67%) of the samples were collected from Muscovy ducks as few were present on the sampling days. Twenty samples were collected from chickens at each of the five live-bird markets ie 100 birds in total. Sterile cotton swabs were inserted and rubbed on the mucosa of the trachea and the same swab then inserted into the cloaca and gentle pressure applied while rotating the swab two or three times on the side of the cloaca (Figure 5.2).
5.2.4 Procedure of the test

An Anigen® (Bionote, Korea) rapid test was used in this study as outlined in Chapter 3. Swabs were collected from the cloaca and trachea in this study as these are reported to have the highest sensitivity for viral detection (Bionote, 2009). Following sampling the swab was placed into a sample collection tube containing assay diluent (Figure 5.3).

Figure 5.3 The collected sample was placed into a sample collection tube containing assay diluent

The swab/diluent combination was mixed thoroughly and the tube left to stand until the large particles had settled to the bottom of the tube (approximately one minute). The test was removed from its foil pouch, and placed on a flat, dry surface. Four to five drops were removed from the swab mixture and added to the sample well. As the test reacted, a purple color developed across the window in the center of the test device. If the migration had not appeared after one minute, another drop of the swab mixture was added to the sample well. The test results were interpreted within 15 minutes of adding the swab/diluent mixture.

5.2.5 Interpretation of the test results

Three possible results could be obtained from the test: negative, positive, or an invalid result. A negative result was indicated by the presence of only one band within the result window (Figure 5.4); a positive result was confirmed by the presence of two bands (“T” and “C”) within the result window (Figure 5.5); and an invalid result (Figure 5.6) was reported if the purple coloured band was not visible within the result window after performing the test (Bionote, Republic of Korea available at http://animal21.en.ec21.com/Anigen_Rapid_AIV_Ag_Test--849022_3061459.html).
Figure 5.4 Negative result obtained during testing of samples in West Timor in 2013

Figure 5.5 Positive result of HPAI Anigen® Rapid H5 AIV antigen test

(Source: Bionote, Republic of Korea available at http://www.bionote.co.kr)
5.2.6 Measuring Confidence Level of Freedom from HPAI

The level of HPAI freedom was calculated using FreeCalc in Survey Toolbox (Cameron, 1999). The calculation required information on the test’s sensitivity (Se), specificity (Sp), minimum expected prevalence (MEP), population size, sample size and the number of positive reactors obtained on testing. The Se and Sp for the Anigen® Rapid H5 AIV antigen test was reported to be 69% (95%CI: 56-80) and 98% (95%CI: 93-99), respectively, under Indonesian field conditions (Loth et al., 2008). As there were no OIE guidelines available for the MEP to demonstrate freedom from AI, a value of 20% was used in this study. This value was chosen based on the understanding that the smaller the MEP, the more samples would be required to be collected in order to have a greater chance of detecting the disease, if it was present (Cannon, 2002; Martin and Cameron, 2002). The population size for this study was described in Chapter 3 (1000). Cannon (2002) stated that data from structured quantitative surveys are used to estimate the probability that the negative results of the survey would be achieved if the disease were present at the design prevalence. If the probability is less than the MEP than the population is considered free from the disease (Martin et al., 2007).

Figure 5.6 Invalid result of HPAI Anigen® Rapid H5 AIV antigen test
(Source: Bionote, Republic of Korea available at http://www.bionote.co.kr)
5.3 Results

5.3.1 Location and Tested Poultry Species

In Table 5.1 the location of the birds sampled and their species are summarized. All samples were negative on testing with the Anigen® Rapid H5 AIV test (0%; 95%CI: 0.0-1.2%).

Table 5.1 Location and species of poultry tested

<table>
<thead>
<tr>
<th>District</th>
<th>Sub-district</th>
<th>Village</th>
<th>Market</th>
<th>Number tested</th>
<th>Total Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>The City of Kupang</td>
<td></td>
<td></td>
<td>Chickens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maulafa</td>
<td>Sikumana</td>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Naikolan</td>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pasar Inpres Naikoten</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Alak</td>
<td>Alak</td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Namosain</td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pasar Oeba</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Kelapa Lima</td>
<td>Oesapa</td>
<td></td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lasiana</td>
<td></td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pasar Oesapa</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Oebobo</td>
<td></td>
<td>Pasar Oebobo</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Belu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tasifeto Barat</td>
<td>Naitimu*</td>
<td></td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bakustulama</td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Kota Atambua</td>
<td>Tenukiik</td>
<td></td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Manumutin</td>
<td></td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Atambua</td>
<td>Pasar Baru</td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>292</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

* One sample in Naitimu gave an invalid result and therefore was retested. On retesting the result was negative.
5.3.2 Calculation of level of Confidence of HPAI H5N1 freedom

Based on the analysis for confidence of freedom of HPAI in West Timor it was concluded that West Timor was free from HPAI at a MEP of 20% with a confidence level of 100%. The probability of detecting 0 positive samples in a sample of 300 poultry from an infected population with a HPAI H5N1 prevalence of 20% was 0.0 (95%CI: 0.0-0.00013). From this, it can be concluded with a high level of confidence (1.0 - 0.0 =1.0, 95%CI: 0.99987, 1.0) that this population is not diseased, and these results are adequate to conclude that West Timor is free from HPAI.

5.4 Discussion

5.4.1 Survey to Prove the Absence of HPAI

Targeted surveillance is defined as a sampling approach where samples (animals or sites) are selected for testing based on the presence of characteristics that indicate a higher probability of disease than the rest of the population (Williams et al., 2009). The term 'targeted' surveillance has been used to describe concepts from an epidemiological perspective, which could constitute risk-based surveillance which is useful to support both strategic and operational decision making (Stärk et al., 2006). The term “targeted surveillance” is becoming more widely adopted, and principally refers to focusing sampling for monitoring and surveillance on high-risk populations with specific risk factors (Salman, 2003). This form of surveillance increases the probability of detecting infection, if present. In this study, the high-risk areas included markets and poultry farms (backyard farms). Backyard farms have been shown to play an important role in the transmission of HPAI H5N1 in Thailand (Tiensin et al., 2005). Markets have also been recognized as important places for the maintenance and exchange of avian viruses, including H5N1 (Kung et al., 2003; Sims et al., 2005), and therefore they play an important role as potential reservoir sites for H5N1 (Woo et al., 2006). A study in Guangzhou, China suggested that food or farmers' markets that have live poultry, play a
role as a source for avian influenza infection in which healthy live birds may carry the virus (Wang et al., 2006). Another study in Hanoi Vietnam in 2001 demonstrated that H5N1 virus could be detected in domestic birds in a live bird market (Nguyen et al., 2005). Markets in West Timor are wet markets where poultry (live commercial, and village and backyard free-range chickens) and other animals, as well as other agricultural products and commercial products, are mixed together. Some of the poultry would return to their owner’s home if they were not sold (see Chapter 6), and could potentially transmit virus from the markets back to the farms (Kung et al., 2003; Sims et al., 2005).

5.4.2 Sensitivity and Specificity of the Test
Commercially available rapid antigen tests for influenza A are quick and simple to perform (WHO, 2007). Meseko et al. (2010) used the rapid antigen test to diagnose HPAI (H5N1) virus in Nigeria and concluded that rapid antigen tests were useful for the prompt diagnosis of HPAI allowing early detection and containment of disease, factors that are critical in disease control. During the outbreak of H5N1 in the Republic of Korea in 2004, the Anigen® H5 Avian Influenza Virus (AIV) Antigen Rapid Test kit was also used with a reported farm level sensitivity of 100%, and 82% in individual specimens compared to virus isolation (Sung et al., 2005). Virus isolation is considered the gold standard test for the diagnosis of AI infection (Animal and Plant Health Inspection Service (APHIS), 2013), however the high sensitivity of the Anigen® Rapid H5 AIV antigen test makes it an ideal test for use in a field situation for testing or screening of flocks (Loth et al., 2008) with subsequent confirmation of positive results with tests such as RT-PCR techniques or viral culture (Chua et al., 2007). Loth et al. (2008) evaluated the characteristics of the Anigen® Rapid H5 AIV test under Indonesian field conditions and reported it was a valuable tool for AI control programs as it was quick and reliable for detecting influenza A virus from swabs.
5.4.3 Measuring the Confidence Level of Disease Freedom

Freedom from a disease is usually defined as the true prevalence being below a specified design prevalence with a certain level of confidence (More et al., 2009). A country can be considered free from a disease based on historical evidence (absence of the disease or infection for a certain period of time) (OIE, 2010) along with the results of surveys (Cannon, 2002). Demonstrating freedom from infection has been aimed at providing sufficient evidence to demonstrate, to an acceptable level of confidence, that infection with a specified pathogen is not present in a population, or if it is present, then it is lower than a specified prevalence (OIE, 2010). However proving a population is free from infection is not possible unless every member of the population is tested simultaneously with an assay with 100% sensitivity. In the current study the MEP was set at 20% to provide strong evidence that the disease under study was not present (Martin and Cameron, 2002) and to increase the chance of detecting disease if it was present through a larger sample size (Cannon, 2002). As the disease is known to affect over 50% of a population in an outbreak (Taubenberger and Morens, 2009), selecting a lower value strengthens even further the confidence that the poultry population sampled is free from AI.

As summarized in Chapter 4 no cases of HPAI H5N1 have been reported in West Timor since 2006. Besides the use of historical data, designed targeted surveillance, as reported in this chapter, was used to support the hypothesis that West Timor is free from HPAI. Targeted surveillance was preferable to a structured survey as it increased the level of confidence of detecting disease, if present, and can detect a lower disease prevalence with the same level of confidence (OIE, 2010). Confidence intervals provide a guide to help with the interpretation of research findings in the light of the effects of chance (Davies and Crombie, 2009). In addition, the confidence that a round of testing would detect disease depends on the prevalence at the time of testing, however as the
prevalence is not known, a design prevalence is used to determine the sample size required to detect disease, with a 95% confidence level of detecting disease if it is present (Cannon, 2002). As no positive results were found in this targeted surveillance conducted in West Timor, a high level of confidence in the region being free from H5N1 can be made.

5.4.4 Maintaining Freedom

5.4.4.1 Surveillance, Monitoring and Control Program

Surveillance is described as an essential activity for effective HPAI H5N1 control, and has been suggested to be the most effective measure to control the disease in Thailand (Buranathai et al., 2007). In addition, it is important to undertake routine surveillance as it can increase the likelihood of detecting H5N1 virus (Brooks et al., 2009). Surveillance of wild birds can play an important role in the recognition of and preparation for threats, including HPAI H5N1 outbreaks, and can be used as an early warning system to assess the risk posed by AIV, including H5N1 (Munster et al., 2006). It has been suggested that domestic animals and humans have been infected by AIV from wild birds as these birds serve as reservoirs for the viruses (Boyce et al., 2009). Although HPAI viruses are rarely isolated from wild birds (Alexander, 2000; Curran, 2012), they can play a role in the long distance spread of the viruses around the globe (Boyce et al., 2009; FAO, 2015). For instance, the spill-over of HPAI H5N1 viruses from poultry to migratory and domestic birds in Mongolia, Kazakhstan, Russia, Turkey, Romania and Croatia has increased the risk for the dispersion of virus to an even larger geographical area (Munster et al., 2006).

In West Timor, even though an outbreak of H5N1 occurred in 2004, surveillance of H5N1 was not implemented until 2005 (Personal communication Drh. Heni Lero Kaka, Staff of Provincial Dinas Peternakan of East Nusa Tenggara, and Drh. Cahyo Sunarno, Coordinator of the Participatory Disease Surveillance and Response (PDSR) program of
Nusa Tenggara Timur). This surveillance was performed through the PDSR program, which was designed to strengthen veterinary services and empower communities in order to prevent and control HPAI through participatory disease surveillance (PDS) and participatory disease response teams aimed primarily at the sector 4 poultry industry (household level) (Azhar et al., 2010b). However to date there has been no surveillance undertaken in wild birds and future surveillance activities should include samples from such birds.

The use of vaccines for the control of HPAI is not recommended in non-infected areas; however the use of vaccination to assist in the control of an avian influenza outbreak is recommended in a control area if there is evidence of it spreading from infected farm(s) (Ellis et al., 2004b). Vaccination against HPAI H5N1 in the People's Republic of China, Egypt, Indonesia and Vietnam succeeded in reducing the morbidity and mortality in chickens, thus helping maintain the livelihood of rural communities and preserve food security (Swayne et al., 2011). The government of Indonesia recommended the use of vaccination for the eradication of the disease in June 2004 in Java, Sumatera, Bali and South Sulawesi, with all poultry in sector 4 in priority areas being vaccinated free of charge under the government program. Farms in sector 3 up to a maximum size of 5000 birds were also vaccinated free of charge (Siregar et al., 2007). In contrast to other areas of Indonesia, the eradication of H5N1 in West Timor was reached by the implementation of stamping-out (slaughter) on infected farms (Dinas Peternakan of NTT, 2005). The use of vaccines for the control of HPAI H5N1 in West Timor is not recommended as the island is considered free (Ellis et al., 2004b); however some commercial breeding farms have reportedly vaccinated their chickens (Personal communication with Drh. Heni D. Dewa, Staff of Provincial Dinas Peternakan of East Nusa Tenggara). This practice of using vaccines in West Timor needs to be evaluated as vaccinated chickens cannot be differentiated from infected ones and hence makes the
confirmation of diagnosis challenging (OIE, 2006), unless DIVA (Differentiating Infected from Vaccinated Animals) vaccines are used (Capua et al., 2003a). However the use of DIVA vaccines has not been widely adopted, with most vaccines used in a range of countries being inactivated whole AI vaccines or recombinant H5-AI vaccines (Marangon et al., 2008). In Indonesia the potential use of DIVA vaccines was investigated by Bouma et al. (2008) and they were proposed to be a valuable adjunct to a surveillance program, although to date they have not been widely used or adopted. Another study conducted by Lambrecht et al. (2007) found that a DIVA based on heterologous neuraminidase Ectodomain protein M2 Influenza virus (M2e), which uses antibody against M2e as an infection marker, has a potential benefit for differentiating vaccinated chickens from naturally infected ones. This vaccine was claimed to be of potential use for Indonesia because the marker is conserved across all AIV and a high proportion of infected birds seroconverted, however to date it is not available for field use in Indonesia (Tarigan, 2015). Furthermore DIVA vaccines are likely to be more expensive than the standard vaccines currently used in Indonesia.

Adopting vaccination in a control campaign requires established vaccine banks to ensure appropriate vaccines are used, along with the use of sensitive diagnostic tests (Capua and Marangon, 2003). Vaccination is also useful in limiting secondary spread and possibly preventing the introduction of AIV into susceptible populations (Capua and Marangon, 2007b), as was undertaken in Europe from 2000 to 2006 (Capua et al., 2009).

Good management, including monitoring, improved biosecurity and movement restriction, is essential for a control program (Capua and Alexander, 2008). It is important that routine husbandry and biosecurity practices are evaluated to determine the risk of disease introduction into a population (Pritchard et al., 2005; Costard et al., 2009). However in West Timor, little is known about the routine husbandry and
biosecurity measures adopted by poultry owning households. In order to address this
deficiency, a study on routine husbandry and biosecurity practices adopted by poultry
owning households in West Timor was undertaken and the results of this study are
outlined in the following chapter.

5.5 Conclusions
This study has demonstrated that it is unlikely HPAI H5N1 was present in West Timor
at the time of the study. Active surveillance should be implemented to ensure that West
Timor maintains this disease-free status, including targeted sampling of wild birds.
CHAPTER SIX

Descriptive Analysis of Husbandry and Biosecurity Practices Adopted on Poultry Farms in West Timor

6.1 Introduction

In this chapter the routine husbandry and biosecurity practices adopted by poultry owners in West Timor, Indonesia are outlined. This information is important as it has been shown elsewhere that the probability of disease introduction and spread is determined by a complex combination of factors which include: the number and density of animals; the type of species or breeds present; the number and type of contacts between flocks; and the sanitary measures adopted (Van Steenwinkel et al., 2011).

Contagious diseases, such as HPAI, can affect animal production in three main ways through: direct losses to producers and others involved in the production and marketing of poultry as a result of morbidity and mortality; government intervention of notifiable diseases, which carries a cost borne by both the general public and the producers; and public health risks which could result in reduced demand for the relevant products or disease in the human population (Otte et al., 2008).

The risk of disease to both animals and humans has increased with changes in management and housing arising from increased animal intensification (Fasina et al., 2012). Similarly the diversity within poultry systems (small and large scale farms) affects the potential risk of the introduction and spread of infectious diseases within the industry (Van Steenwinkel et al., 2011). Implementation of appropriate biosecurity practices is recommended to reduce disease risks and to minimise the likelihood of introducing new diseases or pathogens into poultry flocks (Fasina et al., 2012; Van Steenwinkel et al., 2011; Boklund et al., 2004). Biosecurity encompasses those measures which should be adopted to prevent viruses, bacteria, fungi, protozoa,
parasites, insects, rodents and wild birds from entering or surviving and infecting or endangering the well-being of the poultry flock (Tablante et al., 2009; FAO, 2008). Additionally, biosecurity measures are important to protect both herds and a region or nation from the introduction of exotic diseases or exotic strains of pathogens (Van Steenwinkel et al., 2011). Improving the biosecurity of poultry production is a key component of reducing the incidence of many diseases, including AI (Negro-Calduch et al., 2013).

The study reported in this chapter was designed to describe the routine husbandry and biosecurity practices adopted by poultry owning households in West Timor, Indonesia in relation to the risk of the introduction and transmission of HPAI H5N1.

6.2 Materials and Methods

6.2.1 Study area and design and data collection methods
The location of the sites where data were collected, the study design (study type, sampling method, questionnaire design and questionnaire pilot) and methods adopted to collect data (questionnaire administration, recruitment of interviewees) have been outlined in Chapter Three.

6.2.2 Data analysis
Questionnaire data were entered into a spreadsheet in Microsoft Excel 2011 for Mac and exported into a statistical package (IBM SPSS Statistics version 21) for subsequent analysis. Frequency functions were calculated for all variables measured.

6.3 Results

6.3.1 Characteristics of poultry-owning households
The highest level of education attained by participants varied with 13% having attended university level and only 8% never having received any formal schooling (Table 6.1). All the surveyed households raised poultry (chickens and/or ducks) (a criterion for
selection) with three households also owning pet birds. The majority (60%) of the households surveyed kept chickens for their own household use, although 37% kept them for generating income with only 1% keeping them as pets. All the households surveyed occasionally slaughtered chickens for their own household’s consumption, compared with only one farmer (of 10 who owned ducks) occasionally slaughtering ducks for home consumption.

Table 6.1 The Background of the Respondents

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of households</th>
<th>Percentage (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil servant</td>
<td>18</td>
<td>18 (11.0, 26.9)</td>
</tr>
<tr>
<td>Entrepreneur</td>
<td>19</td>
<td>19 (11.8, 28.1)</td>
</tr>
<tr>
<td>Butcher</td>
<td>1</td>
<td>1 (0, 5.4)</td>
</tr>
<tr>
<td>Retired</td>
<td>7</td>
<td>7 (2.9, 13.9)</td>
</tr>
<tr>
<td>Driver/Laborer</td>
<td>13</td>
<td>13 (7.1, 21.2)</td>
</tr>
<tr>
<td>Housewife</td>
<td>24</td>
<td>24 (16.0, 33.6)</td>
</tr>
<tr>
<td>Farmer</td>
<td>17</td>
<td>17 (10.2, 25.8)</td>
</tr>
<tr>
<td>Nuns/monks</td>
<td>1</td>
<td>1 (0, 5.4)</td>
</tr>
<tr>
<td><strong>Highest educational level achieved</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never attended school</td>
<td>8</td>
<td>8 (3.5, 15.2)</td>
</tr>
<tr>
<td>Primary School</td>
<td>30</td>
<td>30 (21.2, 40.0)</td>
</tr>
<tr>
<td>Junior High School</td>
<td>21</td>
<td>21 (13.5, 30.3)</td>
</tr>
<tr>
<td>Senior High School</td>
<td>28</td>
<td>28 (19.5, 37.9)</td>
</tr>
<tr>
<td>University</td>
<td>13</td>
<td>13 (7.1, 21.2)</td>
</tr>
<tr>
<td><strong>Primary reason for keeping poultry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For own consumption</td>
<td>60</td>
<td>60 (49.7, 69.7)</td>
</tr>
<tr>
<td>For generating income</td>
<td>37</td>
<td>37 (27.6, 47.2)</td>
</tr>
<tr>
<td>For fighting cocks</td>
<td>2</td>
<td>2 (0.2, 7.0)</td>
</tr>
<tr>
<td>As pets</td>
<td>1</td>
<td>1 (0.0, 5.4)</td>
</tr>
</tbody>
</table>

6.3.2 Ownership of chickens and other animals

Sixty-eight (68%, 95%CI: 56, 79) of the households kept hens (village chickens) with an average of 4 owned (range 1 to 20) (Table 6.2). Approximately two thirds of the interviewed households owned cocks (65%, 95%CI: 53, 76) with an average of 3.5 owned (range 1-16). Approximately half of the surveyed households (52%) owned village hens for laying eggs with an average of 1.8 owned (range 1 to 11). Only 18
households owned broilers, however an average of 549 was owned (range 1 to 5000) (Table 6.2).

Few households (n = 10) kept ducks. Similarly few households (3%, 95%CI: 0.6, 8.5) kept pet birds (including song birds). Besides poultry, some households also kept other species of animals with 26 households owning dogs (average 2.15, range 1 to 6), 27 households having pigs (average 3.22, range 1 to 13), 14 owning cattle (average 4.29, range 1 to 12), 8 owning goats (average 3.63, range 1 to 9) and one household kept a horse (Table 6.2).

Table 6.2 Ownership of Poultry and Other Animals

<table>
<thead>
<tr>
<th>Poultry species</th>
<th>% of farmers</th>
<th>Average number</th>
<th>Range (Minimum-Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village chickens (VC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>43</td>
<td>12.2</td>
<td>1-100</td>
</tr>
<tr>
<td>Cockerels</td>
<td>16</td>
<td>4.3</td>
<td>1-20</td>
</tr>
<tr>
<td>Pullets</td>
<td>27</td>
<td>6.7</td>
<td>1-20</td>
</tr>
<tr>
<td>Cocks</td>
<td>65</td>
<td>3.5</td>
<td>1-16</td>
</tr>
<tr>
<td>Hens</td>
<td>68</td>
<td>3.9</td>
<td>1-20</td>
</tr>
<tr>
<td>Hens for laying eggs</td>
<td>52</td>
<td>1.8</td>
<td>1-11</td>
</tr>
<tr>
<td><strong>Total # of households owning VC</strong></td>
<td><strong>72</strong></td>
<td><strong>5.4</strong></td>
<td></td>
</tr>
<tr>
<td>Broilers</td>
<td>18</td>
<td>549.2</td>
<td>1-5000</td>
</tr>
<tr>
<td>Eggs and Survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of eggs laid in the last 12 months</td>
<td>50</td>
<td>31.0</td>
<td>5-360</td>
</tr>
<tr>
<td>Number of eggs hatched in the last 12 months</td>
<td>47</td>
<td>21.0</td>
<td>5-68</td>
</tr>
<tr>
<td>Number of chickens that survived in the last 12 months</td>
<td>46</td>
<td>9.1</td>
<td>0-30</td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of eggs laid in the last 12 months</td>
<td>6</td>
<td>12</td>
<td>4-57</td>
</tr>
<tr>
<td>Number of eggs hatched in the last 12 months</td>
<td>6</td>
<td>12</td>
<td>4-27</td>
</tr>
<tr>
<td>Number of ducklings surviving in the last 12 months</td>
<td>6</td>
<td>12</td>
<td>4-20</td>
</tr>
</tbody>
</table>
### Poultry species

<table>
<thead>
<tr>
<th>Poultry species</th>
<th>% of farmers</th>
<th>Average number</th>
<th>Range (Minimum-Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>3</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>≥3-12 months</td>
<td>1</td>
<td>3.0</td>
<td>2-5</td>
</tr>
<tr>
<td>&gt;12 months</td>
<td>10</td>
<td>4.7</td>
<td>4-5</td>
</tr>
<tr>
<td><strong>Total households owning ducks</strong></td>
<td><strong>10</strong></td>
<td><strong>3.2</strong></td>
<td></td>
</tr>
<tr>
<td>Pet birds owned</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.0</td>
<td>1-4</td>
</tr>
<tr>
<td>Other animals owned</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>26</td>
<td>2.2</td>
<td>1-6</td>
</tr>
<tr>
<td>Pigs</td>
<td>27</td>
<td>3.2</td>
<td>1-13</td>
</tr>
<tr>
<td>Cattle</td>
<td>14</td>
<td>4.3</td>
<td>1-12</td>
</tr>
<tr>
<td>Horses</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Goats</td>
<td>8</td>
<td>3.6</td>
<td>1-9</td>
</tr>
<tr>
<td><strong>Total households owning other animals</strong></td>
<td><strong>50</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6.3.3 Poultry Husbandry Practices

#### 6.3.3.1 Housing

Most households in this survey (65/72 - 90.3%, 95%CI: 81, 96) allowed their village chickens to range freely in the daytime but the birds were caged at night, four households caged their chickens all the time, two households let their chickens roam freely at all times and one household tied up their chickens at or near their house (Table 6.3). Of the 10 households who owned ducks only six (60%, 95%CI: 26.2, 87.8) allowed them to range freely during the daytime although they were caged at night, while four (40%, 95%CI: 12.2, 73.8) kept their ducks in cages all the time. Just over half of the households who kept broilers (55.6%, 95%CI: 30.8, 78.5) kept their chickens in cages, with eight (44.4%, 95%CI: 21.5, 69.2) allowing them to range freely during the daytime while caging them at night (Table 6.3).
Table 6.3 Poultry Husbandry Activities in the Surveyed Households

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of households</th>
<th>Percentage (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where do you keep your village chickens?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free range all the time</td>
<td>2</td>
<td>2.8 (0.3, 9.7)</td>
</tr>
<tr>
<td>Caged all the time</td>
<td>4</td>
<td>5.6 (1.5, 13.6)</td>
</tr>
<tr>
<td>Free range in day but caged at night</td>
<td>65</td>
<td>90.3 (81.0, 96.0)</td>
</tr>
<tr>
<td>Tied up</td>
<td>1</td>
<td>1.4 (0.0, 7.5)</td>
</tr>
<tr>
<td>Where do you keep your broiler chickens?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caged</td>
<td>10</td>
<td>55.6 (30.8, 78.5)</td>
</tr>
<tr>
<td>Free range in day but caged at night</td>
<td>8</td>
<td>44.4 (21.5, 69.2)</td>
</tr>
<tr>
<td>Where do you keep your ducks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caged all the time</td>
<td>4</td>
<td>40 (12.2, 73.8)</td>
</tr>
<tr>
<td>Free range in day but caged at night</td>
<td>6</td>
<td>60 (26.2, 87.8)</td>
</tr>
<tr>
<td>Do you graze ducks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0 (0.0, 30.8)</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>100 (69.2, 100)</td>
</tr>
</tbody>
</table>

6.3.3.2 Feed and drinking water

Approximately two thirds of the households (68%, 95%CI: 61.1, 74.4) fed their chickens a ration of mixed feed (commercial feed, kitchen leftovers and agricultural feed such as corn, cassava root, nuts or tofu waste). Approximately a quarter of the households (23.5%, 95%CI: 17.8, 30.0) let their poultry find their own feed, while only 8.5% (95%CI: 5.0, 13.3) of the households fed their chickens solely on commercial poultry feed. The sources of drinking water for poultry in the surveyed households was from reticulated tap water (66%, 95%CI: 55.8, 75.2), private wells (24%, 95%CI: 16.0, 33.6) or from community wells or collected rain water (7%, 95%CI: 2.9, 13.9 and 3%, 95%CI: 0.6, 8.5, respectively).

