

**Epidemiology and Control of
Avian Influenza H5N1 Virus in Thailand**

Thanawat Tiensin

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Epidemiology and control of avian influenza H5N1 virus in Thailand

Epidemiologie en controle van H5N1 vogelgriep in Thailand
(met een samenvatting in het Nederlands)

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Thanawat Tiensin
geboren op 11 september 1972,
te Yala Province, Thailand

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These works are dedicated to my grandparents,
my parents and my family.

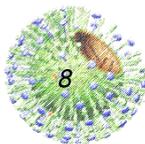
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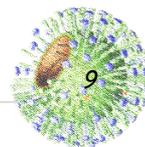
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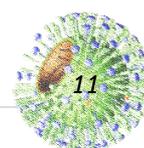


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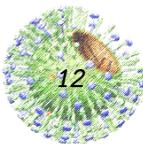
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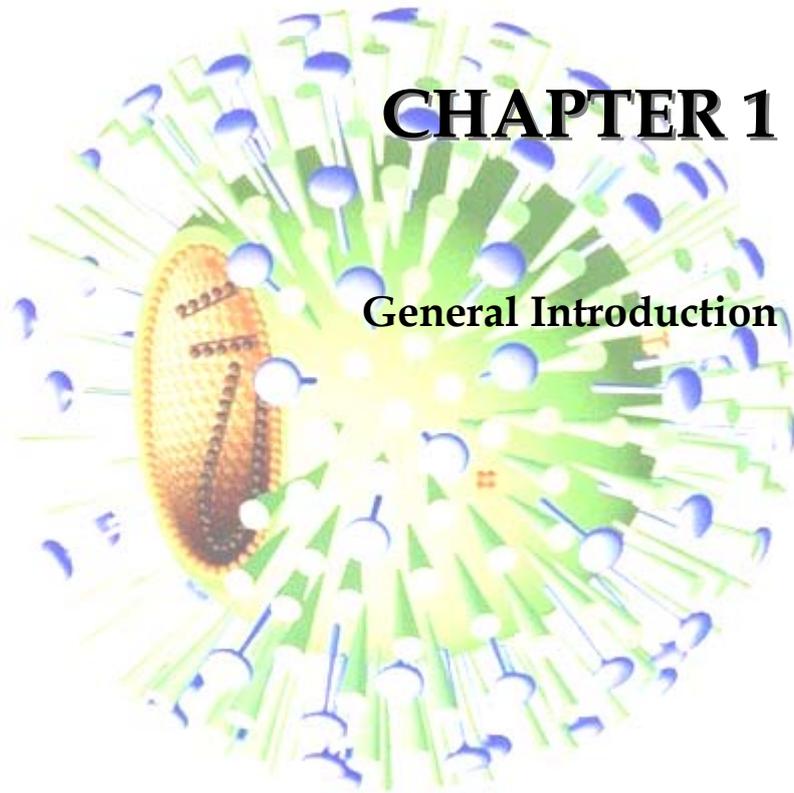




ABBREVIATIONS

AIC	Akaike's Information Criterion
AIV	Avian influenza virus
CI	Confidence interval
DLD	Department of Livestock Development, Thailand
EB	Empirical Bayes
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographical Information System
HI	Hemaagglutination inhibition
HPAI	Highly pathogenic avian influenza
LBM	Live-bird market
LPAI	Low pathogenic avian influenza
MOPH	Ministry of Public Health
NESDB	National Economic and Social Development Board
NIAH	National Institute of Animal Health, Thailand
OIE	World Organisation for Animal Health (Office International des Épizooties)
OR	Odds ratio
R_0	Basic reproduction number
RR	Relative risk
RT-PCR	Reverse transcriptase-polymerase chain reaction
RRT-PCR	Real time reverse transcriptase-polymerase chain reaction
WHO	World Health Organization





CHAPTER 1

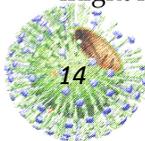
General Introduction



1. OVERVIEW OF AVIAN INFLUENZA

Avian influenza viruses (AIV) are enveloped, single stranded negative RNA viruses that belong to the *Orthomyxoviridae* family (Alexander, 2000). AI viruses are characterized, as type A by the nucleoprotein and matrix protein antigens. Type A influenza viruses can infect a broad range of hosts, including humans (Swayne and King, 2003). Avian influenza viruses of low virulence (LPAI) are perpetuated in wild birds, especially aquatic species, as well as in poultry (Alexander and Brown, 2000; Olsen et al., 2006; Webster et al., 2006). LPAI viruses mainly cause respiratory illnesses in poultry and generally result in low mortality. Upon introduction into poultry flocks, H5 and H7 subtypes may mutate to become highly pathogenic avian influenza (HPAI) (Alexander, 2007a; Munster et al., 2007; Lvov et al., 2010; Winker and Gibson, 2010). HPAI viruses can cause systemic disease in poultry (Alexander, 1995; Alexander and Brown, 2000; Capua and Alexander, 2004), resulting in severe morbidity and high mortality in poultry, restrictions of domestic and international trade of poultry products, and huge impact on farmers' livelihoods and socioeconomics (Capua and Alexander, 2004; Stegeman et al., 2004; Webster et al., 2006; Tiensin et al., 2007; Minh et al., 2009; OIE, 2011).

The reemergence of HPAI H5N1 viruses since 2003 has seriously affected the poultry industries throughout Asia (Tran et al., 2004; Tiensin et al., 2005; Pfeiffer et al., 2007; Minh et al., 2009; Basuno et al., 2010). Moreover, the wide geographic spread of HPAI H5N1 across Asia, Europe, Africa, and the Middle East has increased concern over the potential emergence of a pandemic strain that can effectively transmit among humans (Alexander, 2007b; OIE, 2011; Rios-Soto et al., 2011; Van Kerkhove et al., 2011; WHO, 2011). Several control measures were implemented in affected countries, such as stamping-out, pre-emptive culling, movement restrictions, disinfection, active surveillance, improvement of hygienic measures, restructuring of poultry production system, application of compartmentalization, and vaccination (Ellis et al., 2004; Stegeman et al., 2004; Tiensin et al., 2005; Capua and Marangon, 2006; Kung et al., 2007; Pfeiffer et al., 2007; Biswas et al., 2008; Tiensin et al., 2009; Yupiana et al., 2010). Strategies for containing an emerging avian influenza and human pandemic require controlling avian influenza at the source (Ferguson et al., 2005; Webster and Hulse, 2005), however, the effectiveness of control measures in Asia is often unclear. More knowledge of the epidemiology of HPAI in Asia might help the control and eradication of HPAI virus.



2. HPAI H5N1 EPIDEMICS

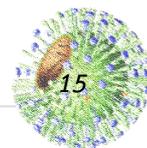
H5N1 virus was first isolated from sick geese in the Guangdong Province of China in 1996 (A/Goose/Guandong/1/96) (Xu et al., 1999). In the late 1990s, HPAI H5N1 began circulating in Hong Kong SAR. In May 1997, a 3-year-old boy in Hong Kong was infected with H5N1 virus and subsequently died from respiratory failure (Suarez et al., 1998). By the end of 1997, 18 people had been infected. The reports of early epidemic investigations of human cases demonstrated an association between infection and their exposure to live bird markets (LBMs) in Hong Kong. Then, all LBMs were closed and their poultry was culled. Subsequently, reports of human cases ceased when LBMs were closed in Hong Kong (Shortridge et al., 1998; Mounts et al., 1999; Snacken et al., 1999).

2.1 Spatial and temporal distribution after the 1997 HPAI H5N1

Since 1997, HPAI H5N1 viruses have been circulating in Hong Kong, China, and the Far East and Southeast of Asia in domestic poultry and wild birds, providing a heterogeneous gene pool for viral reassortment (Cauthen et al., 2000; Hoffmann et al., 2000; Guan et al., 2002b; Tumpey et al., 2002; Ellis et al., 2004).

In May 2001, a severe increase in mortality in chickens due to H5N1 virus was reported in Hong Kong (Sims et al., 2003). Over a million chickens were culled to halt the spread of disease (Guan et al., 2002a). However, outbreaks have occurred in poultry in Hong Kong every year since 2001, usually in the winter months, coinciding with an increase in imported poultry to meet the demand due to festive Chinese New Year (Ellis et al., 2004; Smith et al., 2006; Kung et al., 2007). In late 2002, high mortality rates were observed in free-flying wild waterfowl (geese, ducks and swans) and other captive and wild birds in two parks in Hong Kong (Ellis et al., 2004). Surveillance of avian influenza virus in waterfowl during 1999–2002 in Mainland China yielded 21 H5N1 isolates from apparently healthy ducks in Southern China (Chen et al., 2004).

In addition, in May 2001, an avian H5N1 influenza A virus was isolated from frozen duck meat that had been imported to South Korea from Mainland China (Lu et al., 2003; Tumpey et al., 2003; Mase et al., 2005). An H5N1 influenza A virus was also isolated from duck meat processed for human consumption, imported to Japan from Shandong Province, China in 2003 (Mase et al., 2005).





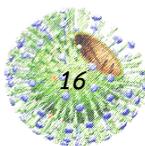
During 2001, surveillance in urban live-bird markets (LBMs) in Hanoi, Vietnam yielded several subtypes of AIV, including an H5N1 isolate from an apparently healthy goose. These results demonstrated that HP H5N1 viruses with properties similar to viruses isolated in Hong Kong and mainland China circulated in Vietnam as early as 2001, suggest a common source for H5N1 viruses circulating in these Asian countries (Nguyen et al., 2005).

2.2 Reemergence of HPAI H5N1 in 2003

In 2003, human cases of H5N1 resurfaced in Vietnam in 2003, which was the first sign of another epidemic in Southeast Asia. Since 2003, H5N1 virus has spread beyond Asia into the Middle East, Europe and Africa (OIE, 2011). Figure 1-1 shows geographical areas or countries reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds in 68 countries worldwide since 2003. From the end of 2003 to March 2011, more than 6,900 outbreaks of HPAI H5N1 in poultry were reported to the OIE from 51 countries (Figure 1-2), and other 17 countries only found H5N1 infections in wild birds.

From October 2003 to April 2011, 549 laboratory-confirmed human cases of Avian Influenza A (H5N1) from 15 countries were reported to the World Health Organization. There was a 58% case-fatality rate (320 fatal cases) of 549 documented human infections (WHO, 2011). Figure 1-3 shows geographical areas or countries reporting laboratory-confirmed cases of H5N1 avian influenza in humans since late 2003.

In addition to humans, domestic poultry and waterfowl, the infected host species for H5N1 has expanded to many species of wild birds, canines, felines and swine (Keawcharoen et al., 2004; Olsen et al., 2006; Songserm et al., 2006a; Songserm et al., 2006b; Starick et al., 2008; OIE, 2011; Siengsan-an-Lamont et al., 2011).



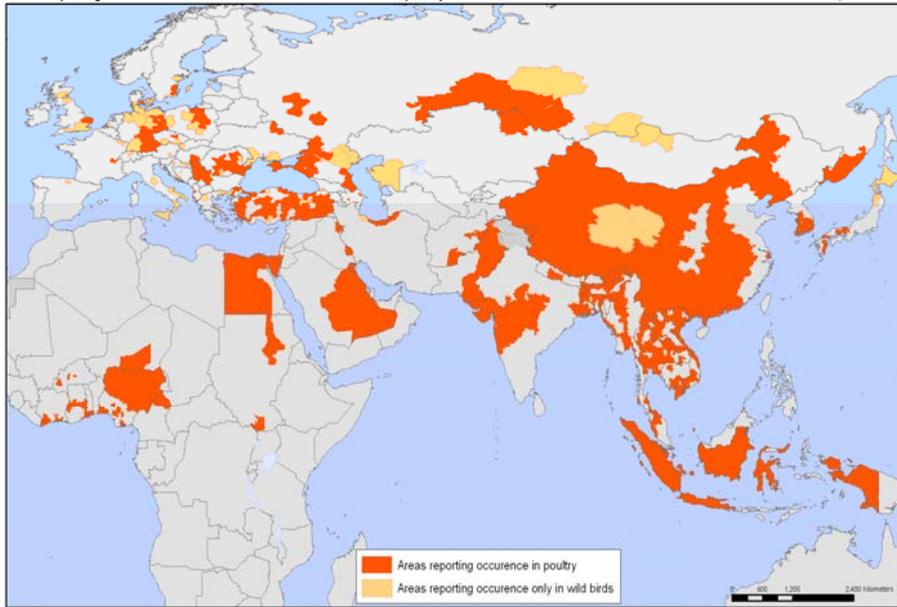


Figure 1-1. World map of areas or countries reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 2003 until 12 April 2011
Source: Modified from the World Health Organization (WHO) and the World Organisation for Animal Health (OIE)



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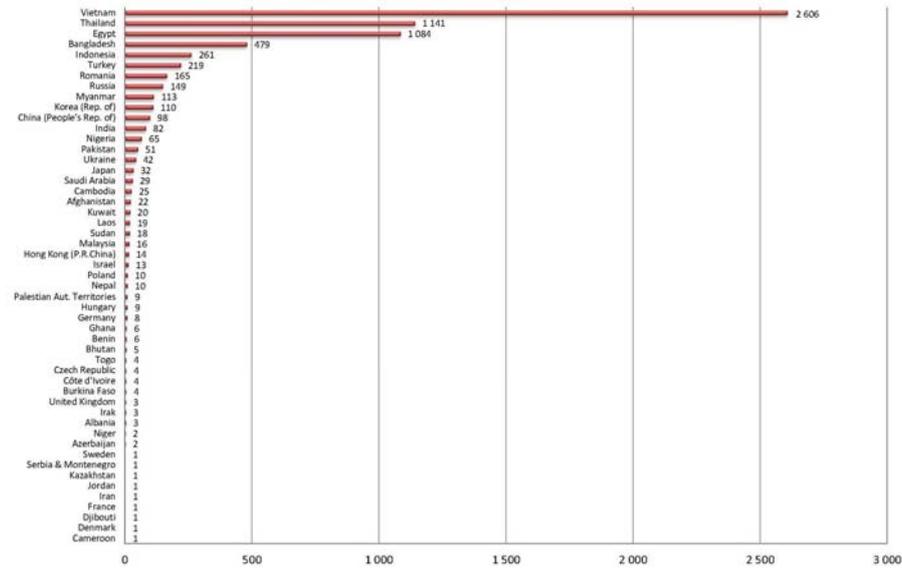
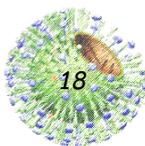


Figure 1-2. Outbreaks of Highly Pathogenic Avian Influenza (subtype H5N1) in poultry reported to the World Organisation for Animal Health (OIE), from the end of 2003 to 30 March 2011

Source: World Organisation for Animal Health (OIE), Last updated 30 March 2011 Available at http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/graph_avian_influenza/graphs_HPAI_30_03_2011.pdf



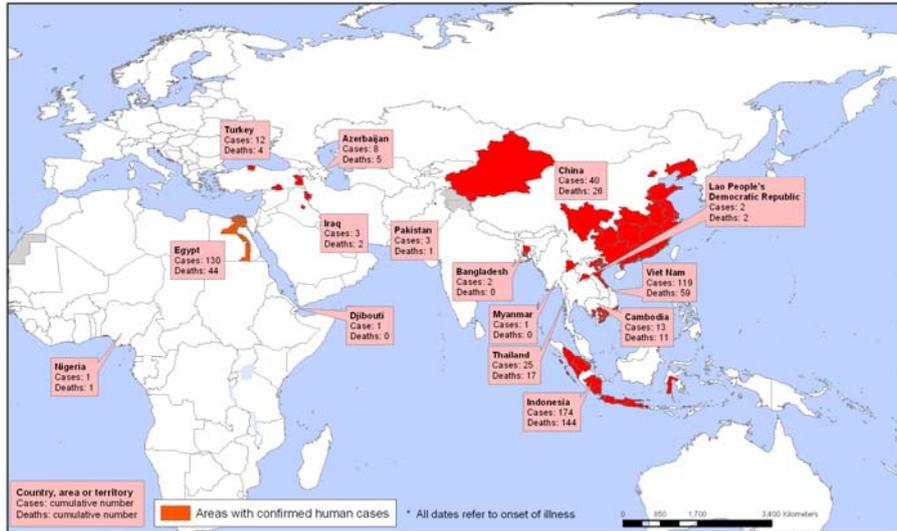


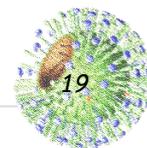
Figure 1-3. World map of areas or countries reporting confirmed cases of H5N1 avian influenza in humans since late 2003 until 16 March 2011

Source: World Health Organization (WHO). Last updated 16 March 2011 Available at http://gamapserver.who.int/mapLibrary/Files/Maps/Global_H5N1inHumanCUMULATIVE_FIMS_20110316.png

3. AIMS AND SCOPE OF THE STUDIES

The overall objective of this thesis was to investigate epidemiological features, risk factors, and transmission dynamics of H5N1 avian influenza in Thailand. The specific objectives of the studies described in this thesis are:

- To perform a descriptive analysis of the 2004 HPAI epidemic in Thailand,
- To quantify the within-flock transmission of HPAI H5N1 virus in infected poultry flocks in Thailand,
- To investigate ecologic risk factors of the clusters of HPAI H5N1, and
- To study risk factors associated with HPAI H5N1 outbreaks in poultry units.





The results obtained from the above-mentioned objectives are described as follows:

Chapter 1 provides a brief introduction of avian influenza and describes aims and scope of the thesis.

Chapter 2 describes epidemiological features, general economic impacts, and control measures implemented during the 2004 HPAI H5N1 epidemic in Thailand.

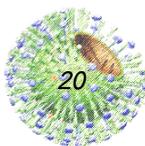
Chapter 3 presents the quantification of within-flock transmission of H5N1 HPAI virus using daily mortality data from infected chicken flocks in Thailand. The transmission rate parameter (β) and the basic reproduction number (R_0) were estimated.

Chapter 4 identifies the spatial and temporal clusters of HPAI H5N1 outbreaks at the subdistricts level in Thailand, 2004-2005. Risk factors with clustering were assessed by a spatial-based case control study.

Chapter 5 demonstrates potential risk factors associated with HPAI H5N1 infections in poultry flocks. A questionnaire-based retrospective case-control study was carried out to quantify the risk factors in different types of poultry flocks in Thailand during 2004-2005.

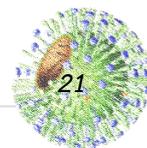
Chapter 6 generally discusses the results, implications, and critical elements of the studies presented in this thesis, and

Chapter 7 concludes with a summary of the thesis in two languages, i.e., English and Dutch.



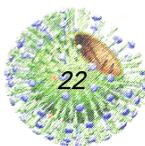
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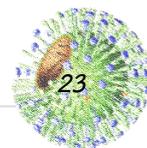




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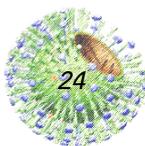


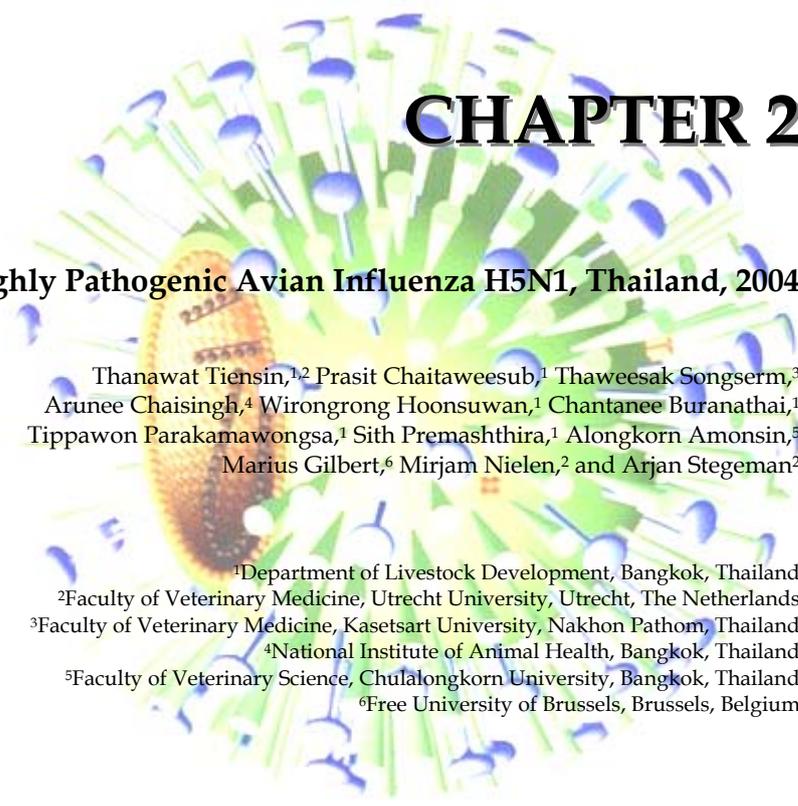
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CHAPTER 2

Highly Pathogenic Avian Influenza H5N1, Thailand, 2004

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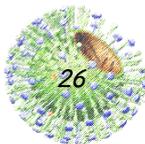
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Abstract

In January 2004, highly pathogenic avian influenza (HPAI) virus of the H5N1 subtype was first confirmed in poultry and humans in Thailand. Control measures, e.g., culling poultry flocks, restricting poultry movement, and improving hygiene, were implemented. Poultry populations in 1,417 villages in 60 of 76 provinces were affected in 2004. A total of 83% of infected flocks confirmed by laboratories were backyard chickens (56%) or ducks (27%). Outbreaks were concentrated in the Central, the southern part of the Northern, and Eastern Regions of Thailand, which are wetlands, water reservoirs, and dense poultry areas. More than 62 million birds were either killed by HPAI viruses or culled. H5N1 virus from poultry caused 17 human cases and 12 deaths in Thailand; a number of domestic cats, captive tigers, and leopards also died of the H5N1 virus. In 2005, the epidemic is ongoing in Thailand.



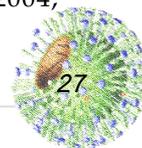
INTRODUCTION

Highly pathogenic avian influenza (HPAI) is a devastating disease in poultry; it is associated with a high death rate and disrupts poultry production and trade (Capua and Alexander, 2004a; OIE, 2004). HPAI viruses may be transmitted from birds to humans (Alexander and Brown, 2000; Koopmans et al., 2004), and they are a potential source of future human influenza pandemics (Capua and Alexander, 2004b). HPAI outbreaks were relatively rare until 1990 but occurred in many countries in the last decade (Capua and Alexander, 2004a). In Asia, since the HPAI H5N1 epidemic in Hong Kong in 1997, HPAI viruses have been isolated continuously through routine surveillance in Hong Kong (Sims et al., 2003; Ellis et al., 2004), South Korea (Tumpey et al., 2002), and China (Liu et al., 2003; Chen et al., 2004; Li et al., 2004). In Thailand, no evidence of HPAI infection was recorded before 2004 (Chaisingh et al., 2003). In 2003 and 2004, HPAI H5N1 outbreaks were reported in several Asian countries (South Korea, Vietnam, Japan, Thailand, Cambodia, Hong Kong, Laos, Indonesia, China, and Malaysia) (Capua and Alexander, 2004a; OIE, 2005), and these outbreaks were not easily halted (FAO, 2004; Li et al., 2004). Furthermore, H5N1 viruses crossed from birds to humans and caused 116 laboratory-confirmed cases in Vietnam, Thailand, Cambodia, and Indonesia with 60 deaths (as of September 29, 2005) (Li et al., 2004; WHO, 2005a). We describe epidemiologic features of the HPAI H5N1 epidemic in Thailand in 2004, with focus on introduction of the virus, distribution of disease in Thai poultry, control measures, and consequences.