6.3.3.3 Presence of Wild Birds

Two-thirds (66%, 95%CI: 55.8, 75.2) of the households had observed wild birds close to their poultry in the 12 months preceding the survey. The majority of these (98.5%, 95%CI: 91.8, 100) reported the presence of wild house sparrows (Passer domesticus) and only one household observed another species, the scaly-breasted munia (Lonchura punctulata), near their poultry. Over half (63.6%, 95%CI: 50.9, 75.1) of the households
that observed wild birds near their poultry stated that these birds were seen throughout the year, although they were more commonly observed in the months of January and March. Most (60/66 - 90.9%, 95%CI: 81.3, 96.6) of the households who saw wild birds stated they see more than 10 wild birds at a time. All owners who had observed wild birds reported that these wild birds came close to, or mixed with, their chickens, although none of the wild birds had been observed to show any unusual behaviors or clinical signs of disease.

6.3.4 Poultry Trading

Nineteen (76%, 95%CI: 54.9, 90.6) of the 25 households who sold village chickens sold them at the age of 3 months or older with an average of 23.9 (range from 2 to 88) chickens sold in a 12 month period. Six (24%, 95%CI: 9.4, 45.1) households sold village chickens less than 3 months of age with an average of 10 sold. In contrast those farmers who sold broilers (18 farmers) sold more birds than farmers who didn’t own broilers, with an average of 9343 birds sold in a year (range 30 - 55000). The average price of the village chickens sold was IDR 75,000 (range 50,000 to 150,000) compared to IDR 29,562 for broilers (range 25,000 to 35,000).

In Table 6.4 the activities undertaken by the participants with respect to their poultry is summarized. Most households (60% 95%CI: 49.7, 69.7) never sold chickens as they were kept for their own consumption. Twenty one percent of all households sold chickens when in need of funds, and only 4% of households sold chickens every day. Only one household provided chickens for offerings at specific events and a few (13%, 95%CI: 7.1, 21.2) gave birds away to other people. If chickens were sold, half of the households sold them from their home, while 15% sold them at permanent markets and a similar proportion sold them to poultry traders. Twenty percent of households that sold chickens sold them to other households in their own or other villages. Two (33.3%, 95%CI: 4.3, 77.7) of the households who sold chickens to permanent markets took the
birds to the market themselves. If any birds remained unsold at the end of the market day then most households (95%, 95%CI: 75.1, 99.9) in this situation would bring those birds back to their home. Birds were sold by households for three major events: graduation ceremonies (51.9% of households selling birds), religious ceremonies (such as Christmas and Easter) (44.4%) and traditional ceremonies (such as funerals, crop harvesting) (3.7%). Seventy percent of the markets where birds were sold were over two kilometers from the seller’s homes. Nearly half (47.5%, 95%CI: 31.5, 63.9) of the households who sold poultry had a family member working at the markets, while a similar percentage (42.5%) had a friend or friends working in the markets.

Most households (98.6%) who purchased village chickens (n = 72) purchased these from markets (Table 6.4). Most of these households (88.3%, 95%CI: 80.0, 94.0) purchased new chickens on an as-needs basis with only 11.7% (95%CI: 6.0, 20.0) regularly buying new chickens each month. Approximately three quarters (77.8%, 95%CI: 52.4, 93.6) of the households purchased day old chickens (Table 6.4). Four owners of ducks (40%, 95%CI: 12.2, 73.8) purchased ducks (Table 6.4). Most households (88.9% 95%CI: 65.3, 98.6) with broilers (n = 18) purchased new broilers as DOC from sellers’ houses outside their village with the remainder purchasing them from markets from another village.

When the surveyed households were asked how they made sure that the chickens they purchased were free from HPAI, 33% (95%CI: 23.6, 43.4) responded that they were not concerned about the disease but were only interested in the price of the birds. In contrast, 26.6% (95%CI: 18.0, 36.7) bought chickens from “safe places”, 22.3% (95%CI: 14.4, 32.1) purchased birds only from sellers who they knew and trusted, and only 18.1% (95%CI: 10.9, 27.4) examined the chickens to confirm that they were apparently clinically healthy before they purchased them (Table 6.4). Only 15.9% (95%CI: 9.2, 25) of households always separated newly purchased chickens from their
existing birds with 2.1% (95%CI: 0.3, 7.5) of households sometimes separating newly acquired chickens from their existing birds (Table 6.4).

Table 6.4 Poultry Trading (Selling and Purchasing) Activities Conducted by the Surveyed Households

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of households</th>
<th>Percentage (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How often do you sell poultry?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>60</td>
<td>60 (49.7, 69.7)</td>
</tr>
<tr>
<td>Daily</td>
<td>4</td>
<td>4 (1.1, 9.9)</td>
</tr>
<tr>
<td>Weekly</td>
<td>5</td>
<td>5 (1.6, 11.3)</td>
</tr>
<tr>
<td>Monthly</td>
<td>10</td>
<td>10 (4.9, 17.6)</td>
</tr>
<tr>
<td>When we are in need of cash</td>
<td>21</td>
<td>21 (13.5, 30.3)</td>
</tr>
<tr>
<td>How often do you offer poultry?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>99</td>
<td>99 (94.6, 100)</td>
</tr>
<tr>
<td>Religious, traditional and/or graduation ceremonies</td>
<td>1</td>
<td>1 (0.0, 5.4)</td>
</tr>
<tr>
<td>How often do you give away poultry?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>86</td>
<td>86 (77.6, 92.1)</td>
</tr>
<tr>
<td>Religious, traditional and/or graduation ceremonies</td>
<td>1</td>
<td>1 (0.0, 5.4)</td>
</tr>
<tr>
<td>When someone is in need</td>
<td>13</td>
<td>13 (7.1, 21.2)</td>
</tr>
<tr>
<td>Where (who) do you sell poultry (to)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent market</td>
<td>6</td>
<td>15 (5.7, 29.8)</td>
</tr>
<tr>
<td>Poultry trader</td>
<td>6</td>
<td>15 (5.7, 29.8)</td>
</tr>
<tr>
<td>Household in the same village</td>
<td>3</td>
<td>7.5 (1.6, 20.4)</td>
</tr>
<tr>
<td>Household in other villages</td>
<td>5</td>
<td>12.5 (4.2, 26.8)</td>
</tr>
<tr>
<td>At home (someone comes and picks them up)</td>
<td>20</td>
<td>50 (33.8, 66.2)</td>
</tr>
<tr>
<td>Who takes the poultry to the permanent market?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myself</td>
<td>2</td>
<td>33.3 (4.3, 77.7)</td>
</tr>
<tr>
<td>My partner/spouse</td>
<td>1</td>
<td>16.7 (0.4, 64.1)</td>
</tr>
<tr>
<td>My neighbour</td>
<td>1</td>
<td>16.7 (0.4, 64.1)</td>
</tr>
<tr>
<td>My relative</td>
<td>2</td>
<td>33.3 (4.3, 77.7)</td>
</tr>
<tr>
<td>Do you bring any unsold poultry back to your home?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>5.0 (0.1, 24.9)</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>95.0 (75.1, 99.9)</td>
</tr>
<tr>
<td>How old are the poultry you usually sell?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day old chickens</td>
<td>5</td>
<td>12.5 (4.2, 26.8)</td>
</tr>
<tr>
<td>1-3 months</td>
<td>25</td>
<td>62.5 (45.8, 77.3)</td>
</tr>
<tr>
<td>&gt;3-12 months</td>
<td>10</td>
<td>25.0 (12.7, 41.2)</td>
</tr>
<tr>
<td>How old are the poultry you usually give away?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 months</td>
<td>1</td>
<td>7.1 (0.2, 33.9)</td>
</tr>
<tr>
<td>&gt;3 months</td>
<td>13</td>
<td>92.9 (66.1, 99.8)</td>
</tr>
<tr>
<td>What major events do you usually sell poultry for?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Religious ceremony</td>
<td>25</td>
<td>62.5 (45.8, 77.3)</td>
</tr>
<tr>
<td>Graduation ceremony</td>
<td>14</td>
<td>35 (20.6, 51.7)</td>
</tr>
<tr>
<td>Traditional ceremony</td>
<td>1</td>
<td>2.5 (0.1, 13.2)</td>
</tr>
<tr>
<td>How far is it to the closest poultry market to your home?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 Km</td>
<td>3</td>
<td>7.5 (1.6, 20.4)</td>
</tr>
<tr>
<td>≥1-2 Km</td>
<td>7</td>
<td>17.5 (7.3, 32.8)</td>
</tr>
<tr>
<td>&gt;2 Km</td>
<td>30</td>
<td>75.0 (58.8, 87.3)</td>
</tr>
<tr>
<td>Do you have a family member, a neighbour or a relative working in a market?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, family member(s)</td>
<td>1</td>
<td>2.5 (0.1, 13.2)</td>
</tr>
<tr>
<td>Yes, neighbour(s)</td>
<td>3</td>
<td>7.5 (1.6, 20.4)</td>
</tr>
<tr>
<td>Yes, relative(s) who visits me</td>
<td>19</td>
<td>47.5 (31.5, 63.9)</td>
</tr>
<tr>
<td>Yes, friend(s) who visits me</td>
<td>17</td>
<td>42.5 (27.0, 59.1)</td>
</tr>
<tr>
<td>Variables</td>
<td>Number of households</td>
<td>Percentage (95% CI)</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>----------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>How often do you buy new poultry (chickens or ducks)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least once a month</td>
<td>11</td>
<td>11.7 (6.0, 20.0)</td>
</tr>
<tr>
<td>When required (once in 6 months)</td>
<td>83</td>
<td>88.3 (80.0, 94.0)</td>
</tr>
<tr>
<td>Where do you usually buy new village chickens?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Market in another village</td>
<td>71</td>
<td>98.6 (92.5, 100)</td>
</tr>
<tr>
<td>House in another village</td>
<td>1</td>
<td>1.4 (0.0, 7.5)</td>
</tr>
<tr>
<td>Where do you usually buy/acquire new ducks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed myself</td>
<td>6</td>
<td>60 (26.2, 87.8)</td>
</tr>
<tr>
<td>House in another village</td>
<td>4</td>
<td>40 (12.2, 73.8)</td>
</tr>
<tr>
<td>Where do you usually buy new broilers?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Market in another village</td>
<td>2</td>
<td>11.1 (1.4, 34.7)</td>
</tr>
<tr>
<td>House in another village</td>
<td>16</td>
<td>88.9 (65.3, 98.6)</td>
</tr>
<tr>
<td>How old are the village chickens you buy? (months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>15.3 (7.9, 25.7)</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>23.6 (14.4, 35.1)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.4 (0.0, 7.5)</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>59.7 (47.5, 71.1)</td>
</tr>
<tr>
<td>How old are the ducks you buy? (months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>50 (6.8, 93.2)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>50 (6.8, 93.2)</td>
</tr>
<tr>
<td>How old are the new chickens (village and broilers) you buy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>70</td>
<td>77.8 (67.8, 85.9)</td>
</tr>
<tr>
<td>≥1 month</td>
<td>20</td>
<td>22.2 (14.1, 32.2)</td>
</tr>
<tr>
<td>How do you make sure that the poultry you buy are free from AI?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I know the seller and trust them</td>
<td>21</td>
<td>22.3 (14.4, 32.1)</td>
</tr>
<tr>
<td>I check the birds are healthy</td>
<td>17</td>
<td>18.1 (10.9, 27.4)</td>
</tr>
<tr>
<td>I buy from “safe places”</td>
<td>25</td>
<td>26.6 (18.0, 36.7)</td>
</tr>
<tr>
<td>My concerns are only the price not disease</td>
<td>31</td>
<td>33.0 (23.6, 43.4)</td>
</tr>
<tr>
<td>Are newly purchased poultry separated from existing ones?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never separated</td>
<td>61</td>
<td>64.9 (54.4, 74.5)</td>
</tr>
<tr>
<td>Rarely separated</td>
<td>16</td>
<td>17.0 (10.1, 26.2)</td>
</tr>
<tr>
<td>Sometimes separated</td>
<td>2</td>
<td>2.1 (0.3, 7.5)</td>
</tr>
<tr>
<td>Always separated</td>
<td>15</td>
<td>15.9 (9.2, 25.0)</td>
</tr>
</tbody>
</table>

### 6.3.5 Poultry Health and Disease Status, Risk factors, Prevention and Disease Awareness.

Most (59%, 95%CI: 48.7, 68.7) people interviewed stated that they had experienced some disease in their poultry in the 12 month period immediately preceding the survey (Table 6.5). Of those households who had experienced disease in their poultry, 39% (95%CI: 15.2, 32.5) reported disease in chickens between the age of 0 and 3 months of age with an average of 29.7 birds affected (range 1 to 170, median of 13) and 22% of households reported disease in pullets (4 to 5 month-old female chickens) with an average number of 16.1 affected (range 1 to 78, median of 10) (Table 6.6).

Of the 59 households that reported disease in poultry, 47.5% said that the disease affecting their birds was Newcastle disease (ND) (Table 6.5). Just over half (58.5%) of
these farmers recognized ND in their poultry based on the presenting clinical signs. The remainder presumed it was ND as they had heard of the disease from friends or neighbours. Approximately a quarter of the households (27%) reported that they had poultry die suddenly in the two years preceding the survey (Table 6.5). Most households (77.8%) with deaths had observed blue combs in dead birds; however no households observed dead birds with blue wattles. Approximately one-fifth (22.2%) of the 27 households who had dead poultry reported seeing birds trembling, 48.2% reported observing birds with difficulty breathing and 18.5% had birds with diarrhoea. Only one household with sick poultry in the two years preceding the survey reported the presence of their sick birds to the Local Dinas Peternakan. Most households slaughtered birds that survived a disease episode for their own consumption (66.7%, 95%CI: 46.0, 83.5), while 25.9% (95%CI; 11.1, 46.3) sold these surviving poultry with the remainder (7.4%, 95%CI: 0.9, 24.3) retaining the poultry in their flock (Table 6.5). More households reported sick poultry in the month of July, although some illness was observed between March and December. All the surveyed farmers stated that they did not vaccinate their chickens against ND because vaccines were not available. In relation to disease risks, the majority (94%) of surveyed households believed that their neighbour’s poultry posed the biggest risk to their own poultry becoming sick (Table 6.5). Only 6% of households considered that newly introduced poultry posed a risk of disease introduction to their flock. The majority of households considered that reducing the contact between their own poultry and those owned by others would prevent their flock from becoming infected. With respect to disease awareness, all interviewed households (100%) had heard of avian influenza or flu burung in Indonesian (bird flu) with the source of this information being the television or radio (Table 6.5). Nearly all (98%) households reported that they would bury dead chickens if they suspected the birds had AI.
Table 6.5 Poultry Health and Disease Status, Risk Factors and Disease Awareness

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of households</th>
<th>Percentage of households with disease status (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did any poultry get sick in the last 5 years?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>41 (31.3, 51.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>59</td>
<td>59 (48.7, 68.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What was the name of the disease?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newcastle disease</td>
<td>28</td>
<td>47.5 (34.3, 60.9)</td>
</tr>
<tr>
<td>Other disease</td>
<td>13</td>
<td>22 (12.3, 34.7)</td>
</tr>
<tr>
<td>Not sure</td>
<td>18</td>
<td>30.5 (19.2, 43.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If ND or another disease, how did you know it was this disease?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I knew from the signs</td>
<td>24</td>
<td>58.5 (42.1, 73.7)</td>
</tr>
<tr>
<td>It was a guess</td>
<td>17</td>
<td>41.5 (26.3, 57.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have any poultry died suddenly in the last two years?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27</td>
<td>27 (18.6, 36.8)</td>
</tr>
<tr>
<td>No</td>
<td>73</td>
<td>73 (63.2, 81.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have any of the dead poultry displayed blue combs in the last two years?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>77.8 (57.7, 91.4)</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>22.2 (8.6, 42.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have any poultry that died had blue wattles during the last few years?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0.0 (0.0, 12.8)</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>100.0 (87.2, 100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did any of the poultry that died tremble prior to death?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>22.2 (8.6, 42.3)</td>
</tr>
<tr>
<td>No</td>
<td>21</td>
<td>77.8 (57.7, 91.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did any of the poultry that died have difficulty breathing prior to death?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>48.2 (28.7, 68.1)</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>51.8 (31.9, 71.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did any of the poultry that died have diarrhoea prior to death?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>18.5 (6.3, 38.1)</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>81.5 (61.9, 93.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What did you do with the surviving poultry when some poultry died?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kept them in the flock</td>
<td>2</td>
<td>7.4 (0.9, 24.3)</td>
</tr>
<tr>
<td>Sold them</td>
<td>7</td>
<td>25.9 (11.1, 46.3)</td>
</tr>
<tr>
<td>Slaughtered for own consumption</td>
<td>18</td>
<td>66.7 (46.0, 83.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you report sick or dead poultry to Dinas Peternakan?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>98.3 (90.9, 100)</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1.7 (0.0, 9.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What factors do you think increases the risk of poultry becoming sick?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly introduced poultry</td>
<td>6</td>
<td>6 (2.2, 12.6)</td>
</tr>
<tr>
<td>Neighbour’s poultry</td>
<td>94</td>
<td>94 (87.4, 97.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What do you see as necessary to prevent or control poultry disease?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccines more readily available</td>
<td>7</td>
<td>7 (2.9, 13.9)</td>
</tr>
<tr>
<td>Variables</td>
<td>Number of households</td>
<td>Percentage of households with disease status (95% CI)</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>----------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Reduce contact between my birds and those of other households</td>
<td>78</td>
<td>78 (68.6, 85.7)</td>
</tr>
<tr>
<td>Regular visit from the Veterinary Department</td>
<td>15</td>
<td>15 (8.6, 23.5)</td>
</tr>
<tr>
<td>Have you ever heard of AI?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>100</td>
<td>100 (96.4,100)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>0.0 (0.0, 3.6)</td>
</tr>
<tr>
<td>Where did you get information about AI?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radio and TV</td>
<td>100</td>
<td>100 (96.4,100)</td>
</tr>
<tr>
<td>What would you do if you suspected your poultry had AI?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bury dead birds</td>
<td>98</td>
<td>98.0 (93.0, 99.8)</td>
</tr>
<tr>
<td>Burn</td>
<td>1</td>
<td>1.0 (0.0, 5.4)</td>
</tr>
<tr>
<td>Report to the Dinas Peternakan</td>
<td>1</td>
<td>1.0 (0.0, 5.4)</td>
</tr>
</tbody>
</table>

Table 6.6 Occurrence of Clinical Disease Reported by Households in Their Poultry in the last 12 months

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of Farmers</th>
<th>Percent of farmers (95%CI)</th>
<th>Average number of birds affected (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmers with sick poultry during the preceding year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village Chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 months</td>
<td>23/59</td>
<td>39.0 (26.5, 52.6)</td>
<td>29.7 (1-170)</td>
</tr>
<tr>
<td>Pullets</td>
<td>6/27</td>
<td>22.2 (8.6, 42.3)</td>
<td>16.1 (1-78)</td>
</tr>
<tr>
<td>Cocks</td>
<td>4/65</td>
<td>6.2 (1.7, 15.0)</td>
<td>10 (0-40)</td>
</tr>
<tr>
<td>Hens</td>
<td>9/68</td>
<td>13.2 (6.2,23.6)</td>
<td>4.5 (2-7)</td>
</tr>
<tr>
<td>Ducks</td>
<td>7/10</td>
<td>70 (34.8, 93.3)</td>
<td>10 (0-10)</td>
</tr>
<tr>
<td>Broilers</td>
<td>10/18</td>
<td>55.6 (30.8, 78.5)</td>
<td>104.8 (5-700)</td>
</tr>
<tr>
<td>Farmers with some poultry dying during the preceding year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 months</td>
<td>2/59</td>
<td>3.4 (0.4, 11.7)</td>
<td>24.3 (0-70)</td>
</tr>
<tr>
<td>Pullets</td>
<td>6/27</td>
<td>22.2 (8.6, 42.3)</td>
<td>8.5 (0-61)</td>
</tr>
<tr>
<td>Cocks</td>
<td>4/65</td>
<td>6.2 (1.7, 15.0)</td>
<td>10 (0-40)</td>
</tr>
<tr>
<td>Hens</td>
<td>5/68</td>
<td>7.4 (2.4, 16.3)</td>
<td>2.5 (1-4)</td>
</tr>
<tr>
<td>Ducks</td>
<td>1/7</td>
<td>10 (0.3, 44.5)</td>
<td>7 (0-9)</td>
</tr>
<tr>
<td>Broilers</td>
<td>9/18</td>
<td>50 (26.0, 74.0)</td>
<td>84.5 (3-700)</td>
</tr>
<tr>
<td>Farmers with surviving poultry after disease occurred in the preceding year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village chickens (0-3 months)</td>
<td>21/23</td>
<td>91.3 (72.0, 98.9)</td>
<td>6.3 (0-28)</td>
</tr>
<tr>
<td>Hens</td>
<td>4/9</td>
<td>44.4 (13.7, 78.8)</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>Ducks</td>
<td>6/7</td>
<td>85.7 (42.1, 99.6)</td>
<td>6 (0-9)</td>
</tr>
<tr>
<td>Broiler</td>
<td>1/10</td>
<td>10 (0.3, 44.5)</td>
<td>2.4 (0-10)</td>
</tr>
</tbody>
</table>
6.4 Discussion

6.4.1 Characteristics of Poultry-Owning Households

Similar to other developing countries rearing poultry in West Timor is an important component of small farm enterprises (Sonaiya and Swan, 2004; Sonaiya, 2008). These animals, through their eggs and meat, provide valuable nutrients to the family, as well as being a source of income (Guèye, 2000b). A smallholder production system has advantages and disadvantages for farmers. Farmers do not need to spend a large amount of money providing feed as the poultry mostly are fed on kitchen leftovers and other feed items that the birds source themselves whilst roaming freely, however when an outbreak of infectious disease occurs they are at a high risk of becoming infected (Tiensin et al., 2005; Biswas et al., 2009; Paul et al., 2011).

Billah et al. (2013) reported that in Bangladesh households with a better educational background had a higher level of productivity in their poultry. In addition, it has been shown that in rural areas of Nigeria, less educated households are less likely to adopt innovative animal husbandry practices (Ogunlade and Adebayo, 2009) or to be involved in extension programs (Dolberg, 2004) than more educated households. In the current study almost half of farmers who had never attended school (40.7%; 95%CI: 22.4, 61.2) reportedly had had chickens die compared to only 3.7% (95%CI: 0.9, 24.3) of farmers who graduated from university (P=0.024). Importantly in the current study the educational background of the poultry owning households varied greatly and this needs to be considered when developing any educational package or extension program to improve the health and productivity of poultry (Alders and Bagnol, 2007). Education and training have been found to enhance a farmers’ ability and willingness to make changes to their management practices (Kilpatrick, 2000; Kilpatrick and Rosenblatt, 1998). Julien and Thomson (2011) reported that interactive training and awareness programs could result in farmers implementing biosecurity practices. Similarly a
community-based education program for farmers in rural Cambodia was shown to improve the adoption of biosecurity practices by backyard poultry operators (Conan et al., 2013).

In most households interviewed in the current study, the rearing of poultry was not the primary occupation. This may influence their understanding about disease control and animal husbandry. A study in Ethiopia similarly demonstrated that low productivity and high mortality of chickens and generally poor disease control and husbandry was present in poultry owned by households whose primary occupation was not the production of poultry (Fentie et al., 2013). As well as poultry, households in West Timor also owned other animals, including pigs, and the ownership of these animals has the potential to increase the risk of HPAI H5N1 virus in their poultry through transmission of the virus between species (Songserm et al., 2006a; Kaplan and Webby, 2013; Ito et al., 1998; Scholtissek, 1990; Ma et al., 2009). This is particularly relevant in a subsistence-farming situation where different species of livestock frequently have both direct and indirect contact with each other.

6.4.2 Poultry Husbandry Practices

Domesticated poultry are more likely to interact with other birds, animals or people if they roam freely (Chantong and Kaneene, 2011). Additionally, Alders et al. (2014) highlighted the role of intensive poultry in the evolution of HPAI. Others have highlighted the increased risk of disease transmission between animals (Gilbert et al., 2007; Songserm et al., 2006c; Tiensin et al., 2007), and backyard poultry, including free-grazing ducks, have been incriminated in outbreaks of AI in Thailand (Chantong and Kaneene, 2011). In this study it was demonstrated that biosecurity measures were rarely implemented in the backyard flocks. This has the potential to perpetuate virus circulation (if present), and therefore be a potential source of virus for other poultry (Capua and Marangon, 2007a; Alders et al., 2014). Poultry in West Timor are primarily
reared in a traditional manner receiving minimal housing, management and husbandry inputs. In this survey, the majority (81.8%) of households allowed their village chickens to roam freely during the day. This practice has been recognized as one of three major risk factors for the occurrence, maintenance and spread of HPAI (Gutiérrez et al., 2009).

Poultry require nutrients and energy to maintain their physiological state, and to facilitate weight gain and egg production (Ravindran, 2013). However, poultry reared under a traditional system are usually provided with limited food and often must scavenge to obtain their daily nutrients through consumption of household waste/kitchen leftovers, insects, worms, seeds and crop residues. Consequently rearing poultry in rural areas is a low cost venture requiring minimal resources and input (Guèye, 2000b; Guèye, 2002; Guèye, 2005; Ahlers et al., 2009). Water is essential to assist in feed digestion, absorption of nutrients, excretion of waste products and regulation of body temperature; however it is rarely deliberately provided to poultry in a rural setting (Ravindran, 2013). Certain sources of water, such as rainwater, can pose a risk for poultry for the introduction and transmission of H5N1 as the virus has been found to survive in these sources (Horm et al., 2012). Several studies have also shown that backyard flocks roaming in or near wetlands have a greater risk of exposure to a virus-contaminated environment or AI infected wild birds (Jansen et al., 2009; Zheng et al., 2010). In addition, ponds or canals have been identified as increasing the risk of HPAI outbreaks in villages in Vietnam, Thailand and Nigeria, resulting in spread of infection to neighbouring villages (Paul et al., 2011; Desvaux et al., 2011; Fasina et al., 2011).

6.4.3 The Role Played by Wild Birds in the Transmission of HPAI H5N1

Wild birds can play a key role in the transmission of diseases, including AI, and are considered to be natural reservoirs for LPAI viruses (Olsen et al., 2006; Tracey et al.,
Brown et al. (2009b) demonstrated that house sparrows were highly susceptible to H5N1 HPAI virus. Infected house sparrows could excrete virus via the oropharynx and/or cloaca for several days prior to the onset of clinical signs and consequently it was concluded that these birds could play a major role as a bridge host in the dissemination of H5N1 HPAI virus to poultry (Caron et al., 2015; Brown et al., 2009b). Owners of poultry surveyed in this study commonly reported the presence of house sparrows near their poultry. These potentially may play a role in the transmission of HPAI if it were introduced into West Timor. Although the households did see wild birds in close proximity to their poultry, there is a need to investigate the migration or movement of wild birds from areas of Indonesia infected with AI to West Timor to evaluate the risk of disease introduction to the Province.

6.4.4 Poultry Trading

Similar to farmers from other parts of Indonesia, farmers in West Timor regularly sell chickens and these are sold at a variety of ages. As anticipated more broiler chickens were sold than village chickens as broilers are reared commercially for sale, although only by a few farmers. Although village chickens command a higher price at sale than broilers, few were sold. Interestingly, over half (60%) of the households never sold poultry, keeping the birds only for their own consumption. Ketaren et al. (2010) also described that poultry, especially village chickens, were commonly raised by rural households in Indonesia as a source of eggs and meat for families, and as a “bank” which could be sold when funds were needed for specific purchases or household activities.

In this study it was found that most (92.2%) households purchased chickens directly from local markets rather than from farms. This may be due to accessibility and a range of size, price and age of chickens available at the markets. However live bird markets (LBMs) can play a significant role in the maintenance and distribution of AIV (Kung et
and surveillance in LBM in Hong Kong indicated that 20% of the chickens at the markets were infected with H5N1 virus (Shortridge, 1999). In Indonesia, 47% of 83 LBMs in three provinces were found to have evidence of H5N1 infection based on swabs collected from live birds at the markets (Indriani et al., 2010). As many species of birds (both wild and domestic) are kept at markets, the practice of purchasing birds from LBM can facilitate the exchange and spread of viruses between species, and therefore movement of contaminated equipment and infected birds between markets and households constitutes a major disease risk (Sims et al., 2005). The risk of markets was further highlighted by the fact that most households interviewed would take any unsold birds home. These returning birds could potentially have been exposed to virus and may subsequently introduce virus into a clean (virus free) flock (Sims et al., 2005; Kung et al., 2003; Shortridge et al., 1998).

For those households which purchased broilers, most preferred to buy them directly from suppliers (homes or farms), and 94.1% purchased DOCs from homes located outside their village. This may be because DOCs are primarily sourced from the island of Java, which is the main source of parent stock for broilers in Indonesia. The practice of not isolating newly introduced chickens from existing poultry could further the potential for transmission of HPAI within birds on a farm.

6.4.5 Health Status and Disease Prevention

An understanding of how poultry owning households perceive the importance of applying specific husbandry and biosecurity measures on their farms can help government and poultry practitioners to deliver suitable services and provide advice on methods to optimize the productivity and health of both the poultry and their associated households. Investment in training and education to improve management practices has been shown to play a significant role in the control and eradication of H5N1 from rural environments (Cristalli and Capua, 2007).
Most poultry owning households in West Timor had experienced some form of disease in their poultry flocks in the five years preceding the survey. The diseases affected chickens of all age groups, with almost all chickens displaying clinical signs dying. As the most common disease reported was Newcastle Disease (ND), given that the clinical signs of ND and HPAI can be similar (Swayne and Suarez, 2000), this may lead to an outbreak of HPAI being overlooked or not reported. Although most households surveyed were aware of AI and were familiar with the clinical signs of the disease, the presence of sick birds in a flock were rarely reported to the authorities.

In West Timor, the use of vaccines as a control strategy for AI is prohibited (Personal Communication with Dr. Maria Geong, Former Head of Animal Health Division of Provincial Dinas Peternakan of NTT, 10 May 2016). In contrast to HPAI, vaccination against ND has been encouraged to limit the disease spread. The disease is a significant problem for the village poultry industry, including West Timor, where it can cause up to 60% mortality in village chickens (Alexander, 2001). However in this study vaccination against ND was not undertaken by households due to vaccines not being available. With respect to HPAI H5N1, the disease can be prevented by adopting good management and husbandry practices to avoid contact between infected and susceptible birds. Spread from animals to humans has also been reduced through limiting exposure of humans to sick and/or dead poultry (Dinh et al., 2006; Hien et al., 2004; Mounts et al., 1999; Bridges et al., 2002). Adopting measures to control ND (other than vaccination) should also have a positive effect in controlling AI in flocks (Alders et al., 2010).

**6.4.6 Disease Risk**

The raising of poultry by smallholders (backyard farming) is a common practice in West Timor as the inputs are minimal and the outputs through eggs and meat are an important contribution to the nutrition and wealth of a family. Pym et al. (2006) described that 80% of poultry flocks in the world are backyard flocks; however this type
of flock can become exposed to a number of infectious diseases, including ND and AI, primarily because of their low biosecurity level (free-ranging, multi-age flocks) (Conan et al., 2013; Sultana et al., 2012). During the current survey the majority (94%) of the households stated that neighbour’s birds were a key risk of disease for their own poultry. Introducing new poultry onto a farm or into a flock is a risk for the introduction and transmission of HPAI, with households in southern Cambodia that purchased live poultry being almost four times more likely to have had H5N1 in their flock than households that did not buy live chickens (Vong et al., 2006). Although households considered neighbouring birds were important for disease, only 6% of households reported that their poultry became sick after contact with newly introduced poultry.

HPAI (H5N1) virus in village chickens also poses a serious threat to public health because of the frequent and close contacts between poultry and humans (Biswa et al., 2009) and close contact with infected village poultry has been associated with many human cases of H5N1 (Sultana et al., 2012; Van Kerkhove et al., 2011; Chotpitayasunondh et al., 2005; Bridges et al., 2002; Rabinowitz et al., 2010; Hien et al., 2004). Exacerbating the risk to human health (Jiang et al., 2014) was the practice of some farmers housing poultry inside the farmer’s house during the evening (Sultana et al., 2012). Furthermore, most cases of human infection of AI result from contact or close contact with infected poultry or their products or surfaces/fomites contaminated with secretions or excretions from infected birds (CDC, 2005a; Areechokchai et al., 2006; Dinh et al., 2006; Zhou et al., 2009). Consequently it is important for those working with poultry to avoid contact with infected or dead poultry. Others have reported that farmers often ignore basic hygiene measures (Iqbal, 2009) and improving these and biosecurity measures would not only reduce the likelihood of HPAI entering but also help to control or prevent other infectious diseases.
6.4.7 Disease Awareness

People involved in the poultry industry (such as village/backyard and commercial farms, poultry buyers and sellers, poultry workers) need to be informed through a public awareness campaign by the use of media or farmer meetings of information about HPAI H5N1 and methods to reduce the impact of this disease and the likelihood of it entering their flocks. The media has been known to play an important role in improving people’s knowledge, attitudes and behaviours towards AI (Maton et al., 2007). In their study they found that people who had received information from the media had better attitudes towards and practices of AI prevention and control, compared with those who had not received such information. The current study found that radio and television are the common media forms used in Indonesia to distribute information about AI during an outbreak, where all interviewed households had heard about the disease through these sources. Any future educational campaign to raise awareness about AI should include these media sources.

6.4.8 Disease Reporting

This survey found that almost all of the farmers surveyed would not report cases of disease in their poultry to the local authorities or veterinarians. This behaviour would potentially delay the early detection of a disease outbreak. It is considered that prompt disease reporting is critical for the rapid detection of a disease and that farmers play a key role in this process (Palmer et al., 2009). Disease identification without reporting potentially allows spread of a disease (Welte et al., 1998). This aspect requires improving in West Timor and inclusion of this in any educational campaign is critical to enhance the likelihood of early detection of an outbreak in the region.

6.5 Conclusions

In this chapter the husbandry and management practices adopted by a sample of poultry owning households in West Timor Indonesia are described. Most poultry are reared
using a traditional system in backyard/village settings. The rearing of poultry was an adjunc
to other occupations; nevertheless chickens play an important role in households and are a valuable source of protein, as well as a source of additional income.

Although HPAI H5N1 is currently absent from West Timor, there is a potential risk of the disease being introduced and transmitted through the movement of poultry, poultry products and fomites. To determine the likely method of entry and transmission a social network analysis was conducted to identify the likely route of entry and dispersal and the results of this study are outlined in the following chapter (Chapter Seven).
CHAPTER SEVEN

Social Network Analysis of Poultry Movement in West Timor

7.1 Introduction

It is widely considered that the global spread of H5N1 has been associated with the marketing of poultry, in particular through live birds markets (LMBs), and the movement of migratory birds (Fournié et al., 2013; Gauthier-Clerc et al., 2007). Additionally, the movement of poultry, poultry products and people have facilitated the introduction and transmission of HPAI H5N1 into a country or poultry population previously free of disease (Fang et al., 2008; Martin et al., 2005; Paul et al., 2011; Eagles et al., 2009; Chen et al., 2006a). A study conducted by Kilpatrick et al. (2006) identified the potential synergism between trade and movement of wild birds in the emergence and pandemic spread of H5N1 in Asia, Europe and Africa. Movement of live animals is one of the most important ways that contagious diseases can spread between holdings (Nöremark et al., 2011) and the movement of poultry through markets is a key factor in the circulation and spread of HPAI (Van Kerkhove et al., 2011).

One method to investigate live animal movements is through network analysis (Nöremark et al., 2011). Social network analysis (SNA) has been used to describe relationships among elements within a group or network (Wasserman, 1994), where social network is a term that refers to elements in a group that may or may not be related (Poolkhet et al., 2013). Social network analysis has become an increasingly popular method within veterinary epidemiology to investigate animal movements (Li et al., 2007; Green et al., 2009). It has a major advantage when compared to other analytical approaches: the ability to handle relations that are bi-directional, such as contacts between individuals, trade, or animal movements (Martínez-López et al., 2009). In preventive veterinary medicine, SNA is an approach that offers benefits for exploring the nature and extent of the contacts between animals or farms, which
ultimately leads to a better understanding of the potential risk for disease spread in a susceptible population (Martinez-López et al., 2009). When SNA is applied to epidemiology, it has typically been assumed that links between network nodes are static and therefore represent fixed, persistent contacts during an individual's infectious period (Nickbakhsh et al., 2013). In the animal sector, it has been used to: characterize the pattern of animal movements in relation to the epidemic of foot and mouth disease (FMD) in the UK in 2001 (Ortiz-Pelaez et al., 2006); describe the movement networks of cattle in Italy in 2007 (Natale et al., 2009); and characterize the movement network of cattle and swine in Denmark to evaluate the risk potential for disease spread (Bigras-Poulin et al., 2006; Bigras-Poulin et al., 2007). By understanding the social network paradigm, a set of concepts and methods can be developed to study the structure of a population through which infectious agents are transmitted during close personal contact and allow an opportunity to develop improved disease control programs (Klovdahl et al., 1994). For this study, the approach will help to understand how poultry move from one place to another through the action of sellers and buyers. Such movements facilitate the spread of infection and understanding these movements can aid in the development of methods to control the introduction and spread of the disease. Live bird markets are integral in the poultry supply chain in Indonesia (Roche et al., 2014), including West Timor. Therefore, movement of poultry through markets is potentially important in the introduction, spread and circulation of HPAI H5N1. However, prior to the current study little was known by the research community about poultry market chains in West Timor. Consequently this study was designed to describe and assess movement and trading patterns of poultry in West Timor, to provide insight into how live poultry are traded and how HPAI could potentially be transmitted through their movement.
7.2 Materials and Methods

7.2.1 Respondent Data Sources

The total number of individuals interviewed for this study was 50 (25 sellers and 25 buyers of both village and commercial chickens).

7.2.2 Poultry Market Details

Buyers and sellers from five live-bird poultry markets were included in the analysis. In Figure 7.1 the location of the four markets in the district of Kota Kupang and the market in the district of Belu are displayed.

![Figure 7.1 Map of West Timor showing the location of the markets where buyers and sellers were surveyed](image)

7.2.3 Population Under Study

Data were collected from surveys conducted at markets (Figure 7.2) and on farms (Figure 7.3). For the market survey, anyone present at the marketplace on the day of interview, either sellers or buyers, and who were willing to be interviewed were the
eligible population. A seller was defined as an individual who was selling poultry in the market on the day of interviewing and a buyer was defined as an individual purchasing poultry on the interview day. The respondents were selected if they agreed to be interviewed (refer to Materials and Methods in Chapter Three). All the respondents agreed to participate during the survey.

Figure 7.2 Broiler chickens at market place in Belu District
Structured interviews with sellers and buyers took place during field visits to the five markets. Interviews were conducted before the animals were sampled for targeted surveillance, as outlined in Chapter 5. Information was collected from buyers on their background, the quantity of birds purchased, whether live birds, chicken meat or eggs were purchased, how the purchased chickens were transported and their destination, as well as their (buyers) knowledge on avian influenza and poultry health (Questionnaire for poultry buyers - Appendix 2). Information was collected from sellers on their background, the management and husbandry procedures adopted for their poultry, the pattern of selling birds and eggs, how chickens were transported and their (sellers) knowledge on avian influenza and poultry health (Questionnaire for poultry Sellers - Appendix 3). The Murdoch University Human Ethics Committee and local authorities had approved the questionnaires before the interviews were conducted (Approval numbers see section 3.4.3 in Chapter Three).
7.2.5 Managing and Analyzing the Data

Transportation of poultry from the original premise (farm or market) to the destination premise (farm or market) was defined as a movement event; and the direction (such as from market to village or from village to market) of the poultry movement was defined as a link. Each respondent was asked to state the district, sub-district and village from where the poultry originated from and their destination.

Data from the survey (sellers and buyers) were initially entered into an excel spreadsheet (Microsoft excel® for Mac 2011 version 14), and then exported into Ucinet (version 6.579 Analytic Technologies, http://www.analytictech.com/products.htm) for subsequent social network analyses.

The movement network of live poultry was analyzed using social network methods (Wasserman, 1994; Scott, 2012). Data on the purchase of live chickens (age, breed and number of chickens purchased, the lowest and highest demand periods for chickens, origin of purchase, destination of sale, and weekly quantities of live chickens and ducks traded between locations), purchase of meat products, pattern of egg purchases, poultry health, transportation and destination of chickens, knowledge about AI, and slaughtering of chickens were used to create a directed network of the poultry movements by market sellers and buyers within and into West Timor. NetDraw (version 2.153 Analytic Technologies, http://www.analytictech.com/products.htm) was used to illustrate the networks as described by Borgatti (2002). Ucinet was used to measure the connectedness of the nodes (locations) within the network, the in-degree (quantity of birds terminating at the node) and out-degree (quantity of birds originating from the node) for each node in the poultry networks, as well as cohesion of the network including geodesic distance and clustering coefficient in order to understand the distance and how connected the nodes were in the network (Borgatti et al., 2002) (Table 7.1).
Due to the sampling approaches used for the buyers and sellers (Chapters 3) it was not possible to calculate several SNA parameters namely closeness, reachability and small world network analysis.

Table 7.1 Parameters Included in the Analysis of the Movement of Poultry between selected Markets and Villages in West Timor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definitions</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of Network</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of nodes</td>
<td>The total number of network members, the unit of interest in a network</td>
<td>(Aznar et al., 2011; Dubé et al., 2009;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Martinez-López et al., 2009)</td>
</tr>
<tr>
<td><strong>Density of Network</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (directed)</td>
<td>The number of ties in the network expressed as a proportion of the number of</td>
<td>(Aznar et al., 2011; Izquierdo and</td>
</tr>
<tr>
<td></td>
<td>all possible ties that could be present</td>
<td>Hanneman, 2006)</td>
</tr>
<tr>
<td>Number directed links</td>
<td>The total number of connections between nodes</td>
<td>(Aznar et al., 2011)</td>
</tr>
<tr>
<td><strong>Degree of Nodes (Measure of</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>centrality)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-degree</td>
<td>The count (number) of contacts a node receives, or the node that receives</td>
<td>(Wey et al., 2008; Martinez-López et al.,</td>
</tr>
<tr>
<td></td>
<td>the highest number of contacts</td>
<td>2009)</td>
</tr>
<tr>
<td>Out-degree</td>
<td>The count of individual recipients obtaining animals from a particular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>market or village location</td>
<td></td>
</tr>
<tr>
<td>Normalised in- and out-degree</td>
<td>It is necessary for the in- and out-degree value to be normalised in order</td>
<td>(Dubé et al., 2009; Wey et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>to be made across networks</td>
<td>(Aznar et al., 2011)</td>
</tr>
<tr>
<td>In-degree centralization</td>
<td>An estimate of the deviation of the largest values of In-degree from the</td>
<td>(Aznar et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>value computed for all other nodes in the network</td>
<td></td>
</tr>
<tr>
<td>Out-degree centralization</td>
<td>An estimate of the deviation of the largest values of Out-degree from the</td>
<td>(Aznar et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>value computed for all other nodes in the network</td>
<td></td>
</tr>
<tr>
<td>Network diameter</td>
<td>The total number of links in the longest pathway between two nodes</td>
<td>(Aznar et al., 2011; Martinez-López et al.,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2009; White and Borgatti, 1994)</td>
</tr>
<tr>
<td>Betweenness centrality</td>
<td>Node where information is more likely to pass through than the other nodes</td>
<td>(Molano and Polo, 2015; Boutilier et al.;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White and Borgatti, 1994; Borgatti and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Everett, 1997)</td>
</tr>
<tr>
<td><strong>Cohesion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geodesic distance</td>
<td>The shortest path length between two nodes</td>
<td>(Narayanan et al., 1983; Scott, 2012;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hanneman and Riddle, 2005)</td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>The clustering coefficient of a vertex measures how connected the neighbours</td>
<td>(Dubé et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>of that vertex are to each other</td>
<td></td>
</tr>
</tbody>
</table>
7.3 Results

Social Network Analysis for the Movement of Poultry from Selected Markets in West Timor

Table 7.2 Calculated Parameters of the Social Network Analysis of Poultry Movement

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Network Size</strong></td>
<td></td>
</tr>
<tr>
<td>Number of sellers/buyers nodes(^a)</td>
<td>50</td>
</tr>
<tr>
<td>Number of market nodes(^a)</td>
<td>5</td>
</tr>
<tr>
<td>Number of directed links (ties)(^b)</td>
<td>415 (304 Kota Kupang, 111 Belu)</td>
</tr>
<tr>
<td>Size(^c)</td>
<td>549 (456 for Kota Kupang; 93 for Belu)</td>
</tr>
<tr>
<td>Average density(^d)</td>
<td>7.5%</td>
</tr>
<tr>
<td>Diameter(^e)</td>
<td>5</td>
</tr>
<tr>
<td><strong>Measure of Centrality</strong></td>
<td></td>
</tr>
<tr>
<td>Mean In-degree (Standard deviation/SD)(^f)</td>
<td>7.6 (3.9)</td>
</tr>
<tr>
<td>Mean Out-degree (SD)(^g)</td>
<td>7.6 (2.9)</td>
</tr>
<tr>
<td>Normalised Out-degree (SD)(^h)</td>
<td>13.9 (5.4)</td>
</tr>
<tr>
<td>Normalised In-degree (SD)(^i)</td>
<td>13.9 (7.2)</td>
</tr>
<tr>
<td>In-degree centralization(^j)</td>
<td>23.5%</td>
</tr>
<tr>
<td>Out-degree centralization(^k)</td>
<td>14.1%</td>
</tr>
<tr>
<td>Betweenness centrality (with the highest value)(^l)</td>
<td></td>
</tr>
<tr>
<td>Pasar Oebobo</td>
<td>339.4</td>
</tr>
<tr>
<td>Node No.1</td>
<td>227.6</td>
</tr>
<tr>
<td>Node no.5</td>
<td>225.4</td>
</tr>
<tr>
<td>Node no.11</td>
<td>215.9</td>
</tr>
<tr>
<td><strong>Measure of Cohesion</strong></td>
<td></td>
</tr>
<tr>
<td>Density (SD)(^m)</td>
<td>0.14 (0.35)</td>
</tr>
<tr>
<td>Average geodesic distance (SD)(^n)</td>
<td>2.29 (0.8)</td>
</tr>
<tr>
<td><strong>Clustering Coefficient</strong></td>
<td></td>
</tr>
<tr>
<td>Overall clustering coefficient(^o)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\) Number of nodes - the total number of network members.
\(^b\) Number of directed links - the total number of connections made between nodes.
\(^c\) Size - the total number of possible unique node pairs.
\(^d\) Density - the number of realized ties divided by the number of possible ties in the network.
\(^e\) Diameter - the number of links in the largest possible pathway between two nodes.
\(^f\) Mean In-degree - the count of contacts a node receives (movements to a location).
\(^g\) Mean Out-degree - the number of contacts from a node (movements from a location).
\(^h\) Normalized Out-degree - the number of contacts to a node divided by the maximum number of possible contacts.
\(^i\) Normalized In-degree - the number of contacts from a node divided by the maximum number of possible contacts.
\(^j\) In-degree centralization - an estimate of the deviation of the largest values of In-degree from the value computed for all other nodes in the network.
\(^k\) Out-degree centralization - an estimate of the deviation of the largest values of Out-degree from the value computed for all other nodes in the network.
\(^l\) Betweenness centrality - It reflects the amount of control that this node exerts over the interactions of other nodes in the network.
\(^m\) Density - the proportion of all contacts that could be present that actually are.
\(^n\) Average geodesic distance - the shortest path length between two nodes.
\(^o\) Overall clustering coefficient- a ratio \(N / M\), where \(N\) is the number of edges between the neighbours of \(n\), and \(M\) is the maximum number of edges that could possibly exist between the neighbours of \(n\).
Table 7.3 Most Relevant Market and Nodes with the Highest In and Out-Degree Centrality Measures

<table>
<thead>
<tr>
<th>Node or Market (pasar)</th>
<th>Out-degree</th>
<th>In-degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasar Oebobo</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Node No.11</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Node No.5</td>
<td>7</td>
<td>20</td>
</tr>
</tbody>
</table>

The overall networks representing formal poultry movements were comprised of five nodes for the market network and 50 nodes for village nodes (Table 7.2). The size of the network was 549, with the size of the network of Kota Kupang being bigger than that of Belu.

A total of 415 directed links were identified for the overall market network highlighting the potential movement for poultry to different districts in West Timor. Kupang was the most prominent node in the poultry trade network of West Timor (Figure 7.4). In addition, Kupang had 304 direct ties of sellers and buyers, while Belu had only 111 direct ties (Table 7.2). Moreover, four nodes in Kupang were found to have the highest hubs (betweeness centrality) in the network (Table 7.2).

The density of the market network as a whole was 0.14, indicating that 14% of the nodes have a tie or relation (Personal Communication - Steve Borgatti, developer of Analytic Technologies Social Network Analysis software), where ties play a role in connecting social groups as networks of nodes (Wey et al., 2008) or networks of sellers and buyers in the context of market networks in West Timor. The average density degree was 7.5%. The diameter of the market network was five (Table 7.2), providing a minimum figure for movement pathways of the nodes across the network. The average grade of the whole network for both out-bound and in-bound movements was approximately eight (Table 7.2).
The in- and out-degree values demonstrated that node 1 (seller) from Kupang had the highest normalized out-degree. This node is regarded as the most influential, and represents a high-risk potential in the spread of disease to other nodes. Poultry from this seller were found to be transported to other sellers and buyers both in Kota Kupang and in Belu district. The highest value of out-degree was found on Node 11 in Kota Kupang, meaning that this node played an important role in transmitting information to all other nodes in the network (Figure 7.4). Node 1 in Kota Kupang was found to have the highest in-degree value of the nodes, suggesting that this node had a greater potential to become infected than did other nodes (Figure 7.5).