POULTRY IN THAILAND AND HPAI VIRUS INTRODUCTION

Before 2004, Thailand was among the world's major poultry exporters and produced \approx 1 billion chickens per year (NESDB, 2004b); >400,000 persons were employed in the poultry industry (Sirimongkolkasem, 2002). Aside from commercial hybrid broilers and layers, backyard poultry are raised for food in most villages (DLD, 2004) (Table 2-1). The poultry population is concentrated in the Central and Eastern Regions of Thailand (Figure 2-1). Table 2-2 categorizes Thai poultry production into 4 sectors on the basis of farm management, biosecurity, and market orientation (FAO, 2004).

In late 2003, poultry farms in the Central and Northern Regions of Thailand experienced large-scale die-offs (ProMED-mail, 2004;





Keawcharoen et al., 2004; Simmerman et al., 2004; Chotpitayasunondh et al., 2005). Beginning in mid-December 2003, H5N1 outbreaks were reported in South Korea, Vietnam, and Japan. Meanwhile, Vietnam confirmed the first human death from H5N1 (OIE, 2005). In December 2003, a nationwide surveillance program was initiated to detect human cases in Thailand (Chotpitayasunondh et al., 2005). Subsequently, the surveillance program was strenuously implemented for poultry in mid-January 2004. Cloacal swabs were collected from poultry flocks throughout Thailand, and all samples were tested for avian influenza by virus isolation (OIE, 2004) at national and regional laboratories of the Thai Department of Livestock Development (DLD).

On January 23, 2004, the Thai national reference laboratory (National Institute of Animal Health [NIAH]) officially confirmed the presence of an H5 HPAI virus in a layer chicken farm in Suphanburi Province (OIE, 2005). The route by which this virus was introduced could not be traced. The virus was characterized as the H5N1 subtype (OIE, 2005), a member of the 2000 avian influenza lineage; most of its genetic sequences were closely related to influenza A/Duck/China/E319.2/03 (Viseshakul et al., 2004); it belonged to genotype Z (Li et al., 2004). That same day, the Thai Ministry of Public Health (MOPH) announced 2 laboratory-confirmed cases of H5N1 virus in children from Suphanburi and Kanchanaburi Provinces; the children eventually died (Puthavathana et al., 2005; WHO, 2005b).

SPREAD OF THE EPIDEMIC

The onset of H5N1 human cases (Chotpitayasunondh et al., 2005) showed that the H5N1 virus was already introduced into Thailand by the end of 2003 (Figure 2-2), before the first identification of the virus. In addition, the 149 reported outbreaks in poultry in 144 villages in 32 of the 76 provinces during the first week of the epidemic indicated that the virus had been widespread throughout the country. The epidemics in Thailand took place in 2 distinct periods, January–May 2004 (termed P1 or the first wave) and July–December 2004 (termed P2 or the second wave) (Figure 2-2). The epidemic is ongoing in Thailand in 2005, but the current analysis includes only outbreaks from January to December 2004.

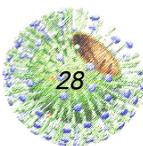


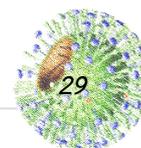
Table 2-1. Poultry population categorized by geographic region in Thailand in 2003

Poultry population	North	Central	East	South	Northeast	Total	RR (95% CI)*
Backyard chickens							
Birds	18,067,529	9,312,042	3,880,535	6,280,375	25,551,093	63,091,574	
Flocks	543,793	143,829	81,804	241,886	1,125,352	2,136,664	
Infected flocks	491	296	107	31	94	1,019	
Incidence, %	0.0903	0.2058	0.1308	0.0128	0.0084	0.0477	1.0
Layers							
Birds	2,288,485	7,682,667	8,304,081	2,113,035	3,924,255	24,312,523	
Flocks	4,209	6,396	3,941	7,666	14,264	36,476	
Infected flocks	29	42	14	1	6	92	
Incidence, %	0.6890	0.6567	0.3552	0.0130	0.0421	0.2522	5.3 (4.4–6.4)
Broilers							
Birds	12,442,797	70,414,281	53,681,571	6,565,161	22,210,976	165,314,786	
Flocks	4,588	6,242	6,507	6,166	22,274	45,777	
Infected flocks	44	54	8	3	2	111	
Incidence, %	0.9590	0.8651	0.1229	0.0487	0.0090	0.2425	5.1 (4.3–6.1)
Ducks							
Birds	2,567,666	8,026,701	6,110,934	1,777,466	5,317,325	23,800,092	
Flocks	58,606	33,607	17,917	95,216	478,483	683,829	
Infected flocks	85	355	29	9	13	491	
Incidence, %	0.1450	1.0563	0.1619	0.0095	0.0027	0.0718	1.5 (1.3–1.7)
Quails							
Birds	199,357	2,920,216	189,342	302,291	81,597	3,692,803	
Flocks	147	324	114	1,797	211	2,593	
Infected flocks	12	26	–	1	1	40	
Incidence, %	8.1633	8.0247	0	0.0556	0.4739	1.5426	32.4 (26.5–39.5)
Geese							
Birds	8,098	154,723	101,465	9,980	34,401	308,667	
Flocks	1,650	1,870	923	2,596	7,646	14,685	
Infected flocks	4	8	3	–	1	16	
Incidence, %	0.2424	0.4278	0.3250	0	0.0131	0.1090	2.3 (1.4–3.7)
Other							
Infected flocks	10	14	6	–	10	40	
Total							
Birds	35,573,932	98,510,630	72,267,928	17,048,308	57,119,647	280,520,445	
Flocks†	612,993	192,268	111,206	355,327	1,648,230	2,920,024	
Infected flocks‡	665	781	161	45	117	1,769	
Incidence, %	0.1085	0.4062	0.1448	0.0127	0.0071	0.0606	
RR (95% CI)	1.0	3.7 (3.4–4.1)	1.3 (1.1–1.6)	0.1 (0.01–0.15)	0.05 (0.06–0.08)		

*Relationship between cumulative incidence and relative risk (RR) of influenza H5N1 epidemic. Cumulative incidences of infected flocks of backyard chickens and the Central Region were assigned an RR of 1.0.

†Included only the number of flocks of backyard chickens, layers, broilers, ducks, quails, and geese. Some flocks are mixed types of poultry on site.

‡Included only the number of infected flocks of backyard chickens, layers, broilers, ducks, quails, and geese.





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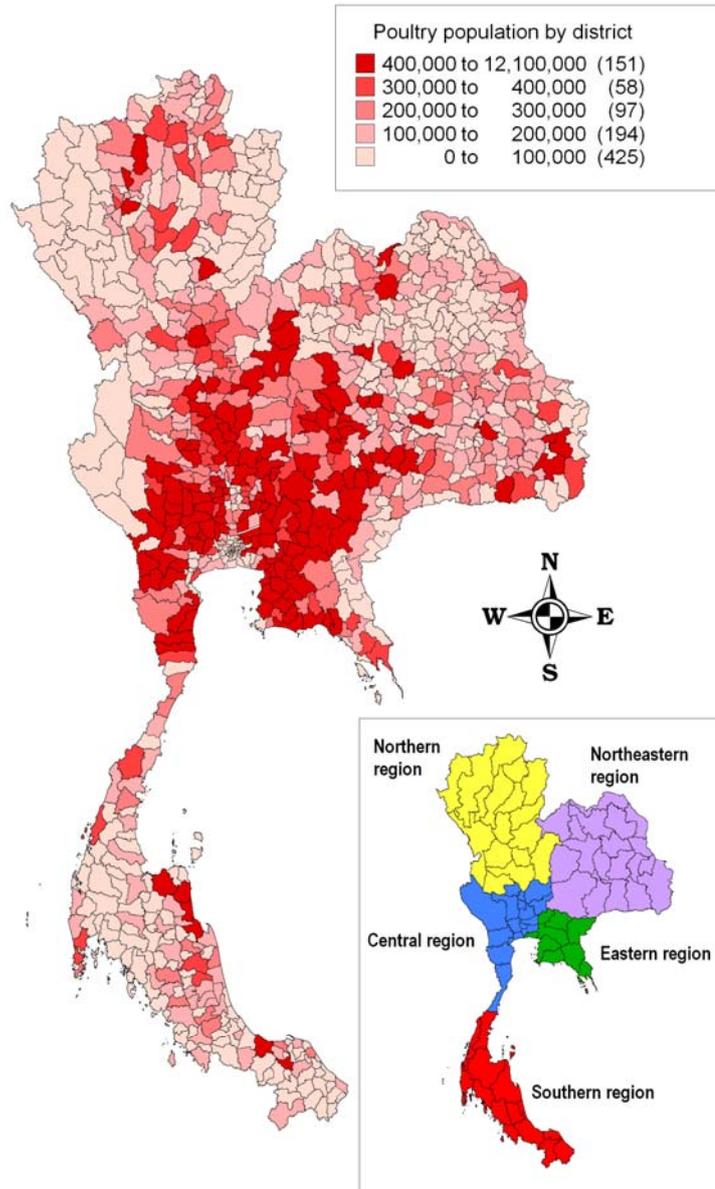


Figure 2-1. Distribution of poultry population in Thailand in 2003.

**Table 2-2.** Poultry production system in Thailand*

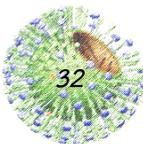
Poultry production	Biosecurity	Market orientation	Example
Sector 1	High	Commercial	Industrial integrated system: all components of the production chain (e.g., hatchery, feedmill, poultry farm, slaughterhouse, processing plant, transportation) owned by company with strictly implemented procedures for biosecurity
Sector 2	Moderate to high	Commercial	Semivertical integrated system (or contract farming system): poultry houses owned by the farmer but chicks, feed, and veterinary service supplied by private company. Birds kept indoors with basic physical barriers and hygiene to prevent contact with other animals
Sector 3	Low	Commercial, local, or live-bird market	Layer farm with caged birds in open sheds or free-roaming birds that spend time outside the shed
Sector 4	None	Local	Village or backyard poultry: birds freely roam the village around people and other animals, including cockfighting

*Source: Food and Agriculture Organization (FAO, 2004).

From January to May 2004, HPAI infections were detected in 188 villages in 42 of 76 provinces throughout Thailand (Table 2-3). The outbreaks occurred in all parts of Thailand but particularly in the Central, the southern part of the Northern, and the Eastern Regions. The last outbreak of the first wave was reported on May 24, 2004, from a layer farm in Chiangmai Province (OIE, 2005).

On July 3, 2004, the recurrence of HPAI was confirmed in layer farms in Ayudthaya and Pathumthani Provinces, north of Bangkok. These viruses were characterized as the H5N1 subtype, with genetic sequences similar to the H5N1 isolated in January 2004 (Buranathai et al.). During P2, HPAI infections were detected in 1,243 villages in 51 provinces (Table 2-3), which were concentrated in the same 3 regions (Figure 2-3). From July 3 onward, \approx 1-5 cases per day were detected in the first weeks of the epidemic. It reached a peak of 61 cases per day in mid-October 2004 (Figure 2-2B).

The geographic distribution of the second wave differs markedly from that of the first wave, and the number of confirmed outbreaks was 8 times higher. Most HPAI outbreaks were found in the Central and Northern Regions where chicken and duck flocks are relatively more abundant. In the Northern Region, 99% of infected flocks were detected in the southern part. Figure 2-3 shows that HPAI was sporadic in the Southern, the northern part of the Northern, and the Northeastern Regions, which



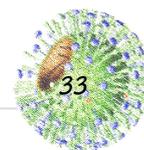
have a lower number and density of poultry populations. Figure 2-2 shows a dramatic increase in HPAI-positive flocks in January and October 2004, which coincided with the nationwide surveillance programs implemented at that time. Also, the number of infected flocks, particularly of backyard chickens and ducks, increased markedly in these months (Figure 2-4).

Type of Poultry Affected

Table 2-1 shows the various types of poultry in HPAI-positive flocks in 2004. Eighty-three percent of infected flocks were backyard chickens (56%) or ducks (27%); the rest were broilers (6%), layers (5%), quails (2%), and other birds (3%). From field studies in early 2004, ducks were determined to be silent carriers of HPAI virus (Chen et al., 2004; Songserm et al., 2005b). Accordingly, the proportion of infected ducks diagnosed during P2 markedly increased when compared to the number diagnosed during the early epidemic (P1) because more samples from ducks were submitted to laboratories.

Figure 2-4 shows epidemic curves by species; consistent dissemination of infection was confined to backyard chickens and ducks. Figure 2-5 illustrates the percentage of HPAI-infected poultry by region. More than 50% of infected flocks were of the backyard type in all regions except the Central Region (\approx 40% of infected flocks were backyard), which suggests that backyard chickens played a crucial role in the epidemic. However, during P2, 46% of infected flocks in the Central Region were ducks, which shows that they also contributed substantially to the epidemic. Free-grazing ducks are common in the Central Region (Table 2-1), with its abundance of wetlands and rice paddies. In 102 flocks (6.05%), HPAI was detected in >1 species (mixed farms).

Table 2-1 also shows cumulative incidence and relative risk (RR) of HPAI outbreaks. RRs of a flock's becoming infected were 3.7 and 1.3 times higher, respectively, in the Central and Eastern Regions compared to the Northern Region. Moreover, risks for HPAI infection were 5.3, 5.1, 1.5, 32.4, and 2.3 times higher, respectively, in layers, broilers, ducks, quails, and geese compared to backyard chickens.





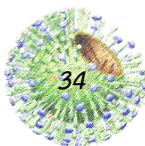
Spread to Other Species

In the early epidemic, domestic cats, captive tigers, and leopards also died from H5N1 viruses, which indicates that avian influenza can cross species barriers (Keawcharoen et al., 2004; OIE, 2005; WHO, 2005b). In October 2004, the infection of H5N1 viruses was confirmed in captive tigers at Sriracha tiger zoo in Chonburi Province, eastern Thailand (Thanawongnuwech et al., 2005). A total of 147 of 441 tigers kept in the zoo died or were euthanized to prevent possible spread to other zoo animals. Fresh chicken carcasses used to feed the zoo animals, contaminated with HPAI viruses, were considered to be the most plausible source of the infection (Thanawongnuwech et al., 2005).

Table 2-3. Number of detections of highly pathogenic avian influenza H5N1 outbreaks in each administrative division during epidemic in Thailand, 2004 (n = 1,685 flocks, record with missing data excluded)

Administrative division	No. detections (Jan–May)	No. detections (Jul–Dec)	Total (Jan–Dec)*
Province (N = 76)	42	51	60
District (N = 926)	89	264	305
Subdistrict (N = 7,409)	146	781	903
Village (N = 71,864)	188	1,243	1,417

*Some HPAI outbreaks during P2 (Jul–Dec) occurred repeatedly in the same administrative division as during P1 (Jan–May).



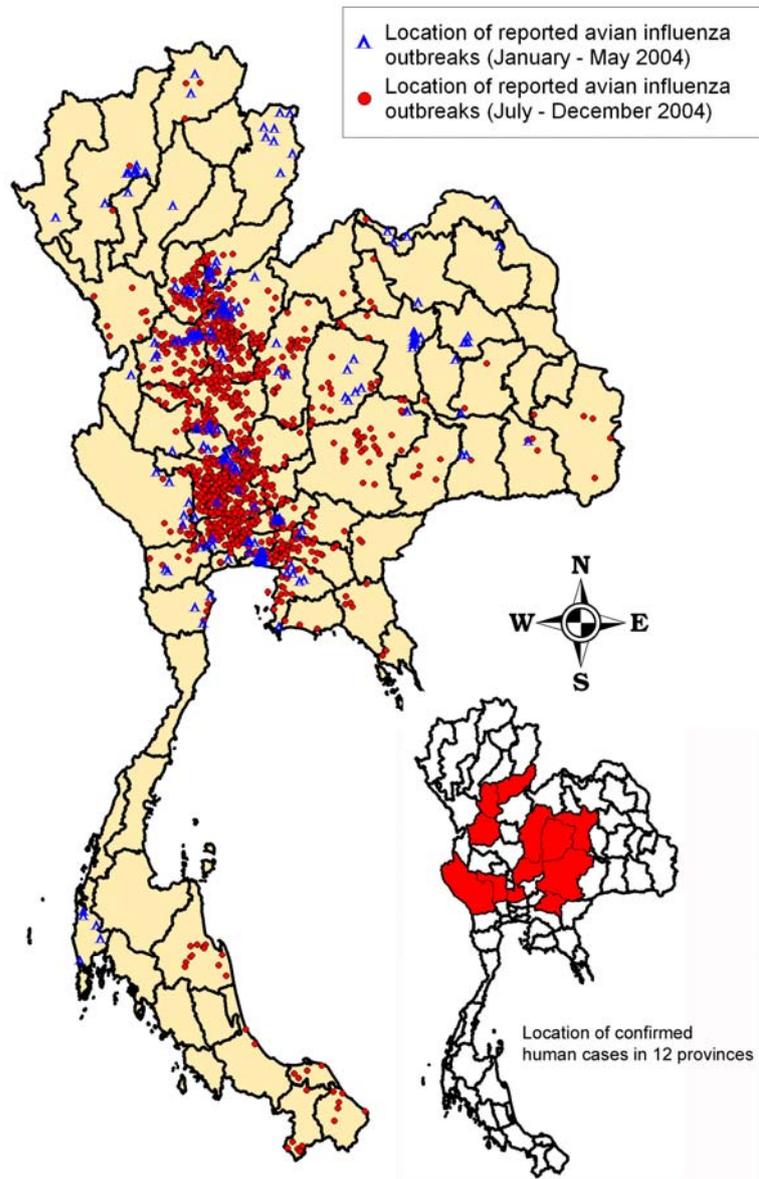


Figure 2-3. Distribution of reported highly pathogenic avian influenza H5N1 outbreaks in villages in Thailand, January–May 2004.



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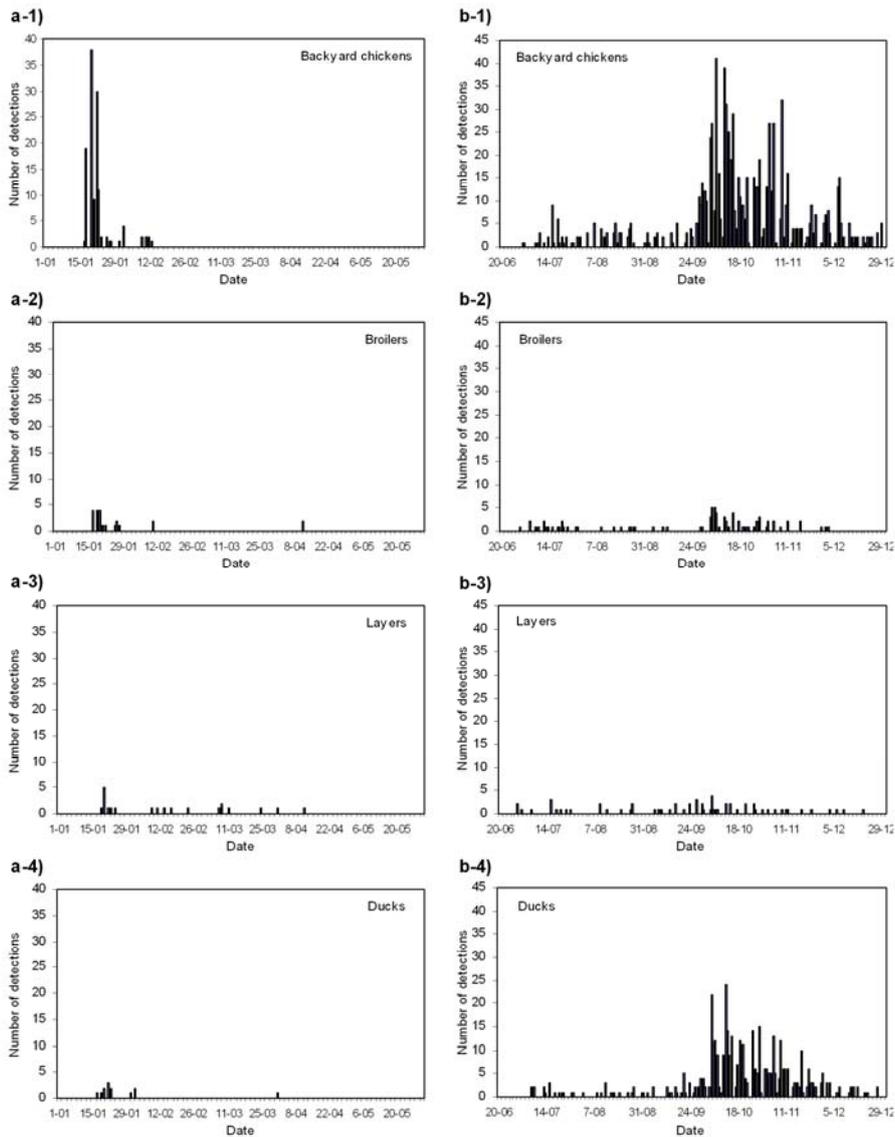


Figure 2-4. Infected flocks by day of detection and type of poultry, January–May 2004 and July–December 2004.