Figure 7.4 Sellers and buyers network based on out-degree of formal movement of poultry

The node size indicates its Out-degree value (larger nodes represent higher Out-degree values). The thickness of the arrow’s head indicates its tie strength (the thicker the head of the arrows the stronger its tie connectedness)
Figure 7.5 Sellers and buyers network based on in-degree of formal movement of poultry.

Circles: buyers and sellers nodes, Squares: market nodes. Edges or ties linking nodes illustrate the direction of poultry movement as indicated by arrows and tie strength is indicated by the thickness of the head of arrows (i.e., the thicker the head of an arrow, the more poultry passing between the two locations).

From Figure 7.6 and Table 7.3 it can be seen that Pasar Oebobo had the highest in-degree and out-degree value of 15, indicating a high degree of poultry entering and exiting this market. Four nodes were found to have the highest betweenness centrality values with a mean value of 69.6 (SD 2.4). Pasar Oebobo, and node numbers 1, 5, and 11 had the highest degree of betweenness centrality with 339.4, 227.6, 225.4, and 215.9, respectively, meaning that they had the most control over the information and communication that flowed through the network.
In relation to the Cohesion measure, the average geodesic distance was found to be approximately 2, suggesting localised movement with poultry moving between two consecutive nodes, commonly a neighbouring node within the same area (village/subdistrict). The network of poultry in West Timor can be classified as low connected with an overall clustering coefficient of 0.15 (Table 7.2).

7.4 Discussion

This study has demonstrated that poultry movement could enable the dissemination of disease which could subsequently circulate through households or farms with poultry. Several studies have found that the movement of chickens for trade or to fight in fighting cock’ competitions have played an important role in the spread of HPAI (Roche et al., 2014; Magalhães et al., 2010; Van Kerkhove et al., 2009). H5N1 has been shown to circulate in and can be regularly isolated from traditional LBMs (Abdelwhab et al., 2011). In Phnom Penh, Cambodia, the market network has been found to be a
potentially important hub for the transmission and circulation of H5N1 (Van Kerkhove et al., 2011). The current study found that market sellers and buyers are actively moving through the market network, and therefore could potentially contribute to the spread of HPAI, if an outbreak occurred in the region. However, the network size of Kota Kupang was larger than Belu, suggesting that Kota Kupang plays a bigger role in the network in terms of the number of nodes and in the event of an outbreak more resources need directing to this location.

In a social network analysis density is the relationship between the number of existing connections and the maximum number of possible connections; and average density shows the number of relationships of a network (Molano and Polo, 2015; Wasserman, 1994). In addition, the density of a network explains the diffusion speed of information among the nodes, and the extent to which nodes have high levels of social capital and/or social constraint (Hanneman and Riddle, 2005). Moreover, a density score of 1 indicates that all nodes in the network are directly tied to one another, conversely a density score of 0 indicates the network is fully disconnected (Prell et al., 2009). The density of the network in this study in West Timor (0.14) was low, indicating a low connection or relationship of the poultry network members (network of sellers and buyers). So if an outbreak did occur it is unlikely to be able to reach the entire population rapidly (Keeling and Eames, 2005) from any starting point in West Timor. This would mean that implementing a control program early after the detection of the disease may be able to prevent widespread distribution of the disease in West Timor.

The in and out-degree values in the current study suggest that poultry were moving in and out of the areas through the movement of sellers and buyers. Out-degree is usually a measure of how influential the node is in the network (Hanneman and Riddle, 2005). In the event of an outbreak, nodes with the highest Out-degree scores have the potential to distribute disease widely (Aznar et al., 2011). This study found that Node 11 in Kota
Kupang had the highest value of out-degree, indicating that this node plays a key role in transmitting information to all other nodes in the network of poultry in West Timor. This would mean that this node has the potential to spread the disease to other nodes if an outbreak occurred, and hence extra resources should be provided to monitor this node and control any disease occurring in it.

Highly central nodes with regards to their outgoing movement are also highly central in terms of incoming movements, indicating the high probability of disease incursion from incoming movements (Natale et al., 2009). As Kota Kupang is the major poultry trade centre in West Timor, with poultry being transported into and out of the area, Kota Kupang could play a key role in the transmission of the disease, if it entered the region. Therefore, it is important surveillance is increased in this district, particularly through the targeting of high-risk areas, specifically markets and villages where sellers and buyers originate from. Use of risk-based surveillance in this situation is a cost-effective means of undertaking disease surveillance to maximize the likelihood of early recognition of the incursion of a disease (Stärk et al., 2006).

This study also found that there was potential movement of poultry in different districts in West Timor through the directed links or ties, which could contribute to the transmission and spread of HPAI. In relation to disease transmission, ties are important for connecting different groups so that information can spread throughout a population (Granovetter, 1983). In addition, people pass rumors, information, or diseases when they interact with others who later pass these resources (or risks) to others through their relations (contacts) (Moody, 2002). This indicated that HPAI could potentially be spread through the movements and contacts of poultry and traders and/or sellers in this study area as interactions between sellers and buyers exist.

Regarding the betweenness centrality, four nodes had the highest degree of betweenness, which indicates that all other nodes (sellers/buyers) would go through
these nodes, as they were the most central and influential nodes or persons in the network (Narayanan, 2005). This suggests that if HPAI H5N1 entered West Timor, it would go through these nodes before it was introduced and transmitted across the network. Consequently identifying these nodes is critical for controlling disease and targeting disease surveillance funds and activities.

The result of the geodesic distance in the current study suggested a localised movement of poultry between two consecutive nodes on average. This shows a number of relations in the shortest possible walk (walking distance/distance) from one node to another for both directed and undirected network data; and when the geodesic distance of a network is small, the information may travel very quickly in the network (Hanneman and Riddle, 2005). This indicates how quickly the disease spreads in the population, meaning that if a HPAI outbreak occurred in West Timor, it could spread quickly but locally to two to three farms, as indicated by the localised movement of poultry between two consecutive nodes.

In the current study the overall network clustering coefficient of 0.15 was low. The clustering coefficient is the amount of interrelationship that exists between all the nodes that are directly connected to one specific node of interest (Bollobás and Riordan, 2003). The low clustering coefficient in West Timor indicated low interrelationship between nodes, indicating that sellers or buyers did not really know each other, and connection between them was low, implying that if an outbreak of HPAI occurred in West Timor it would spread slowly. Dubé et al. (2009) highlighted that if all holdings/farms/flocks are connected to each other, then one large cohesive group is present and the network is fully connected (Dubé et al., 2009), which did not apply to the present study.

Understanding the movement of poultry through the market chain is important in predicting disease transmission within a region and this study provided information on
the potential for disease transmission through the network of sellers and buyers. However it is critical that the risk of disease introduction is also understood. In the next chapter (Chapter 8) the likelihood of the introduction of HPAI into West Timor through the importation of live poultry (DOC) is investigated.

**7.5 Conclusions**

Social network analysis provided a useful tool for understanding the movement and trading patterns of poultry in West Timor. Even though the nodes (poultry sellers and buyers) were not all connected, it is suggested that special attention should be given to the trading pattern and movement of poultry between markets and villages (especially households), as there was potential movement of poultry from one district to another.

The results of this analysis indicate that the disease would be spread throughout the market network. Therefore, control measures should be applied to the pathway between markets and poultry owning houses to control HPAI H5N1 and to prevent it spreading to backyard farmers.
CHAPTER EIGHT

Risk Analysis of the Entry and Transmission of HPAI into West Timor

8.1 Introduction

In 2014 the total population of poultry in West Timor was 4,660,396 with the majority being village chickens and only 15% being commercial broilers and layers (BPS NTT, 2014). West Timor mainly relies on Java and other islands to supply live chickens (DOC) for replacement purposes. However the movement of these birds does pose a risk for the introduction of HPAI H5N1, as well as other infectious diseases. Medical epidemiologists have defined risk as the probability of disease developing in an individual in a specified time interval (Rothman and Greenland, 1998). Risk analysis is a structured, science-based and transparent approach to risk management (Pfeiffer, 2010), and its components have been defined in the OIE’s Terrestrial Animal Health Code to include hazard identification, risk assessment, risk management and risk communication (Vallat, 2008).

Martin et al. (2007) described that quantitative analysis of the results of structured representative surveys or qualitative assessments of multiple sources of evidence (including complex non-representative sources) were the preferred methods to demonstrate zone or country freedom from disease. Classification of poultry sites, based on the risk for disease introduction and spread, is an important step in the development of risk based surveillance strategies, policies and recommendations for farmers, as well as for modelling purposes (Niemi et al., 2009).

The objective of the study described in this chapter was to quantify and estimate the likelihood of the introduction and establishment of HPAI H5N1 in West Timor through the legal importation of DOC from Java.
8.2 Methodology

8.2.1 Study Design and Data Collection

The study design (study type, sampling method,) and data collection methods adopted (questionnaire design, pilot and administration) have been outlined in detail in Chapter Three.

8.2.2 Interview of experts

Twenty four experts (12 veterinarians from the Livestock Service Offices, eight quarantine officers, and four border patrol staff) were selected based on their role in the poultry sector (Chapter 3) and a questionnaire administered in person. The Livestock Service and Quarantine officers were asked their opinion regarding: the risk of West Timor having an outbreak of HPAI H5N1; the likelihood of HPAI infection, survival and establishment via various pathways; the possible risk of each route of HPAI introduction from other Indonesian islands or countries into West Timor and each district in West Timor; the likelihood of villages and poultry in each district in West Timor becoming infected by HPAI; and the likelihood of involvement of veterinarians or District Dinas Staff (DDS) to a report from farmers of an outbreak of HPAI. The Border patrol staff (BPS) were asked their opinion regarding: the likelihood of the BPS detecting infected chickens; the likelihood of infected chickens being confiscated; and the likelihood of confiscated chickens being submitted to a quarantine office (Questionnaire for quantification of risk of entry - Appendix 4).

8.2.3 Hazard Identification

The hazard identified in this study was the introduction and establishment of HPAI H5N1 virus by the movement of DOC into West Timor. The virus could possibly be introduced from Java to West Timor at any time through a number of ways, such as through the importation of infected frozen chickens, live chickens or eggs. As data on
the importation of frozen chickens, eggs and the movement of fomites were not available; this risk assessment only evaluated the risk from the importation of live DOC.

8.2.4 Risk Questions

8.2.4.1 Risk Question 1. Release Assessment

The release assessment evaluated the likelihood of HPAI infection, survival and establishment via the importation of live chickens (DOC).

Specific questions included:

- What is the likelihood of HPAI H5N1 entering, surviving and establishing in West Timor via live DOC imported from Java?
- What is the likelihood of DOC from Java being infected with H5N1 before coming into West Timor?

8.2.4.2 Risk Question 2. Exposure Assessment

The exposure assessment evaluated the probability of local chickens being exposed to virus introduced by the imported chickens.

The specific question was:

- What is the probability of the imported chickens (DOC) being released with the local chickens in West Timor?

8.2.4.3 Risk Question 3. Consequence Assessment

The consequence assessment evaluated the probability of West Timor having an outbreak of HPAI.

The specific question asked was:

- What is the probability of West Timor and its districts having an outbreak of HPAI H5N1?
8.2.5 Risk Quantification Method

The risk assessment was performed quantitatively using a probability scenario tree analysis (Martin et al., 2007). The scenario tree covered all relevant components influencing the risk of HPAI H5N1 with values for each input parameter based on expert opinion and other relevant sources.

8.2.6 Risk Pathway: Possible Pathways for the Introduction of HPAI through the Importation of Live Chickens (DOC) from Java

A scenario tree model was developed to show the most likely pathways for the introduction of HPAI H5N1 into West Timor through the legal movement of live chickens (DOC) from Java. The possible pathways for the introduction of HPAI into West Timor through the importation of DOC from Java are summarized in Table 8.1.
Table 8.1 Possible pathways for the introduction of HPAI through the importation of DOC from Java

<table>
<thead>
<tr>
<th>Node code</th>
<th>Node</th>
<th>Parameter</th>
<th>Node type</th>
<th>Branches</th>
<th>Next node</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Infected</td>
<td>P(Inf1)</td>
<td>Infection</td>
<td>Infected</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not infected</td>
<td>No entry</td>
</tr>
<tr>
<td>b</td>
<td>Asymptomatic infected chickens</td>
<td>P(Asympinf)</td>
<td>Risk</td>
<td>Yes</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>category</td>
<td>No</td>
<td>No entry</td>
</tr>
<tr>
<td>c</td>
<td>Infected chickens detected</td>
<td>P(Det)</td>
<td>Detection</td>
<td>Yes</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>HPAI entry</td>
</tr>
<tr>
<td>d</td>
<td>Confiscated</td>
<td>P(Conf)</td>
<td>Risk</td>
<td>Yes</td>
<td>e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>category</td>
<td>No</td>
<td>HPAI entry</td>
</tr>
<tr>
<td>e</td>
<td>Chickens destroyed</td>
<td>P(Dest)</td>
<td>Risk</td>
<td>Yes</td>
<td>f</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>category</td>
<td>No</td>
<td>No entry</td>
</tr>
<tr>
<td>f</td>
<td>Exposed local chickens</td>
<td>P(Exp)</td>
<td>Risk</td>
<td>Yes</td>
<td>g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>category</td>
<td>No</td>
<td>No exposure/outbreak</td>
</tr>
<tr>
<td>g</td>
<td>Infected local chickens</td>
<td>P(Inf2)</td>
<td>Infection</td>
<td>Yes</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>No outbreak</td>
</tr>
<tr>
<td>h</td>
<td>Vets collect samples from</td>
<td>P(Vecosa)</td>
<td>Risk</td>
<td>Yes</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>sick/dead poultry</td>
<td></td>
<td>category</td>
<td>No</td>
<td>j</td>
</tr>
<tr>
<td>i</td>
<td>Vets test samples collected</td>
<td>P(Vetest)</td>
<td>Risk</td>
<td>Yes</td>
<td>j</td>
</tr>
<tr>
<td></td>
<td>from sick/dead poultry</td>
<td></td>
<td>category</td>
<td>No</td>
<td>No HPAI outbreak</td>
</tr>
<tr>
<td>j</td>
<td>Infected chickens infect other</td>
<td>P(Inf3)</td>
<td>Infection</td>
<td>Infected</td>
<td>Further HPAI outbreak</td>
</tr>
<tr>
<td></td>
<td>chickens/farms</td>
<td></td>
<td></td>
<td>Not infected</td>
<td>No further HPAI outbreak</td>
</tr>
</tbody>
</table>
Figure 8.1 Scenario tree for the introduction of HPAI through the importation of DOC from Java.
Figure 8.2 Scenario tree for the introduction of HPAI through the importation of DOC from Java (continued).
8.2.7 Scenario Tree Calculation Methods for the HPAI Introduction through the Importation of Live Chickens from Java.

There were two possible branches for each node; for example for infection nodes there were the two possible branches: infected and not infected. Every branch of a node was assigned a probability value based on the relevant source data after the scenario trees were drawn. The assessments included entry, exposure and consequence assessments. In the entry assessment, there were two possible terminal outcomes: presence of asymptomatic infected chickens and no asymptomatic infected chickens present. The asymptomatic infected chicken node had two possible outcomes: infected chickens detected or infected chickens not detected. The detected chicken node had the two outcomes of confiscated or not confiscated. Confiscated chickens were either destroyed or were not destroyed. Birds destroyed would not result in entry of HPAI, in contrast to those not destroyed, which could introduce HPAI. Infected chickens that were not confiscated could result in HPAI entry. Similarly, infected chickens that were not detected could result in HPAI entry. Once the infected chickens have entered the island, chickens that were not destroyed and were released with local chickens will have two possible outcomes: local chickens are exposed to the virus or local chickens are not exposed to the virus and hence no outbreak will occur. The exposed local chicken node had two branches: local chickens became infected (disease outbreak) or they were not infected (no outbreak). The infected local chickens could have two possible outcomes: veterinarians would collect samples from affected birds or they would not collect samples. The veterinarians who collected samples could either have the samples tested for virus or the samples were not tested. If the samples are tested then infected chickens would be detected and destroyed, which would result in no further outbreak of HPAI. However if the veterinarians did not collect samples then there are two outcomes: the
infected chickens infect other chickens (another outbreak occurs) or they do not infect other chickens (no further outbreak occurs).

8.2.8 Risk Estimation

The probability (risk) of disease entry leading to outbreak was calculated as follows:

**Branches** = the probability of live chickens (DOC) being infected just prior to importation into West Timor leading to an outbreak, where:

A1. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times P(\text{Det}) \times P(\text{Conf}) \times (1-P(\text{Dest})) \times P(\text{Exp}) \times P(\text{Inf2}) \times P(\text{Vecosa}) \times (1-P(\text{Vetesa})) \times P(\text{Inf3}) \)

A2. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times P(\text{Det}) \times P(\text{Conf}) \times (1-P(\text{Dest})) \times P(\text{Exp}) \times P(\text{Inf2}) \times (1-P(\text{Vecosa})) \times P(\text{Inf3}) \)

B1. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times P(\text{Det}) \times (1-P(\text{Conf})) \times P(\text{Exp}) \times P(\text{Exp}) \times P(\text{Inf2}) \times P(\text{Vecosa}) \times (1-P(\text{Vetesa})) \times P(\text{Inf3}) \)

B2. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times P(\text{Det}) \times (1-P(\text{Conf})) \times P(\text{Exp}) \times xP(\text{Exp}) \times P(\text{Inf2}) \times (1-P(\text{Vecosa})) \times P(\text{Inf3}) \)

C1. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times (1-P(\text{Det})) \times P(\text{Exp}) \times P(\text{Inf2}) \times P(\text{Vecosa}) \times (1-P(\text{Vetesa})) \times P(\text{Inf3}) \)

C2. \( P(\text{Inf1}) \times P(\text{Asyminf}) \times (1-P(\text{Det})) \times P(\text{Exp}) \times P(\text{Inf2}) \times (1-P(\text{Vecosa})) \times P(\text{Inf3}) \)

Therefore, the risk estimation = \( \sum \ (A1+A2+B1+B2+C1+C2) \).

8.2.9 Data Sources for the Risk Quantification Method

Expert opinion was used to calculate the probability of HPAI H5N1 entering West Timor via live DOC. Twenty-four experts were interviewed using face-to-face interviews with structured questionnaires (Appendix 5). The questions related to their role as government staff and their opinions regarding the risks of entry, transmission and establishment of HPAI H5N1. For each question, information on minimum, average (most likely) and maximum likelihood values were collected and calculated.

8.2.10 Running the simulation and the statistical methods used

The simulation of the scenario tree model was run in Excel add-in PopTools (Version 3.5) (http://www.poptools.org/functions/) with 1000 iterations used and the
A Pert distribution as dPertDev(min, most likely, max, 4) was used to describe the stochastic data for proportion of the expert opinions. A binomial distribution was used to calculate the final probability outcome from the result of the Pert distribution. The binomial distribution was calculated as dBinomDev(x,p), where x is a vector of selected values and p is the frequency or probability. The probabilities of entry, exposure, establishment and an outbreak of H5N1 for all branch outcomes were calculated after calculating the probability of each branch outcome. In this study, there were two possible branch outcomes: one for the probability of disease entry and one for the probability of a disease outbreak. Examples of the outcomes of HPAI H5N1 in the scenario tree branches are listed in Equation 8.1.

\[
P(\text{FirstOutcome}) = P(\text{Inf1}) \times P(\text{Asyminf}) \times P(\text{Det}) \times P(\text{Conf}) \times \left(1 - P(\text{Dest})\right) \times P(\text{Exp}) \times P(\text{Inf2}) \times P(\text{Vecosa}) \times \left(1 - P(\text{Vetest})\right) \times P(\text{Inf3}) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \text{Equation 8.1}
\]

8.2.11 Formulation of HPAI Risks

8.2.11.1 Probability of Having an Outbreak

The probability of “n” infected chickens that had exposed local chickens resulting in an outbreak was also calculated using a binomial formula (Cameron and Baldock, 1998) as seen in Equation 8.2. This was calculated after determining the result of the probability of all input parameters resulting in an outbreak of HPAI H5N1 P(Outbr), by summing the values of the scenario tree branches that would have resulted in an outbreak. The binomial method (Cameron and Baldock, 1998) was used to calculate the probability of n infected DOC imported from Java.

The probability of all n infected chickens that had successfully entered and had contact (exposure) with local chickens resulting in an outbreak in West Timor was
\[ P(\text{Outbr})^n \] \hspace{1cm} \text{Equation 8.2}

The probability that no infected chickens that had successfully entered and had contact with local chickens resulting in an outbreak in West Timor

\[ [1 - P(\text{Outbr})]^n \] \hspace{1cm} \text{Equation 8.3}

Therefore the probability of at least one infected chicken that had successfully entered and had contact with local chickens resulting in an outbreak in West Timor was

\[ 1 - [1 - P(\text{Outbr})]^n \] \hspace{1cm} \text{Equation 8.4}

8.2.12 Sensitivity analysis methods

To assess the sensitivity of the input parameters to the output probabilities the analysis was conducted using the Spearman Rank correlation coefficient test in @risk 7 (Risk analysis add-in for Microsoft excel version 7.0.1, 2015 Palisade Corporation).

8.3 Results

8.3.1 Results of the Experts Interviewed

In Table 8.2 the probabilities of entry and outbreak of HPAI H5N1 calculated using the scenario tree as described earlier in this chapter are summarized. These input variables were used to quantify the risk of introducing HPAI H5N1 into the study area.
### Table 8.2 Summary of the scenario tree probability branches used to analyze the risk of entry of HPAI into West Timor through the importation of DOC from Java

<table>
<thead>
<tr>
<th>Input variables</th>
<th>Source</th>
<th>Mean</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Probability of infection P(Inf1)*</td>
<td>Assumption based on references and discussion with staff of Provincial Dinas Peternakan of East Java</td>
<td>9.8x10^{-8}</td>
<td>6.3x10^{-9}</td>
</tr>
<tr>
<td>Probability of infecting local chickens P(inf2)**</td>
<td>Literature</td>
<td>6.9x10^{-5}</td>
<td>4.3x10^{-5}</td>
</tr>
<tr>
<td>Probability of importing asymptomatic infected chickens P(Asympinf)#</td>
<td>Literature</td>
<td>0.0026</td>
<td>0.0022</td>
</tr>
<tr>
<td>Probability of Animal Quarantine detecting infected live chickens P(Det)</td>
<td>Expert</td>
<td>0.82</td>
<td>0.72</td>
</tr>
<tr>
<td>Probability of confiscation P(Conf)</td>
<td>Expert</td>
<td>0.97</td>
<td>0.92</td>
</tr>
<tr>
<td>Probability of live chickens destroyed P(Dest)</td>
<td>Expert</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>Probability of chickens released and exposed to local chickens P(Exp)</td>
<td>Expert</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>Probability of veterinarians collecting samples of sick or dead chickens P(Vecosa)</td>
<td>Expert</td>
<td>0.84</td>
<td>0.74</td>
</tr>
<tr>
<td>Probability of veterinarians testing samples of sick or dead chickens P(Vetest)</td>
<td>Expert</td>
<td>0.85</td>
<td>0.81</td>
</tr>
<tr>
<td>Probability of infected chickens infecting other chickens P(Inf3)</td>
<td>Expert</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of DOC imported from Java each month</td>
<td>DLS</td>
<td>124,026</td>
<td>53,855</td>
</tr>
</tbody>
</table>

Sources: * P(Inf1): (Biswas et al., 2008; OIE, 2007; Conan et al., 2012; Christie, 2007)  
** P(Inf2): Biswas et al. (2008); #P(Asympinf): Chen et al. (2006c)
8.3.2 Risk Assessment

8.3.2.1 Chicken Infection Status (Prevalence of DOC in East Java, the Place of Origin of DOC)

This probability is the infection status of chickens in the place of origin (Java Island). The probability of infection \( P(\text{Inf1}) \) of DOC from any source in East Java was estimated to be \( 0.000001 \) (\( 1 \times 10^{-6} \)). This value was selected based on several literature reports: firstly, DOC are very unlikely to be infected by HPAI H5N1 (OIE, 2007); secondly, most commercial farms implement high biosecurity measures (Conan et al., 2012), providing them a high protection to outside threats resulting in a low risk to and hence prevalence of HPAI; and thirdly the Provincial Dinas Peternakan of Nusa Tenggara Timur only allows the importation of DOC into NTT from high biosecurity level commercial, closed farms in Java (Christie, 2007), which are extremely unlikely to be infected by HPAI H5N1. Therefore, it was calculated that the likelihood of an individual DOC being infected and introduced into West Timor was \( 9.8 \times 10^{-8} \) (95%CI: \( 6.3 \times 10^{-9}, 3.1 \times 10^{-7} \)).

8.3.2.2 Probability of Local Chickens Being Exposed

This node describes the probability of the local chickens being exposed by DOC imported from outside the area (in this case from Java). Based on expert opinion, the probability of the chickens in West Timor being exposed (having contact) to imported chickens was 0.14 (95% CI: 0.03, 0.27).

8.3.2.3 Probability of Backyard Chickens Being Infected

This node explains the probability of chickens (backyard chickens) being infected with H5N1. The probability of backyard chickens being infected by H5N1 was \( 6.9 \times 10^{-5} \) (95%CI: \( 4.3 \times 10^{-5}, 9.6 \times 10^{-5} \)).
8.3.2.4 Probability of Detecting Infected Chickens

This node describes the probability of infected chickens being detected by animal quarantine officers and/or veterinarians. This study found that the probability of infected chickens being detected was 0.82 (95%CI: 0.72, 0.93) based on the opinions of the surveyed experts.

8.3.2.5 Probability of Confiscation

This node describes the probability of infected chickens being confiscated upon detection. This study found that the probability of chickens being confiscated was 0.97 (95%CI: 0.92, 0.99) based on the opinions of the surveyed experts.

8.3.2.6 Probability of Confiscated Chickens Being Destroyed

This node describes the probability that infected chickens are destroyed after being confiscated. This study found that the probability of chickens being destroyed by quarantine staff was 0.94 (95%CI: 0.92, 0.95) based on the opinions of the experts.

8.3.2.7 Probability of Veterinarians Collecting Samples

This node describes the probability of local government veterinarians collecting samples of chickens in West Timor. The study found that the probability of veterinarians collecting samples was 0.84 (95%CI: 0.74, 0.92) based on the opinions of surveyed experts.

8.3.2.8 Probability of Veterinarians Testing Samples

This node describes the probability of local government (veterinarians) testing samples of chickens in West Timor. The study found that the probability of vets testing samples was 0.85 (95%CI: 0.81, 0.88) based on the opinions of the surveyed experts.
8.3.2.9 Probability of Infected Chickens Infecting Other Chickens and Causing Further Outbreaks

This node describes the probability of infected chickens infecting other chickens and causing further outbreaks in West Timor. The study found that the probability of infected chickens infecting other chickens was 0.005 (95%CI: 0.001, 0.008)

8.3.2.10 Probability of Having an Outbreak of HPAI

This node describes the probability of the chicken population having an outbreak of H5N1 when the virus enters and chickens are exposed to it. Using a nested formula in Excel, summing all probability values in the scenario tree branches that would lead to an outbreak, the probability of one chicken leading to an outbreak of H5N1 was estimated at 0.00009 (95%CI: 0.00000001, 0.00064).

By using this value, the probability of n imported chickens that had contact with local chickens causing an outbreak was calculated using the Binomial method as described by (Cameron and Baldock, 1998) (Equations 8.2 to 8.4). The results show that the probability of local chickens being exposed to no infected chickens and hence the probability of an outbreak not occurring in West Timor was 0.999910004 (95% CI: 0.999857209, 0.999962799). Therefore the probability of at least one infected chicken resulting in an outbreak in the area in a year (=1-0.999910004) was calculated as 0.000089996 (95%CI: 0.000037201, 0.000142791).

8.3.3 Sensitivity Analysis

8.3.3.1 Parameters of Risk of Entry, Exposure and their Consequences

After conducting a sensitivity analysis eight parameters had a positive correlation on the probability of an outbreak of HPAI H5N1 into West Timor. These were: the probability of importing infected DOC P(Inf1); the probability of importing asymptomatic infected
chickens $P(\text{Asyminf})$; the number of DOC imported; the probability of local chickens getting infected $P(\text{Inf2})$; the probability of infected chickens infecting other chickens $P(\text{Inf3})$; the probability of veterinarians collecting samples of sick or dead chickens $P(\text{Vecosa})$; and the probability of veterinarians testing samples of sick or dead chickens $P(\text{Vetest})$. However, only one input parameter (the probability of importing infected DOC - $P(\text{Inf1})$) had a high positive correlation on the probability of an outbreak (Figure 8.3). Three parameters had a negative correlation on the probability of an outbreak of HPAI H5N1: the probability of detection $P(\text{Det})$; the probability of confiscation $P(\text{Conf})$; and the probability of live chickens being destroyed $P(\text{Dest})$. All of these parameters had a low negative correlation on the Spearman correlation test (Figure 8.3).

![Figure 8.3](image-url)  
Figure 8.3 Sensitivity analysis results of the input parameters to the probability of a HPAI outbreak.
8.4 Discussion

8.4.1 Risk of Entry of HPAI H5N1

8.4.1.1 Probability of Infection in DOC

This is the first quantitative risk assessment undertaken for the introduction and transmission of HPAI H5N1 into the chicken population of West Timor via imported live DOC. The current study demonstrated that the risk of virus being introduced through legally imported DOC was very low (9.8x10^-8; 95%CI: 6.3x10^-9, 3.1x10^-7).

The movement of live poultry, predominantly through trading, has been shown to play an important role in the spread of H5N1 (Sims et al., 2005; Olsen et al., 2006). Introduction of live poultry into West Timor is mainly driven by the demand for DOC from Java for the small broiler industry. DOC for replacement layers and broilers in West Timor had also previously been sourced from Bali, however to reduce the likely introduction of AI, the importation of DOCs into NTT was restricted in 2004 to DOC originating from only one breeding farm located in the Surabaya region of Java. This farm was selected because of its high level of biosecurity and management (Personal Communication – Dr Maria Geong, Former Head of Animal Health Department-Provincial Dinas Peternakan NTT, 10 June 2016). The need to import DOC from Java, rather than acquire them locally, is due to the lack of a parent stock supplier in NTT large enough to supply the local demand. In 2014, the number of DOC imported from Java was, on average, 104,090 per month (data from Livestock Services of NTT, 2015).

The importation of DOCs into West Timor offers both advantages and disadvantages for the local poultry industry. Imported DOCs help meet the demand for poultry meat in the region and provide access to a genetically superior, in terms of growth rate and feed conversion rate, line of birds as compared with local (village) chickens. However, the importation of chickens could result in the introduction of new diseases, including
HPAI H5N1, which could have a devastating effect on the local industry (Magalhães et al., 2010; Van Kerkhove et al., 2009). Restricting importations to DOC from high biosecurity farms helps minimize this disease importation risk (OIE, 2007). In addition, commercially produced DOC are less likely to be infected with H5N1 viruses when they leave the incubator than are older birds, given the limited likelihood that eggs from infected hens would hatch and the poor prospects for survival of exposed virus at 37°C in incubators (FAO, 2016). However, there is the possibility that birds may have had contact with contaminated fomites prior to or during transportation (Drh. Budi Kusworo, Staff of Animal Health Department of Provincial Livestock Services of NTT, Personal Communication, 16 June 2015). To minimise this risk DOC are only sourced from the one high biosecurity farm in Java and chickens are isolated in their travelling cartons from all other poultry during transport.

8.4.1.2 Probability of the Detection and Confiscation of Infected Chickens.

Quarantine has been recognised by others to play an important role in the strategic control and prevention of both mild and highly pathogenic AI (Swayne and Suarez, 2000; Pittman et al., 2007). Early warning, detection and response to outbreaks have been identified as critical in the prevention and control of HPAI (Tiensin et al., 2005). In West Timor, examination of DOC is performed by Animal Quarantine Officers on arrival of these birds at Kupang airport. If infected chickens were detected, they would be confiscated and subsequently destroyed, which helps to minimize the probability of HPAI H5N1 entering West Timor. In the current study it was estimated that the Animal Quarantine Section could detect 82% of infected DOC. However this does mean that there is an 18% chance that the officers will fail to detect disease, if present, and virus could potentially enter the region. The study also found that the probability of infected chickens being confiscated was high at 0.97 (95%CI: 0.92, 0.99).
Furthermore although birds could be infected with HPAI virus they would be unlikely to develop clinical signs until 4 to 7 days post-infection (Hulse-Post et al., 2005), and sometimes may be asymptomatic (Aviaire, 2014). Consequently it is extremely unlikely that DOC will display detectable clinical signs of HPAI. Fortunately importation of DOC from a flock tested negative for HPAI and with high biosecurity status, and controlled movement of the birds from the originating farm to Kupang greatly reduces the likely introduction of virus to the region.

It is important that the veterinary authorities, including quarantine, laboratories and animal health centres, are well equipped in order to detect and diagnose a disease incursion at the borders, ports and in the field of West Timor. Webster et al. (2006) highlighted that many of the countries in South East Asia affected with HPAI have weak veterinary infrastructure and this needs strengthening for the early detection and control of the disease. Additionally, veterinary services in endemically infected countries often have insufficient capacity to identify and respond to all cases of infection due to: limited numbers of adequately trained staff; a lack of guidance and direction to provincial and district animal health and production services from the central (national) government levels; and an inadequate budget for animal health from local authorities (FAO, 2011a).

This study only examined the risk of disease introduction through the legal importation of DOC, similar quantitative risk assessments should be conducted to evaluate the risk of disease introduction through illegal imports, as was undertaken in Timor Leste (Cardoso, 2011) or through wild bird movements as was undertaken in northern Australia (Curran, 2012).
8.4.1.3 Probability of Destruction of Infected Chickens.

In relation to the destruction of infected chickens, elimination of the source of infection, including infected birds, is believed to be the most effective infection control measure for H5N1 (de Jong and Hien, 2006). Destruction of affected birds played a key role in the successful eradication in Hong Kong, Canada and the Netherlands (Tweed et al., 2004; Chan, 2002; Koopmans et al., 2004). However, the implementation of culling in Indonesia has been accompanied with other measures, including vaccination and improved biosecurity measures (Sumiarto and Arifin, 2008; OIE, 2016). In West Timor, during the outbreak of HPAI in 2004 to 2006, selected slaughter was applied on affected farms as outlined in Chapter Four. Rapid destruction and disposal of affected and high-risk in-contact birds minimises the potential for virus to spread and infect other susceptible birds and other animals (Hinrichs et al., 2006).

8.4.2 Risk of Exposure and Establishment

From the scenario tree, it can be seen that if infected chickens were introduced to the island, then chickens that were not destroyed would be released with local chickens potentially resulting in disease in local birds. The result of the study found that the probability of local chickens being exposed to the imported chickens was 0.14 (95% CI: 0.03, 0.27). As HPAI H5N1 is a contagious viral disease (de Jong and Hien, 2006; Swayne and Suarez, 2000) and chickens in a naïve population are highly susceptible to the disease (Martin and Cameron, 2002), contact between infected and susceptible chickens is likely to result in a disease outbreak (de Jong and Hien, 2006).

The probability of local poultry being exposed to or having contact with imported birds is dependent on whether the newly introduced chickens are separated or released with the existing population. Most households in West Timor do not separate newly acquired chickens from their existing flock, with only 15% (95%CI: 8.6, 23.5) of households
always separating newly purchased chickens from their existing birds (Chapter 6). This practice poses a risk for introducing HPAI into the existing poultry population (OIE, 2007). The risk of introducing a virus to poultry or other birds can be reduced by good biosecurity and hygiene, which includes minimising contact with other domesticated poultry (Aviaire, 2014).

Once local chickens are exposed to the virus, it is highly likely that they will become infected if they are susceptible to HPAI, especially if they have not been vaccinated against the disease. H5N1 outbreaks were reported in unvaccinated chicken farms in late December 2002 and January 2003 in Hong Kong (Ellis et al., 2004b). However in the current study the probability of local chickens being infected by imported infected DOC was extremely low at 6.9x10^{-5} (95%CI: 4.3x10^{-5}, 9.6x10^{-5}) and the probability of an outbreak occurring through the importation of DOC in a year was less than one in 10,0000 (8.9996 x10^{-5}; 95%CI: 3.7201 x 10^{-5}, 1.42791 x 10^{-4}).

Sample collection and subsequent laboratory investigation of suspected cases are important components of a surveillance system in order to detect disease and for future control and eradication programmes at the regional and national levels (OIE, 2014a). The need to control AI has been highlighted to minimise the potential for a pandemic to occur, to retain the profitability of the local poultry industry, and to guarantee food security in developing countries (Capua and Marangon, 2006). Farmers and veterinarians represent the first line of defence against the pandemic threat and for the prevention and control of this infection in poultry (Capua and Marangon, 2007a). In Indonesia, government veterinarians are responsible for data collection and epidemiological and laboratory investigation of animal diseases, including AI. Although the results of this study revealed that the probabilities of veterinarians collecting and then testing samples were high (0.84, 95%CI: 0.74, 0.92; 0.85, 95%CI: 0.81, 0.88,
respectively), there is still room for improving these values. Critical to this is an understanding of the constraints for the control of HPAI in Indonesia, particularly arising from insufficient human resources (veterinarians and veterinary assistants) and funds, and the lack of movement control of birds during outbreaks (Bappenas-National Agency for Plannings and Developments, 2005).

8.4.3 Impact of the Input Parameters to the Probability of Outbreak

The sensitivity analysis conducted as part of this study demonstrated that, not surprisingly, the probability of importing infected DOC or $P\text{(inf1)}$ had the greatest impact on the likelihood of an outbreak in West Timor. In order to minimize $P\text{(inf1)}$, imported DOC must only be sourced from farms that apply a high level of biosecurity measures and which are also known (tested to a high level of confidence) to be free from HPAI. Strict quarantine during the importation process needs to be maintained to minimise the potential for DOC to have contact with infected/contaminated fomites or birds, particularly asymptomatic infected chickens (Chan, 2002). Parameters such as the detection, confiscation and destruction of birds were found to have a negative correlation on the likelihood of an outbreak. Increasing the probabilities of detecting, confiscating and destroying affected birds would result in a further reduction in the likelihood of HPAI entering West Timor and have been described as key components of a control strategy for avian influenza (Swayne and Akey, 2005).

8.4.4 Communicating and minimizing the risks of introducing HPAI H5N1 into West Timor

Stakeholders, including local governments, importers, quarantine offices, local livestock services and farmers need to be informed about the likelihood of the disease entering and causing an outbreak in West Timor through the movement of poultry for trade. Di Giuseppe et al. (2008) highlighted the need for educational programs in the
dissemination and widespread adoption of preventive measures. Information and education campaigns are popular policy tools for increasing public awareness (Yakhshilikov et al., 2009). Along with education, it is important to carry out routine monitoring, surveillance and diagnosis in all ports in Kupang and the borders with Timor Leste in order to increase the likelihood of detecting disease incursion and to keep West Timor free from HPAI H5N1. Enhanced biosecurity has been used as the first line of defence against AI and has been reported to be highly successful in the prevention and control of the disease on commercial farms throughout the world (Halvorson, 2002). The potential for distribution of H5N1 virus once it has entered West Timor is high as live chickens are extensively traded across the island and there is a general low level of adoption of on-farm biosecurity (Chapter 6). In order to minimise the transmission of virus into the area strict quarantine is required and the processes adopted in West Timor for the importation of DOC sourced only from high biosecurity, low risk farms for HPAI plays a key role in significantly reducing the risk of virus introduction through this pathway (Kung et al., 2007).

8.5 Conclusions

This study has analyzed parameters likely to impact on the probability of an outbreak of HPAI in West Timor through the importation of live chickens (DOC) from Java. The study identified that only one factor, the probability of infection of HPAI H5N1 in DOC in Java island P(Inf1), had a large impact on the outcome of the model. The risk of having an outbreak in West Timor through imported DOC is highly dependent upon the prevalence of the disease in Java, where the disease is now endemic. To reduce the risk of disease introduction, several factors need to be considered on-farm in West Timor including avoiding exposure of new DOC to existing chickens on a farm and
undertaking surveillance (sample collection and testing) on a regular basis, particularly targeting the newly introduced DOC and in-contact birds.

Based on the findings from this study it is recommended that the local government should communicate the risk of HPAI H5N1 entering the island through dialogue with stakeholders, especially importers and other persons involved in the poultry business, to reduce the likelihood of a future outbreak.
CHAPTER NINE
Discussion, Conclusions and Recommendations

9.1 Discussion

9.1.1 Introduction
This study was designed to: validate the free status of West Timor from HPAI H5N1; analyse the pattern of movements of poultry and poultry products through a market chain analysis; determine the potential impact of the disease on farmers and the poultry industries if the disease entered; identify potential risk factors that may be associated with the introduction and transmission of the disease; and develop plans for suitable prevention programs for HPAI. The information obtained from the study will provide valuable information on the likely route of HPAI introduction to the region enabling appropriate preventive measures to be initiated and targeted surveillance programs developed.

9.1.2 History of HPAI H5N1 in West Timor
Based on phylogenetic analysis, it has been suggested that a single introduction of H5N1 clade 2.1 into Indonesia (Java) occurred around November 2003 through either the migration of wild birds or from the trade in poultry or their products (Kilpatrick et al., 2006; Smith et al., 2006; Olsen et al., 2006; Lam et al., 2012). The virus was believed to have originated from southern China where it was first observed in 1996 (Smith et al., 2006; Martin et al., 2006). After the initial outbreak of disease in Indonesia, the virus spread to 27 provinces in Indonesia by June 2006 and the disease is now considered endemic in the country (Lam et al., 2008; Daniels et al., 2013; FAO, 2011b). Smith et al. (2006) suggested that within Indonesia, H5N1 was most likely
transmitted through the movement of poultry rather than via bird migration. These authors suggested that this route resulted in the entry of the virus into West Timor in 2004. This belief is supported by the observation that commercial poultry were sourced from Java (Christie, 2007; Sumiarto and Arifin, 2008). In West Timor, the virus resulted in the culling of more than 8,000 chickens in the City of Kupang during a two week period in 2004 (Lelin, 2007). The disease was subsequently reported in five districts in West Timor, with Kota Kupang being the most affected district with a prevalence of 21.4% (95%CI: 18.6, 24.5) (Chapter 4).

In response to outbreaks of avian influenza in NTT, the provincial government launched the program “Nine steps to avian influenza prevention”. This program included aspects of: enhancement of biosecurity; selective depopulation in infected areas; vaccination; replacement of stock (restocking) with disease free animals; culling of affected birds in newly infected areas/flocks; control of the movement of poultry, poultry products, poultry waste and vaccines; traceability and surveillance; public awareness; monitoring, reporting and evaluation (de Fortuna, 2007). This program was designed to eradicate the disease from NTT. Based on the results of the retrospective study reported in Chapter 4, no cases of H5N1 were reported in the province during the period from 2007 to 2013. In addition, the results of the testing of samples described in Chapter 5 in 2013 provided further evidence to support the freedom of disease in the province.

9.1.3 Veterinary Services and Government Regulations, Husbandry and Biosecurity Measures Adopted by Households Owning Poultry in West Timor.

Veterinary services, including infrastructure, laboratory capacity and personnel (veterinarians), play a key role in the prevention and control of animal diseases, such as HPAI H5N1 (Jebara, 2004). Veterinarians working for the poultry industry or for the public sector represent the first line of defence against the pandemic threat of HPAI and
for the prevention and control of infection in both poultry and wild birds (Capua and Alexander, 2006; Chaddock, 2006). Raising public awareness of HPAI H5N1, accompanied with frequent disinfection of farms, monitoring livestock movement and control of the importation of poultry and eggs, can decrease the impact of a number of viruses during a surveillance program (Amosin et al., 2006; Chutinimitkul et al., 2007; Buranathai et al., 2007). Jordan (1990) described that good husbandry practices should include providing adequate feed, suitable housing, an appropriate stocking, good ventilation, the appropriate disposal of waste materials, and the cleaning and disinfection of the premises to minimise the risk of infections entering an establishment and spreading. In relation to housing, most households in this study (90.3%) allowed their village chickens to range freely in the daytime, although the birds were caged at night. Free roaming poultry would allow potential contact with other poultry or wildlife, in particular wild birds, and increase the risk of introducing AI into a flock or region, as has been found in other parts of Asia, Europe, Africa and Australasia (Alexander and Brown, 2009; Alexander, 2007b; Songserm et al., 2006c; Gilbert et al., 2006).

In this study over half (61%) of the interviewed households fed their poultry kitchen leftovers (Chapter 6). Feeding chickens with leftovers can be a risk for the transmission of AI through contaminated feed (de Jong and Hien, 2006). However offset against this risk is the low cost associated with this source of food (Aini, 1990; Sonaiya, 1995), which is a valuable source of nutrition for poultry available to subsistence farmers (Mwalusanya et al., 2002).

Contaminated water sources have also been shown to result in transmission of AI as the virus can survive for up to three days in water at 28°C with salinity of 30 parts per thousand (ppt) (Brown et al., 2007). Most households interviewed in the current study sourced water for their poultry from tap water, with only 3% using rainwater.
Contaminated rainwater can result in infection (Hinshaw et al., 1980; Hinshaw et al., 1979) and minimising the contamination of this water source from bird faeces is critical in reducing the risk from this source.

Introduction of HPAI H5N1 into a flock or region can occur through both direct and indirect contact. In particular the movement of infected poultry, contaminated equipment, fomites or vehicles plays a key role in disease spread. However implementing a biosecurity plan at the farm level can minimise these risks (Capua and Marangon, 2007a). Currently biosecurity, particularly in village chickens, in West Timor is weak resulting in the risk of disease introduction. Therefore, it is important to design appropriate biosecurity and preventive disease control programs that can be adopted at a farm level, as well as at a regional and provincial level (Capua and Alexander, 2008).

Vaccination has been shown to be a powerful tool to support eradication programmes when used in conjunction with other control methods by increasing the resistance of the birds to field challenge, reducing the level of virus shedding in vaccinated birds and influencing the transmission dynamics of the disease (van der Goot et al., 2005a; Capua et al., 2003a). The use of vaccination in the control of AI has been recommended by some authors, particularly if the disease is endemic, with the need for establishing vaccine banks to enable effective control programs (Capua and Marangon, 2003). However, the use of vaccines for H5N1 in West Timor is prohibited as the island is considered free from the disease (Dr. Maria Geong, Personal communication, 10 May 2016) and while it remains free the use of vaccination in flocks would be questionable other than to protect birds from a future outbreak of AI virus cross-reactive with the subtype in the vaccine.
Control of the disease through stamping out measures has been successful in some countries, although not in others. Stamping out was used to successfully eliminate the disease from Japan, Malaysia and South Korea and also in West Timor (Dinas Peternakan of NTT, 2005), however it has not been successful in China, Egypt and Vietnam (FAO, 2011a). The successful eradication of HPAI from West Timor was linked to several factors including: West Timor is a small island compared to Java, making logistical operations easier; the outbreak in West Timor affected a smaller number of chickens than outbreaks on other islands of Indonesia, resulting in less funds being required to compensate farmers for culled birds; and only a small number of chickens were imported each month into the island (100,000 - Chapter 8) allowing better control in preventing future disease introduction through effective quarantine services. Furthermore the local government in West Timor adopted intensive monitoring and evaluation; targeted investigation and surveillance of avian infectious disease with the expectation that the Provincial Dinas Peternakan would investigate all instances of avian infectious disease in the province; and raising awareness of the disease in both the general population and those associated with the poultry industries (Ratnawaty et al., 2005).

Animal movements and disease transmission are strongly correlated and control of animal to animal transmission has been regarded as a key feature in the epidemiology of infectious diseases (Fèvre et al., 2006). As the movement of poultry and people in West Timor is significant (Chapter 7), it is important to design appropriate movement controls for both the legal and potentially illegal poultry movements that may occur (Fèvre et al., 2006; Gilbert et al., 2006). For legal movement, it is important to enhance biosecurity, surveillance and monitoring, along with the culling of infected poultry and providing a suitable compensation for destroyed birds and property. Proper hygiene, use
of personal protective equipment (PPE) and improved biosecurity on farms have also been recommended to be adopted to control disease (Apisarnthanarak and Mundy, 2008; Dowd et al., 2013). However implementing sanitation and wearing PPE are challenging to adopt in subsistence farming systems, especially in the tropical climates (Yupiana et al., 2010). Other control measures proposed by FAO for Indonesia were aimed at supporting the development of a comprehensive program regarding surveillance, prevention and outbreak control in villages, commercial farms and markets through local government and private sector based initiatives (FAO, 2011a). These measures included: supporting healthier poultry by establishing a certification program for poultry; and strengthening veterinary services by supporting the development of a national veterinary service and building the capacity of animal health services to effectively address zoonotic diseases of concern, including HPAI H5N1. It has been highlighted by the OIE that countries with well-developed veterinary services with strong early disease detection and response capacities can effectively control and eliminate HPAI H5N1, on the other hand, those with weak veterinary capacities and those that face major risk factors, such as high poultry population densities with poor biosecurity, would have difficulty in achieving effective control (OIE, 2007).

The illegal movements of poultry and poultry products across local, regional and international boundaries, which is predominantly driven by price differential, has been linked with large-scale transboundary epidemics of HPAI during the last two decades (FAO, 2001; FAO, 2011a; Cardoso, 2011). To overcome such movements, it is recommended that border controls are strengthened in collaboration with neighbouring provinces and countries, sufficient well trained quarantine officers are employed and the quarantine facilities are well equipped in order to rapidly detect any suspected infection or disease incursion.
This study found that most of the interviewed households in West Timor believed that their neighbour’s poultry were a risk for disease introduction to their own flock. This highlights the frequent contacts that occur between poultry originating from different households through free roaming poultry. Between-farm contacts (both commercial and non-commercial farms) have been identified as a key reason for the spread of HPAI (Fiebig et al., 2009; Le Menach et al., 2006). However such contacts would almost be impossible to prevent in a free-roaming subsistence system adopted by most farmers in West Timor. A more effective means for preventing disease entry would be to treat West Timor as a single entity/enterprise and implement effective quarantine on the entry of new poultry to the region as a whole. The FAO recommended zoning was an effective method to maintain international market access for an infected country by focusing control strategies on infected zones and establishing disease free zones (FAO-OIE, 2005). In addition, the FAO and OIE, in collaboration with the WHO, have developed a global strategy for the progressive control of HPAI, where it was suggested that public awareness was critical in promoting practices to limit the risk of the transmission of HPAI and to reduce the risk of exposure of humans to the virus (FAO et al., 2007). These organizations recommended a global strategy for the prevention and control of HPAI H5N1 which focuses on supporting countries in planning and implementing their plans for HPAI prevention and control, including surveillance, diagnosis, epidemiology, management of disease information and improved veterinary services.