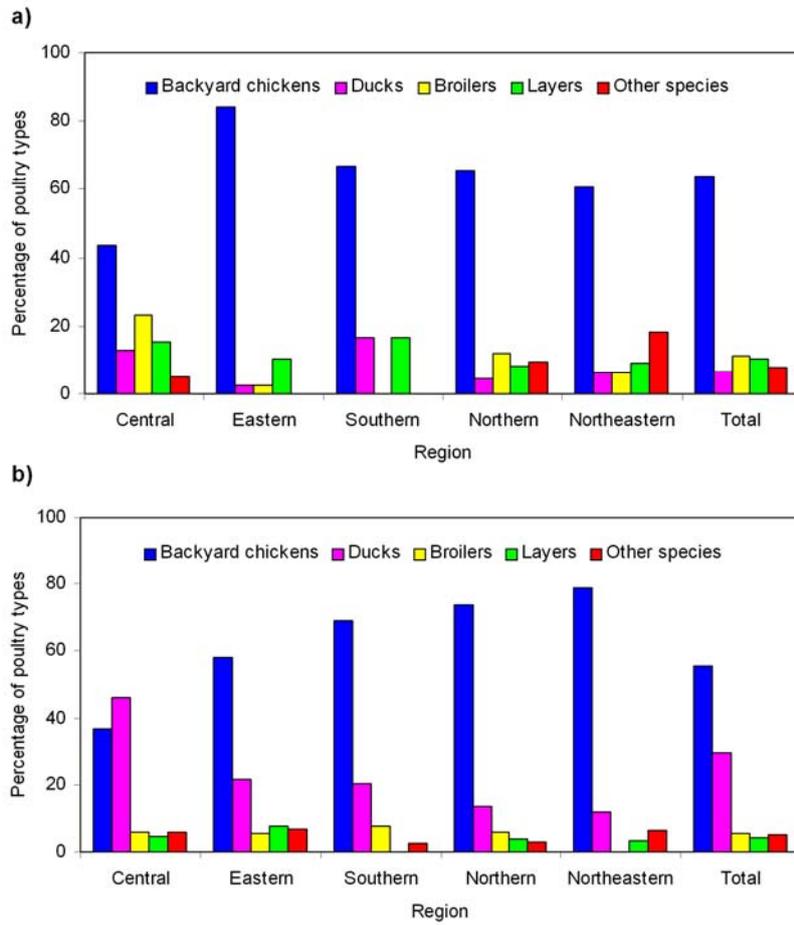


Figure 2-5. Percentage of main poultry types in infected flocks by region during the 2004 HPAI H5N1 epidemic in Thailand



CONTROL MEASURES

Basic Control Measures

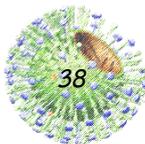
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Several measures were taken after the first isolation of HPAI virus in January 2004. Initially, all poultry, their products, feed, bedding, waste, and manure from infected flocks were destroyed immediately by the veterinary authorities. Culling infected birds in each flock was generally completed 1–2 days after the virus was confirmed by virus isolation (confirmatory diagnosis took \approx 2–8 days after submission of samples). Meanwhile, a restriction on moving poultry and their products within a 5-km radius around the infected flocks was enforced by DLD inspectors in collaboration with local police, and control checkpoints were temporarily established in these areas. Moreover, infected premises and equipment were cleaned and disinfected.

In July 2004, DLD implemented a series of control measures to enable quick action. Specifically, if the poultry death rate in any facility was $>10\%$ within a single day, all birds, their products, and other potentially contaminated materials had to be destroyed without delay. Cloacal swabs of affected flocks were then collected for laboratory confirmation. Subsequently, neighboring flocks were destroyed immediately or quarantined until H5N1 laboratory confirmation. Upon a confirmative laboratory result, quarantined flocks were culled. Furthermore, movement of poultry and their products was restricted within a 1- to 5-km radius around the infected area.

Preemptive Culling

In January 2004, contiguous flocks were preemptively culled as quickly as possible within a 5-km radius of a confirmed outbreak. After July 2004, preemptive culling was implemented only within a village, within an area of 1 km around an outbreak, or on suspected farms. This new strategy was adopted because the density of poultry flocks decreased after the massive culling during P1. Negative public perception of massive culling was another reason that this strategy was revised.

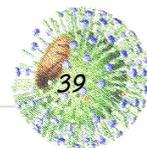


Surveillance and Diagnosis

In mid-January 2004, DLD launched a nationwide surveillance program to detect possible HPAI infections in poultry. Cloacal swabs were randomly collected from 4 flocks in each village (5 birds per flock). Swab samples were placed in tubes that contained virus transfer medium; usually 5 swabs were pooled per tube. During P1, >100,000 tubes of swab samples were tested for avian influenza virus. During P2, ≈130,000 tubes of swab samples and 72,000 serum samples were collected for diagnosis.

Swab samples as well as sick or dead bird specimens were submitted to NIAH or regional laboratories. All samples were processed for virus isolation in embryonated chicken eggs (≈1–2 days) (OIE, 2004); 2 serial passages in embryonated chicken eggs were performed before a specimen was regarded as negative (≈8 days). In January 2004, the first avian influenza isolate was sent to the University of Hong Kong to identify the virus and serotype hemagglutinin (HA) and neuraminidase (NA) antigens. Thereafter, NIAH itself established the necessary facilities to identify and serotype virus. Furthermore, real-time reverse transcription–polymerase chain reaction analyses for avian influenza were used to detect the virus at all laboratories to reduce the time of diagnosis. Hemagglutination inhibition (HI) test was used to detect antibodies to avian influenza virus in serum samples (OIE, 2004).

A nationwide comprehensive surveillance program (known as "x-ray survey") was implemented October 1–31, 2004. The goal of this survey was to detect HPAI infection in any village. In close collaboration among the Ministry of Agriculture and Cooperatives, MOPH, and provincial governors, volunteer public health MOPH workers and DLD livestock workers searched for and reported sick and dead poultry in villages. Through the surveillance program, farmers were also persuaded to report sick or dead poultry in their flocks to authorities. In 2005, x-ray surveys were implemented continuously every 6 months. Moreover, commercial poultry flocks will spend ≈8 days waiting for the results of cloacal swab or blood tests; only if birds are free of the virus will their owners be allowed to move them to slaughterhouses or new areas.



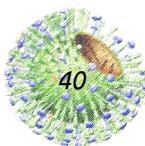


Other Supportive Measures

A public awareness campaign was started to educate the public on avian influenza and to bolster consumers' confidence that poultry was safe. In addition, the so-called "Big Cleaning Week" was promoted from March 1 to 7, 2004, to encourage relevant parties to be aware of HPAI and to disinfect their facilities, e.g., poultry houses, farm equipment and vehicles, slaughterhouses, and retail markets. Soaps, detergents, alkalis, acids, aldehydes, chlorine, and quaternary ammonium compounds were used as disinfectants. Poultry exhibition and cockfighting were prohibited (since early 2004). A violation of this regulation is subject to fine. Additionally, the practice of allowing ducks to freely graze was discontinued. Because of traditional farming styles, however, these practices are unlikely to change in a short period of time. After an affected flock was culled, a wait of ≥ 60 days in broiler farms and ≥ 90 days in layer farms and backyard chickens was imposed before a new flock could be established. Farmers must also improve sanitary measures in their farms to meet DLD's requirements.

Consequences of Epidemic

In early 2004, lack of information and communication with regard to HPAI caused the public to lose confidence in poultry products. The decrease in domestic consumption and bans on Thai poultry products by importing countries damaged the poultry industry. In addition, H5N1 virus from poultry caused 17 human cases with 12 deaths in 12 provinces (Figures 2-2 and 2-3) (Chotpitayasunondh et al., 2005; WHO, 2005a). The Thai government used a stamping-out policy to control HPAI outbreaks and compensated farmers for their losses. According to the Animal Epidemic Act, farmers are entitled to compensation of 75% of the value of animals that are destroyed. However, 100% compensation was provided during P1 because the epidemic was widespread and devastating to Thai farmers; compensation was reduced to 75% during P2. Compensation per bird was (in US dollars) \$0.38–\$65, depending on the type of poultry (\$0.38 for quail; \$1.13 for broiler; \$2 for meat duck; \$2.25 for backyard chicken; \$3.5 for layer chicken, layer duck, or goose; \$7.25 for turkey; and \$65 for ostrich).



Approximately 62 million birds were either killed by H5N1 viruses or culled for disease control and animal welfare reasons. The government allocated a budget of ≈5.3 billion Thai baht (US \$132.5 million) for direct compensation to affected farmers (DLD, 2005). All costs of implemented basic measures were covered by the government. As of March 2004, the HPAI epidemic had an estimated effect on the national gross domestic product of 0.39%. These losses amount to 25.24 billion Thai baht (US \$631 million) (NESDB, 2004a).

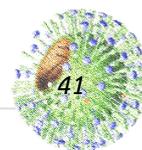
CONCLUSIONS

Detection and Early Distribution of HPAI Virus

Epidemiologic data from the early epidemic indicate that the period between the introduction of the virus into Thailand and its conclusive identification was too long. The route of virus introduction could not be traced. Also the delay between primary infection, first diagnosis, and finding the initial case allowed widespread dissemination of the virus and contributed to the large scale of the epidemic (Capua and Marangon, 2000; Gibbens et al., 2001). Early warning, early detection, and early response are essential to prevent and control HPAI. In view of potential public health implications of HPAI, notifying and collaborating with public health authorities is equally important.

Geographic Regions, Affected Species, and Incidence

The epidemic differed by region. The Central and Northern Regions contained 82% of the total outbreaks (Table 2-1). Infections were prominent in backyard chicken flocks in the southern part of the Northern Region and in free-grazing ducks in the area adjacent to the Central Region. Backyard chickens and free-grazing ducks played essential roles as H5N1 hosts (Figure 2-4); 83% of confirmed flocks were backyard chickens or ducks. Because of improved surveillance during P2, disease detections increased markedly (Figure 2-2). The difficulty of clinically detecting HPAI in ducks (Songserm et al., 2005a; Songserm et al., 2005b) and free-ranging backyard chickens and ducks also made controlling the disease difficult.

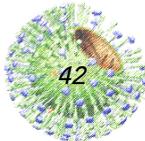




The 2004 cumulative incidence and RR also show a higher number of detections in the Central, Northern, and Eastern Regions relative to other parts of Thailand (Table 2-1). This finding reflects that the high density of poultry, the local geography (e.g., wetlands, water reservoirs, and rice paddies), and farming practice in these regions might be risk factors for outbreaks. Other studies showed a strong association between free-grazing duck populations and the practice of free-grazing farming with spread of the virus in the Central Region (M. Gilbert et al., pers. comm.). In our exploratory analysis, the RR for HPAI infection in Thai poultry production could not be clearly elucidated. The RR for HPAI infection was high in broilers, layers, quails, geese, and ducks compared to backyard chickens (Table 2-1). The number of detections in these types of poultry increased substantially in backyard chickens and ducks when national surveillance was implemented in January and October 2004. This observation suggests that when larger-scale farmers observed suspected cases in layer and broiler farms, they immediately reported them to local authorities, encouraged by the compensation that they received. In contrast, small farmers most likely did not report their few dead poultry. Consequently, the number of outbreaks in small farmers may have been underestimated. Additionally, size of flock may be a confounding factor in the higher risk for infection in broiler, layer, and quail flocks (Rothman and Greenland, 1998; Dohoo et al., 2003).

Course of the Epidemic

The epidemic curve during P1 shows a steep rise in the first week; detections decreased sharply after control measures were taken (Figure 2-2A). In the early epidemic, samples of culled flocks were not tested during massive culling. Undoubtedly, the quantity of infected flocks was underestimated, thus obscuring the effectiveness of control measures to stem the outbreaks. High numbers of HPAI detections coincided with low temperatures in Thailand from October to February, when wild birds from central and northern Asia migrate into Thailand (Ellis et al., 2004). Therefore, seasonal conditions and bird migration might have contributed to the introduction of HPAI virus. Furthermore, the lower temperature supports survival of the virus in the environment and facilitates transmission (Songserm et al., 2005a). In addition, several festivals, which are associated with raising, selling, and transporting



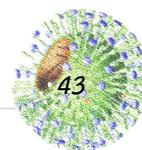
poultry, occurred around the end of the year. Illegal transportation and cockfighting may have worsened the HPAI situation.

Effectiveness of Control Measures

Because of differences and changes to control measures and surveillance programs during P1 and P2, HPAI outbreak data are difficult to compare. The start of the outbreak was an emergency period, during which epidemiologic data could not be effectively or completely collected. However, our results indicate that although several measures were implemented in 2004, the epidemic could not be controlled. HPAI outbreaks can be controlled rapidly with highly restrictive measures by totally depopulating all poultry in the entire areas in some countries (Capua and Marangon, 2000; Sims et al., 2003; Stegeman et al., 2004). However, given that HPAI was widespread in all parts of Thailand, total depopulation was not a practical option. But a combination of depopulation with improved early detection and response practiced during P2, combined with the culling rigor practiced during P1, may be a realistic option.

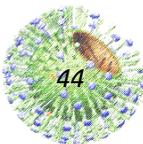
The Thai epidemic shows that the virus continues to circulate in the country. The immediate challenge is, therefore, to control avian influenza in free-ranging animals in rural areas, particularly in backyard chickens and free-grazing ducks. However, control of outbreaks in these types of poultry is difficult because of traditional farming practices. Control could be achieved by improving biosecurity of poultry farms and changing farming practices (Capua et al., 2002; Sims et al., 2003; Stegeman et al., 2004). Meanwhile, educating farmers and staff on early detection and the basic concepts of biosecurity may be the most critical way to eliminate avian influenza virus (Capua and Marangon, 2000).

Since January 2004, a stamping-out policy has been used to control avian influenza outbreaks in Thailand; vaccination has been not allowed. According to the Office International des Épizooties Terrestrial Code 2005, 2 broad vaccination strategies exist, inactivated whole avian influenza viruses and hemagglutinin expression-based vaccines. Thus, vaccination may be worthwhile to consider as an additional control measure (Stegeman et al., 2004). Vaccination significantly reduces excretion of viruses (Swayne et al., 2000; Swayne et al., 2001), which may reduce viral load in the environment and decrease the risk for human exposure. However, HPAI infection could become endemic if vaccination is not managed appropriately (Capua and Marangon, 2004).



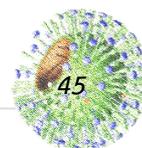


Early detection of all cases was essential to rapidly implement control measures. Meanwhile, comprehensive veterinary surveillance and long-term control measures are required (Li et al., 2004). The success of HPAI elimination, therefore, depends on a collaboration of all stakeholders, including farmers, industries, veterinarians, public health authorities, academic institutions, media, and the government (Capua et al., 2002; Stegeman et al., 2004).



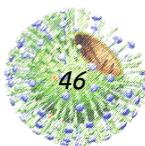
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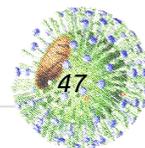


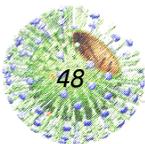


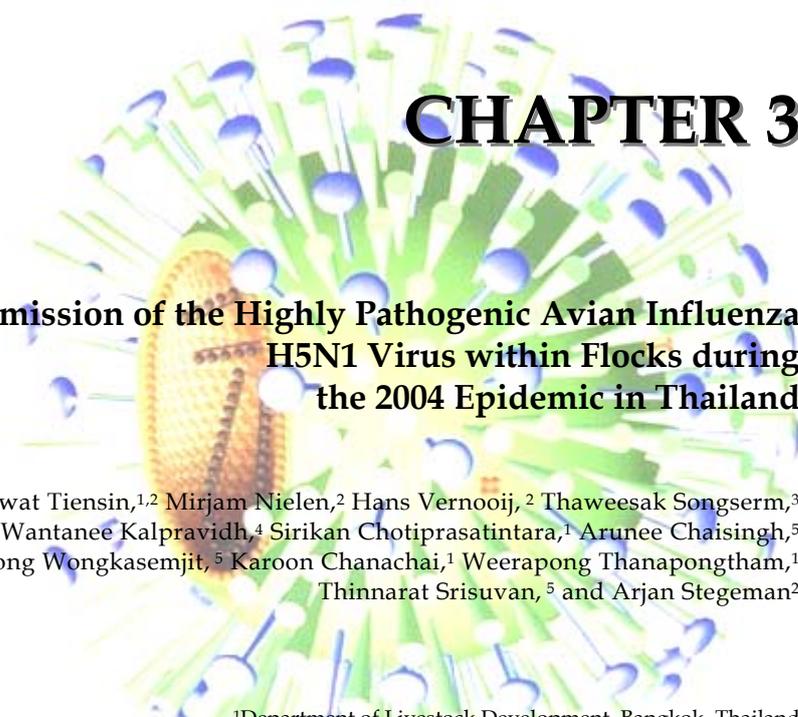
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CHAPTER 3

Transmission of the Highly Pathogenic Avian Influenza H5N1 Virus within Flocks during the 2004 Epidemic in Thailand

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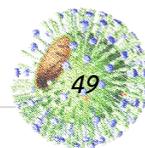
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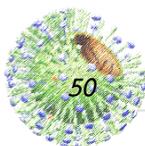
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Abstract

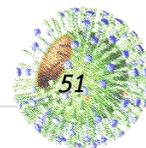
This present study is the first to quantify the transmission of avian influenza virus H5N1 within flocks during the 2004 epidemic in Thailand. It uses the flock-level mortality data to estimate the transmission-rate parameter (β) and the basic reproduction number (R_0). The point estimates of β varied from 2.26/day (95% confidence interval [CI], 2.01–2.55) for a 1-day infectious period to 0.66/day (95% CI, 0.50–0.87) for a 4-day infectious period, whereas the accompanying R_0 varied from 2.26 (95%CI, 2.01–2.55) to 2.64 (95% CI, 2.02–3.47). Although the point estimates of β of backyard chickens and fighting cocks raised together were lower than those of laying hens and broiler chickens, this difference was not statistically significant. These results will enable us to assess the control measures in simulation studies. They also indicate that, for the elimination of the virus, a critical proportion of the susceptible poultry population in a flock (i.e., 80% of the population) needs to be vaccinated.



INTRODUCTION

Subtype H5N1 of the highly pathogenic avian influenza A (HPAI-A) virus is having a serious impact on poultry production, human health, the livelihood of farmers, wildlife conservation, and other socioeconomic factors in Asia, Africa, and Europe (WHO, 2005; OIE, 2006). Several measures to stop the spread of HPAI-A virus (e.g., large-scale culling, movement restrictions, vaccination, and hygienic measures) have been implemented (Capua et al., 2003; Stegeman et al., 2004; Tiensin et al., 2005). However, with the exception of continental Europe and Japan, the measures have not yet resulted in the elimination of HPAI-A virus (Stegeman et al., 2004; Nishiguchi et al., 2005; Tiensin et al., 2006). It has been suggested that a better understanding of the transmission dynamics of HPAI-A virus within flocks could help improve the effectiveness of control measures (Stegeman et al., 1999a; Stegeman et al., 2004). In fact, quantification of the within-flock transmission could be useful for (1) determination of the time of introduction of the virus into a flock, (2) estimation of the proportion of animals requiring vaccination, and (3) assessment of the effect of intervention strategies (Anderson and May, 1992; Stegeman et al., 2004; Le Menach et al., 2006). Within-flock transmission can be quantified using the basic reproduction number (R_0) and the transmission rate parameter (β). R_0 is the expected number of secondary cases produced by a typical primary case in an entirely susceptible population (Anderson, 1992; Savill et al., 2006). R_0 has a threshold value that indicates whether an infection will spread or fade out: if $R_0 > 1$, a disease can spread; if $R_0 < 1$, chains of transmission will inevitably fade out (Anderson and May, 1992; Diekmann and Heesterbeek, 2000). Vaccination, one of the tools available to public health officials and veterinarians, helps reduce R_0 to a value < 1 , where the critical proportion of a susceptible population to be immunized is $1-1/R_0$ (Anderson and May, 1992; Ferguson et al., 2005). β determines the average rate at which susceptible animals become infected and hence spread a disease within a population. R_0 and β can be quantified from transmission experiments in which a small number of birds in a flock are inoculated with a virus. The status of all birds in the flock is then carefully monitored to record the spread of the virus (van der Goot et al., 2003; van der Goot et al., 2005).

However, outbreaks under field conditions can differ considerably from transmission experiments with a few animals under controlled conditions, in term of numbers of animals, contact structure, housing, and management.





Especially when $R_0 > 1$, which is expected in an unvaccinated population, estimates derived from transmission experiments are often imprecise (van der Goot et al., 2003; van der Goot et al., 2005). In the present study, the within-flock transmission of avian influenza virus H5N1 in infected chicken flocks was quantified by use of mortality data collected during the HPAI-A subtype H5N1 epidemic in Thailand. HPAI has a mortality close to 100%, and mortality data recorded in field situations are a very valuable source in its epidemiological analysis.

Here we quantified the transmission of HPAI-A virus subtype H5N1 within flocks during the 2004 epidemic in Thailand. We also investigated whether the within-flock transmission of H5N1 virus differed between flocks of backyard chickens, broilers, fighting cocks, or laying hens.

3

MATERIALS AND METHODS

Data and model assumptions. The study population included Thai chicken flocks that had tested positive for H5N1 virus by virus isolation during July–November 2004 (Tiensin et al., 2006). In 139 of these flocks, mortality (number of dead birds per day) was recorded for at least 2 days before all animals were culled. These data were collected by official veterinarians of the Thai Department of Livestock Development. The 139 flocks include flocks of backyard chickens, broiler chickens, fighting cocks, and laying hens, groups that have different housing systems: backyard chickens are mixed indigenous chickens (~20–50 birds a flock) that mingle freely in the village; broiler chickens are kept in either a closed house or an open house with netting, with large numbers of birds per housing unit, without subdivisions; laying hens are kept in battery cages in an open house with or without netting, with a large number of cages housed together; and fighting cocks are either raised with backyard chickens or sometimes kept in individual cages.

This present study was conducted under the implicit assumptions that (1) the chickens had no immunity to H5N1 virus at the time of its introduction, (2) all susceptible chickens were equally susceptible, (3) all infected chickens were equally infectious and spread the virus throughout the flock, and (4) all H5N1-infected chickens eventually died from the disease (Tian et al., 2005; Webster et al., 2006b).

Data-set construction for statistical model. The transmission data set was constructed on the basis of back-calculation, by an approach illustrated in

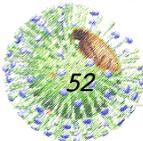
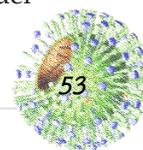


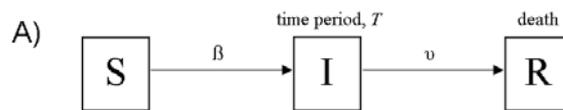
figure 1 (de Jong, 1995; Stegeman et al., 1999a; Diekmann and Heesterbeek, 2000). Specifically, before infection, all animals are susceptible (S), and, after infection, they become infectious (I) to other susceptible animals. Then they are removed (R) from the population, in this case because of death (Diekmann and Heesterbeek, 2000; Heffernan et al., 2005). On the basis of the literature, we assumed that chickens that had been infected started to excrete the virus 1–2 days after infection and died within 2–6 days (Lee et al., 2005; Tian et al., 2005; Swayne et al., 2006; Webster et al., 2006b). Estimation of β requires knowledge of both the number of susceptible chickens (S) and the number of infectious chickens (I) per day (Stegeman et al., 1999a; Stegeman et al., 2004). Because HPAI-A is highly lethal to chickens (Capua and Alexander, 2004; Li et al., 2004), we presumed that the number of dead chickens per day, in a flock, would be the number of newly infected chickens (cases) at an earlier time (C) when we back-calculated the data sets. In the present analysis, the mortality data on a flock were back-calculated into the format $C(t)$, $S(t)$, $I(t)$, and $R(t)$, for numbers of birds in each category at time t (days 0, 1, 2, 3, etc.). The total number of chickens in a flock at a specific time t was also designated as $N(t) = S(t) + I(t)$. On the basis of experimental infection studies of H5N1 virus (Lee et al., 2005; Tian et al., 2005; Swayne et al., 2006; Webster et al., 2006b), we constructed data sets by assuming that the chickens died after an infectious period of 1, 2, 3, or 4 days (Table 3-1).

Statistical analysis. According to this SIR assumption, the number of newly infected cases (C) per day has an expected value, $E(C)$, that depends on β and the number of S and I animals, in the total flock, per day. The equation for $E(C)$ is shown in Figure 3-1 (Becker, 1989; Dohoo et al., 2003). Thus, $\ln(\beta)$ could be estimated by a generalized linear model (GLM) using the statistical program R (version 2.2.0) (McCullagh and Nelder, 1989; Dohoo et al., 2003; R Development Core Team, 2006). At this stage, the back-calculated data sets (exemplified in Table 3-1) on 139 flocks were used to estimate β . In this analysis, C was a dependent variable, and $\ln(S(t)I(t)/N(t))$ was included as an offset variable. Flock types (i.e., backyard chickens, broiler chickens, laying hens, and fighting cocks) were added as a categorical, fixed effect, and flock was added as a random effect. The latter was needed because more observations from the same flock were included in the data set (McCullagh and Nelder, 1989; Dohoo et al., 2003). We also used the negative binomial distribution, instead of a binomial or Poisson distribution, to correct for overdispersion of the outcome of the model





(Dohoo et al., 2003; Motulsky and Christopoulos, 2004). Akaike's information criterion (AIC) was used to select the best-fitting model. The model with the lowest AIC value received the most support from the data (Akaike, 1973; Motulsky and Christopoulos, 2004). Subsequently, the R_0 was calculated as $R_0 = \beta T$, the product of the infectious period T (days) and β (individuals per day) (Anderson and May, 1992; Stegeman et al., 2004).



B)

$$E(C) = \frac{\beta SI}{N} \Delta t$$

Figure 3-1. Stages in the SIR model used to estimate avian influenza A subtype H5N1's dynamics of transmission between individual birds within a flock. *A*, SIR model: a bird is first susceptible (S), becomes infected (according to transmission-rate parameter [β]), and stays for a time (t) in the infectious stage (I) before it dies of H5N1 (R, removed). *B*, Equation for the expected value for newly infected cases, used for statistical analysis [12, 18].

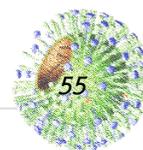
Table 3-1. Example of data-set construction of a laying hen flock, based on SIR model, at hypothetically different infectious periods of 1, 2, or 3 days.

Infectious period	Back-calculation based on SIR model					
	Day	C ^a	S	I	R	N
1 day	0	55	4100	0	0	4100
	1	115 ^b	4045	55	0	4100
	2	203 ^b	3930	115	55	4045
	3	415 ^b	3727	203	115	3930
	4	837 ^b	3312	415	203	3727
	5		2475	837	415	3312
	6				837	2475
2 days	0	55	4100	0	0	4100
	1	115	4045	55	0	4100
	2	203 ^b	3930	170	0	4100
	3	415 ^b	3727	318	55	4045
	4	837 ^b	3312	618	115	3930
	5		2475	1252	203	3727
	6		2475	837	415	3312
	7				837	2475
3 days	0	55	4100	0	0	4100
	1	115	4045	55	0	4100
	2	203	3930	170	0	4100
	3	415 ^b	3727	373	0	4100
	4	837 ^b	3312	733	55	4045
	5		2475	1455	115	3930
	6		2475	1252	203	3727
	7		2475	837	415	3312
	8				837	2475
9					2475	

NOTE. C, number of newly infected chickens (cases) at an earlier time when we back-calculated the data sets; SIR, before infection, all animals are susceptible (S), and, after infection, become infectious (I) to other susceptible animals, and are removed (R) from the population, in this case because of death.

^a Assumes that the number of dead animals represents the number of newly infected animals at an earlier time after introduction of the virus into a flock.

^b After back-calculation of raw data, records containing complete data in C, S, I, and N columns were used to estimate the transmission-rate parameter (β).

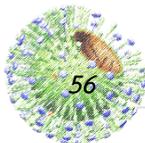




RESULTS

Characteristics of the H5N1-infected flocks. The characteristics of the 139 H5N1-infected chicken flocks used in the present study are summarized in Figure 3-2. Most of these flocks (67%) were backyard chickens of relatively small size; whereas the laying-hen and broiler-chicken flocks were generally much larger: the flock size of the backyard chickens was 8–300 birds/flock, that of the laying hens was 197–7604 birds/flock, and that of the broiler chickens was 400–21,500 birds/flock. Cumulative mortality in the 139 infected flocks was 2% within 1 day of the appearance of clinical signs, with 100% mortality within 6 days and average daily mortality progressing from 1% to 36%.

Within-flock transmission. Table 3-2 presents the outcomes of the statistical model, stratified by length of infectious period and type of chicken flock. The estimated varied between the different infectious periods (1, 2, 3, and 4 days). Depending on the assumed infectious period for all flock types included in the model, the point estimates of varied from 2.26/day (95% confidence interval [CI], 2.01–2.55/day) for a 1-day infectious period to 0.66/day (95% CI, 0.50 – 0.87/day) for a 4-day infectious period. The respective R_0 values varied from 2.26 (95% CI, 2.01–2.55) to 2.64 (95% CI, 2.02–3.47) (Table 3-2). The values of β and R_0 for the different types of chicken flocks at the specified infectious periods are also shown in Table 3-2. Although the point estimates of β and R_0 for backyard chickens and fighting cocks raised together were lower than those for laying hens and broiler chickens, the difference was not statistically significant.



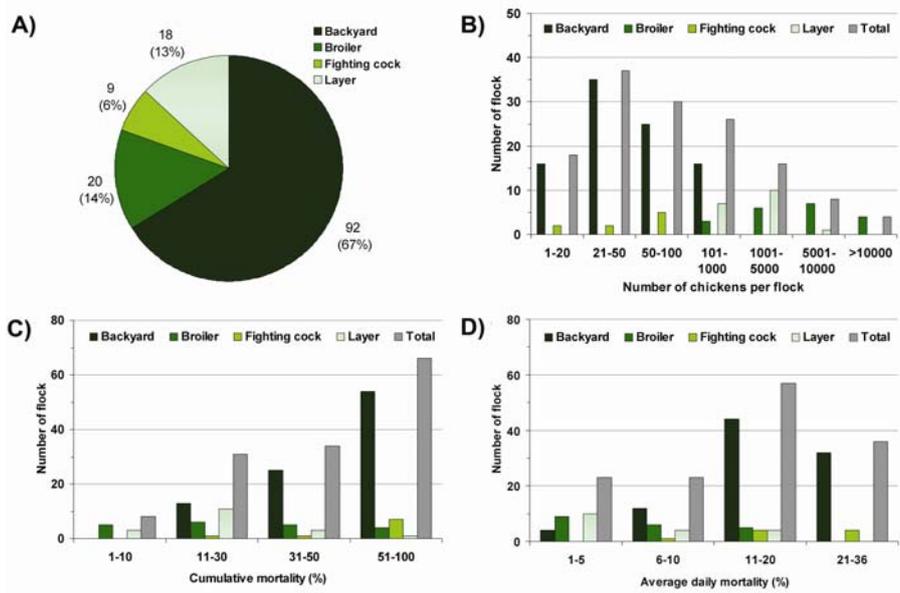
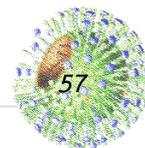


Figure 3-2. Characteristics of 139 chicken flocks infected with the highly pathogenic avian influenza A virus subtype H5N1 during the 2004 Thai epidemic that are used in the present study: Number and percentage of flock type (A), flock size (B), cumulative mortality (C), and average daily mortality (D).

DISCUSSION

Estimates of β and R_0 . In the present study, we quantified the within-flock transmission of HPAI-A virus subtype H5N1 in infected chicken flocks during the 2004 epidemic in Thailand, for different types of chicken operations. The values of β resulting from this study are 0.66 -2.26/day (Table 3-2); these values are much lower than the point estimate of 33/day for a 6-day infectious period that van der Goot et al. (van der Goot et al., 2005) have reported for HPAI-A virus subtype H7N7. However, their estimate was based on the observations of only 2 contact-infected chickens, resulting in a CI of 1.3- ∞ for R_0 , which includes our estimates of R_0 within the range of 2-5. Our value for R_0 is also much lower than that used by Savill et al. (Savill et al., 2006). In this case, comparison is difficult because their R_0 estimates were modeled on the basis of data from experiments with individually challenged birds, and therefore were not quantified on the basis



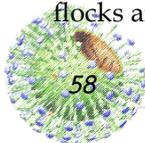


of either transmission data or data originating from H5N1-infected flocks in field conditions, as in our study. However, the estimate of R_0 in the present study is higher than estimates for human flu pandemics (e.g., 1.89 for the human pandemic A [H3N2] in Hong Kong (Rvachev and Longini, 1985) and 2–3 for the 1918 human pandemic A [H1N1] in the United States (Mills et al., 2004).

Table 3-2. Estimates of transmission-rate parameter (β) and basic reproduction number (R_0), with 95% confidence interval in parentheses, based on a generalized linear model (GLM) of data on 139 chicken flocks infected with the highly pathogenic avian influenza A virus subtype H5N1 during the 2004 Thai epidemic, with flock as a random effect and with results from the GLM with 2 groups of flock types added.

Flock type and values	Infectious period, days			
	1	2	3	4
All flocks				
β , per day	2.26 (2.01–2.55)	1.23 (1.11–1.36)	0.87 (0.75–1.02)	0.66 (0.50–0.87)
R_0	2.26 (2.01–2.55)	2.46 (2.23–2.72)	2.62 (2.25–3.07)	2.64 (2.02–3.47)
Laying hens and broiler				
β , per day	2.30 (1.92–2.76)	1.43 (1.20–1.71)	1.16 (0.90–1.50)	0.79 (0.50–1.25)
R_0	2.30 (1.92–2.76)	2.86 (2.41–3.41)	3.49 (2.70–4.50)	3.17 (2.01–5.00)
Backyard and fighting cock				
β , per day	2.18 (1.94–2.46)	1.15 (1.02–1.30)	0.75 (0.63–0.91)	0.60 (0.43–0.84)
R_0	2.18 (1.94–2.46)	2.31 (2.05–2.60)	2.26 (1.88–2.72)	2.40 (1.71–3.36)

We think it is very likely that the transmission of HPAI-A virus subtype H5N1 differed between the various types of flocks, because of the contact-structure differences between them, due to age, flock size, breed, management, and possibility of contact. Moreover, we expected that the differences in the density at which the chickens are housed would have an impact on transmission (Stegeman et al., 1999a; Klinkenberg et al., 2002; Keeling et al., 2003; Stegeman et al., 2004). However, in the present study, no significant within-flock transmission difference between flock types was found. Nevertheless, the resulting point estimates support the intuitive suggestion that within-flock transmission in backyard chickens and fighting cocks raised together is lower than that in broiler chickens and laying hens. In addition, the model with 3 groups of flocks—laying-hen and broiler-chicken flocks and backyard chickens and fighting cocks raised together— shows

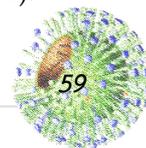


that point estimates of transmission is higher in broiler chickens than in laying hens or backyard chickens (results not shown). This would reflect the effect that contact-structure differences have on transmission. Broiler chickens are kept as 1 group, with a high possibility for mutual contact. Laying hens are kept in battery cages, with a large number of cages housed together. Backyard chickens and fighting cocks mingle freely in the village and may have lower possibility for contact than do broiler chickens and laying hens.

Bias, data validity, and back-calculation assumptions. Records of dead birds might not have been kept with the same accuracy by all poultry keepers. Moreover, record keeping might have differed depending on flock type. The number of chicken houses of large commercial poultry units, which was not known, may have caused an underestimation of the β if the disease occurred in only 1 house in a commercial unit with several houses. Data such as the total number of animals and the number of deaths per day really should be recorded per house, to facilitate epidemiologic analysis.

In our assumptions, all chickens within a flock were equally infectious for a designated period. This may differ from actual biological conditions, leading to overly precise results in the present study. Moreover, on the basis of the lethality of HPAI-A virus subtype H5N1, we assumed that all infected chickens eventually died. However, during the outbreak, culling had to be performed immediately after the infection was recognized. As a consequence, mortality data on the day of culling might not have been accurate. In our analysis, a latent-infection period (i.e., infected but not yet infectious) was not included, because, when using 1-day time interval, we assumed that an infected individual became immediately infectious (Diekmann and Heesterbeek, 2000). Experimental studies of H5N1 have not yielded clear results on the existence or length of a latent infection period (Lee et al., 2005; Tian et al., 2005; Swayne et al., 2006; Webster et al., 2006b). Also, assuming an SIR model instead of an SEIR model allowed better fitting of the data (results not shown).

Application of modeling to intervention strategies. A goal of control strategies is to reduce R_0 to < 1 . In general, R_0 reduction in animal populations can be achieved in 3 ways, by reducing the (1) contact rate between animals, by isolation or separation; (2) infectiousness of infected animals, by treatment or vaccination; or (3) susceptibility of still uninfected animals, by vaccination (Stegeman et al., 1999a; Stegeman et al., 1999b; Stegeman et al., 2004).



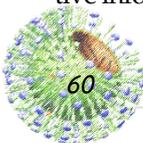


When an HPAI-A epidemic in an avian species is quickly controlled, the risk of human infection is reduced (Le Menach et al., 2006). Vaccination of domestic poultry against the H5N1 subtype of avian influenza has been used in several countries. Using vaccination to reduce the transmission rate could provide an alternative to mass culling, by reducing both the susceptibility of healthy flocks and the infectiousness of infected flocks. However, incomplete protection at the flock level can cause the silent spread of the virus within and between flocks. If vaccination is used but is not appropriately managed as part of a control strategy, elimination of the disease will not be achieved, and the concomitant public health risk will still be present (Capua and Marangon, 2004; Savill et al., 2006; Webster et al., 2006a).

In the present study, the upper limit of the R_0 estimate was 5.0 (Table 3-2), on the basis of the model with broiler chickens and laying hens used as a categorical variable (in the worst-case scenario). Therefore, the present study indicates that if at least 80% of the birds in a flock are effectively vaccinated, a major spread of the virus will not occur, on the basis of the fraction of $1-1/R_0$ (Anderson and May, 1992). In commercial flocks, it is feasible to apply and maintain a vaccination coverage for 80% of a total flock. However, vaccination coverage of 80% might be more difficult in backyard chickens. Another problem is that vaccine efficacy is seldom 100% (van der Goot et al., 2005; Swayne et al., 2006; Webster et al., 2006b). In that case, the vaccination coverage will need to be higher than the estimated value of $1-1/R_0$ (i.e., > 80%) but not as high as suggested by those who assume a much higher R_0 (van der Goot et al., 2005; Savill et al., 2006).

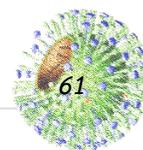
For other species (e.g., quails, geese, and domestic ducks), the data available are insufficient to allow transmission to be estimated. In addition, the mortality-based approach cannot be used for domestic ducks, because they show very little or late mortality (Sturm-Ramirez et al., 2005; Songserm et al., 2006). We suggest that transmission studies in domestic ducks be based on longitudinal serological results. Nowadays, mathematical modeling is widely used and has become a tool for preparedness planning and for modeling of new disease outbreaks (Ferguson et al., 2005; Savill et al., 2006). The results of our analyses seemed quantitatively robust, so our β values can be used in simulation models to estimate the effectiveness of intervention measures.

In conclusion, β and R_0 of HPAI-A virus subtype H5N1 were estimated by the use of mortality data collected at the flock level, from the field. This quantitative information can be used to plan a future program for control of HPAI-A.



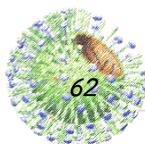
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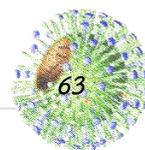


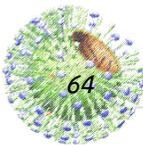


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CHAPTER 4

Ecologic Risk Factor Investigation of Clusters of Avian Influenza A (H5N1) Virus Infection in Thailand

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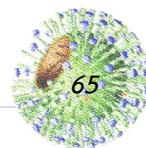
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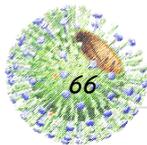
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Abstract

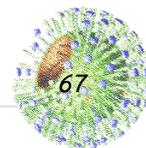
This study was conducted to investigate space and time clusters of highly pathogenic avian influenza A (H5N1) virus infection and to determine risk factors at the subdistrict level in Thailand. Highly pathogenic avian influenza A (H5N1) was diagnosed in 1890 poultry flocks located in 953 subdistricts during 2004–2007. The ecologic risk for H5N1 virus infection was assessed on the basis of a spatial-based case-control study involving 824 case subdistricts and 3296 control subdistricts from 6 study periods. Risk factors investigated in clustered areas of H5N1 included human and animal demographic characteristics, poultry production systems, and wild birds and their habitats. Six variables remained statistically significant in the final model: flock density of backyard chickens (odds ratio [OR], 0.98), flock density of fighting cocks (OR, 1.02), low and high human density (OR, 0.60), presence of quail flocks (OR, 1.21), free-grazing duck flocks (OR, 2.17), and a poultry slaughterhouse (OR, 1.33). We observed a strong association between subdistricts with H5N1 virus-infected poultry flocks and evidence of prior and concomitant H5N1 infection in wild birds in the same subdistrict.



INTRODUCTION

Since 2003, outbreaks of highly pathogenic avian influenza (HPAI) H5N1 virus infection have resulted in a high number of affected animals, losses in domestic and international trade of poultry products, socioeconomic impacts, impacts on farmers' livelihoods, and public health consequences. The disease spread widely in >60 countries across Asia, Europe, Africa, and the Middle East (Alexander, 2007; OIE, 2008). As of 10 November 2008, H5N1 virus transmitted from infected birds had caused 387 human cases, 245 of which were fatal (Webster et al., 2006; OIE, 2008; WHO, 2008). Outbreaks of HPAI H5N1 have emerged and persisted mainly in East and Southeast Asia - southern China (Li et al., 2004), central Thailand (Tiensin et al., 2007), northern and southern Vietnam (Pfeiffer et al., 2007), and Indonesia (Sedyaningsih et al., 2007). Therefore, it is of interest to study the risk factors associated with disease occurrence in specific locations (cluster areas) and to determine whether they may be present in certain places only at certain times, taking into account bias in H5N1 case detection, control measures, or changes in demographic characteristics in at-risk populations. Risk factors within cluster areas have been investigated for other infectious diseases (e.g., bovine spongiform encephalopathy (Stevenson et al., 2000), severe acute respiratory syndrome (Lai et al., 2004), sleeping sickness (Berrang-Ford et al., 2006), and West Nile virus (Ruiz et al., 2004)). However, despite the fact that HPAI remains a major threat for animal and public health worldwide (WHO, 2008), the risk factors in H5N1 clusters have remained largely unexplored.

The HPAI H5N1 epidemics in Thailand during 2004–2007 provided epidemiological data for the study of risk factors associated with the reemergence, spread, and persistence of H5N1 in cluster areas. Although the extent and location of clusters was not well described, field data have allowed for an epidemiological study of H5N1 virus infection in poultry and wild birds. We investigated a number of potential risk factors for H5N1 clusters, including differences and variety in agro-ecology, human and animal demographic characteristics, poultry production systems, and wild birds and their habitats. The latter has not yet been epidemiologically and systematically studied, although it is increasingly acknowledged that the association between infected wild birds and poultry plays a critical role in the maintenance and spread of influenza A virus (Songserm et al., 2006; Amonsin et al., 2008; Uchida et al., 2008).



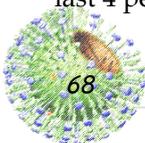


In this study, we examined the spatial and temporal clusters of outbreaks of HPAI H5N1 at the subdistrict level in Thailand during 2004–2005. We identified risk factors associated with H5N1 virus infection in high-cluster areas with use of a spatial-based case-control study. We also performed a retrospective subdistrict-level analysis of the data from 2004–2005 for the presence of sick or dead wild birds with laboratory evidence of HPAI H5N1 in case and control subdistricts.

MATERIALS AND METHODS

Study population

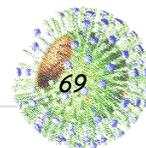
Data on outbreaks of H5N1 in Thailand have been collected since January 2004 by the Department of Livestock Development, Thailand. The virus was confirmed in sick or dead birds and cloacal samples from poultry and wild birds by diagnostic laboratories with use of reverse-transcriptase polymerase chain reaction and virus isolation (Tiensin et al., 2005). The data at the subdistrict level included H5N1 detection date, location and species of dead or sick poultry, animal and human demographic characteristics, evidence of H5N1 virus infection in wild birds, wild bird habitats, and location of poultry establishments (i.e., slaughterhouses, feed mills, and poultry farms). The animal population census data from 2004 were used; the spatial distribution of poultry and flock density in Thailand are shown elsewhere (Tiensin et al., 2007). The outbreak data were based on both the mandatory clinical disease reporting system and the nationwide active surveillance program known as the “X-ray survey,” which is described in detail elsewhere (Tiensin et al., 2005; Tiensin et al., 2007); these 2 systems are complementary and ensure disease detection. To detect H5N1 virus infection in wild birds, dead birds that met selection criteria (i.e., the bird died recently and had little apparent decay or trauma) and cloacal samples from live birds were collected for testing for influenza A virus as part of the ongoing wild bird surveillance program. Approximately 10,580 samples were collected during 2004–2005, including either cloacal swab samples or dead wild birds. For the temporal analysis, we used the data aggregated during 2004–2005 for the 6 following study periods: January–February 2004 (period 1), July–August 2004 (period 2), October–November 2004 (period 3), January–February 2005 (period 4), July–August 2005 (period 5), and October–November 2005 (period 6); the last 4 periods were based on the national active surveillance programs. For



evidence of H5N1 virus infection in wild birds, we included wild bird infection detected within the month before and the month after the outbreak among poultry in each subdistrict for the 6 study periods. During 2006–2007, H5N1 occurred sporadically in Thailand. This period was excluded from further analysis, because no cluster pattern was detected.