In relation to raising public awareness, in this study most households acquired information about HPAI through television. This fact can be useful when developing health education programs, ensuring that the suitable vehicle is used in dispersing the message and it is developed in a way that households can understand. Raising
awareness of risk factors for HPAI H5N1 in backyard poultry farmers has been shown to help reduce disease transmission in Bangladesh (Biswas et al., 2009). It was suggested that in order to effectively communicate with the rural community and producers, awareness campaigns should incorporate training materials appropriate to the socio-demographic and economic characteristics of the farmers (Yakhshilikov et al., 2009). Maton et al. (2007) highlighted that the knowledge, attitudes and practices (KAP) of farmers regarding AI play key roles in the control of the disease, therefore farmers must be informed about the causative agent, its mode of transmission, risk groups affected, methods to prevent transmission and control the disease including measures such as washing hands after contact with poultry, using PPE and environmental management around the house. In order to disseminate information to poultry households in West Timor, farmer groups could be used to raise the awareness of farmers about the disease. Farmer groups have been established on the island of Alor in NTT for the control of CSF and through education have resulted in a significant reduction in the deaths of pigs from CSF, as well as increased adoption of new management and improved husbandry practices (available on http://aciar.gov.au/project/ah/2004/020).

9.1.4 Social Network Analysis of Poultry Movement

The SNA study of poultry movement in West Timor identified that the movement of poultry through the market network could potentially contribute to the spread of HPAI in West Timor. It was demonstrated that the Kota Kupang network was larger than that of Belu with extensive poultry movements within Kota Kupang and from Kota Kupang to other districts in West Timor. This indicates that Kota Kupang plays a large role in the network in terms of the number of sellers and buyers. Therefore it needs to be given priority in order to prevent the entry and spread of AI in West Timor. In this study Kota
Kupang was identified as potentially a key port of entry for the disease as suggested by the results of the In- and Out-degree values of the SNA study in Chapter 7. Greater attention should be given to sellers and buyers in West Timor, as poultry movement from, to and between markets can spread disease. Sellers and buyers should be involved in an educational and training program that focuses on the prevention and control measures for the introduction of HPAI H5N1. Markets (especially LBM) play an important role in the transmission of HPAI H5N1 to other birds (Martin et al., 2011), as well as to humans (Panigrahy et al., 2002; Bulaga et al., 2003; Liu et al., 2003). Additionally, Van den Berg (2009) highlighted that informal or illegal movement of domesticated poultry, fighting cocks and wild birds is extensive in South East Asia, with this type of movement posing a significant risk of transmitting HPAI H5N1. Therefore it is very important that the movement of live poultry is monitored to reduce the spread of disease. All DOCs must be obtained only from registered/licensed importers or breeders which are known to have a high level of biosecurity and disease freedom as certified by the government.

9.1.5 The Risk of Introduction and Establishment of HPAI H5N1 in West Timor

The movement of poultry and poultry products has contributed to the spread of HPAI H5N1 (Kilpatrick et al., 2006). A case control study conducted in China revealed that the transportation of poultry and their products along highways potentially contributed to the long-distant national spread of the disease (Fang et al., 2008). A survey of HPAI in LBM in Vietnam found that most traders entered farms with their vehicles/crates, and handled birds themselves without any additional protection, therefore potentially spreading infection (Phan et al., 2013). Restricting the entry of people to flocks can reduce the risk of introducing infectious agents, including HPAI, to the flocks (Henning et al., 2009; Alhaji and Odetokun, 2011). Similar to other places in South East Asia, the
farms in West Timor are primarily backyard/village-operated settings with poor adoption and application of biosecurity measures. In this setting poultry and their products, as well as traders, are free to move in and out of the farms, increasing the potential for disease introduction and transmission (Conan et al., 2012). Movement of people can facilitate the introduction of HPAI into flocks, hence it has been recommended to restrict contact of people with flocks to reduce the risk of disease introduction (Alhaji and Odetokun, 2011; Henning et al., 2009). However, this measure may be difficult to adopt in West Timor as most buyers are relatives or friends of the poultry-owning households. Provision of foot-baths/shoe cleaning stations helps in reducing the introduction of pathogens onto poultry enterprises (Meroz and Samberg, 1995), however this would only be feasible if poultry were confined. Confinement would also offer the benefit of reducing contact between the farmer’s birds and those belonging to other farmers and wildlife, although confinement requires farmers to spend money on establishing a barrier and would require providing feed to poultry. These may be beyond the financial resources of subsistence farmers. In contrast on large commercial poultry farms biosecurity measures are generally higher (Conan et al., 2012) and the entry of people and birds is usually restricted, minimizing the probability of disease entry (Bavinck et al., 2009).

In relation to the role that wild birds play in the transmission of infectious diseases, water-birds, especially members of the Anatidae family, have been found to act as natural reservoirs for low-pathogenic AIV and be the primary source of infection in low-pathogenic AI outbreaks in Australia (Tracey et al., 2004). Experimentally, it has been shown that several species of wild birds can survive infection and shed the H5N1 virus without displaying any apparent disease signs (Sturm-Ramirez et al., 2004). Similarly several studies have highlighted the role of wild birds in the transmission of
AI including H5N1 in Hong Kong in 1997 (Claas et al., 1998a), H7N3 viruses in Australia (Forsyth et al., 1993; Westbury, 1998) and H5N2 in Italy (Capua et al., 1999). Little is known on the role of wild birds in the transmission of AI in Indonesia, especially in West Timor. Therefore, there is a need for further study, including a risk assessment, on the movement of wild birds from infected areas to West Timor. The seasonal and daily movement of birds should be confirmed, samples tested from wild birds to identify high risk species (Curran, 2012) and ecological studies undertaken to understand the potential for contacts between wild birds and domesticated chickens. This information will help expand the knowledge on the likely introduction of virus into West Timor.

**9.2 Recommendations**

**9.2.1 Education, Training, and Dissemination of Information**

Further work should be undertaken in order to develop suitable educational material for small-holder poultry owners in West Timor with materials focusing on increasing awareness of HPAI H5N1 and the adoption of husbandry and biosecurity measures suitable for village farms. Education and training should be focused on developing suitable prevention, recording and reporting systems. Poultry owners should be trained on how to restrict access to their property, prevent contact between their birds and wild birds and to practice enhanced biosecurity. They also should be trained about recording and reporting systems and advised on the importance of immediately reporting deaths in their flocks to their local veterinarian.

Farmer groups should also be formed and regular meetings held to help promote dialogue about HPAI H5N1 as well as other poultry diseases. Similar groups have been demonstrated to be successful in other regions of NTT with small-holder pig farmers.
having a reduced occurrence of CSF and improved productivity after establishing farmer groups (Christie, 2007).

As most poultry are imported from outside of the province through legal importation, it is important to monitor and evaluate the health status of the source farms in Java regularly (at least once every 12 months) in order to ensure that only healthy poultry are imported into West Timor and other islands in NTT. Highly sensitive tests should be used to ensure the disease freedom of these exporting flocks.

**9.2.2 Status of West Timor towards HPAI H5N1**

Based on the findings of the research presented in this thesis it is considered that, at the time of the study, West Timor was free from HPAI H5N1. To maintain this freedom it is recommended that: routine monitoring and surveillance be undertaken; implementation of strict control of the movement of live poultry be adopted, with poultry only being imported from certified free farms or from uninfected areas; educational programs be developed to improve the awareness of farmers about HPAI and other diseases of significance to the local poultry industry.

**9.2.3 Further Research**

Further research should be conducted in relation to the role of wild birds in the introduction and transmission of HPAI H5N1 in West Timor, in particular, and in NTT in general. A thorough understanding of the risk of disease introduction will help in the development of risk-mitigation protocols.

The illegal movement of birds has not been investigated in this study and further study is required on such movement which has previously been identified as a significant risk factor in the transmission of HPAI H5N1 in other regions and countries (Fèvre *et al.*, 2006; Tiensin *et al.*, 2005; Yee *et al.*, 2009).
9.2.4 Limitation of the Study

The potential for recall bias may have affected the responses of farmers, particularly given that they were asked to reflect on events that happened up to 12 months prior to survey.

9.3 Conclusions

This thesis has provided important information to: demonstrate the prevalence of HPAI H5N1 in West Timor between 2004 and 2006, with the disease being most prevalent in 2004; support the current free status of HPAI H5N1 in West Timor; describe the husbandry and biosecurity practices adopted by poultry households in West Timor; analyse the movement pattern of poultry through the market network of West Timor; and analyse the risk of entry and transmission of the disease into the island. Prevention of the entry of HPAI into West Timor requires involvement at many levels from the Government, market and farmers and maintaining the disease freedom of West Timor will produce economic, social and health benefits to the poultry industry and the general community.
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Appendix 1. Village Farmer Questionnaire

QUESTIONNAIRE FOR TARGETED SURVEILLANCE TO DEMONSTRATE FREEDOM FROM AVIAN INFLUENZA H5N1 VIRUS

VILLAGE FARMER QUESTIONNAIRE

Participant consent

“You have been selected to participate in this questionnaire due to your involvement with poultry in West Timor. We would like you to answer a few questions as this will help us to understand any problems related to bird flu, as well as help us understand the management and health status of poultry in West Timor. Your involvement is voluntary and you are free to pass on any question you do not want to answer or to stop the interview at any time. Everything you do say will be confidential and used for project purposes only. The questionnaire should take approximately 25 minutes. We are also collecting a blood sample from birds as we would like to see if they are healthy. Once we have this information it will allow us to understand the best way we can help farmers and their animals.”

I. GENERAL INFORMATION.

Farmer Identification No: 
Code: T for Timor
* Code for village 1-?
* Respondent No. (2 digits) 01-?
Example Code Identification No: T101 (T=Timor, 1=village 1, 01=respondent 1)
Name of Interviewer: ____________________
age: _______ years old
Interviewer’s mobile number: ____________________ Gender: M/F
Date of interview: ______/____/____ (dd/mm/yyyy)
Sub-district: _____________________ Village: _____________________
Name of farmer: _____________________
Main Occupation: _____________________
Highest education: _____________________
Number of people in the household: □ Male □ Female

Firstly, I would like to ask some questions about the number and type of animals you have in your household.

1. Do you raise any birds in your household? □ Yes □ No
2. How many head of poultry do you own now?
A. Please complete the following table

<table>
<thead>
<tr>
<th>Species</th>
<th>Total # Heads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicks (&lt;3 months)</td>
</tr>
<tr>
<td>Village Chickens</td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
</tr>
<tr>
<td>Muscovy</td>
<td></td>
</tr>
<tr>
<td>Other please specify….</td>
<td></td>
</tr>
</tbody>
</table>

B. From the number of mature female poultry in the above table, how many of them are currently laying eggs?
   a. Village chicken:............
   b. Ducks:........................
   c. Muscovy:........................

3. Why do you keep poultry/birds? (tick all that are applicable)
  ☐ For eating
  ☐ For selling birds
  ☐ For selling eggs
  ☐ For selling chicks/ducklings
  ☐ For offerings
  ☐ Keep for fighting cocks
  ☐ Keep as pets
  ☐ Other reasons: please explain:.................................

Chickens
4. Did your hens lay any eggs during the last 12 months?
   ☐ Yes ☐ No

5. If YES, approximately how many eggs did a hen lay in the last year? ....................

6. How many eggs hatched in the last 12 months?............How many chicks survived in the last 12 months?..............

7. If all your chicks did not survive, what caused the losses?
   ☐ Disease
   ☐ Predators
   ☐ Bad weather,
   ☐ accident

8. How often are chickens slaughtered for consumption?
   ☐ weekly
   ☐ monthly
   ☐ other.................(specify)

Ducks
9. Did your ducks lay eggs during the last 12 months? ☐ Yes ☐ No

10. If YES, approximately how many eggs did a duck lay in the last 12 months?
    ☐ Muscovy.................. eggs  ☐ Laying ducks................ eggs
11. How many eggs hatched in the last 12 months?

<table>
<thead>
<tr>
<th>Eggs Survive (ducklings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscovy</td>
</tr>
</tbody>
</table>

12. If all your ducklings did not survive, what caused the losses? (Tick all that are applicable)
- ☐ Disease
- ☐ Predators
- ☐ Bad weather
- ☐ Other, (specify) ……………

13. How often are ducks slaughtered for consumption?
- ☐ Weekly
- ☐ Monthly
- ☐ Other, (specify) …………………………………

Other types of animals raised
14. What other type of animals do you have in your household and how many do you have? (please complete the table below).

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Sex</th>
<th>Total # head</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Doves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E Pet birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F Dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G Cats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H Pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J Horses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K Goats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L Other, please specify…..</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

II. HOUSING.
For Interviewers: Make sure you clean your boots between farm visits.

Now, may I see where you keep your poultry?

15. Interviewer: please tick where poultry/birds are kept.

<table>
<thead>
<tr>
<th>Village chickens</th>
<th>Ducks (layer)</th>
<th>Muscovy ducks</th>
<th>Other…</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paddy field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free range in day but caged at night</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

III. HUSBANDRY PRACTICES
Now, I would like to ask some questions about how you take care of your poultry and your crops.

16. Feeding practices.
(A) What do you feed your chickens? (Tick all that are applicable)
- ☐ Premixed commercial feed
- ☐ Rice and kitchen leftovers
- ☐ Graze paddy fields
- ☐ Let them find own feed
- ☐ Other: please specify: ……………
(B) If you keep ducks (Muscovy or layers), what do you feed your ducks? (tick all that are applicable)
☐ Premixed commercial feed
☐ Rice and kitchen leftovers
☐ Graze paddy fields
☐ Let them find own feed
☐ Other: please specify: ........................

(C) If you graze ducks, please describe
a. How often (e.g. daily) do your ducks graze? ....................
b. What time of the year (e.g. whole year or certain months) do you graze ducks? ................................................
c. Can you name the village(s) where you graze your ducks?: ........................
d. Do ducks from other households or villages usually graze in the same paddy area with your ducks?
☐ Most of the time
☐ Sometimes
☐ Never
e. How do you bring your ducks to the paddy?
☐ Walk my ducks
☐ Transport my ducks in a vehicle
☐ Ducks wander by themselves

17. Water. What is the source of drinking water for your poultry?
☐ Tap water
☐ River water
☐ Own well
☐ Community well
☐ Collected rain water
☐ Other source: please specify: ........................

IV. POULTRY ACTIVITIES (Selling, Buying and Biosecurity practices)
Now, I would like to ask some questions about selling or buying poultry.
18. Selling, offering or giving away poultry/birds.
(A) How often do you sell your poultry/birds?
☐ Never, I only keep them for our own consumption
☐ Every (please specify): ....... days
☐ Every (please specify): .......weeks
☐ Every (please specify): .......months
☐ Other (please specify): ................................

(B) How often do you offer your poultry/birds?
☐ Never, I only keep them for our own consumption
☐ Every (please specify): .......days
☐ Every (please specify): .......weeks
☐ Every (please specify): .......months
☐ Other (please specify): ................................
(C) How often do you give away your poultry/birds?
☐ Never, I only keep them for our own consumption
☐ Every (please specify): ........days
☐ Every (please specify): ........weeks
☐ Every (please specify): ........months
☐ Other (please specify):............................................

(D) Where do you sell, offer or give away your poultry/birds? (tick all that are applicable)
☐ Weekly market
☐ Permanent market
☐ Poultry trader
☐ Household in same village
☐ Household in other villages
☐ Other: please specify............................................

(E) Who takes the poultry/birds to these places? ........................................

(F) If all the birds are not sold, do you bring unsold birds back to your farm?
☐ Yes ☐ No

(G) At what age do you sell, offer or give away your poultry/birds?

<table>
<thead>
<tr>
<th>Age</th>
<th>Chickens (Village)</th>
<th>Ducks (Layer)</th>
<th>Ducks (Muscovy)</th>
<th>Fighting cocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Give away</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(H) Do you ever sell, offer or give away your poultry during festivals or religious ceremonies?
  a. Sell ☐ Yes ☐ No
  b. Offer (traditional ceremony) ☐ Yes ☐ No
  c. Give away ☐ Yes ☐ No

(I) If yes, what are these (major) festivals and when are they observed?
  a. Sell at..........................................
  b. Offer at..........................................
  c. Give away at.....................................

19. Approximately how many poultry did you sell last year?

<table>
<thead>
<tr>
<th>Species</th>
<th>Average Price/head (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicks &lt;3months</td>
<td></td>
</tr>
<tr>
<td>Cockerels 3-12 months</td>
<td></td>
</tr>
<tr>
<td>Cocks &gt;12 months</td>
<td></td>
</tr>
<tr>
<td>Hens &gt;12 months</td>
<td></td>
</tr>
</tbody>
</table>
20. Poultry/bird market.
   a. Please name the sub district or village where the nearest market is located. ......................
   b. Approximately how far away is the market from your household?.....km
   c. Do you have family, neighbours, relatives or friends who work in any poultry/bird markets?
      ☐ Yes, my family members
      ☐ Yes, my neighbours
      ☐ Yes, my relatives who visit me
      ☐ Yes, my friends who visit me
      ☐ No, none of the above

   (A) Where do you usually buy new chickens, ducks or fighting cocks?
      | Chicken (native) | Ducks (layer and Muscovy) | Fighting cocks |
      | Breed myself ☐  | Breed myself ☐             | Breed myself ☐ |
      | Market ☐ where: | Market ☐ where:            | Market ☐ where: |
      | Wholesaler/ dealer ☐ | Wholesaler ☐             | Wholesaler ☐ |
      | House in same village ☐ | House in same village ☐ | House in same village ☐ |
      | House in another village ☐ | House in another village ☐ | House in another village ☐ |
      | Other:........... | Other:...........         | Other:........... |
   (B) How often do you buy new poultry/birds?
      ☐ Never
      ☐ Every (please specify): ..........days
      ☐ Every (please specify): ..........weeks
      ☐ Every (please specify): ..........months
      ☐ Other (please specify):.................................
   (C) At what age do you usually buy new poultry/birds?
      | Chicks (Native) | Ducks | Muscovy ducks | Broiler Chickens |
      | Age:          | Age: | Age: | Age: |
      | days          | days | days | days |
   (D) How do you make sure these new poultry/birds are free of disease?
      ☐ I know the seller and trust him/her
      ☐ I check the birds are healthy
      ☐ I buy from safe places
      ☐ My concern is only the price but not disease
      ☐ Other: please specify:.................................

22. Poultry replacement (what do you do when you buy new chickens or ducks).
   (A) Are all new birds kept separate from the rest of your birds upon arrival?
      ☐ Never separate (I put new birds with the rest of my birds)
      ☐ Rarely separate
      ☐ Sometimes separate
      ☐ Always separate
   (B) If you separate the new birds, for how long do you keep the new birds separate from the rest of your birds?
      ☐ Separate all the time
      ☐ Separate for first ............... days

23. Eggs.
   (A) Did you sell any chicken or duck eggs last year? ☐ Yes ☐ No
   (B) If you sold eggs last year, how many did you sell and what was the average price per egg?
      | Species     | Number of eggs sold | Price per eggs (average) |
      | Village chicken |                   |                            |
      | Duck/Muscovy   |                   |                            |
      | layer         |                   |                            |
      | others        |                   |                            |
(C) Please tick if you sell:
☐ Eggs for consumption
☐ Fertile eggs for hatching

(D) Did you lose any eggs last year?  ☐ Yes ☐ No

(E) If YES, what caused the losses?
☐ Broken
☐ Eaten by predator
☐ Stolen
☐ Other (specify) …………..

(F) Please describe where you sell your eggs: .................................................................
........................................................................................................................................
........................................................................................................................................

(G) Please tick if you buy:
☐ Eggs for eating
☐ Fertile eggs for hatching

(H) Please describe where you buy these eggs from: ...................................................
........................................................................................................................................
........................................................................................................................................

(I) How often do you consume eggs from your own poultry?.
☐ daily ___ eggs
☐ weekly ___ eggs
☐ monthly ___ eggs

(A) Do you own any songbirds?  ☐ Yes ☐ No
If yes, what species of songbirds do you have and how many?..................

<table>
<thead>
<tr>
<th>No</th>
<th>Song bird species</th>
<th>Number of species owned</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25. Poultry waste (manure, litter and feathers, other parts of poultry that are not eaten)
Please describe what you do with poultry manure and litter and feathers after birds are slaughtered?
☐ Thrown outside house, Please specify where... ................................. distance from house:......(m/km)
☐ Buried or composted
☐ Burnt
☐ Spread onto fields
☐ Spread around house garden
☐ Leave where it is
☐ Other, Please specify: ..........

26. Cleaning cages (if farmers keep poultry in cages or sheds).
(A) How often do you clean or wash your poultry cages or sheds?
☐ Every day
☐ Every week
☐ Every month
☐ Less often than once a month
☐ Never
(B) Please specify if any chemicals are used for cleaning, disinfecting or washing?.................................................................................................................................

V. WILD BIRDS.
Now, I would like to ask some questions about wild birds near your household.
27. Have you ever seen wild birds within your household or farm in the last 12 months? ☐ Yes ☐ No
   a. If yes, do you know which type of birds? Common name: .............
   b. What month(s) are these wild birds present?:.........................
   c. Approximately how many wild birds are present at a time?
      ☐ <10
      ☐ Up to 50
      ☐ 50–200
      ☐ More than 200
   d. Have you seen wild birds mix or come close (within several metres) to your poultry?
      ☐ Yes ☐ No
   e. Has there been any unusual behaviour in these wild birds e.g. sick birds or deaths
      ☐ Yes ☐ No
   f. If unusual behaviour: When was the incident ..............and please describe the incident ...............

VI. Poultry disease.
Now, I would like to ask some questions about your experience with poultry diseases
28. What are the names of common poultry diseases in your own village? List: .................................................................................................................................
29. (A) Have your poultry/birds had any of the following diseases in the last few years?
      ☐ Newcastle disease
      ☐ Other poultry/bird diseases, if known, please specify:..............
      ☐ No disease in last few years
      ☐ Do not know
      (B) If you answered Newcastle disease or other poultry/bird diseases, please explain how you knew it was Newcastle disease or another disease?
      .................................
      ...............................................................
      ...............................................................

30. Have any of your poultry/birds displayed any of the following problems in the last few years?
   a. Sudden death ☐ Yes ☐ No ☐ Don’t know
   b. Blue combs ☐ Yes ☐ No ☐ Don’t know
   c. Blue wattles ☐ Yes ☐ No ☐ Don’t know
   d. Difficult breathing ☐ Yes ☐ No ☐ Don’t know
   e. Trembling ☐ Yes ☐ No ☐ Don’t know
   f. Diarrhoea ☐ Yes ☐ No ☐ Don’t know
   If yes to Q28(A)(a) or Q29, can you recall
      a. Which year and month did the birds get sick? .................................

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b. How many were sick? Complete the following table

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Poultry Sick # heads</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicks (1-3 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cockerels* (young male 3-12 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pullets* (young hens 3 to 12 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cocks &gt;12 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hens &gt;12 months</td>
<td></td>
</tr>
<tr>
<td>Village chickens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fighting cocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

c. How many died? Complete the following table

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Poultry Sick # heads</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicks (1-3 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cockerels* (young male 3-12 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pullets* (young hens 3 to 12 months)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cocks &gt;12 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hens &gt;12 months</td>
<td></td>
</tr>
<tr>
<td>Village chickens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fighting cock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d. What were the clinical signs.................................
e. How long after you noticed your chicken(s) were sick did it (they) die?.....
f. Which birds were affected?
  ☐ chickens,
  ☐ ducks,
  ☐ other, please specify........
g. How many were sick, how many died, and how many birds never got sick? Please complete the table below:

<table>
<thead>
<tr>
<th>Species</th>
<th># Sick</th>
<th># Died</th>
<th># remained alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Village Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Broiler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other….</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

h. What did you do with the sick, dead, and the remaining live birds? Please complete the table below:

<table>
<thead>
<tr>
<th>Species</th>
<th>Sick</th>
<th>Died</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Village Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Broiler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other….</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
31. Reporting poultry diseases.
(A) If your poultry/birds got sick or died, would you report it immediately to the authorities?
☐ Yes
☐ No
☐ Don’t know

(B) If yes in (A), to whom would you report? (tick as applicable)
☐ Village livestock workers
☐ Neighbours, friends or relatives
☐ Local authority (village or community leaders)
☐ Government veterinarian
☐ Other: please specify

32. (A) Have there ever been any outbreaks of poultry disease in your village?
☐ Yes
☐ No
☐ Don’t know

(B) If yes in (23A), can you recall (as detailed as possible) when did the outbreak occur? and what happened? 

VII VACCINATION
Now, I would like to ask some questions about vaccination of your poultry/birds.
33. (A) Have your poultry/birds been vaccinated for any diseases?
☐ Yes, Please specify for what disease:____
☐ No
☐ Do not know

(B) If your birds have been vaccinated for ND are they?
☐ All vaccinated
☐ Only some vaccinated, how many......
☐ None vaccinated......
☐ Do not know

(C) If not all poultry/birds vaccinated in (B), please describe which birds were not vaccinated (e.g. ducks, chicken etc)? 

(D) If not all poultry/birds were vaccinated in (B), why not? Do not read answers. Encourage respondent to answer.
☐ Vaccines not available
☐ Vaccines not enough for vaccination
☐ Vaccinator did not come
☐ Difficult to catch birds
☐ Inconvenient to bring birds
☐ Other: please explain

(E) If poultry/birds have been vaccinated against ND, many doses/drops did each bird receive?
☐ One
☐ Two
☐ Three
☐ Other: please specify

(F) If poultry/birds vaccinated for ND, what month was the vaccination(s) carried out? (as exact date as possible) 

(G) Please indicate who performed the vaccinations? 

(H) If you vaccinated the birds yourself, where did you obtain the vaccine from? 