Disease mapping and cluster detection methods

Thailand is administratively divided into 76 provinces (each with its own veterinary services) and is additionally partitioned into 926 districts and 7327 subdistricts (mean area, 70.37 km²; median area, 45.59 km²; range, 0.13–2383 km²); subdistrict data were used in this analysis. To minimize errors in mapping, the locations of H5N1 virus-infected flocks were cross-referenced with the subdistrict names and specific location identification numbers. Arc-GIS, version 9.2 (ESRI), was used for mapping and visualization. Incidence (crude rate estimate) was calculated and shown spatially for all subdistricts. An empirical Bayes (EB)–smoothing method was applied to H5N1 outbreak data; this smoothing technique adjusted disease rates, especially in areas with small population estimates, toward the overall mean of the study areas (Clayton and Kaldor, 1987). To examine the spatial clustering of H5N1 outbreaks at a subdistrict level, global and local spatial autocorrelation analyses were performed. First, the spatial pattern of feature values formed by incidence rates and EB-smoothed rates was measured. Global Moran’s I spatial autocorrelation statistic using GeoDa, version 0.9.5-I (Arizona State University, 2008) was used to quantify spatial dependence (Moran, 1950; Anselin, 1995). This technique simply reveals whether there is spatial aggregation of the incidence or the EB estimates or whether high or low values of the data are interspersed. It provides a global measure of spatial autocorrelation but can only identify the presence of a cluster, not its specific location. Second, 2 local cluster detection methods were applied to localize the specific clusters as hot spots or cold spots of H5N1 outbreaks. The local spatial autocorrelation analysis was conducted using the GeoDa cluster detection program to generate Anselin’s Local Moran test statistics. Local Moran’s statistics assess spatial autocorrelation and identify clustering subdistricts with disease rates statistically similar to or dissimilar from their neighbors on the basis of aggregated data (Anselin, 1995, 2005). A spatial scan test by SaTScan, version 7.0.3 (SaTScan, 2008), was then used to fully examine our hypothesis that HPAI would show spatial clustering over subdistricts



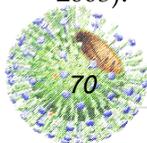


(Kulldorff and Nagarwalla, 1995). The spatial scan statistic test assesses disease distribution with use of centroids and a circular scan. Because different analytical methods may identify different underlying spatial patterns (Jacquez and Greiling, 2003), we sought more robust results with consistent findings. The same cluster subdistricts should be identified with 2 methods.

Case-control selection process and statistical analysis.

According to Tobler's first law of geography, everything is related to everything else, but near things are more related than distant things (Tobler, 1970). Case and control subdistricts were selected on the basis of the aforementioned spatial cluster analysis. Different buffers (radius, 10–60 km) were created for the case-control selection process. The eligible buffer for selection of case and control subdistricts was defined as a 50-km radius buffer around the centroid of identified cluster subdistricts for the 6 different periods during 2004–2005. Subsequently, all subdistricts within the 50-km radius buffer per period were candidates for case-control selection. The subdistricts with ≥ 1 flock infected with HPAI H5N1 virus during a period were defined as case subdistricts. All remaining subdistricts within the 50-km buffer were candidates for control subdistricts, which were randomly selected with a case-control ratio of 1:4.

All variables were individually tested for an association with the case-control status of a subdistrict by univariable logistic regression analysis for each study period and then for all periods combined. Variables that were statistically significant at $P \leq 0.2$ were included for further analysis. To take into account possible nonlinear relations, all continuous variables were categorized using a decile classification scheme. When appropriate, variables were categorized before further analysis (Dohoo et al., 2003). When possible, data were reclassified into plausibly biological appropriate categories. Subsequently, multivariable logistic regression analysis was used to assess an association between the independent variables and the dependent variable (which was either case or control status of a subdistrict). Finally, the 6 study periods were added as fixed variables. A final model was fitted using a backward stepwise procedure. Statistical significance of risk factors was assessed using the likelihood ratio test based on $P \leq 0.05$. Collinearity was assessed by correlation coefficients ($|\rho| < 0.5$) among all covariates to be considered for inclusion in the final model (Dohoo et al., 2003). We then assessed model fit with use of the Hosmer-Lemeshow

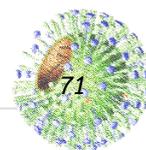


goodness-of-fit test and the ratio of the deviance to the degree of freedom. Regression coefficients were converted into odds ratios (ORs; e^{β}) and their 95% confidence intervals (CIs) (Hosmer and Lemeshow, 2000).

RESULTS

Study population

From January 2004 through December 2007, a total of 1890 poultry flocks with laboratory-confirmed H5N1 were detected in 953 (13%) of the 7327 subdistricts; 1323 (70%) of the 1890 H5N1 virus-infected flocks were found mostly in central and northern Thailand. The distribution and number of infected flocks by subdistrict during 2004–2005 are shown in Figure 4-1A and 4-1B. The median number of infected flocks per subdistrict was 1 (range, 1–16 flocks); there were 398 subdistricts in which ≥ 1 infected flock was reported. Of the 7327 subdistricts, 48 subdistricts were excluded because of lack of available data; this resulted in a study sample of 7279 subdistricts. On the basis of the cluster analysis, 824 case subdistricts and 3168 control subdistricts were selected for inclusion in this study. Figure 4-1C shows the distribution and location of H5N1 virus-infected wild birds found in 40 subdistricts in Thailand during 2004–2005. The epidemic curve of H5N1 outbreaks from January 2004 through December 2007 and a curve of the total number of subdistricts infected per week are shown in Figure 4-2. The latter curve gives an indication of the spatial extent of HPAI H5N1, and the former curve indicates the magnitude of the epidemic. The dramatic increase in incidence from October and November 2004 through January and February 2005 is related to the effort of the nationwide active surveillance programs. The number of cases and subdistricts with infected flocks decreased in 2005, and outbreaks occurred sporadically during 2006–2007.



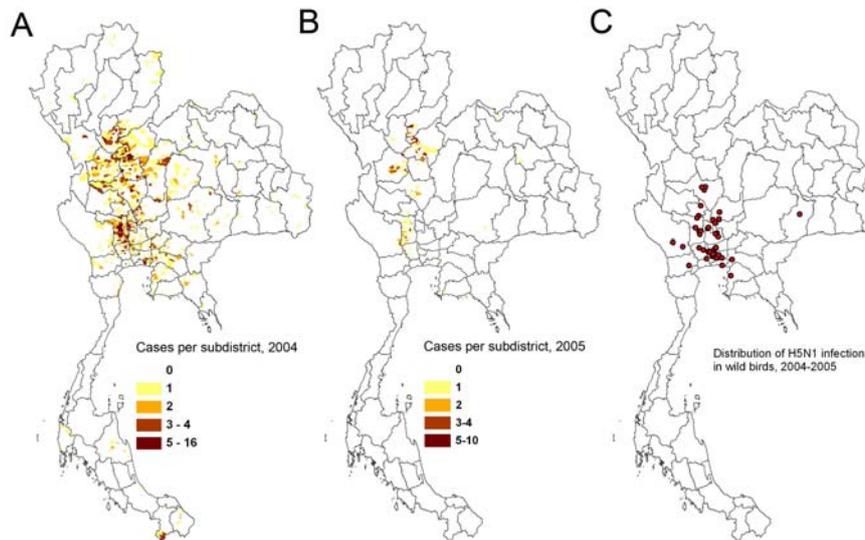
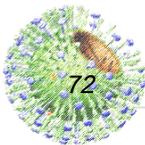


Figure 4-1. Number of flocks of domestic poultry infected with highly pathogenic avian influenza A (H5N1) virus, by subdistrict in Thailand. *A*, Outbreaks during 2004. *B*, Outbreaks during 2005. *C*, Distribution of H5N1 virus infection among wild birds during 2004–2005 (province boundaries are also shown).

Disease mapping and cluster detection

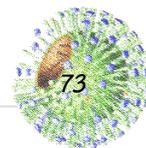
Figure 4-3 shows maps of EB-smoothed incidence of HPAI per subdistrict in each of the 6 study periods. As suggested in the EB-smoothed rate map and by local cluster detection, EB rates of H5N1 virus infection for clusters at high risk ranged from 1.0 to 73.7 infected flocks per 1000 flocks from July through August 2004 and from 1.7 to 62.4 infected flocks per 1000 flocks from October through November 2004. Global spatial autocorrelation was investigated using Moran's I statistics (Table 4-1); we found that the Z score increased during 2004, which indicates that outbreaks became more geographically fixed. The spatial pattern showed strong clustering during 2 periods: from October through November 2004 and from January through February 2005 ($P < 0.001$). On the basis of the local Moran statistics test, various multicentered clusters of high HPAI risk were detected from January 2004 through February 2005, and 2 multicentered clusters were



detected from July through August 2005 (Figure 4-3). The spatial scan test indicated that the primary multiclusters were located in the central region; the relative risk was 26.9 during July–August 2004 and 38.6 during October–November 2004 ($P < 0.001$). Secondary clusters were identified in the areas adjacent to the central region; the relative risk was 39.2 during July–August 2004 and 5.5 during October–November 2004 ($P < 0.001$ for both). The clusters at high risk of HPAI that were detected in GeoDa were either included in or overlapped the clusters identified using SaTScan. The most likely low-risk areas were located in southern and northeastern Thailand ($P < 0.001$). All low-risk areas were statistically consistent with use of both methods. Although several individual sub-districts appeared to have elevated risk with use of the EB-smoothing method, compared with incidence rate, these were not identified as a statistically significant cluster by either the Local Moran test or the spatial scan test.

Risk factors based on the subdistrict level analysis

A total of 824 case subdistricts with flocks with H5N1 virus infection were identified in a 50-km radius buffer around the centroid of high-cluster subdistricts during the 6 study periods. In addition, a total of 3296 control subdistricts were identified among candidate subdistricts during each study period (Table 4-1). Table 4-2 shows the variables that yielded a P value ≤ 0.2 in the univariable analysis. Eleven of 31 variables were considered for inclusion in the multivariable logistic regression model. In the univariable analysis, the findings reveal that subdistricts with commercial poultry flocks (i.e., broilers; OR, 1.31 [95% CI, 1.10–1.55]), laying chickens (OR, 1.38 [95% CI, 1.12–1.70]), quails (OR, 1.31 [95% CI, 1.10–1.55]), and flock density of meat and laying ducks (OR, 1.07 [95% CI, 1.01–1.15]) were more likely to have infected flocks than were other subdistricts. Subdistricts with free-grazing duck flocks showed an association with H5N1 virus infection (OR, 2.48 [95% CI, 2.12–2.90]). We found a strong association between case subdistricts and evidence of prior and concomitant H5N1 virus infection in wild birds in the same subdistrict (OR, 4.00 [95% CI, 1.62–9.85]; $P < 0.001$). However, only 5 subdistricts had H5N1 detected in wild birds prior to the study periods. Period was forced as a covariate in the model, but it was not statistically significant. We found a statistically significant relationship between the evidence of HPAI in wild birds in subdistricts per period.



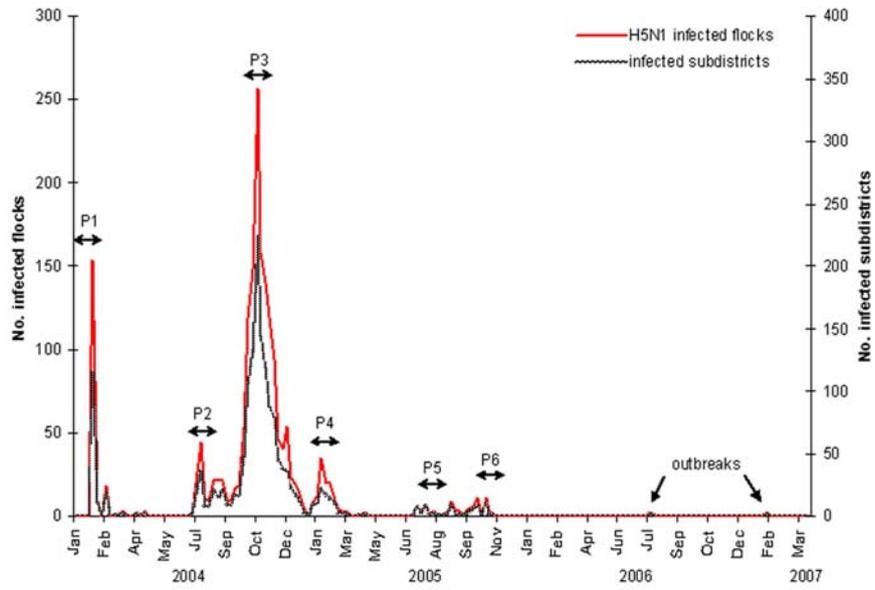
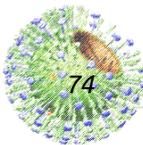


Figure 4-2. Weekly epidemic curve of the number of flocks infected with highly pathogenic influenza A (H5N1) virus and the number of subdistricts with infected flocks, Thailand, 2004–2007. P1, January–February 2004; P2, July–August 2004; P3, October–November 2004; P4, January–February 2005; P5, July–August 2005; P6, October–November 2005.



However, wild birds were not included in the multivariable analysis because of incomplete records (i.e., for many subdistricts, no wild birds were tested). The results of the multivariable logistic regression model, corrected for period, are shown in Table 4-3. Six variables remained in the final model: (1) flock density of backyard chickens (as protective factor), (2) fighting cocks, (3) human density, (4) presence of quail flocks, (5) free-grazing duck flocks, and (6) poultry slaughterhouse. The Hosmer-Lemeshow statistics value for goodness-of-fit of the final model was 8.7 (degree of freedom, 8; $P = 0.37$), which was considered to be acceptable (Hosmer and Lemeshow, 2000). No

Table 4-1. Results of the global spatial autocorrelation by Moran's I statistics, based on the incidence of highly pathogenic influenza A (H5N1) virus infection, by subdistricts, for the 6 study periods and combined during 2006–2007.

Study periods	Moran's I statistic	Z-score	P	Pattern	No. of case subdistricts	No. of control subdistricts
January–February 2004	0.02	4.58	0.006	clustered	101	404
July–August 2004	0.05	9.71	0.002	clustered	87	348
October–November 2004	0.11	18.42	0.001	clustered	536	2016
January–February 2005	0.06	10.12	0.002	clustered	54	216
July–August 2005 (X-ray 3 survey)	0.05	9.33	0.003	clustered	24	96
October–November 2005 (X-ray 4 survey)	0.01	1.65	0.1	less clustered ^a	22	88
January 2006–October 2007	–0.007	–0.05	0.78	random

NOTE. The data from 2006–2007 were excluded from additional case-control study.

^a Compared with the study periods during January 2004–August 2005.

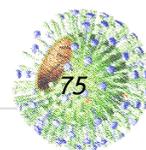




Table 4-2. Univariable analysis of potential risk factors for highly pathogenic influenza A (H5N1) virus infection in domestic poultry at the subdistrict level, Thailand, 2004 –2005 (with 6 study periods combined).

Variables	No. (%) of case subdistricts (n=824)	No. (%) of control subdistricts (n=3168)	OR (95% CI)*	P
Backyard chicken flock density (flocks per km ²)	0.99 (0.98–1.00)	0.1
Fighting cock flock density (flocks per km ²)	1.02 (1.01–1.03)	0.005
Meat and laying duck flock density (flocks per km ²)	1.07 (1.01–1.15)	0.032
Broiler flock in subdistrict				
Present	603 (73)	2140 (68)	1.31 (1.10–1.55)	0.002
Absent	221 (27)	1028 (32)	...	
Laying hen flock in subdistrict				
Present	696 (84)	2526 (80)	1.38 (1.12–1.70)	0.002
Absent	128 (16)	642 (20)	...	
Quail flock in subdistrict				
Present	444 (54)	1496 (47)	1.31 (1.12–1.52)	0.001
Absent	380 (46)	1672 (53)	...	
Free-grazing duck flock in subdistrict				
Present	496 (60)	1199 (38)	2.48 (2.12–2.90)	<0.001
Absent	328 (40)	1969 (62)	...	
Human density (persons per km ²)				
<60	48 (6)	306 (10)	0.52	<0.001
60–430	655 (79)	2164 (68)	1	<0.001
>430	121 (15)	698 (22)	0.57	<0.001
Household density (houses per km ²)				
<15	42 (5)	286 (9)	0.49	<0.001
15–124	655 (79)	2190 (69)	1	<0.001
>124	127 (15)	692 (22)	0.61	<0.001
Slaughterhouses in subdistrict				
Yes	164 (20)	484 (15)	1.38 (1.13–1.68)	0.001
No	660 (80)	2684 (85)	...	
Evidence of H5N1 infection in wild birds ^a				
Yes	15 (2)	8 (0.003)	4.00 (1.62–9.85)	0.003
No	75 (9)	160 (5)	...	
Unknown	734 (89)	3000 (95)	...	

NOTE. Variables with $P < 0.2$ were used in multivariable analysis. CI, confidence interval; OR, odds ratio.
^a Data are for 258 subdistricts.

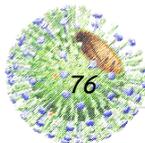


Table 4-3. Results of a multivariable logistic regression model for highly pathogenic influenza A (H5N1) status per subdistrict, Thailand, 2004–2005, corrected for period.

Variables	Adjusted OR (95% CI)	P
Backyard chicken flock density (flocks per km ²)	0.98 (0.96–0.99)	0.001
Fighting cock flock density (flocks per km ²)	1.02 (1.01–1.04)	0.008
Quail flock in subdistrict	1.21(1.03–1.42)	0.02
Free-grazing duck flocks	2.17(1.84–2.56)	<0.001
Human density (persons perkm ²)		
<60	0.57(0.41–0.80)	0.009
60–430	1	<0.001
>430	0.63 (0.49–0.80)	0.001
Slaughterhouse in subdistrict, yes	1.33 (1.08–1.63)	0.007

NOTE. H5N1 virus infection in wild birds by subdistrict was excluded from the final model, because the it is a subset of all observations (subdistricts). The Hosmer-Lemeshow statistics value for goodness-of-fit of the final model was 8.7 (degree of freedom, 8; $P = 0.37$). CI, confidence interval; OR, odds ratio.

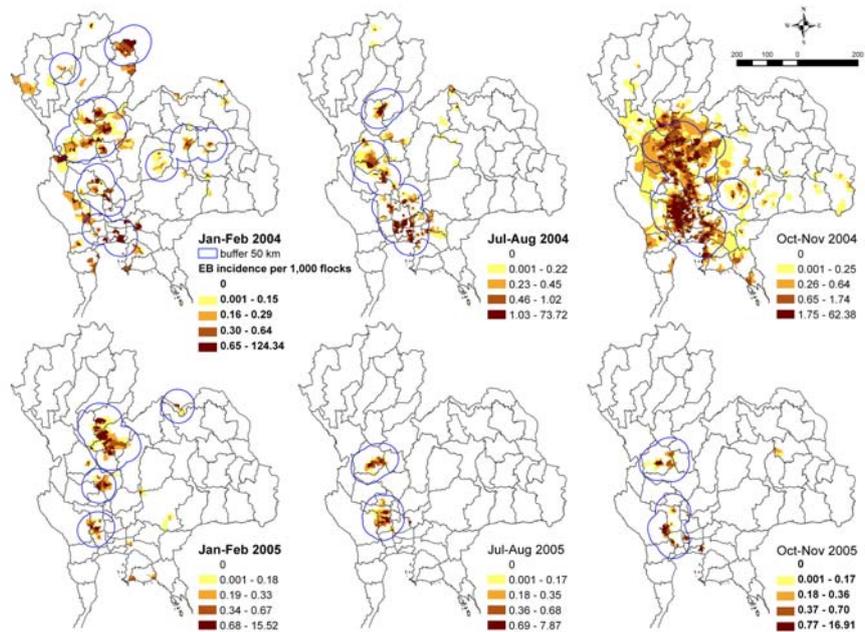
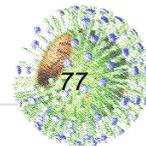


Figure 4-3. Empirical Bayes (EB)-smoothed incidence of highly pathogenic influenza A (H5N1) virus infection, by subdistrict, for the 6 periods with epidemics during 2004–2005. A 50 -km -buffer around identified cluster subdistricts is shown. Legend categories vary between maps; province boundaries are also shown.





DISCUSSION

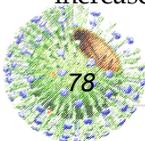
Disease mapping and spatial clustering

Our study detected significant spatial clusters of HPAI at a subdistrict level in Thailand during 2004–2005 with use of 2 local cluster detection methods: Anselin's Local Moran test and the spatial scan statistic test. Both of these cluster detection methods identified approximately the same clusters at high risk and low risk of HPAI, which suggests that our results are robust. Consistent results of both the incidence of HPAI outbreak data and the EB rate-smoothing technique also improved our understanding of the geographic distribution of HPAI by indicating that the disease was likely to occur in areas surrounding hot spots.

Figure 4-3 shows that H5N1 clusters were scattered all over the country during January–February 2004. This may indicate long-distance spread of the disease through transportation of poultry and poultry products. After control measures had been implemented (i.e., restrictions on transportation of commercial chickens, free-grazing ducks, and fighting cocks and pretesting for H5N1 infection in poultry flocks before movement) (Tiensin et al., 2007), the outbreaks mostly occurred in high-cluster areas in central Thailand (Figures 4-1 and 4-3).

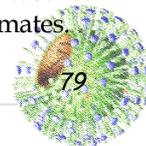
Risk factors

We used a novel approach to select control subdistricts, such that they originated from a high-risk area. The identified risk factors, therefore, would truly differentiate between case and control status. The origin of H5N1 epidemics is complex and multifactorial. Although many of the risk factors in the current study appear to be plausible, an association does not equal causation. As the hypothesized causal pathway in Figure 4-4 shows, multiple factors can cause the disease. This pathway was used to identify potential risk factors in our study at the ecologic subdistrict level. The results show that subdistricts with a high flock density of fighting cocks and with quail and free-grazing duck flocks had higher infection rates than did subdistricts with low flock density of these poultry types. Subdistricts with poultry slaughterhouses were more frequently infected than were those without slaughterhouses. Subdistricts with poultry slaughterhouses may have increased activities related to transportation of poultry that increase the likelihood that the virus will be brought into these subdistricts.



Live-bird markets were not included in this study as a risk factor (although they were included in previous studies (Mounts et al., 1999; Dinh et al., 2006; Kung et al., 2007), because there are very few live-bird markets in Thailand. The sale of live chickens or ducks in the retail market is neither practical nor popular in Thailand, compared with other East and Southeast Asian countries (Liu et al., 2003; Dinh et al., 2006; Amonsin et al., 2008). A poultry slaughterhouse functions as a dissemination point for H5N1 infection, because poultry from many sources (from duck, chicken, and quail farms and free-grazing ducks) are brought there for slaughter, and the movement of cages to and from such slaughterhouses may disseminate virus back to farms. Such mechanisms have been postulated to occur in relation to live poultry markets. Understanding interactions between animals and humans is critical in preventing outbreaks of zoonotic diseases. Our study reveals that the odds of H5N1 virus infection in a subdistrict were significantly higher in the subdistricts with commercial poultry flocks than in subdistricts with backyard flocks. Also, our analysis indicates that subdistricts with lower or higher human population densities had a lower incidence of infection than did those with medium density. This may reflect the fact that both subdistricts with low human density and those with high human density have smaller poultry populations and fewer poultry production-related activities. It seems that certain poultry farming activities (e.g., commercial poultry farming, local slaughtering, and cock fighting) increase infection risk at the subdistrict level. Therefore, biosecurity of those activities should be revisited. Understanding the relationship between subdistricts with infected flocks and the presence of free-grazing duck flocks is crucial, and our findings agree with those of previous studies (Tiensin et al., 2005; Gilbert et al., 2006). We observed a significant association between subdistricts with H5N1 virus-infected flocks and evidence of H5N1 virus infection in wild birds in the same subdistrict. Although significantly associated with infection in the univariable analysis, the presence of infected wild birds could not be included in the final model because of missing data on the subdistrict level. Of interest, the presence of infected wild birds within the subdistricts was a significant risk factor, but we did not find an association between wild bird habitats and subdistricts with infected flocks. Therefore, the continued monitoring of influenza A virus infection in wild birds is essential.

Over the 2-year study period, all H5N1 outbreak data were collected systematically across the country, minimizing the chance that unmeasured localized events either temporally or spatially confounded the risk estimates





We found no statistical relationship between H5N1 virus infection in subdistricts and period. Undetected or underreported cases in backyard poultry may have occurred during the early outbreaks in 2004, leading to misclassification of the control status of subdistricts and also influencing incidence estimates in each subdistrict studied (Rothman, 1986). Because of the clustered nature of the epidemics, case subdistricts in one period could be control subdistricts in another period and vice versa. This will have caused nondifferential misclassification in our risk factor analysis (Dohoo et al., 2003). Such misclassification could only bias the results toward the null effect, suggesting even stronger ecologic relations than those reported.

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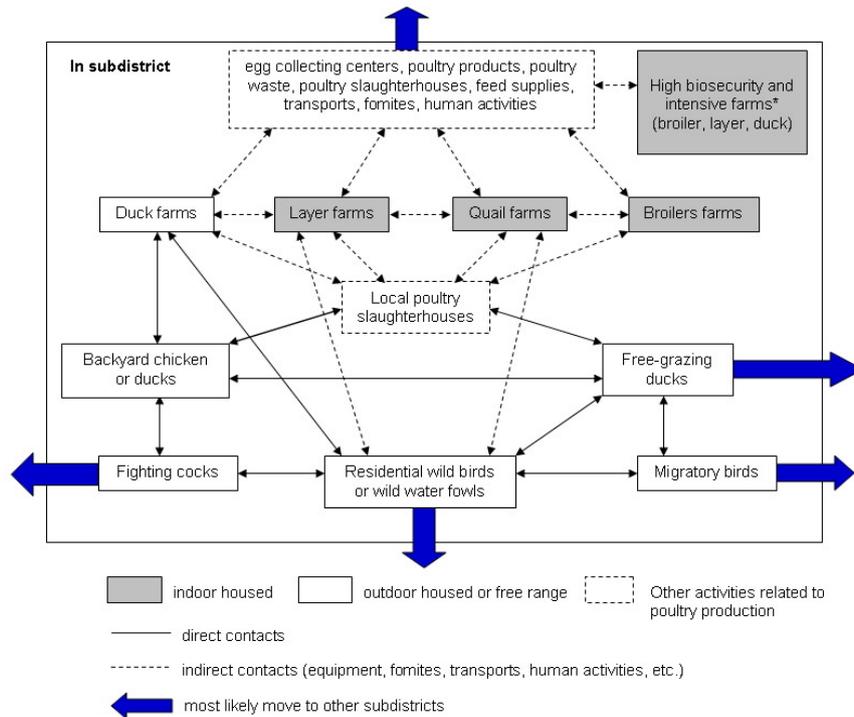
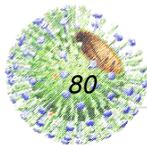


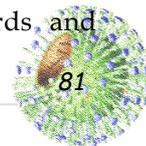
Figure 4-4. Hypothetical causal pathways of highly pathogenic influenza A (H5N1) virus transmission within and between subdistricts in Thailand.



Prevention and control implications

Real-time geographic cluster analysis of H5N1 outbreaks could provide a basis for targeted public education and surveillance activities. Poultry production in Thailand varied from small-scale to large-scale or industrial-scale operations. The larger, integrated commercial chicken farms, operating with modern facilities, tend to be located in the eastern and northeastern regions. Such farms had fewer contacts, combined with enhanced biosecurity, compared with other farms. Of note, these farms were located outside the main affected areas. However, many commercial poultry farms in Thailand operate as small or medium family-run businesses (Songserm et al., 2006; Tiensin et al., 2007), which were associated with subdistricts with a high risk of infection in our study. There is substantial evidence of pathogen movement between and among such farming facilities and release to the external environment (McQuiston et al., 2005; Thomas et al., 2005; Graham et al., 2008). These ecologic data suggest that efforts to control H5N1 must consider risk factors related to the long distance movement involved with certain poultry activities (i.e., fighting cocks and free-grazing ducks) and also with commercial poultry production (i.e., transportation of poultry, poultry products, equipment, waste, and by-products). Similarly, our findings reveal that subdistricts with slaughterhouses more frequently had infected flocks than did those without slaughterhouses. Subdistricts with slaughterhouses, therefore, could be targeted as locations needing protective and monitoring measures. These data also suggest that successful strategies to prevent and control HPAI outbreaks must consider risk factors specific to certain types of poultry production. However, the underlying disease dynamics take place at a between-flock level (Stegeman et al., 2004) and not at a between-subdistrict level. Therefore, additional study of flock-level risk factors is needed.

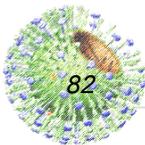
Our analysis demonstrated an association between H5N1 infection in wild birds concomitant with the outbreak among poultry in subdistricts. This may reflect spill-over from the infected poultry to affected wild birds, rather than implicate wild birds as the vector introducing the virus to poultry. When H5N1 virus infection is identified in poultry or wild birds, a clustering pattern may provide additional confirmation of an ongoing epizootic and help define the geographic area of increased animal and human risk. The geographic analysis of H5N1 virus-infected dead bird reports may sometimes provide an early warning of viral activity among wild birds and subsequent domestic poultry infection.



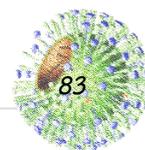


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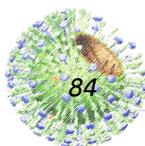


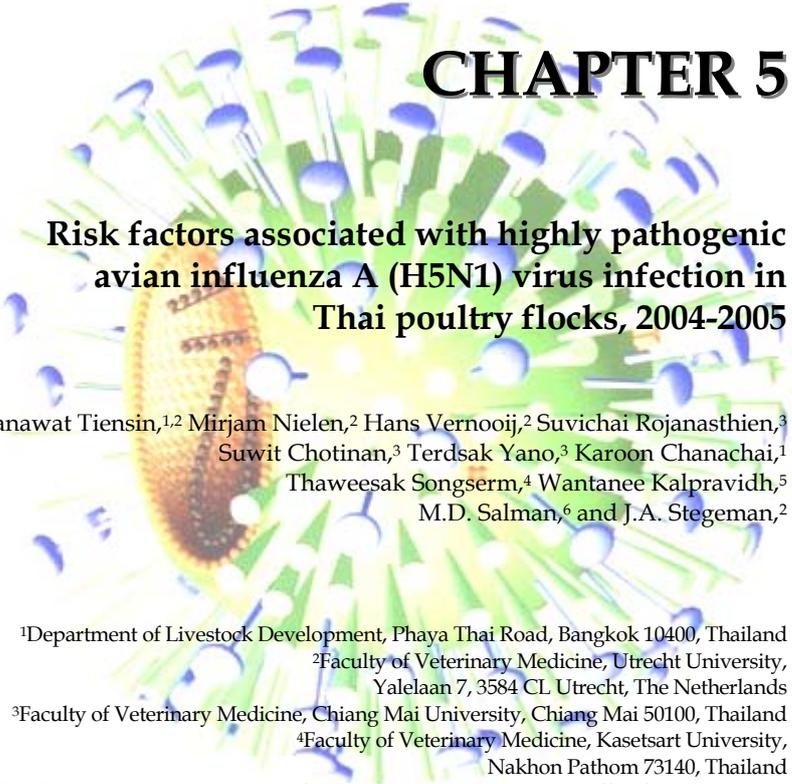
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CHAPTER 5

Risk factors associated with highly pathogenic avian influenza A (H5N1) virus infection in Thai poultry flocks, 2004-2005

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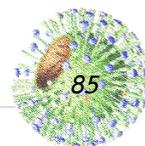
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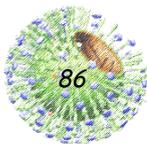
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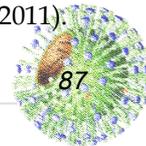
Abstract

Outbreaks of highly pathogenic influenza A (H5N1) virus occurred in Thailand between 2004 and 2008. To investigate potential risk factors associated with HPAI H5N1 infection in poultry flocks, a matched case-control study was conducted in northern Thailand between 2004 and 2005. Questionnaire data on H5N1 exposures was collected from 172 case flocks with H5N1 infection and 456 randomly selected control flocks. A final multivariable conditional logistic regression model indicated that HPAI H5N1 was more likely to occur in flocks which had fighting cocks on the premise (Odds Ratio (OR)=5.81), shared feed with other flocks (OR=3.92), vehicles could enter the premise (OR=13.07), were close to a previous outbreak within 5 km (OR=18.61), or of which farmers had visited suspected flocks (OR=4.95). In contrast, HPAI H5N1 was less likely to occur in flocks of farmers which used tap water (OR=0.08) or buried dead birds on their premise (OR=0.08). Knowledge about their risk levels may help farmers prevent future HPAI dissemination, even if they cannot influence their farm location, the other risk factors are under control of the farmer.



INTRODUCTION

The spread of highly pathogenic avian influenza A (H5N1) virus in the last decennium in poultry and wild birds has resulted in infections among avian species, mammalian species and humans in a large area of the world (Alexander, 2007; OIE, 2011; WHO, 2011). HPAI H5N1 outbreaks caused serious socio-economic consequences through high mortality of poultry, costs associated with disease control, impact on human health and national and international trade restrictions (OIE, 2011; WHO, 2011). Several measures were implemented to stop the spread of the disease in affected countries, i.e., culling of infected flocks, a ban of bird transportation, vaccination, pre-emptive culling of contiguous flocks, active surveillance, hygienic measures, and compartmentalization (Tiensin et al., 2005; Pfeiffer et al., 2007). H5N1 viruses continue to circulate and evolve among poultry, and recurrences of HPAI H5N1 outbreaks have been reported in several countries particularly in Asia (OIE, 2011). Thus, knowledge of introduction and spread of H5N1 infections at flock level in high-risk areas needs to be explored. Previous reports quantified risk factors for influenza (H5N1) infections at flock level in Asia. A case-control study conducted in smallholder farmers in Vietnam found higher risk of an outbreak of HPAI H5N1 in unvaccinated flocks and in flocks that had family and friends visiting (Henning et al., 2009). Studies conducted in Bangladesh (Biswas et al., 2009a; Biswas et al., 2009b) found that offering slaughter remnants of purchased chickens to backyard chickens, having a nearby body of water, and having contact with pigeons were the most significant risk factors for HPAI H5N1. In parallel, in commercial chickens in Bangladesh, accessibility to feral and wild animals and use of a footbath at entry were positively associated with H5N1 infection (Biswas et al., 2009a). An Indonesian study described that transport of poultry in or out of villages was a risk factor for outbreaks in household chickens and ducks (Santhia et al., 2009). In Hong Kong, retail marketing of live poultry was the main route of exposure to infection on chicken farms (Kung et al., 2007). As Thailand is a poultry exporting country, the structure and practice of poultry production is different from other Asian countries (Tiensin et al., 2007). As a result, findings from other Asian studies may be less relevant to the HPAI H5N1 outbreaks in Thailand. In addition, the role of commercial and backyard operations in spread of HPAI H5N1 in Thailand is unclear, as is the importance of wild birds (Tiensin et al., 2009; Li et al., 2010; Reperant et al., 2010; Prosser et al., 2011; Siengsanon-Lamont et al., 2011).





This study reports the results of a matched case-control study to determine risk factors associated with HPAI H5N1 infection in poultry units in high-risk areas in northern Thailand between 2004 and 2005.

MATERIALS AND METHODS

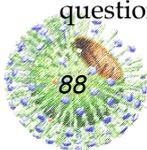
Study population

In Thailand, all outbreaks of HPAI H5N1 in poultry flocks were reported to the Department of Livestock Development, Ministry of Agriculture and Cooperatives through a national animal disease reporting system. From January 2004 through December 2007, 1,890 poultry flocks were confirmed with H5N1 infections; 1,323 (70%) of the 1,890 H5N1 virus-infected flocks were located in central and northern Thailand (Tiensin et al., 2009). Our case-control study areas included nine provinces in the Northern Region of Thailand recognized as H5N1 high-risk areas (Tiensin et al., 2005; Tiensin et al., 2007). The unit of observation of this study was the poultry flock or farm. The study population included 628 commercial and backyard poultry flocks. One hundred seventy-two case flocks were confirmed as HPAI H5N1 infection from September 2004 to October 2005 based on clinical signs and laboratory confirmation by hemagglutination inhibition test, reverse-transcriptase polymerase chain reaction, or virus isolation, as described elsewhere (Tiensin et al., 2007).

Study design and survey method

In the matched case-control design, each case flock was matched with three control flocks (1:3 matched) by flock location at the subdistrict level at the time of outbreak occurrence. Within the subdistrict, potential control flocks were obtained from a list of poultry flocks provided by the District Livestock Office. Three potential control flocks were selected randomly from the list of eligible control flocks. Control flocks had no abnormal deaths or clinical signs of HPAI H5N1, but were not confirmed negative with laboratory diagnosis. Eligible control flocks were poultry flocks that were present in the same subdistrict as the matched case flock for at least 3 months before the date of HPAI H5N1 confirmation in the subdistrict.

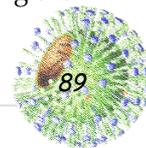
Information of H5N1 infected case flocks and matched control flocks was collected by interview of the poultry owner with use of a standardized questionnaire. After a pretest in 10 flocks, the questionnaire contained 60



questions (with 89 variables) on potential risk factors regarding HPAI status, farm profile, production characteristics (broilers, layer chickens, backyard chickens, meat or layer ducks, or quails), house types, farm management, hygienic and biosecurity measures, poultry stock, feed sources, water sources, direct and indirect contact with poultry. The interviews were carried out by trained veterinarians or animal health assistants from Thailand's Department of Livestock Development and Faculty of Veterinary Medicine, Chiang Mai University. Data collection was carried out in May-November 2005 in the nine provinces in northern Thailand. Data were obtained from representatives (farmers, farm owners or farm managers) from either case or control flocks. Ninety-two of the 720 selected flocks were excluded from this study because of incomplete data. Eventually, only 628 eligible flocks were included for further analysis.

Data analysis

An association between the case and control status and the explanatory variables was first analyzed using univariable conditional logistic regression. Variables with $P \leq 0.2$ were retained for further multivariable analysis. To avoid collinearity in the analysis, each pair of variables was assessed for correlation by Spearman correlation coefficients for continuous variables (Hosmer and Lemeshow, 2000; Dohoo et al., 2003). When variables were highly correlated ($|\rho| > 0.5$), the more biologically plausible variable was chosen for the model (Dohoo et al., 2003). Some continuous variables were categorized before analysis, with categories selected on the basis of the distribution of those variables. Subsequently, a multivariable conditional logistic regression model was used to assess the association between the explanatory variables and the case and control status of a poultry flock. A final model was fitted by backward stepwise procedure, removing the variable with the highest p-value. The new model was then compared with the previous one by likelihood-ratio test. Statistical significance of risk factors was assessed using the likelihood ratio test based on $P \leq 0.05$. Only biological plausible two-way interactions were tested. To assess confounding, the coefficients (β s) were observed by adding the potential confounding variable to the model as explanatory variable. If the β s of the variables of interest in the model changed more than 30%, it was considered that the variables had a confounding relationship with the included variable. Regression coefficients were converted into ORs and their 95% confidence intervals (CIs) (Dohoo et al., 2003). We then assessed model fit using the





Hosmer-Lemeshow goodness-of-fit test and the ratio of the deviance to the degree of freedom (Hosmer and Lemeshow, 2000). The statistical analyses were conducted using SPSS version 16. As the Thai poultry production system may be categorized into 4 sectors (Tiensin et al., 2007), a comparison of risk factors for avian influenza (H5N1) infection among different poultry production sectors was tested.

RESULTS

Table 5-1 describes the number of outbreaks by province during 2004-2005 and the 172 case (infected) and 456 control (uninfected) poultry flocks by province enrolled in this study. Of 172 H5N1 infected flocks, the majority was backyard chickens (74%). Among 628 interviewed case and control flocks, backyard chickens were raised in 559 households (89%). Table 5-2 describes the characteristics of all the flocks included in this study.

In the univariable analyses with 89 variables, 29 met the inclusion criteria ($P \leq 0.2$) (Table 5-3). The multivariable conditional logistic regression with backward procedure resulted in a final model with six risk factors and two protective factors (Table 5-4). The risk factors were 1) having fighting cocks on the premise, 2) sharing feed with other flocks, 3) flocks where vehicles could enter the premise, 4) flock location close to a previous outbreak, 5) flocks of which farmers visited suspected flock. In contrast, HPAI H5N1 was less likely to occur in flocks where farmers used tap water or buried dead birds on their premise (Table 5-4). No significant two-way interaction terms or confounding factors were observed during the model building process.

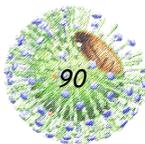


Table 5-1. Number of poultry flocks reported with HPAI H5N1 infection during the 2004-2005 outbreaks and number of case and control flocks enrolled in the study in the 9 provinces of northern Thailand, 2004-2005

Province	No. of infected flocks in 2004-2005	No. of case flocks enrolled	No. of control flocks enrolled
Chiang Mai	10	10 (100%)	30
Chiang Rai	4	4 (100%)	12
Kamphaeng Phet	104	31 (30%)	80
Lamphang	1	1 (100%)	3
Lamphun	1	1 (100%)	3
Mae Hong Son	1	1 (100%)	3
Nan	8	8 (100%)	24
Phitsanulok	220	72 (33%)	186
Uttaradit	44	44 (100%)	115
Total	393	172	456

Table 5-2. Characteristics of the 628 poultry flocks enrolled in the case-control study in nine provinces in Thailand, 2004-2005

Type of poultry	Poultry production sector*	n**	Min	25 Percentile	Median	75 Percentile	Max	Mean
Broilers	2, 3	13	1,000	3,682	4,800	6,100	9,000	4,920
Layer chicken	2,3	19	500	1,800	3,120	5,100	80,000	8,700
Fighting cock	4	74	1	8	25	60	500	46
Backyard chicken	4	559	2	15	24	38	400	35
Layer duck	3, 4	20	2	5	21	152	40,000	2,189
Meat duck	3, 4	18	6	19	30	66	2,000	159
Quail	2, 3	7	500	500	3,000	6,000	20,000	5,000

* Categorization of Thai poultry production into four sectors based on farm management, biosecurity, and market orientation. (A) Sector 1—a closed house using EVAP with high level of biosecurity; (B) Sector 2—a closed house using EVAP or an open house with netting (semi-closed house), moderate level of biosecurity; (C) Sector 3—a caged layer, broiler, or duck farm in open houses, free-grazing ducks, low level of biosecurity; and (D) Sector 4—backyard chickens and ducks, no biosecurity (Tiensin et al., 2007).

** Some flocks have more than one species on the premises, resulting in 710 units.

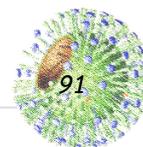
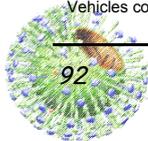




Table 5-3. Descriptive statistics and results of univariable logistic regression analysis of the 28 selected ($P < 0.2$) risk factors for highly pathogenic influenza A (H5N1) virus infection at the poultry flock level, Thailand, 2004–2005

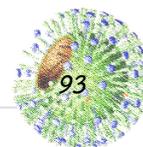
Risk factors	No. (%) of case (N = 172)	No. (%) of control (N = 456)	Conditional Odds Ratio (95% CI)	P
Type of poultry species on premise				
Broiler chickens, Yes	8 (4.7)	5 (1.1)	6.10	0.008
No	164 (95.3)	451 (98.9)	(1.60-23.40)	
Layer chickens, Yes	12 (7.0)	7 (1.5)	6.55	0.001
No	160 (93.6)	449 (98.5)	(2.08-20.59)	
Fighting cocks, Yes	45 (26.2)	29 (6.4)	5.76	<0.001
No	127 (73.8)	427 (93.6)	(3.27-10.16)	
Backyard chickens, Yes	127 (73.8)	432 (94.7)	0.12	<0.001
No	45 (26.2)	24 (5.3)	(0.06-0.24)	
Duck, Yes	10 (5.8)	8 (1.8)	4.50	0.007
No	162 (94.2)	448 (98.7)	(1.50-13.54)	
Quail, Yes	5 (2.9)	2 (0.4)	6.36	0.028
No	167 (97.1)	454 (99.6)	(1.23-32.99)	
Have wild birds on the premise, Yes	8 (4.7)	4 (0.9)	5.10	0.008
No	164 (95.3)	452 (99.1)	(1.53-17.03)	
Flock size, > 27 birds (median)	120 (69.8)	182 (39.9)	3.50	<0.001
< 27 birds	52 (30.2)	274 (60.1)	(2.37-5.16)	
All-in all-out, Yes	11 (6.4)	17 (3.7)	1.79	0.17
No	161 (93.6)	439 (96.3)	(0.78-4.10)	
Source of poultry stock (purchase), Yes	21 (12.2)	14 (3.1)	5.35	<0.001
No	151 (87.8)	449 (96.9)	(2.42-11.82)	
Source of feed (purchase), Yes	97 (56.4)	105 (23.0)	9.60	<0.001
No	75 (43.6)	351 (77.0)	(5.54-16.65)	
Sharing of feed source, Yes	98 (57.0)	126 (27.6)	4.21	<0.001
No	74 (43.0)	330 (72.4)	(2.79-6.34)	
Source of water (tap water), Yes	57 (33.1)	298 (65.4)	0.10	<0.001
No	115 (66.9)	158 (34.6)	(0.05-0.17)	
Sharing of water source, Yes	59 (34.3)	59 (12.9)	10.89	<0.001
No	113 (65.7)	397 (87.1)	(5.27-22.50)	
Use of perimeter fencing, Yes	15 (8.7)	23 (5.0)	2.27	0.045
No	157 (91.3)	433 (95.0)	(1.02-5.04)	
Distance to poultry flocks within 1 km, Yes	90 (52.3)	103 (22.6)	19.46	<0.001
No	82 (47.7)	353 (77.4)	(8.83-42.88)	
Distance to community within 5 km, Yes	127 (73.8)	298 (65.4)	2.22	0.006
No	45 (26.2)	158 (34.6)	(1.26-3.93)	
Semi-close or open house, Yes	26 (15.1)	17 (3.7)	6.24	<0.001
No (no house)	146 (84.9)	439 (96.3)	(2.89-13.50)	
Number of persons involved on raising (continuous variable)	1.13	0.019
(1.02-1.25)				
Personal disinfection, Yes	76 (44.2)	358 (78.5)	0.03	<0.001
No	96 (55.8)	98 (21.5)	(0.01-0.08)	
No. of vehicles visited premises, Yes (≥ 1)	124 (72.1)	181 (39.7)	7.55	<0.001
No (0)	48 (27.9)	275 (60.3)	(4.51-12.62)	
No. of vehicles owned by farmers, > 2	67 (39)	82 (18)	2.99	<0.001
< 2	105 (61)	374 (82)	(2.00-4.45)	
No. of persons visited the premise a month (continuous variable)	1.06	0.007
(1.02-1.10)				
Vehicles could enter the premises, Yes	73 (42.4)	119 (26.1)	3.99	<0.001
No	99 (57.6)	337 (73.9)	(2.33-6.81)	