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VIII. DISEASE RISKS.
Now, I would like to ask some questions about your views on the risks of poultry having diseases.
34. What factors do you think pose a risk of your poultry becoming sick?
Do not read answers. Encourage respondent to answer. (Tick all that are applicable)
☐ New poultry
☐ New eggs
☐ People, equipment and vehicles entering household
☐ Wild birds near household
☐ Fighting cocks
☐ Paddy ducks
☐ Contaminated feed
☐ Contaminated water sources
☐ Neighbours’ poultry
☐ Other (please specify):

35. What do you see as necessary to prevent or control poultry disease? Do not read answers. Encourage respondent to answer. (Tick all that are applicable)
☐ Early disease detection in poultry/birds
☐ Higher compensation for culled poultry
☐ Clean feed and water
☐ More education and awareness on disease prevention
☐ Safe source of poultry/birds
☐ Vaccines more easily available
☐ Better vaccines
☐ Someone to advise me when my birds are sick
☐ Control poultry movement from infected areas
☐ Reduce contact between my birds & other households
☐ Regular visits from veterinary department
☐ Other (please specify):

IX Disease awareness
Now, I would like to ask some questions about bird flu in general.
36. Have you ever heard of bird flu?
☐ Yes
☐ No
☐ Not sure
37. If yes where have you obtained information about Bird flu? (tick all that are applicable)
☐ Village livestock workers
☐ Veterinarians
☐ Village or community leaders
☐ Radio & Television
☐ Newspapers
☐ Pamphlets/brochure/poster
☐ Neighbours
☐ Friends
☐ Family
☐ Wholesalers or dealers
☐ Other, please specify:
38. Please explain what you would do if you suspected your poultry/birds had Bird flu? Do not read answers. Encourage respondent to answer. (Tick all that are applicable)
☐ Treat myself, type of medications used........
☐ Throw dead birds away (where?):...........................
☐ Eat sick birds ourselves & share with friends
☐ Feed birds to other animals, what animals?........
☐ Give away, to who?............................
☐ Sell birds, which market?..................
39. Are you doing anything special to prevent you and your family from getting Bird flu? Do not read answers. Encourage respondent to answer. (Tick all that are applicable)

☐ Not eat poultry that fall sick or die
☐ Not eat poultry that die
☐ Eat only well-cooked poultry or eggs
☐ Bury dead poultry
☐ Burn dead poultry
☐ Wash hands with soap after handling poultry or manure

☐ Change clothes after handling poultry or manure
☐ Not letting children play with poultry
☐ Disinfect household regularly
☐ Do nothing
☐ Other, please specify:..........

Thank You very much for your participation in this questionnaire
Appendix 2. Poultry Buyer Questionnaire

QUESTIONNAIRE FOR MARKET CHAIN AND BIOSECURITY PRACTICES SURVEYS

Poultry Buyer Questionnaire

Part A: Respondent demographics
A.1 Name (not compulsory):

A.2 Year of birth: _______________ OR □ Unknown

A.3 Gender: □ Male □ Female

A.4 Address: District _______________ Subdistrict _______________
Village __________________________________________

A.5 Education - Please tick the highest year of school completed:

A.6 Primary Occupation: _______________ Secondary Occupation: _______________

Part B: Respondent Background
B.1 Are you a poultry buyer at this market??

B.2 What other animal and animal products do you buy at the market?

Part C: Purchasing live chicken
C.1 How many chickens are you purchasing today?

<table>
<thead>
<tr>
<th>Chicken Age</th>
<th>Chicken breed (circle all relevant breeds)</th>
<th>Number of chicken purchased</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>Village chicken</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commercial chicken</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>Village chicken</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commercial chicken</td>
<td></td>
</tr>
<tr>
<td>Adult chicken (hen)</td>
<td>Village chicken</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commercial chicken</td>
<td></td>
</tr>
<tr>
<td>Adult chicken (rooster)</td>
<td>Village chicken</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Commercial chicken</td>
<td></td>
</tr>
</tbody>
</table>

C.2 What months do you purchase the highest number of chickens? (Circle all relevant months)

Jan   Feb   Mar   Apr   May   Jun   Jul   Aug   Sept   Oct   Nov   Dec

OR □ Not sure (go to question C.4)

(i) Please explain WHY you think these months have higher demand for chickens:
_____________________________________________________________________
_____________________________________________________________________

C.3 During high demand periods, how many chickens do you purchase per week?

□ per week OR □ Not sure

C.4 What months do you purchase the lowest number of chickens? (Circle all relevant months)

Jan   Feb   Mar   Apr   May   Jun   Jul   Aug   Sept   Oct   Nov   Dec
Please explain WHY you think these months have lower demand for chickens:

_____________________________________________________________________
_____________________________________________________________________  

C.5 During low demand periods, how many chickens do you purchase per week?

___ per week  OR  □ Not sure

C.6 What are the chickens purchased at this market used for?

□ Home consumption
□ Keep at home on property/farm
□ Sell the chicken
□ Purchased to fill a customer order/s
□ Religious festival
□ Traditional festival
□ Other (please specify) ____________________

C.7 Why do you choose this market to purchase chickens?

□ Close to your residence
□ It has the type of chickens you want to purchase
□ The market is open on days that suit you
□ Other (please specify)

C.8 Do you purchase live chickens from another market place?

□ Yes  □ No (go to question C.9)

(i) If YES, please specify the location of the market you predominantly use other than this market:

District_________________________Subdistrict_________________________

Village_________________________Market name_________________________

C.9 What is the most common source used to purchase live chickens?

□ Market
□ Chicken farmer (purchase chickens directly from farm)
□ From another chicken seller
□ Other (please specify)

C.10 Please rank the following factors from 1-4 in order of importance when purchasing a live chicken, with 1 being not important and 4 being very important

Price ___________
Chicken health ___________
Age/Size ___________
Breed ___________

C.11 When purchasing live chickens, what time do you go to the market? (Please tick the most appropriate time period and fill in the time at the market example 7-10:30am)

Morning ________________________  All day ________________________
Lunch ________________________  All night ________________________
Afternoon ________________________
Night ________________________
C.12 Do you do anything to ensure the chickens you purchase are free from disease?

□ Yes □ No (go to Part D: Purchase of chicken meat products)

(i) If YES, please specify:

____________________________________________________________________
____________________________________________________________________

Part D: Purchase chicken meat products

D.1 How much chicken meat are you buying today?

Kg, Price per kg Rp………….

D.2 What months do you purchase the highest amount of chicken meat? (Circle all relevant months or tick below)

Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec

OR □ Not sure

(i) Please explain WHY you think these months have the highest demand for poultry meat products:

____________________________________________________________________
____________________________________________________________________

(ii) During these high demand months, how much meat do you purchase per week?

kg per week OR □ Not sure

D.3 What months have the lowest demand for chicken meat? (Circle all relevant months or tick below)

Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec

OR □ Not sure

(ii) Please explain WHY you think these months have the lowest demand for chicken meat products:

____________________________________________________________________
____________________________________________________________________

D.4 During months of low demand for chicken meat, how many kilograms do you purchase per week?

kg per week OR chicken OR □ Not sure

D.5 What is the most common source used to purchase poultry meat and other poultry meat products?

□ Market
□ Chicken farmer (purchase chickens directly from farm)
□ Other (please specify)……..

D.6 Does purchase location of live chickens or chicken meat change depending on whether it is a low or high demand period?

□ Yes □ No (go to question D.7)

(i) If YES, please specify WHY you use alternative locations:

____________________________________________________________________
____________________________________________________________________

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D.7 Which product is more suitable for specific occasions?

<table>
<thead>
<tr>
<th>Occasion</th>
<th>Live chicken</th>
<th>Chicken meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Birthday celebrations</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>2. Religious celebrations</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>3. Weddings</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>4. Traditional celebrations</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>5. Funerals</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

D.8 When purchasing chicken meat products, what time do you go to the market? (Please tick the most appropriate time period and fill in the time at the market example 7-10:30am)

- Morning: 
- Lunch: 
- Afternoon: 
- Night: 
- All day: 
- All night: 

D.9 Are you concerned if the meat you purchase is free from disease?

- □ Yes (go to section (i) below)
- □ Not sure (go to Part E: Pattern of poultry egg)
- □ No

(i) If YES, please specify why you are concerned?
__________________________________________________________________
__________________________________________________________________

Part E: Pattern of egg purchases

E.1 What poultry egg products do you currently buy at this market?

<table>
<thead>
<tr>
<th>Species’ egg</th>
<th>buy at market</th>
<th>buying price for 1 egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quail eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck eggs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.2 Do you sell eggs laid by your own poultry/birds?

- □ Yes  □ No

E.3 How often do you buy poultry eggs in this market & how many?

<table>
<thead>
<tr>
<th>Frequency of buying</th>
<th># eggs bought</th>
<th>Total bought</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other…please specify…</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
E.4 What is the lowest and highest price of eggs you buy in the last six months?

<table>
<thead>
<tr>
<th>Species</th>
<th>Lowest price</th>
<th>Highest price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck/Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.5 What months have the highest demand for poultry/chicken eggs? (Circle all relevant months or tick below)

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec

OR  □ Not sure (go to question E.6)

(i) Please explain WHY you think these months have the highest demand for poultry/chicken eggs:

_____________________________________________________________________
_____________________________________________________________________

E.6 During months of high demand for poultry/chicken eggs, how many do you buy per week?

                          kg per week, OR  eggs, OR  □ Other, please specify………

E.7 What months have the lowest demand for poultry/chicken eggs? (Circle all relevant months or tick below)

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec

OR  □ Not sure (go to Part F: poultry health)

(i) Please explain WHY you think these months have the lowest demand for poultry eggs:

_____________________________________________________________________
_____________________________________________________________________

E.8 During months of low demand for poultry eggs, how many do you buy per week?

                          kg per week, OR  eggs, OR  □ other….please specify………

Part F: Poultry Health

F.1 Are there any symptoms you are aware of that indicate that a chicken is sick?

□ Yes  □ No (go to Part F: Transportation and destination of chickens)

(i) If YES, please specify from the options below (Please tick only what the respondent states, multiple answers are possible)

□ Sudden death
□ Severe depression with ruffled feathers
□ Inappetence
□ Drastic decline in egg production
□ Edema of head and neck
□ Swollen and cyanotic combs and wattles
□ Other..please specify………

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Part G: Transportation and destination of chickens

G.1 Following purchase of live chickens at this market, where do you transport them to?

☐ your home
☐ Another market. please specify location below)

District ________________ Subdistrict __________________

Village ________________ Market name __________________

☐ Other please specify location below)

District ________________ Subdistrict __________________

Village ________________ Market name __________________

G.2 How do you transport live chickens?

☐ Car
☐ Truck
☐ Motorbike
☐ Bus
☐ Other, please specify…………………

(i) Please specify the owner of the vehicle?

☐ Own vehicle
☐ Borrowed (go to question G.3)
☐ Rented (go to question G.3)
☐ Ojek (go to question G.4)
☐ Other, please specify…………………

(ii) How often do you clean your vehicle?

☐ After each use
☐ Never (go to question G.3)

(iii) What product/s are used for cleaning the vehicle?

☐ No products are used
☐ Water
☐ Disinfectant (Karbol, Lysol, Creolin)
☐ Soap (Detergent)
☐ Other (please specify)…………………

G.3 How are the chickens restrained during transport?

☐ Not restrained
☐ Tied up
☐ In shed
☐ In bag
☐ Other, please specify…………………

Those who did NOT answer ‘IN CAGE’ go to question G.6

G.4 How often are the cage/s cleaned?

☐ After each use
☐ Never
G.5 What product/s are used for cleaning the cage/s?
- □ No products are used
- □ Water
- □ Disinfectant (Karbol, Lysol, Creolin)
- □ Soap (Detergent)
- □ Other (please specify)

G.6 Do you keep chickens at your house?
- □ Yes  □ No

(i) If YES, what is the purpose for keeping chickens?
- □ Selling for income
- □ Religious ceremonies
- □ Traditional activities
- □ Keep as a money bank source
- □ To sell when your children go to school
- □ Other (please specify) ………………………………..

G.7 Have you heard of the term biosecurity?
- □ Yes  □ No (go to section (ii) below)

(i) If YES, please specify what you believe biosecurity is:
_____________________________________________________________________
_____________________________________________________________________

(ii) If No, are any management practices put in place to reduce the introduction of disease at your home?
- □ Yes  □ Not sure (Go to Part H. Avian Influenza/Bird Flu) □ No (Go to Part H. Avian Influenza/Bird Flu)

Part H: Avian Influenza/Bird Flu

H.1 Do you know what Avian Influenza/bird flu is?
- □ Yes
- □ Not sure (Go to Part I: Slaughtering of chickens)
- □ No (Go to Part I: Slaughtering of poultry)

H.2 Do you know any clinical signs of bird flu?
- □ Yes  □ No (Go to question H.3)

(i) If YES, please specify below (Please circle only what the respondent states, multiple answers are possible)
- □ Sudden death
- □ Severe depression with ruffled feathers
- □ Inappetence
- □ Other… please specify….
- □ Drastic decline in egg production
- □ Edema of head and neck
- □ Swollen and cyanotic combs and wattles
- □ Other… please specify….
H.3 Please rate how dangerous you believe Bird flu is to your chickens using the numbers 1 to 5, with 1 being not dangerous and 5 being very dangerous. (Circle appropriate response)
   1  2  3  4  5

H.4 To your knowledge, have you ever sold a chicken at the market that showed clinical signs of bird flu?
   □ Yes   □ No (Go to question H.5)
   (i) How long ago did this occur? _______ days/weeks/months/years (please circle appropriate unit)
   (ii) What breed of chicken was sick?
         □ Domestic/village chicken
         □ Commercial breed

H.5 To what extent are you likely to report any suspect bird flu cases?
   □ Very Unlikely
   □ Unlikely
   □ Not sure
   □ Likely
   □ Very Likely
   (i) If UNLIKELY or VERY UNLIKELY, please specify WHY you would not report bird flu cases:

H.6 Please rate how important you believe biosecurity is for the control of bird flu using the numbers 1 to 5, with 1 being not important and 5 being very important. (Circle appropriate response)
   1  2  3  4  5  Not sure

H.7 Where did you get your knowledge about bird flu?
   □ Television
   □ Newspaper/book/magazine
   □ Radio
   □ Vet/Veterinary assistant
   □ Family
   □ Friends
   □ Field Extension Agent
   □ Colleagues at the market place
   □ Other (please specify)……………………
Part I: Slaughtering of chickens

1.1 Do you also slaughter chickens?
   □ Yes  □ No (go to question I.9)

(i) Please specify, are the chickens you slaughter your own or owned by someone else?
   □ Own chickens  or  □ Chickens owned by someone else

1.2 Is an inspection performed on the chicken prior to slaughter to detect any cases of disease?
   □ Yes
   □ Not sure (go to question I.3)
   □ No (go to part (ii) below)

(i) If YES, who performs this examination?
   □ Yourself
   □ Government staff
   □ Other (please specify)

(ii) If NO, why is no examination performed?

1.3 Where do you slaughter the chicken?
   □ Home
   □ Market
   □ Abattoir
   □ Other (please specify)

1.4 What do you do with the poultry meat once the animal has been slaughtered?
   □ Consumed by family
   □ Sell at market
   □ Sell to family/friends in your village
   □ Other (please specify)

1.5 What months are the highest numbers of chickens slaughtered? (Circle all relevant months or tick below)

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec
OR  □ Not sure (go to question I.7)

1.6 During months of increased slaughter, how many are slaughtered per week?

□ blank chickens per week  OR  □ Not sure

1.7 Following slaughter of chickens, which of these waste disposal methods do you use for carcass waste?
   □ Burying  □ Throw away
   □ Burning  □ Other (please specify)
   □ Feed to pigs

1.8 How often is slaughtering equipment cleaned?
   □ Cleaned following every chicken slaughter
   □ Cleaned after use
   □ Other (please specify): per day/week/month (please circle appropriate unit)
   □ Never
I.9 Do you have chickens slaughtered by someone else?

☐ Yes ☐ No (go to Completion of questionnaire)

(i) If YES please specify:

☐ Abattoir

☐ A farmer in your village

☐ By another chicken seller

☐ Other (please specify)

Completion of questionnaire: Would you be willing to provide additional information if we need to contact you for future inquiries?

☐ Yes ☐ No

Thank you very much for your participation in this questionnaire.
Appendix 3. Poultry Seller Questionnaire

QUESTIONNAIRE FOR MARKET CHAIN AND BIOSECURITY PRACTICES SURVEYS

Poultry Seller Questionnaire

Part A: Respondent demographics
A.1 Name (not compulsory): _______________________
A.2 Year of birth: __________________ OR □ Unknown
A.3 Gender: □ Male □ Female
A.4 Address: District________________ Subdistrict________________ Village_____________________
A.5 Education - Please circle the highest year of school completed:
□ School not attended or primary not completed
□ High School
□ Primary School
□ Undergraduate University degree
□ Secondary School
□ Postgraduate University degree
A.6 Primary Occupation: __________________ Secondary Occupation __________________

Part B: Respondent Background
B.1 What is your role with chickens at the market place?
□ Poultry/chicken seller at the market
□ Egg seller at the market (go to egg section)
(i) As a Poultry/chicken SELLER, please specify the following:
□ You sell your own poultry/chicken at the market
□ You are a permanent seller at the market
□ You are a Poultry/chicken collector - please specify your role:
_________________________________________________________________
□ You are a Poultry/chicken trader - please specify your role:
_________________________________________________________________
□ Other (please specify)________________________________________________________________
B.2 How long have you been a Poultry/chicken seller at this market? (Please circle unit or tick)
□ days/weeks/months/years □ Not sur
B.3 Why do you sell Poultry/chickens?

☐ Primary income
☐ Extra income
☐ Family tradition
☐ Other (please specify) ________________________________

B.4 Why do you come to this market to sell Poultry/chickens?

☐ Close to your residence
☐ Popular market place
☐ The market is open on days that suit you
☐ Other (please specify) ________________________________

B.5 Do you sell Poultry/chicken at other markets?

☐ Yes  ☐ No (go to Part C: poultry Management) (i) If YES, please specify the location of the market you predominantly use other than this market:

District __________________ Subdistrict __________________

Village __________________

Market name: ________________________________

**Part C: Live Poultry/Chicken Management**

C.1 What age group of Poultry/chicken are you selling at the market today?

<table>
<thead>
<tr>
<th>Chicken age group</th>
<th># poultry/chicken selling today</th>
<th>Poultry/chicken breed (circle all relevant breed)</th>
<th>Selling price of 1 bird (in Rp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pullet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broiler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rooster</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C.2 How are your Poultry/chicken separated into different cages/sheds at the market?

☐ They are not separated into groups
☐ Age
☐ Sex
☐ Sick and healthy
☐ Breed
☐ Other (please specify) ________________________________
C.3 Are your Poultry/chickens fed while being kept at the market?

□ Yes    □ No (go to question C.4)

(i) If YES, please specify ___________________________

C.4 How often do you clean up animal waste and faeces while keeping Poultry/chickens at the market? (Please circle appropriate unit or tick)

☐ per day/week/month OR  □ Never (go to question C.7)

C.5 Which of these waste disposal methods do you most commonly use for removal of animal waste and faeces at the market?

☐ Bury (go to question C.7)  ☐ Compost (go to question C.7)
☐ Cover waste with soil, sand or ash  ☐ Clean by hand
  (go to question C.7)  ☐ Throw away (go to question C.7)
☐ Burn (go to question C.7)  ☐ Other (please specify)

C.6 Are any product/s used for cleaning up animal waste and faeces?

☐ No
☐ Water
☐ Disinfectant (Carbol, Lysol, Creolin)
☐ Soap (Detergent)
☐ Other (please specify)

C.7 When there are unsold chickens at the end of the day, what do you do?

☐ Take them home
☐ Sell them at a cheaper price (go to question C.8)
☐ Transport them to another market (go to question C.8)
☐ Leave at market until sold (go to question C.8)
☐ Other (please specify) (go to question C.8)

(i) If you answered ‘Take them home’ are these chickens mixed with other chickens that are already kept on your property?

□ Yes    □ No

C.8 Have you heard of the term biosecurity?

□ Yes    □ No (go to section (ii) below)

(i) If YES, please specify what you believe biosecurity is:

_____________________________________________________________________
_____________________________________________________________________

Go to section (iii) below
(ii) If NO, are any management practices put in place to reduce the introduction of disease such as cleaning, vaccination or isolating new animals?

☐ Yes
☐ Not sure (go to Part D: Pattern of live chicken selling)
☐ No (go to Part D: Pattern of live poultry/chicken selling)

(iii) If YES, please specify the measures put in place at the market:

☐ Separate sick and healthy animals
☐ Clean chicken transportation vehicles
☐ Clean equipment in contact with chickens
☐ Footbath at entrance to market
☐ Vaccination (please specify)

☐ The use of gloves/masks/boots when handling chickens
☐ Other (please specify)

Part D: Pattern of Live chicken/bird Selling

D.1 What kind of poultry are you usually selling each day?

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Village chicken</td>
<td></td>
</tr>
<tr>
<td>B. Duck/muscovy</td>
<td></td>
</tr>
<tr>
<td>C. layer</td>
<td></td>
</tr>
<tr>
<td>D. Other species</td>
<td></td>
</tr>
</tbody>
</table>

D.2 Do you sell your own chickens?

☐ Yes
☐ No

D.3 If No, where did the poultry/chicken come from that you are selling today?

<table>
<thead>
<tr>
<th>Poultry age group</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
</tr>
<tr>
<td>Pullet</td>
<td></td>
</tr>
<tr>
<td>Layer</td>
<td></td>
</tr>
<tr>
<td>Broiler</td>
<td></td>
</tr>
<tr>
<td>Hen</td>
<td></td>
</tr>
<tr>
<td>Rooster</td>
<td></td>
</tr>
</tbody>
</table>
D.4 The poultry/chickens you have sold at the market over the past year, where do they come from?

☐ Your own home raised chickens
☐ Another market (please specify location below)
  District: ____________________ Subdistrict: ____________________
  Village: ____________________
  Market Name: ____________________
☐ Other farmer (please specify location below)
  District: ____________________ Subdistrict: ____________________
  Village: ____________________
☐ Other (please specify) ____________________ (please specify location below)
  District: ____________________ Subdistrict: ____________________
  Village: ____________________

D5. How often do you sell poultry in this market & how many do you usually sell each time?

<table>
<thead>
<tr>
<th>Frequency of selling</th>
<th># available for selling</th>
<th>Total sold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other…please specify…</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D6. What is the lowest and highest price you have sold poultry for in the last six months?

<table>
<thead>
<tr>
<th>Species</th>
<th>Lowest price</th>
<th>Highest price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck/Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D.7 What months do you sell the highest numbers of poultry/chickens? (Circle all relevant months or tick below)

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec

OR  ☐ Not sure (go to question D.8)

(i) Please explain WHY you think these months have higher demand for poultry/chicken:

_____________________________________________________________________
_____________________________________________________________________

(ii) During these high demand months, how many poultry/chicken are sold per week?

_______ poultry/chicken per week  OR  ☐ Not sure
D.8 What months have the lowest demand for live poultry/chicken? (Circle all relevant months or tick below)
Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec
OR  □  Not sure (go to question D.9)

(i) Please explain WHY you think these months have lower demand for poultry/chicken:
______________________________________________________________________
______________________________________________________________________

(ii) During months of low demand for live poultry/chicken, how many are sold per week?

□  poultry/chickens per week    OR   □  Not sure

D.9 What is the busiest time of day for selling live poultry/chickens at this market?
(Select appropriate time period and enter time at market place for example 7-10am)
□  Morning  □  Lunch  □  Afternoon  □  Night  □  All day  □  All night

D.10 What is the most common method you use to sell live poultry/chickens?
□  Sell chicken/poultry at market
□  Sell chicken/poultry directly from home
□  Sell chicken/poultry to another chicken/poultry seller
□  Other (please specify)

Part E: Pattern of poultry egg sales

E.1 What poultry egg products do you currently sell at this market?

<table>
<thead>
<tr>
<th>Species’ egg</th>
<th>Sell at market</th>
<th>Selling price for 1 egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quail eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck eggs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.2. Do you sell eggs laid by your own poultry/birds?
□ Yes  □ No
E.3 If No, where have you sourced your poultry egg products from over the past year?

<table>
<thead>
<tr>
<th>Species’ egg</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District</td>
</tr>
<tr>
<td>Chicken eggs</td>
<td></td>
</tr>
<tr>
<td>Quail eggs</td>
<td></td>
</tr>
<tr>
<td>Duck eggs</td>
<td></td>
</tr>
</tbody>
</table>

E.4 How often do you sell poultry eggs in this market & how many?

<table>
<thead>
<tr>
<th>Frequency of selling</th>
<th># available for selling</th>
<th>Total sold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other…please specify…</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.5 What is the lowest and highest price you received for your poultry eggs in the last six months?

<table>
<thead>
<tr>
<th>Species</th>
<th>Lowest price</th>
<th>Highest price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck/Muscovy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E.6 What months have the highest demand for poultry/chicken eggs? (Circle all relevant months or tick below)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
OR □ Not sure (go to question E.7)

(i) Please explain WHY you think these months have the highest demand for poultry/chicken eggs:

_____________________________________________________________________
_____________________________________________________________________

(ii) During months of high demand for poultry/chicken eggs, how many do you sell per week?
□…. kg per week , OR □ …eggs, OR □ Other, please specify…….

E.7 What months have the lowest demand for poultry/chicken eggs? (Circle all relevant months or tick below)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
OR □ Not sure (go to Part F: Management of poultry outside the market)

(i) Please explain WHY you think these months have the lowest demand for poultry eggs:

_____________________________________________________________________
_____________________________________________________________________

(ii) During months of low demand for poultry eggs, how much is sold per week?
□…. kg per week, OR □ …eggs, OR □ Other….please specify…….
Part F: Management Of Poultry/chicken Outside The Market

F.1 Do you look after poultry/chickens outside the market place?

☐ Yes  ☐ No (go to Part G: Poultry Health)

F.2 Where is the farm located? (Tick all appropriate answers)

☐ Your home  OR

☐ Other (please specify below)

District ______________________  Subdistrict ______________________

Village _______________________

F.3 How many poultry do you have in your herd? (Please fill in all appropriate boxes with the number of poultry in each age range)

<table>
<thead>
<tr>
<th>Age Group</th>
<th>DOC</th>
<th>Pullet</th>
<th>Layer</th>
<th>Broiler</th>
<th>Hen</th>
<th>Rooster</th>
</tr>
</thead>
</table>

F.4 What do you do with poultry that are produced outside the market place?

☐ Keep all poultry on your property for home consumption (go to Part G: poultry health)

☐ Sell live poultry

☐ Sell poultry meat products

☐ Other (please specify)

F.5 How do you house your poultry?

☐ A pen adjacent to your house

☐ Tethered next to your house

☐ Free range – poultry are free to roam

☐ Other (please specify)
F.6 What method do you use for selling your poultry produced at home?
☐ Private sale (directly from farm) (go to question F.7)
☐ Sell at the market (go to question F.7)
☐ Sell to another individual who is involved in transportation or selling of your chicken
☐ Other (please specify) (go to question F.7)

(i) Please specify the individual/s involved
☐ Another farmer
☐ A poultry collector
☐ A poultry trader
☐ A permanent seller at the market
☐ Other (please specify)

F.7 What are your poultry fed at home?
☐ Commercially prepared feed
☐ Table scraps from home
☐ Local agriculture product (please specify) (such as corn, cassava, coconut, taro)
☐ Mixed feed (combined commercial and home feed)
☐ Other (please specify)

F.8 What is the disposal method most commonly used for dead birds?
☐ Bury them
☐ Burn them
☐ Sell for consumption
☐ Throw away
☐ Other (please specify)

F.9 Are there any management practices put in place to reduce the introduction of diseases such as cleaning, vaccination or isolating new animals?
☐ Yes
☐ Not sure (go to Part G: Poultry Health)
☐ No (go to Part G: Poultry Health)
(i) If YES, please specify: (Please tick only what the respondent states, multiple answers are possible)

☐ Separate sick and healthy animals
☐ Clean poultry transportation vehicles
☐ Clean equipment and cages in contact with chicken
☐ Footbath at entrance
☐ Vaccination (please specify)
☐ The use of gloves/masks/boots when handling chicken
☐ Other (please specify)

**Part G: Poultry Health**

G.1 Are there any symptoms you are aware of that indicate that a chicken is sick?

☐ Yes  ☐ No (go to question G.3)

(i) If YES, please specify from the options below (Please tick only what the respondent states, multiple answers are possible)

☐ Sudden death  ☐ Edema of head and neck
☐ Severe depression with ruffled feathers  ☐ Swollen and cyanotic combs and wattles
☐ Inappetence  ☐ Other please specify………
☐ Drastic decline in egg production

G.2 If a chicken is sick, what is usually done?

☐ Nothing  by family
☐ Treated with medicine  ☐ Sold live for
☐ Slaughtered and meat is sold  slaughter/consumption
☐ Slaughtered and meat consumed  ☐ Other (please specify)…………..

G.3 How do you usually dispose of dead birds at the market?

☐ Bury them  location)…………
☐ Burn them  ☐ Other (please specify)…………
☐ Sell for consumption  specify)……………
☐ Throw away (please specify)

G.4 Are dead or sick poultry/chicken at the market reported to anyone?

If NOT SURE, go to Part H: Transportation of live chickens If NO, go to section (ii) below)
(i) Please specify who this information is reported to:

- Local Veterinarian
- Market Manager
- Government Officer
- Other (please specify) …………………

(ii) Why are you NOT reporting? ………………..

Please go to Part H: Transportation of live chickens/poultry

**Part H: Transportation of Live chicken**

H.1 How do you transport live birds?

- Car
- Truck
- Motorbike
- Bus
- Animal (please circle)

(i) Please specify the owner of the vehicle?

- Own vehicle
- Borrowed (go to question H.2)
- Rented (go to question H.2)

(ii) How often do you clean your vehicle?

- After each use
- _______ per day/week/month (please circle appropriate unit)
- Never (go to question H.2)

(iii) What product/s are used for cleaning the vehicle?

- No products are used
- Water
- Disinfectant (Carbol, Lysol, Creolin)
- Soap (Detergent)
- Other (please specify)

H.2 Do you use sekam (rice paddy husks) on the surface of your vehicle when transporting chickens?

- Yes
- No (go to question H.3)

(i) If YES, how do you dispose of this when you are finished transporting?

- Bury
- Burn
- Other (please specify)
- Throw away
- Re-use
H.3 How are the chickens restrained during transport?
☐ Not restrained
☐ Tied up
☐ In bags
Those who did NOT answer ‘IN CAGE’ go to Part I: Avian Influenza

H.4 How often are the cage/s or shed/s cleaned?
☐ After each use
☐ per day/week/month
☐ Never (go to Part I: Avian Influenza)

H.5 What product/s are used for cleaning the cage/s or shed/s?
☐ No products are used
☐ Water
☐ Disinfectant (Carbol, Lysol, Creolin)
☐ Soap (Detergent)
☐ Other (please specify)

Part I: Avian Influenza/Bird Flu

I.1 Do you know what Avian Influenza/ bird flu is?
☐ Yes
☐ Not sure (Go to Part J: Slaughtering of chickens)
☐ No (Go to Part J: Slaughtering of poultry)

I.2 Do you know any clinical signs of bird flu?
☐ Yes ☐ No (Go to question I.3)
(i) If YES, please specify below (Please circle only what the respondent states, multiple answers are possible)
☐ Sudden death
☐ Edema of head and neck
☐ Severe depression with ruffled feathers
☐ Swollen and cyanotic combs and wattles
☐ Inappetence
☐ Other…please specify…
☐ Drastic decline in egg production

I.3 Please rate how dangerous you believe Bird flu is to your chickens using the numbers 1 to 5, with 1 being not dangerous and 5 being very dangerous. (Circle appropriate response)
1 2 3 4 5
I.4 To your knowledge, have you ever sold a chicken at the market that showed clinical signs of bird flu?

□ Yes  □ No (Go to question I.5)

(i) How long ago did this occur? [__] days/weeks/months/years (please circle appropriate unit)

(ii) What breed of chicken was sick?

□ Domestic/village chicken  □ pure breed  □ Other (please specify) ………

I.5 To what extent are you likely to report any suspect bird flu cases?

□ Very Unlikely  □ Likely

□ Unlikely  □ Very Likely  □ Not sure

(i) If UNLIKELY or VERY UNLIKELY, please specify WHY you would not report bird flu cases:
_____________________________________________________________________
_____________________________________________________________________

I.6 Please rate how important you believe biosecurity is for the control of bird flu using the numbers 1 to 5, with 1 being not important and 5 being very important. (Circle appropriate response)

1 2 3 4 5  Not sure

I.7 Where did you get your knowledge about bird flu?

□ Television  □ Friends

□ Newspaper/book/magazine  □ Field Extension Agent

□ Radio  □ Colleagues at the market place

□ Vet/Veterinary assistant  □ Other (please specify)

□ Family

Part J: Slaughtering of chickens

J.1 Do you also slaughter chickens?

□ Yes

□ No (go to question J.9)
(i) Please specify, are the chickens you slaughter your own or owned by someone else?

- Own chickens or
- Chickens owned by someone else

J.2 Is an inspection performed on the chicken prior to slaughter to detect any cases of disease?

- Yes
- Not sure (go to question J.3)
- No (go to part (ii) below)

(i) If YES, who performs this examination?

- Yourself
- Government staff
- Other (please specify)

(ii) If NO, why is no examination performed?

J.3 Where do you slaughter the chicken?

- Home
- Market
- Abattoir
- Other (please specify)

J.4 What do you do with the poultry meat once the animal has been slaughtered?

- Consumed by family
- Sell at market
- Sell to family/friends in your village
- Other (please specify)

J.5 What months are the highest numbers of chickens slaughtered? (Circle all relevant months or tick below)

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec

OR  Not sure (go to question J.7)

J.6 During months of increased slaughter, how many are slaughtered per week?

________ chickens per week  OR  Not sure

J.7 Following slaughter of chickens, which of these waste disposal methods do you use for carcass waste?

- Burying
- Burning
- Throw away
- Feed to pigs
- Other (please specify)
J.8 How often is slaughtering equipment cleaned?

- □ Cleaned following every chicken slaughter
- □ Cleaned after use
- □ Other (please specify): per day/week/month (please circle appropriate unit)
- □ Never

J.9 Do you have chickens slaughtered by someone else?

- □ Yes  □ No (go to Completion of questionnaire)

(i) If YES please specify:

- □ Abattoir
- □ A farmer in your village
- □ By another chicken seller
- □ Other (please specify)

Completion of questionnaire: Would you be willing to provide additional information if we need to contact you for future inquiries?

- □ Yes  □ No

Thank you very much for your participation in this questionnaire.
Appendix 4. Expert Opinion: Border Police Unit

QUESTIONNAIRE FOR QUANTIFICATION OF RISK OF ENTRY

Expert Opinion: Border Police Unit

Questionnaire of experts’ opinion for modeling the likelihood of HPAI entry and establishment in West Timor

| Name of Respondent: ___________________________ |
| Position: ________________________________ |
| Address: ________________________________ |
| Date of interview: ______/____/____ (dd/mm/yyyy) |
| Phone number: ________________________________ mobile: ________________________________ |
| Fax: ________________________________ |
| Email address: ________________________________ |

1. In your opinion, approximately how many smugglers are doing business in your patrol areas?
   a. A minimum of..................
   b. An average of..................
   c. A maximum of ..................
   d. If you score yourself on the accuracy of your answer, one being the lowest to five being the highest, what would you score yourself (please circle).....1/2/3/4/5

2. In your opinion, what is the number of chickens smuggled by each smuggler?
   a. A minimum of............
   b. An average of............
   c. A maximum of............
   d. If you score yourself on the accuracy of your answer, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/

3. In your opinion, what is the probability of the BPS detecting smuggled chickens?
   a. A minimum of...............(%) of total smuggled
   b. An average of...............(%) of total smuggled
   c. A maximum of...............(%) of total smuggled
   d. If you score yourself about the accuracy of your answer, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5
4. In your opinion, what is the probability of smuggled chickens being confiscated?
   a. A minimum of.................(%) of total smuggled
   b. An average of..................(%) of total smuggled
   c. A maximum of..................(%) of total smuggled
   d. If you score yourself about the accuracy of your answer, one
      being the lowest to five being the highest, what would you score yourself, (please
      circle).....1/2/3/4

5. In your opinion, what is the probability of submitting confiscated smuggled chickens
   being submitted to quarantine?
   a. A minimum of..................(%) of total confiscated
   b. An average of..................(%) of total confiscated
   c. A maximum of..................(%) of total confiscated
   d. If you score yourself about the accuracy of your answer, one
      being the lowest to five being the highest, what would you score yourself, (please
      circle).....1/2/3/4/5

Thank-you very much for your participation in this survey
Appendix 5. Expert’s Opinion: Dinas and Quarantine Questionnaire

QUESTIONNAIRE FOR ESTIMATION OF THE RISK OF INTRODUCING HPAI INTO WEST TIMOR

EXPERT OPINION: Dinas and Quarantine Questionnaire

Qualitative estimation of the risk of introducing HPAI into West Timor for Livestock, Veterinary, and Quarantine officers Perspectives Respondent’s details

<table>
<thead>
<tr>
<th>Respondent Identification No:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of interview:</td>
<td>/</td>
<td>/ (dd/mm/yyyy)</td>
</tr>
<tr>
<td>Sub-district:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name of officer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Job title:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please note that the time period of your risk estimation is for the next 5 years.

Section 1. In this first section, we would like to know your opinion regarding the risk of an outbreak of HPAI in West Timor.

Please answer each of the following questions by ticking the most appropriate box which represents your opinion to the following statement:

Negligible (0); Very low (1); Low (2); Moderate (3); High (4); Very high (5)

1. What is the probability of Kota Kupang and each of its sub-districts having an outbreak of HPAI H5N1?

<table>
<thead>
<tr>
<th>Probability</th>
<th>Negligible (0)</th>
<th>Very Low (1)</th>
<th>Low (2)</th>
<th>Moderate (3)</th>
<th>High (4)</th>
<th>Very High (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. City of Kupang</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Individual Sub-districts in the city of Kupang</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Alak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Maulafa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Oebobo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Kelapa Lima</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. What is the probability of the District of Kupang and each of its sub-districts having an outbreak of HPAI H5N1?

<table>
<thead>
<tr>
<th>Probability</th>
<th>Negligible (0)</th>
<th>Very Low (1)</th>
<th>Low (2)</th>
<th>Moderate (3)</th>
<th>High (4)</th>
<th>Very High (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. District of Kupang</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Individual Sub-districts in District of Kupang</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Section 2.** In this second section, we would like to know your opinion regarding the likelihood of HPAI infection, survival and establishment via various pathways. Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statements:

<table>
<thead>
<tr>
<th>1</th>
<th>What is the probability of Kota Kupang and each of its sub-districts having an outbreak of HPAI H5N1?</th>
<th>negligible (0)…very high (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>What is the probability of the District of South Central Timor and each of its sub-districts having an outbreak of HPAI H5N1?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. District of South Central Timor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Individual Sub-districts in District of South Central Timor</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>What is the probability of North Central Timor and each of its sub-districts having an outbreak of HPAI H5N1?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. North Central Timor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Individual Sub-districts in North Central Timor</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>What is the probability of the District of Belu and each of its sub-districts having an outbreak of HPAI H5N1?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. District of Belu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Individual Sub-districts in District of Belu</td>
<td></td>
</tr>
</tbody>
</table>

**a** HPAI Infection
1. Legal frozen chickens imported from Java or Bali
2. Live chickens imported from Java or Bali
3. Fomites e.g. cages, egg trays carried from Java or Bali
4. Contaminated local water sources
5. Migratory wild birds transmitting infection to domestic Poultry
6. Legal Importation of eggs from Java or Bali
7. Mutation (LPAI Mutating to HPAI)

What is the probability of HPAI virus surviving in the following pathways?

**b** HPAI virus survival
1. Legal frozen chicken imported from Java or Bali
2. Live chickens imported from Java or Bali
3. Fomites e.g. cages, egg trays carried from Java or Bali
4. Contaminated local water sources
<table>
<thead>
<tr>
<th></th>
<th>What is the likelihood of the following commodities being infected or contaminated with HPAI virus before coming into West Timor</th>
<th>Negligible.....Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Migratory wild birds transmitting infection to domestic Poultry</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Legal Importation of eggs from Java or Bali</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Mutation (LPAI Mutating to HPAI)</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>HPAI surviving in legal frozen chicken importation from Java or Bali &amp; causing infection of.......</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>HPAI surviving in live chickens from Java or Bali and causing infection of............</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>HPAI surviving in Legal Importation of eggs from Java or Bali and causing infection of.............</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>HPAI surviving in contaminated local drinking water sources and causing infection of.............</td>
<td></td>
</tr>
</tbody>
</table>
### Section 3

In this third section, we would like to know your opinion regarding the possible risk of each route of HPAI introduction from overseas/ other islands into West Timor and each district in West Timor.

Please answer each of the following by ticking the most appropriate box which represents your opinion to the following statement:

**Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)**

<table>
<thead>
<tr>
<th>Path of Introduction</th>
<th>Negligible (0)</th>
<th>Very low (1)</th>
<th>Low (2)</th>
<th>Moderate (3)</th>
<th>High (4)</th>
<th>Very High (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of Introduction</td>
<td>Negligible (0)</td>
<td>Very low (1)</td>
<td>Low (2)</td>
<td>Moderate (3)</td>
<td>High (4)</td>
<td>Very High (5)</td>
</tr>
<tr>
<td>1. Natural spread via air</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Legal live poultry importation</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>3. Illegal importation of frozen chickens</td>
<td></td>
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<td></td>
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<tr>
<td>4. Legal importation of commercial poultry feed</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5. Illegal importation of commercial poultry feed</td>
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<td></td>
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</tr>
<tr>
<td>6. Illegal importation of eggs</td>
<td></td>
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<tr>
<td>7. Swill from hotels/restaurants</td>
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<tr>
<td>8. Swill from boats/air craft</td>
<td></td>
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</tr>
<tr>
<td>9. Other fomites (boots, clothes, soil, etc.)</td>
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<td></td>
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<tr>
<td>10. Other (please specify)</td>
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<tr>
<td>a. ................................</td>
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<tr>
<td>b. ................................</td>
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<tr>
<td>c. ...............................</td>
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</tr>
</tbody>
</table>

#### Part A.

If your answer for the probability of the City of Kupang having a primary outbreak of HPAI in section one was negligible (0) please do not answer this question and go to Part B.

Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statement:

**Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)**

<table>
<thead>
<tr>
<th>Path of Introduction</th>
<th>Negligible (0)</th>
<th>Very low (1)</th>
<th>Low (2)</th>
<th>Moderate (3)</th>
<th>High (4)</th>
<th>Very High (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of Kupang</td>
<td>Negligible (0)</td>
<td>Very low (1)</td>
<td>Low (2)</td>
<td>Moderate (3)</td>
<td>High (4)</td>
<td>Very High (5)</td>
</tr>
<tr>
<td>1. Legal frozen chickens imported from Java or Bali</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Live chickens imported from Java or Bali</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3. Fomites e.g. cages, egg trays carried from Java or Bali</td>
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</tr>
<tr>
<td>4. Contaminated local water sources</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Migratory wild birds transmitting infection to poultry</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>6. Imported eggs from Java or Bali</td>
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<tr>
<td>7. Mutation (LPAI Mutate to HPAI)</td>
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<td></td>
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<tr>
<td>8. Natural spread via air</td>
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<tr>
<td>9. Legal importation of live poultry</td>
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<tr>
<td>10. Illegal importation of frozen chickens</td>
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<tr>
<td>11. Legal importation of commercial poultry feed</td>
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</tr>
<tr>
<td>12. Illegal importation of commercial poultry feed</td>
<td></td>
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<tr>
<td>13. Swill from hotels/restaurants</td>
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</tr>
<tr>
<td>14. Swill from boats/air crafts</td>
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<tr>
<td>15. Other fomites (boots, clothes, soil, etc.)</td>
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<tr>
<td>16. Others (please specify)</td>
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<tr>
<td>a. ................................</td>
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<td>b. ................................</td>
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<tr>
<td>c. ...............................</td>
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</tbody>
</table>
Part B. If your answer for the probability of Kupang District having a primary outbreak of HPAI in section one was negligible (0) please do not answer this question and go to Part C.

Please answer each of the following by ticking the box that most appropriately represent your opinion to the following statement:

Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)

<table>
<thead>
<tr>
<th>1</th>
<th>What is the probability of each of the following routes being responsible for causing an outbreak of HPAI in West Timor? Districts in WT</th>
<th>Negligible (0)…Very high(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>District of Kupang</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1. Legal frozen chickens imported from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Live chickens imported from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Fomites e.g. cages, egg trays carried from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Contaminated local water sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Migratory wild birds transmitting infection to poultry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Imported eggs from Java and Bali</td>
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<td></td>
</tr>
<tr>
<td>7. Mutation (LPAI Mutate to HPAI)</td>
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<td></td>
</tr>
<tr>
<td>8. Natural spread via air</td>
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<td>10. Illegal importation of frozen chickens</td>
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<td>11. Legal importation of commercial poultry feed</td>
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<td>12. Illegal importation of commercial poultry feed</td>
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<tr>
<td>13. Swill from hotels/restaurants</td>
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</tr>
<tr>
<td>14. Swill from boats/ air crafts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Other fomites (boots, clothes, soil, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Others (please specify)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. .........................</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. .........................</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. .........................</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Part C. If your answer for the probability of South Central Timor (SCT) having a primary outbreak of HPAI in section one was negligible (0) please do not answer this question and go to section 4.

Please answer each of the following by ticking the box that most appropriately represent your opinion to the following statement:

Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)

<table>
<thead>
<tr>
<th>1</th>
<th>What is the probability of each of the following routes being responsible for causing an outbreak of HPAI in West Timor? Districts in WT</th>
<th>Negligible (0)…Very high(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>District of SCT</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1. Legal frozen chickens imported from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Live chickens imported from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Fomites e.g. cages, egg trays carried from Java or Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Contaminated local water sources</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Migratory wild birds transmitting infection to poultry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Imported eggs from Java and Bali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Mutation (LPAI Mutate to HPAI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Natural spread via air</td>
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<tr>
<td>9. Legal importation of live poultry</td>
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<td></td>
</tr>
<tr>
<td>10. Illegal importation of frozen chickens</td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>12. Illegal importation of commercial poultry feed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Swill from hotels/restaurants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Swill from boats/ air crafts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Part D

If your answer for the probability of North Central Timor (NCT) having a primary outbreak of HPAI in section one was negligible (0) please do not answer this question and go to Section 4.

Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statement:

**Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)**

<table>
<thead>
<tr>
<th></th>
<th>What is the probability of each of the following routes being responsible for causing an outbreak of HPAI in West Timor? Districts in WT</th>
<th>Negligible (0)…Very high(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District of NCT</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1. Legal frozen chickens imported from Java or Bali</td>
<td>0 1 2 3 4 5</td>
</tr>
<tr>
<td></td>
<td>2. Live chickens imported from Java and Bali</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Fomites e.g. cages, egg trays carried from Java and Bali</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Contaminated local water sources</td>
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</tr>
<tr>
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<td>5. Migratory wild birds transmitting infection to poultry</td>
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<td></td>
<td>6. Imported eggs from Java and Bali</td>
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<td></td>
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<td>13. Swill from hotels/restaurants</td>
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</tr>
<tr>
<td></td>
<td>15. Other fomites (boots, clothes, soil, etc.)</td>
<td></td>
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<tr>
<td></td>
<td>16. Others (please specify)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. .........................................................................................................................</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. .........................................................................................................................</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. .........................................................................................................................</td>
<td></td>
</tr>
</tbody>
</table>

### Part E

If your answer for the probability of the District of Belu having a primary outbreak of HPAI in section one was negligible (0) please do not answer this question and go to section 4.

Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statement:

**Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)**

<table>
<thead>
<tr>
<th></th>
<th>What is the probability of each of the following routes being responsible for causing an outbreak of HPAI in West Timor? Districts in WT</th>
<th>Negligible (0)…Very high(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District of Belu</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1. Legal frozen chickens imported from Java or Bali</td>
<td>0 1 2 3 4 5</td>
</tr>
<tr>
<td></td>
<td>2. Live chickens imported from Java and Bali</td>
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</tr>
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<td></td>
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<td>8. Natural spread via air</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9. Legal importation of live poultry</td>
<td></td>
</tr>
</tbody>
</table>
### Section 4.

In this section we would like to know what is the likelihood of villages, and poultry in each district in West Timor getting infected by HPAI. Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statement:

<table>
<thead>
<tr>
<th>Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> What is the likelihood of a village/poultry species in ....... District getting infected by HPAI?</td>
</tr>
<tr>
<td>Village in ....</td>
</tr>
<tr>
<td>a. Kupang City</td>
</tr>
<tr>
<td>b. District of Kupang</td>
</tr>
<tr>
<td>c. District of SCT</td>
</tr>
<tr>
<td>d. District of NCT</td>
</tr>
<tr>
<td>e. District of Belu</td>
</tr>
<tr>
<td>Poultry in ....</td>
</tr>
<tr>
<td>a. Kupang City</td>
</tr>
<tr>
<td>b. District of Kupang</td>
</tr>
<tr>
<td>c. District of SCT</td>
</tr>
<tr>
<td>d. District of NCT</td>
</tr>
<tr>
<td>e. District of Belu</td>
</tr>
</tbody>
</table>

### Section 5.

In this section we would like to know what is the likelihood of your involvement as government veterinarians or District Dinas Staff (DDS) response to the report from farmers about an outbreak of HPAI. Please answer each of the following by ticking the box that most appropriately represents your opinion to the following statement:

<table>
<thead>
<tr>
<th>Negligible (0) ; Very low (1); Low (2); Moderate (3); High (4); Very high (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> What is the likelihood of you as a government officer responding to a report from farmers about sick or dead chickens by .....?</td>
</tr>
<tr>
<td>Collecting samples</td>
</tr>
<tr>
<td>Doing a rapid test</td>
</tr>
<tr>
<td>Sending positive samples (on rapid test) to an Animal Health Laboratory (e.g. BBVet Denpasar)</td>
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QUESTIONNAIRE FOR QUANTIFICATION OF RISK OF ENTRY

Section A: for veterinarians only

1. In your opinion, what is the prevalence of bird flu in chickens in West Timor?
   a. A minimum of..............(%) of total imported
   b. An average of..............(%) of total imported
   c. A maximum of..............(%) of total imported
   d. If you score yourself about the accuracy of your answers, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5

2. In your opinion, what is the probability of imported chickens being released with local chickens?
   a. A minimum of..............(%) of total imported
   b. An average of..............(%) of total imported
   c. A maximum of..............(%) of total imported
   d. If you score yourself about the accuracy of your answers, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5

3. In your opinion, what is the probability of imported chickens infecting other local birds?
   a. A minimum of..............(%) 
   b. An average of..............(%) 
   c. A maximum of..............(%) 
   d. If you score yourself about the accuracy of your answers, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5

4. In your opinion, what is the probability of imported chickens being consumed/eaten?
   a. A minimum of..............(%) 
   b. An average of..............(%) 
   c. A maximum of..............(%) 
   d. If you score yourself about the accuracy of your answers, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5

Section B for quarantine staff only

5. What is the probability of you destroying imported infected chickens?
   a. A minimum of..............(%) of total confiscated
   b. An average of..............(%) of total confiscated
   c. A maximum of..............(%) of total confiscated
   d. If you score yourself about the accuracy of your answers, one being the lowest to five being the highest, what would you score yourself, (please circle).....1/2/3/4/5

Thank You Very Much for Your Participation