Risk factors	No. (%) of case (N = 172)	No. (%) of control (N = 456)	Conditional Odds Ratio (95% CI)	P
Burial of dead birds, Yes	157 (91.3)	447 (98)	0.12	<0.001
No	15 (8.7)	9 (2)	(0.04-0.38)	
Farm visited by family or friends, Yes	148 (86.0)	357 (78.3)	2.42	0.007
No	24 (14.0)	99 (21.7)	(1.28-4.58)	
Distance to previous outbreak within 5 km, Yes	121 (70.3)	86 (18.9)	21.09	<0.001
No	51 (29.7)	370 (81.1)	(11.27-39.46)	
Visit suspected or infected farm, Yes	66 (38.4)	70 (15.4)	4.61	<0.001
No	106 (61.6)	386 (84.6)	(2.83-7.52)	
Type of poultry flocks on premise				
Commercial, Yes (Production Sector 2/3)	27 (15.7)	17 (3.7)	6.56	<0.001
No (Production Sector 4)	145 (84.3)	439 (96.3)	(3.05-14.12)	

Table 5-4. A final multivariable conditional logistic regression model for highly pathogenic influenza A (H5N1) at the flock level in Thailand, 2004–2005

	Coefficient (β)	P	Adjusted Odds Ratio	95% CI
Having fighting cocks on the premise, Yes	1.76	0.001	5.81	2.08 – 16.26
Sharing of feed source, Yes	1.37	<0.001	3.92	1.89 – 8.17
Vehicles could enter the premises, Yes	2.57	<0.001	13.07	4.28 – 39.90
Distance close to previous outbreak, Yes	2.68	<0.001	18.61	8.00 – 43.28
Visit suspected or infected farm before, Yes	1.60	<0.001	4.95	2.15 – 11.41
Source of water (tap water), Yes	-2.54	<0.001	0.08	0.03 – 0.22
Burial dead birds, Yes	-2.49	0.003	0.08	0.02 – 0.42



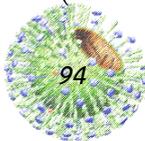


DISCUSSION

Source of infection

The aim of our study was to investigate risk factors associated with HPAI H5N1 infection in poultry units in high-risk areas in northern Thailand during the 2004-2005 outbreaks. Our findings show that flocks in close proximity to outbreaks or other flocks had a greater chance of infection. Many commercial chicken, duck, or quail farms in Thailand operate as small or medium size family-run business that are located in close proximity to each other or near the local community. Chicken and quail eggs are often collected and sold daily or weekly from layer flocks. Consequently, a flock may get infected by vehicles or shared equipment and tools between farms. Egg collection, transport and sale activities among poultry farms can spread the disease to other flocks. Our results also showed that poultry flocks where other vehicles could enter the premise or of which the farmer visited a suspected farm with sick or dead birds had increased risk of HPAI infection. Our findings suggest again that keeping poultry isolated from other flocks may prevent or minimize infections which agreed with other studies showing a strong association between H5N1 outbreak with densely populated areas, short distances to a highway junction, and short distances to large cities (Tiensin et al., 2009; Paul et al., 2010; Yupiana et al., 2010). This indicates that human activities play an important role in the spread of H5N1 in agreement with our univariable results. Factors that require particular attention in risk management include movement of humans (buyers, bird catchers, poultry owners, and friends) and fomites (cages, egg trays, vehicles, manure) between or among flocks because such movements may carry virus over long distances. However, a distance close to other poultry flocks within 1 km was excluded in the final model because the result showed colinearity with other variables.

In this study, poultry flocks sharing feed sources with other poultry were at high risk. In a previous study on ecologic risk factors at the sub-district level in Thailand (Tiensin et al., 2009), a strong association between HPAI infection in wild birds and infected poultry flocks in the same subdistrict was found where birds may share feeding areas and have close contact. Similarly, in Bangladesh a positive association was demonstrated between H5N1 infected flocks and contact with pigeons and wild animals (Biswas et al., 2009a; Biswas et al., 2009b). Sharing of feed or water source is



most common among backyard chickens or ducks which mingle freely in the village. As a result, the outbreak of HPAI in backyard chickens most likely occurs locally in the village.

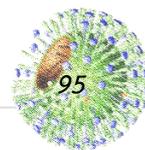
The results showed that the presence of fighting cocks on the premises increased infection risk. Fighting activities within or between villages may cause spread of the disease. This suggests that after returning from a cockfight, the farmer should separate and quarantine those fighting cocks for at least 1-2 weeks to minimize risk of infection from other cocks.

Use of tap water and burial of dead birds on the premise were identified as protective factors, suggesting that those farmers may have better biosecurity and biocontainment on their farm. Burial of dead birds is the most common procedure in both cases and controls. Other procedures could be related to more risk, such as selling or throwing away sick birds to other areas. Use of tap water may avoid contamination of virus from other water sources such as canals or public ponds. Songserm et al. (2005) demonstrated that H5N1 virus is stable in water in rice fields in Thailand.

The risk of avian influenza infection was increased in backyard operations in Asian countries such as Indonesia, Vietnam, and Bangladesh (Biswas et al., 2009b; Minh et al., 2009; Yupiana et al., 2010). In contrast, our univariable findings found that HPAI H5N1 was likely to occur in poultry sector 2 and 3 (commercial operations) which agreed with HPAI outbreaks in Japan (Nishiguchi et al., 2005; Nishiguchi et al., 2007), Republic of Korea (Lee et al., 2008; Kim et al., 2010) (Kwon et al., 2005), the Netherlands (Thomas et al., 2005; Bavinck et al., 2009) and Hong Kong SAR (Kung et al., 2007).

Limitations of the study

The administration of the questionnaire may have been inconsistent between interviewers depending on their experience and interpretation (Rothman and Greenland, 1998). The interviewers were not blinded to the case-control status of the flock owners (interviewees). Some questions may have been interpreted subjectively by persons answering or completing the questionnaire. Serological and virological testing was not conducted among the control flocks because of the high fatality rate among H5N1-infected poultry. This may have caused some misclassification of control flocks because we observed only flocks without abnormal deaths and clinical signs during the outbreaks, and may have biased our results towards the null-effect. Practically, even in farms which applied hygiene



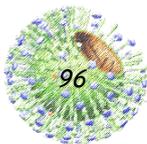


procedures, the same measures were not always applied to visitors or vehicles that entered the farm. The degree of implementation of hygiene measures may be different among poultry farms, but was not studied in our questionnaire.

Practical implications

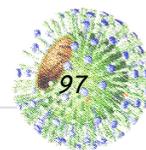
The evidence from this study suggested again that movement of humans, vehicles, and live birds between flocks contributed significantly to the occurrence of the disease, where use of tap water and proper handling of dead birds was protective. Our univariable analysis suggests that commercial poultry production, with an ostensibly more “biosecure” system of production, is not associated with a reduction in risk of HPAI infection at the farm or flock level, compared to subsistence backyard flocks. Although the majority of reported HPAI outbreaks in Thailand in 2004 occurred in the latter, this is primarily due to their relatively greater numbers (Tiensin et al., 2005). However, no infections occurred in Thailand in the poultry sector 1 farms (Songserm et al., 2006; Tiensin et al., 2007). This may indicate that the biosecurity measures might have been breached or incompletely applied somehow resulting in introduction of HPAI H5N1 virus into the flocks of sector 2 and 3 (Otte et al., 2007; Graham et al., 2008).

In conclusion, this study in Thailand suggested that risk of HPAI infection of the flock is manageable, because most factors are under control of the farmers, with the exception of clustering of farms and humans.



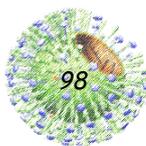
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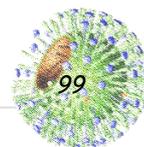


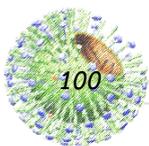


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CHAPTER 6

General discussion



The main goal of this thesis was to better understand epidemiological features, transmission dynamics, and risk factors of highly pathogenic avian influenza (HPAI) H5N1 virus outbreaks in Thailand during 2004-2008. Thailand first reported H5N1 HPAI in 2004, but by then the disease was widespread throughout the country. Several control measures were implemented to contain the outbreaks. The last outbreak of HPAI H5N1 in Thailand was reported in November 2008. Since 2004, major changes in disease surveillance and biosecurity measures have been implemented to prevent and control the disease.

It is important to understand why no cases in poultry or humans have been reported since November 2008. Many of the factors that could lead to persistence of H5N1 HPAI have been eliminated. Therefore, this chapter also reveals approaches to control, prevention and elimination of HPAI H5N1 in Thailand as compared with those of other countries particularly in Asia where HPAI H5N1 remains endemic.

LESSONS LEARNED FROM THE HPAI H5N1 EPIDEMICS IN THAILAND AND OTHER ASIAN COUNTRIES

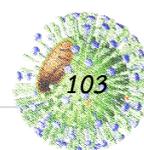
Findings from HPAI H5N1 outbreaks in Thailand

The outbreaks of HPAI H5N1 virus in Thailand during 2004-2008 had serious consequences for poultry production, human health, social community, and farmers' livelihoods (Tiensin et al., 2005; Tiensin et al., 2009). Retrospective analyses of the 2004-2005 HPAI H5N1 outbreaks lead to some considerations. Firstly, the Central Region and the lower part of the Northern Region of Thailand can be considered a high risk area for avian influenza infection (Tiensin et al., 2005; Gilbert et al., 2006; Tiensin et al., 2007a). This could be linked to the high density of the poultry population in these regions and, in particular, the large numbers of free-grazing ducks raised in the rice fields (Gilbert et al., 2007; Tiensin et al., 2009). Domestic ducks likely hampered detection and control of the disease in 2004-2005, because they can excrete HPAI H5N1 virus without signs of illness (Sturm-Ramirez et al., 2005; Songserm et al., 2006). Intensive surveillance programs implemented to detect HPAI H5N1 virus in duck flocks in the abovementioned regions have improved this since 2005. Lastly, the comparison between the 2004 and the 2005 episodes suggested that the incidence of HPAI H5N1 in Thailand was reduced dramatically since stringent control measures had been implemented especially in the Central, Northern and Eastern regions (Tiensin et al., 2007a).

During 2006-2008, HPAI H5N1 outbreaks occurred sporadically, and they could be contained quickly. Besides Thailand, HPAI H5N1 viruses have been reported in 67 countries since 2003. Most of them have managed to eradicate the virus; however, the virus is still endemic in Bangladesh, China, Egypt, India, Indonesia, and Vietnam (OIE, 2011; WHO, 2011).

No HPAI H5N1 outbreak was detected in sector 1 poultry farms (Tiensin et al., 2005; Songserm et al., 2006; Tiensin et al., 2007a), suggesting that virus introduction onto a farm can be prevented by stringent biosecurity and biocontainment measures. However, outbreaks in poultry production sector 2 and 3 were reported, indicating the need to identify risk factors in these poultry units. We found that a high density of poultry, the local landscape (e.g., wetlands, water reservoirs, and rice paddies), and traditional farming practice (e.g. free-grazing) in these regions were risk factors for outbreaks. In addition, virus spread was strongly associated with the presence of free-grazing duck populations and the practice of free-grazing farming (Tiensin et al., 2005; Gilbert et al., 2006; Songserm et al., 2006; Tiensin et al., 2009). Although the probability of infection was lower in backyard flocks than in poultry sector 2/3 flocks, most HPAI H5N1 virus infections were observed in backyard poultry in 2004-2005 (Tiensin et al., 2007a), because they largely outnumber the other flocks. Backyard poultry greatly contribute to local consumption and subsistence agriculture in rural areas. However, the traditional methods of raising backyard chickens and ducks make it quite a challenge to implement effective biosecurity measures.

To allow the prompt detection of HPAI infection followed by quick implementation of control measures, surveillance programs were strengthened. Since 2004, intensive active surveillance programs (known as “X-ray survey”) have been carried out twice a year by randomly collecting cloacal swabs in the poultry population and searching suspected cases of avian influenza in the villages nationwide. Moreover, proactive clinical surveillance of avian influenza has been performed which aimed at reducing the time between infection and implementation of control measures. More specific, all birds, their products, and other potentially contaminated materials had to be destroyed without delay if the poultry death rate in any backyard flock was higher than 5% within 2 days in several flocks in the same village or if in a commercial flock the death rate was higher than 1% within 2 days or food and water intake reduced by 20% within 1 day. There was a financial compensation scheme for culling of the affected flocks.





In the affected flocks, cloacal swabs were collected for laboratory confirmation. Additionally, registration of fighting cocks and free-grazing duck flocks was implemented; cloacal swabs and blood samples from the cocks and ducks were collected every 6 months for disease monitoring. Moreover, biosecurity of the fighting cocks on the premises has also been improved. In addition to movement control, electronic service to register animal movements (called “e-movement”) has been developed. Check points for poultry movement control were established throughout Thailand. To further strengthen surveillance activities at sub-district level, Thai veterinary authority (Department of Livestock Development) recruited more than 2,000 animal health assistants to work in high risk subdistricts. In collaboration with the Ministry of National Resources and Environment, surveillance programs in wild birds were conducted. Cloacal swabs and data of wild birds (e.g. species, age, latitude and longitude of their habitats) were collected and submitted for laboratory diagnosis. Additionally, those birds were also tagged with leg rings for further study on migration flyway. All control measures and surveillance programs abovementioned have been implemented under the National Strategic Plan for Avian Influenza Control and Flu Preparedness.

As mentioned above, the 2004 cumulative incidence and relative risk (RR) of HPAI H5N1 infection showed a higher probability of infection in the Central, Northern, and Eastern Regions relative to other parts of Thailand (Tiensin et al., 2005). In addition, the RR for HPAI infection was high in broilers, layers, quails, geese, and ducks compared to backyard chickens. Otte et al. (2007) revealed that the different regions also have a different mixture of flock types, and they found an interaction between region and species, such that within the Northern and Central regions, backyard flocks had the lowest probability of an outbreak, whereas in the East, there was relatively little difference in the probability of infection between flock types, although layers and geese had the highest probability. Across regions, the probability of infection for quail flocks is by far the highest of all species. In Thailand, quail eggs and meat are commonly sold in the market and moved across the regions. In addition, quail flocks have low biosecurity measures compared to chicken broiler or layer flocks. This may increase the higher probability of infection for quail flocks in Thailand. Taken together, the data do not support the assumption that backyard poultry in Thailand is more at risk for HPAI infection than larger and confined commercial poultry operations (that is either layer, or broiler chickens). However, this conclusion is based on the assumption that detection

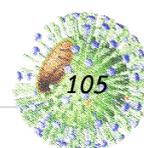
of HPAI H5N1 is equally effective in backyard flocks than in commercial poultry flocks. One of the remarkable observations during the epidemic was that the number of HPAI H5N1 detected in poultry increased during “X-ray surveys” (intensive active surveillance programs) (Tiensin et al., 2007a). This most likely indicates underreporting in the periods between these surveys. Thus, the number of outbreaks in backyard poultry may have been underestimated because backyard farmers may not always have reported small numbers of chicken or duck deaths to the authority. Another reason why we have to pay attention to backyard flocks is that the total number of outbreaks was highest in backyard flocks, because of the high number of backyard flocks present (Tiensin et al., 2005; Otte et al., 2007).

In 2006-2008, H5N1 outbreaks occurred locally and sporadically in only some provinces in the lower part of the Northern Region and the Northeastern Region of Thailand (Tiensin et al., 2009; OIE, 2011).

Comparison with HPAI H5N1 situations in other countries

Our findings of increased risks of HPAI infection in commercial poultry flocks are consistent with other studies conducted in connection with outbreaks of H5N1 in Hong Kong, South Korea, Japan, and Bangladesh (Kwon et al., 2005; Kung et al., 2007; Biswas et al., 2008; Biswas et al., 2009; Sugiura et al., 2009; Ahmed et al., 2010; Kim et al., 2010) and of H7N7 in the Netherlands (Thomas et al., 2005; Bavinck et al., 2009). However, in Indonesia significantly more outbreaks were found in backyard flocks as compared to large-scale commercial poultry operations (Yupiana et al., 2010).

During the epidemics in Thailand, the number of HPAI outbreaks was higher in poultry dense regions than in sparsely populated areas, as was found in studies from other Asian countries (Pfeiffer et al., 2007; Gilbert et al., 2008; Biswas et al., 2009; Minh et al., 2009). Poultry density is most likely associated with the number of contacts between poultry flocks. Transportation of live poultry is one of the most important routes of transmission. Transportation of live birds at the beginning of the outbreaks in a region may have caused wide spread of the outbreaks in affected areas. When an outbreak in a poultry flock starts, to avoid loss of his money, the owner may decide to sell the live chickens not yet showing clinical signs as soon as possible. This action is more associated with commercial poultry flocks than with backyard flocks, because the farmers are dependent on this trade for their income.

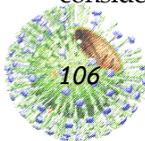




However, this observation is not in accordance with findings from Indonesia, where the number of poultry outbreaks did not increase with an increase in poultry density (Yupiana et al., 2010). The reason may be that the highest poultry population densities in Indonesia are found in areas with industrialized farms that have good management practices, biosecurity and vaccination programs. Another explanation could be the transportation of the sick birds from high to low poultry population density districts. Several districts in Indonesia import live poultry from other areas within or outside the province. When outbreaks in poultry occur, it will affect many chickens in the same location, which either will die or become subclinically infected.

During the outbreaks in Thailand, we found that high numbers of HPAI detections coincided with low temperatures (average 23-27 degree Celsius) in Thailand from October to February, when wild birds from central and northern Asia migrate into Thailand (Ellis et al., 2004; Siengsanant et al., 2009). Seasonal conditions might have been more favorable for the virus in winter and, moreover, bird migration might have contributed to this pattern. The lower temperature in winter supports survival of the virus in the environment and facilitates transmission (Songserm et al., 2005). In addition, several festivals, which are associated with raising, selling, and transporting poultry, occurred around the end of the year (Tiensin et al., 2009) further facilitating spread of the virus. In Vietnam, the temporal pattern of the reported HPAI outbreaks also occurred around the Vietnamese New Year holiday period from January till February. During this period, there is an increased demand for poultry and poultry products, and therefore trade of poultry prior and during the festival period is a likely explanation for the virus spread (Pfeiffer et al., 2007; Minh et al., 2009).

Retail marketing of live poultry on live-bird markets (LBM's) was implicated as the main source of exposure and spread of H5N1 virus across Asia. Repeated outbreaks of HPAI H5N1 outbreaks in poultry occurred in LBMs within the Hong Kong Special Administrative Region since 1997 (Guan et al., 2002; Sims et al., 2003a; Sims et al., 2003b; Kung et al., 2007). Moreover, several studies indicated that H5N1 viruses circulated among geese, ducks, and chickens in LBMs in affected Asian countries since 2003 (Nguyen et al., 2005; Jadhao et al., 2009; Liao et al., 2009; Van Kerkhove et al., 2009; Indriani et al., 2010). However, in Thailand there are very few live-bird markets. The sale of live chickens or ducks in the retail market is considered neither practical nor popular in Thailand (Tiensin et al., 2009).

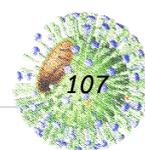


However, a surveillance program for influenza A viruses (H5N1) was conducted in LBMs and food markets in central Thailand during 2006–2007. The subtype H5N1 viruses circulating in the markets were genetically related to those that circulated in Thailand during 2004–2005 (Amonsin et al., 2008). A possible explanation for virus contamination in LBMs and food markets may be animal movement from outbreak areas to the markets as well as an attempt to sell infected (dead or dying) birds, especially quail, as bird meat. In addition, most animals or meats in the markets came from backyard farms or farm sector 3. Under the National Strategic Plan for Avian Influenza Control and Flu Preparedness, a surveillance program for influenza A viruses (H5N1) has been conducted in LBMs in Thailand. Together with the limited number of LBM's in Thailand this may have given Thailand a better starting point for eradication of H5N1 virus than other Asian countries.

Different control measures and practices in H5N1 affected countries

Infected countries have implemented varying control measures such as stamping-out infected flocks, restrictions on poultry movement, cleaning and disinfecting, and vaccinating poultry (Capua and Marangon, 2000; Stegeman et al., 2004; Tiensin et al., 2005; Kung et al., 2007; Pfeiffer et al., 2007; Chen, 2009; Sugiura et al., 2009; Yupiana et al., 2010; OIE, 2011).

Control measures implemented in Thailand were stamping-out, pre-emptive culling, movement restrictions, disinfection, active surveillance, improvement of biosecurity and biocontainment, restructuring of poultry production system, education and public awareness campaign, and application of compartmentalization (dividing the country into compartments). However, vaccination has been prohibited. Additionally, financial compensation was available for stamping-out in Thailand. In 2004, the Thai Government paid full compensation to affected farmers (Tiensin et al., 2005). Rural villages are dependent economically on poultry for food and income; however, in many countries a culling program did not include compensation for their losses. Morris (2006) reported poor compliance to regulations in Cambodia, where there was little incentive for reporting ill or dead poultry to the government as there was no compensation for their losses (Morris, 2006). Consequently, the compensation scheme in Thailand may have been an important factor explaining the success of control in Thailand.





Thailand has been successful in controlling and eliminating HPAI H5N1 by implementing stringent control measures even though it is extremely difficult to control this disease in densely populated poultry areas. However, because export of poultry products is important for Thailand, there was a lot of pressure from the industry to effectively control the disease. Education of farmers on basic concepts of biosecurity and biocontainment is crucial to control avian influenza virus. Moreover, early warning, rapid detection, and quick response are absolutely essential to prevent and control an HPAI epidemic. The success of HPAI elimination, therefore, depends on a collaboration of all stakeholders, including farmers, poultry industries, veterinarians, public health authorities, academic institutions, media, and the government.

Since 2003, HPAI H5N1 outbreaks have been reported in 68 countries (OIE, 2011). Today, most of them have managed to stamp it out, but the virus remains firmly entrenched in Bangladesh, China, Egypt, India, Indonesia, and Vietnam. Some factors may have contributed to the persistence of H5N1 virus in those countries. Firstly, it may relate to the structure of their national poultry sectors. Endemically infected countries usually feature complex production and market chains, with poultry reared and sold under conditions that give little protection to incursion of influenza viruses. Secondly, the quality of public and private veterinary and animal production services is not always able to detect and respond to infections properly or identify and correct underlying structural problems in production and marketing systems. Lastly, the level of commitment to dealing vigorously with H5N1 is not a priority and, consequently, the fear of H5N1 does not result into concrete plans for virus control and elimination (Biswas et al., 2009; Basuno et al., 2010; Soares Magalhaes et al., 2010; Yupiana et al., 2010).

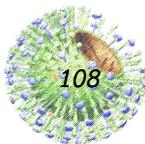
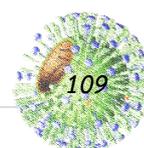


Table 6-1. Vaccination policy of countries infected with H5N1 avian influenza virus. (Source: "Avian influenza vaccines: a practical review in relation to their application in the field with a focus on the Asian experience" and "OIE Update on Avian Influenza in Animals (Type H5 and H7)" (Peyre et al., 2009; OIE, 2011))

Country	Vaccination status (practical application)
Afghanistan	Unknown (not in use)
Albania	Prohibited
Azerbaijan	Prohibited
Burkina Faso	Authorized (not in use)
Cambodia	Unknown (not in use)
Cameroon	Unknown
P.R. China	Authorized (in use)
Ivory Coast	Authorized (not in use)
Egypt	Authorized (in use)
France	Authorized for preventive vaccination on selected birds (in use)
Germany	Prohibited
Hong Kong (SAR)	Authorized (in use)
India	Authorized for emergency vaccination (not in use)
Indonesia	Authorized (in use)
Iraq	Prohibited
Israel	Prohibited Exception for endangered species (in use for ostrich farms regarding EC/94/2004)
Japan	Prohibited
Jordan	Authorized (not in use)
Kazakhstan	Prohibited
Korea (Republic of)	Prohibited
Laos	Unknown (not in use)
Malaysia (peninsular)	Prohibited
Myanmar	Prohibited
Netherlands	Authorized for preventive vaccination on selected birds (in use)
Niger	Authorized (not in use)
Nigeria	Prohibited (but illegal use suspected)
Palestinian Autonomous Territories	Prohibited
Pakistan	Authorized (not in use)
Romania	Authorized (not in use)
Russia	Authorized for emergency vaccination around outbreaks (in use)
Serbia and Montenegro	Prohibited
Sudan	Authorized (not in use)
Sweden	Prohibited
Switzerland	Authorized for preventive vaccination on selected birds (in use)
Thailand	Prohibited
Turkey	Prohibited
Ukraine	Prohibited
Vietnam	Authorized (in use)



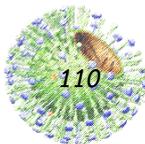


ESTIMATES OF WITHIN-FLOCK TRANSMISSION

In Chapter 3, the within-flock transmission of HPAI H5N1 virus was quantified using daily mortality data from infected chicken flocks during the epidemic in Thailand originating from different types of flocks. The values of the transmission parameter (β) resulting from this study were between 2.3 (for a 1-day infectious period) and 0.6 day⁻¹ (for a 4-day infectious period) (Tiensin et al., 2007b) which are much lower than those observed for H7N7 (33 day⁻¹ (van der Goot et al., 2005) and 4.5 day⁻¹ (Bos et al., 2009)). However, HPAI H7N7 virus transmission (β) in turkeys was 1.43 (for a 2-day infectious period) (Bos et al., 2010). Moreover, the transmission parameter estimated in Vietnam was even lower than the estimate described in this thesis (Soares Magalhaes et al., 2010).

The estimates reported in chapter 3 were based on the results of a SIR model with a default scenario of a 2-day infectious period. Assuming chickens have a short latent period, a SIR model was used instead of an SEIR model which resulted in a better fitting of the model to field data. Bouma et al (2009) showed that (i) the period of latency of H5N1 influenza virus in unvaccinated chickens is short (mean: 0.24 days; 95% confidence interval: 0.099–0.48 days) and (ii) the infectious period of H5N1 virus in unvaccinated chickens is approximately 2 days (mean: 2.1 days; 95%CI: 1.8–2.3 days).

In addition, the estimates of R_0 described in chapter 3 were between 2.2 (95% CI: 1.9–2.5) and 3.2 (95% CI: 2.0–5.0) (Tiensin et al., 2007b), comparable to the estimate of 1.99 (95%CI: 1.87–2.10) for HPAI H5N1 in chickens in Vietnam (Soares Magalhaes et al., 2010) and the estimate of 1.6 (0.90–2.5) for HPAI H5N1 in unvaccinated chickens under experimental conditions (Bouma et al., 2009). Very likely differences in H5N1 HPAI transmission between chickens exist because of differences in contact structure among chicken types, age, flock size, breed, and management. Moreover, differences in the density at which the chickens are housed may have an impact on transmission. However, the result in chapter 3 did not show a significant difference of within-flock transmission among different chicken types (Tiensin et al., 2007b). Nevertheless, the resulting point estimates support the intuitive suggestion that the within-flock transmission of backyard chickens and fighting cocks is lower than that of broilers and layer hens that are kept at higher within-flock densities. The difference of transmission rate between the HPAI H5N1 virus in Thailand and the HPAI H7N7 virus



in the Netherlands (Bos et al., 2009) could have been caused by differences in virus, contact structure within poultry flocks, different susceptibility and/or infectivity of the birds to the virus or a combination of these factors.

Application of modeling to intervention strategies

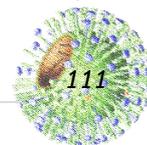
In the study reported in chapter 3, the upper limit of R_0 was 5.0 based on the model with broiler and layer combined as a categorical variable (Tiensin et al., 2007b). Taking this value, if ~80% of a flock can be effectively immunized, a major outbreak can be prevented within a flock, based on the fraction of $1-1/R_0$ (Anderson and May, 1992). This estimate of the vaccination coverage is in agreement with findings of Bouma et al., (2009). It seems feasible in commercial flocks to apply and maintain vaccination coverage at even more than 80% of a total flock. However, vaccination coverage of 80% might be more difficult in backyard chickens. Another problem is that vaccine efficacy is seldom 100%.

Mortality data collected at the flock level was used to quantify transmission of HPAI virus. Nowadays, mathematical modeling is widely used and has become a tool for preparedness planning and for modeling of new disease outbreaks. The results of our analyses seemed quantitatively robust, so our transmission parameter values can be used in simulation models to estimate the effectiveness of intervention measures.

Feasibility of vaccination in Asian situations

Vaccination has proven to be a useful additional measure implemented alongside controlled culling in Italy in 1999-2000 (H7N1), in Mexico in 1995 (H5N2), and in Pakistan in 2003 (H7N3) (Capua and Marangon, 2003; Naeem and Siddique, 2006; Villarreal, 2006). However, vaccination against HPAI is not allowed in most countries (Table 6-1), because of its interference with AI surveillance tests and its negative impact on poultry trade.

Vaccination against H5N1 in Asia has been practiced in China, Hong Kong SAR, Vietnam, and Indonesia (Ellis et al., 2006; Bouma et al., 2008; Chen, 2009; Pfeiffer et al., 2010; Soares Magalhaes et al., 2010; Yupiana et al., 2010; OIE, 2011). When a large proportion of poultry is kept in backyard flocks, vaccine dissemination and coverage is practically difficult. Crucial points to the success of a vaccination campaign are the implementation of strategies to upgrade biosecurity, monitor vaccine coverage, identify





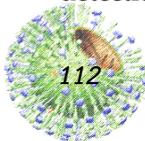
infected flocks, and the appropriate management of infected flocks, regardless of their vaccination status. The use of high quality vaccines and post-vaccination monitoring are also essential for the successful implementation of vaccination. Different vaccination strategies could be applied according to the risk profile and capacity of a country (Capua and Marangon, 2007; Bruschke et al., 2009; Peyre et al., 2009).

- (1) Preventive vaccination may be used in a country free from infection but at high risk of introduction of the virus. For instance, France, The Netherlands, and Switzerland vaccinated zoo birds at high risk.
- (2) Emergency vaccination may be conducted during an outbreak. All unaffected animals within and around an outbreak quarantine zone should be vaccinated. The size of the vaccination zone depends on the transmission rate and initial spread during the high-risk period. However, the efficacy of this strategy depends on various factors including vaccine availability and the feasibility of rapid administration. This represents a major limitation for developing countries.
- (3) Prophylactic vaccination should be applied when the disease has become endemic. Birds are vaccinated systematically against the same HA subtype of the virus circulating in poultry to obtain a minimum protective level within the “at-risk population”. The final goal of the approach is to control (and then to eradicate when possible) the disease within the country. This could be a long-term vaccination plan which should be applied nationwide on all commercial and backyard poultry farms. Strict control measures including stamping-out should be applied to infected flocks

SPATIAL ANALYSIS AND RISK FACTORS

Characterizing spatial and temporal patterns among epidemics in which the mechanism of spread is uncertain is important for generating disease spread hypotheses, which may inform disease control and prevention strategies. Several techniques can be used to investigate temporal, spatial, and spatio-temporal patterns of HPAI outbreaks (Anselin, 1995; Kulldorff and Nagarwalla, 1995; Jacquez and Greiling, 2003; Ghneim et al., 2007; Pfeiffer et al., 2007; Ahmed et al., 2010; Ersbøll and Ersbøll, 2009).

In Chapter 4, spatial clusters of HPAI at sub-district level in Thailand in different time periods during 2004-2007 were detected using two local cluster detection methods: Anselin’s Local Moran (LISA) test and the spatial scan

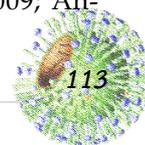


statistic test (Tiensin et al., 2009). The different cluster detection methods both identified roughly the same high risk clusters and low risk areas, suggesting that the results are robust.

H5N1 clusters were scattered all over the country in 2004. This may indicate a long-distance spread of the infection by means of transport of poultry and poultry products. After control measures had been implemented (i.e. restrictions on transport of commercial chickens, free-grazing ducks, and fighting cocks, and pre-testing of H5N1 infection in poultry flocks before movement), the outbreaks occurred locally in high cluster areas in Central Thailand (Tiensin et al., 2009). In Vietnam, HPAI epidemics also occurred in the period of active poultry population buildup immediately prior to the Vietnamese New Year (January-February). Outbreaks were clustered in space and time within both northern and southern deltas, consistent with infection transmission occurring via a combination of local and long-distance spread (Pfeiffer et al., 2007; Minh et al., 2009). Whereas significant primary clusters of HPAI outbreaks in Bangladesh were identified in the central part of the country dominated by commercial production systems and in the northwestern part primarily in backyard production systems (Ahmed et al., 2010).

A spatial-based case-control study was described (in Chapter 4). To elucidate the complex and multi-factorial origins of H5N1 epidemics, a multiple potential pathway was drawn. Figure 6-1 illustrates a scheme of multiple potential pathways for exposure to and transfer of pathogens in small-scale poultry operations in rural areas. It also shows multiple factors which can transmit the infection at subdistrict level (Tiensin et al., 2009). The results of the spatio-temporal analysis show that sub-districts with a high flock density of fighting cocks and presence of quail and free-grazing duck flocks had higher infection rate than those with low flock density of these poultry types. Sub-districts with poultry slaughterhouses were more often infected than those without slaughterhouses. Live-bird markets were not included in this study as a risk factor, because there are very few live-bird markets in Thailand.

Our study revealed that the odds of H5N1 infection were significantly higher in sub-districts with commercial poultry flocks as compared to those with backyard flocks. Therefore, biosecurity and biocontainment of commercial poultry farms must be improved to mitigate HPAI infection. Understanding the relation between infected sub-districts and the presence of free-grazing duck flocks is crucial, and our findings agree with other studies (Gilbert et al., 2007; Pfeiffer et al., 2007; Minh et al., 2009; Ahmed et al., 2010).





In addition, a significant association between H5N1 infected sub-districts and the evidence of H5N1 infection in wild birds in the same sub-district was demonstrated (Tiensin et al., 2009). Therefore, the continued monitoring of influenza A virus infection in wild birds is essential (Siengsanan et al., 2009; Knight-Jones et al., 2010; Li et al., 2010). The dead wild bird surveillance program and the active surveillance activities in wild birds have contributed detailed information about the prevalence, pathology and molecular epidemiology of avian influenza A viruses in a wide variety of wild avian species. This information will raise awareness of avian influenza A viruses in wild birds and alert people to take measures to prevent virus introduction from these birds into domestic poultry. From a temporal and a geographical perspective, HPAI outbreaks did not occur uniformly or randomly across Thailand in 2004, but were shown to be linked to certain agroecological factors (e.g. rice field and wet land areas) and HPAI infection in wild birds such as the extent of wetlands and rice paddies (Gilbert et al., 2007; Tiensin et al., 2009; Siengsanan-Lamont et al., 2010).

Previous studies of HPAI outbreaks in poultry have suggested that several risk factors are involved in the spread and maintenance of this virus. For example, it was shown that areas with ducks and sharing of scavenging areas with ducks had higher infection rates, which were strongly related to agricultural land use and rice crop intensity. Poultry slaughter houses, fighting cocks, quail flocks, vaccination status, and climate (specifically annual precipitation) have also been found to be associated with outbreaks in poultry. These factors could be potential risk factors for the spread and maintenance of HPAI virus in Asian countries including Thailand (Kung et al., 2007; Biswas et al., 2008; Fang et al., 2008; Biswas et al., 2009; Minh et al., 2009; Ahmed et al., 2010; Yupiana et al., 2010). Free-grazing practice may be modified by moving ducks locally with short distance within the village or district. Moreover, monitoring and surveillance program in free-gazing ducks and scavenging chickens must be strengthened.



6

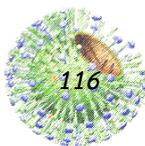
Figure 6-1. A multiple potential pathway for HPAI H5N1 infection within the environment, wild birds, domestic poultry, and humans of small-scale poultry operations in rural areas. (Source: Modified from an educational material produced by UNICEF, FAO, and Myanmar's Livestock Breeding and Veterinary Department)



ANIMAL-HUMAN-ECOSYSTEM INTERFACE AND HPAI H5N1

Understanding the animal-human-ecosystem interface is a critical element in preventing, evaluating and predicting risks of emerging zoonotic diseases, as well as in designing evidence-based programs for prevention and early detection of emerging infectious diseases, such as avian influenza. Poultry production has changed from small-scale methods to industrial-scale operations. There is substantial evidence of pathogen movement between and among these industrial facilities, release to the external environment, and exposure to farm workers, which challenges the assumption that modern poultry production is more biosecure and biocontained as compared with backyard or small holder operations in preventing introduction and release of pathogens (Schlundt et al., 2004; Karesh et al., 2005). The changes in organization, intensity, housing, and waste management may influence the emergence and transfers of avian influenza virus among wild and domestic species, and from avians to human populations. Most importantly, the modern methods of poultry production have changed particularly in broiler and layer chickens. These operations result in high numbers of poultry housed under confined conditions at great density and geographic concentration. These methods of food animal production generate many routes of pathogen transfer among wild and domesticated species and from animals to humans through occupational, peri-occupational, and environmental pathways. Figure 6-2 shows a schematic representation of multiple potential pathways for exposure to and transfer of pathogens within the environment of concentrated animal feeding operations (CAFOs) (Graham et al., 2008; Van Kerkhove et al., 2011).

At the animal-human-ecosystem interface in these operations, there is inadequate protection of workers and their communities, and, more generally, there is incomplete biocontainment to prevent transfers from the animal house to the general environment. For example, it has not been recognized that the necessity for high ventilation of densely confined animals greatly impairs attempts at biocontainment. Moreover, little attention has been given to the generation and lack of management of the millions of tons of animal wastes generated annually. Food animal wastes are largely disposed on land, and this creates an unrecognized magnet for wild avians because of the presence of undigested feed in the waste. There is some use of poultry wastes as bedding for fish ponds, which creates an additional opportunity for wild avian contact (Graham et al., 2008).



An analysis of data from the 2004-2007 HPAI H5N1 in Thailand indicated that the odds of H5N1 outbreaks and infections were significantly higher in large-scale commercial poultry operations as compared with backyard flocks (Tiensin et al., 2005; Tiensin et al., 2009). These data suggest that successful strategies to prevent or mitigate the emergence of pandemic avian influenza must consider risk factors specific to modern industrialized food animal production. The analysis of the Thai HPAI outbreak and surveillance data suggests that commercial poultry production, an ostensibly more “biosecure” system of production, is not associated with a reduction in risk of HPAI infection at the farm or flock level, as compared with that experienced by subsistence backyard producers. Although the majority of reported HPAI outbreaks in Thailand in 2004-2005 occurred in the latter, this increased cumulative risk of HPAI in the backyard sector is primarily due to their absolutely greater numbers of flocks rather than more risky production practices. As a result, some measures to make backyard poultry production safer, including the housing or confinement of backyard chickens and fighting cocks, have been widely introduced in Thailand as part of the restructuring and improvement program of poultry production (Figure 6-3). This may result in an overall reduction of HPAI risks and enhanced biosecurity and biocontainment in backyard poultry.

Additionally, the geographic concentration and housing density of commercial poultry production can greatly augment the spread of HPAI in an infected area. A study of an outbreak in The Netherlands found that the transmission rate of the virus was insufficiently reduced by improved biosecurity and biocontainment measures but the epidemic could only be stopped by complete depopulation of infected areas (Stegeman et al., 2004). This suggests that reducing commercial flock density might reduce the probability of a major epidemic. In Thailand, stamping-out policy was implemented as one of the control measures during the HPAI epidemic (Tiensin et al., 2005; Tiensin et al., 2007a).

In addition, monitoring the poultry population may improve early detection of avian influenza. Moreover, it should be considered that the agricultural workforce constitutes a high-risk population for whom protection from zoonotic disease is important not only for their health but for the health of their communities and the population at large. Finally, improved oversight and management of animal wastes – including transport and sale as well as use in aquaculture or in crop activities – should be included in strategies to reduce risks of pandemic HPAI.

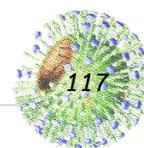




Figure 6-3: A housing or confinement of backyard chickens and fighting cocks has been widely introduced in Thailand as part of the restructuring and improvement program of poultry production.

CONCLUSIONS OF THIS THESIS

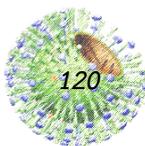
The abovementioned findings in this thesis lead to the following main conclusions.

- The period between the introduction of the virus into Thailand and its conclusive identification was too long resulting in wide spread of HPAI outbreaks in Thailand. Most outbreaks were reported in backyard chickens in the Central, North and East of the country. However, commercial poultry operations, with less than perfect bio-security, apparently were at the highest risks of HPAI infection.



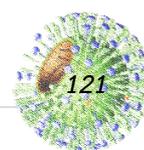
- No significant difference in within-flock transmission between various flock types was found. It is expected that HPAI will not result in a major outbreak if 80% of the birds in a flock are effectively vaccinated.
- Sub-districts with a high flock density of fighting cocks and presence of quail and free-grazing duck flocks had higher infection rate than those with low flock density of these poultry types. Subdistricts with poultry slaughterhouses were more frequently infected than were those without slaughterhouses. A significant association between H5N1 infected sub-districts and the evidence of H5N1 infection in wild birds in the same sub-district was demonstrated.

Knowledge gained from this thesis emphasized that epidemiological analysis provides insight information of past epidemics which can be used for improving control measures and preventing the future epidemic.



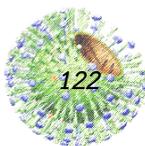
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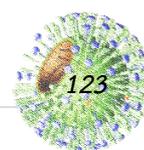




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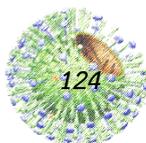


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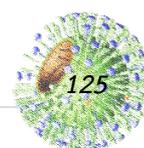


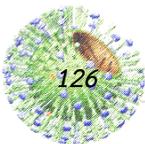


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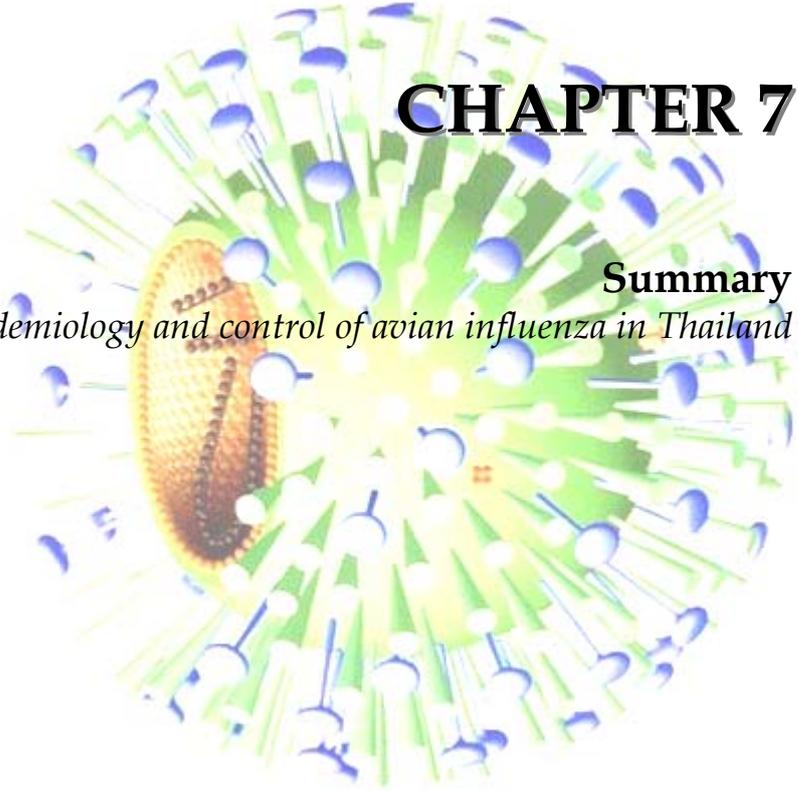




CHAPTER 7

Summary

Epidemiology and control of avian influenza in Thailand





SUMMARY

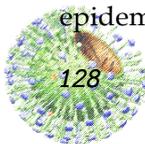
Highly pathogenic avian influenza (HPAI) is a notifiable contagious disease, resulting in severe morbidity and high mortality in poultry, restrictions of domestic and international trade of poultry products, and huge impact on farmers' livelihoods and socioeconomics. To date, the highly virulent influenza A viruses that produce acute clinical disease in chickens, turkeys and other birds of economic importance have been associated only with the H5 and H7 subtypes.

The HPAI H5N1 epidemic in Asia since 2003 had caused serious losses through severe morbidity and mortality in poultry and through losses in human life. HPAI H5N1 widely spread across Asia, Europe, Africa, and the Middle East increased concern over the potential emergence of a pandemic influenza. In addition, current control measures implemented within the poultry farming community also caused large economic and livelihood losses both on the national and community level.

The main objective of this thesis was to gain insight in the epidemiological features, risk factors, and transmission dynamics of avian influenza H5N1 outbreaks in Thailand. To this end, the knowledge that was gained from the studies can be adapted to the Thai situation to develop an overall monitoring and control programme. This may also allows for the improvement of preventive and control measures before a next possible outbreak.

To update the recent situation of HPAI H5N1 worldwide, the course of HPAI since its discovery of the Asian strain H5N1 virus in 1996, modes of disease transmission, and control and prevention strategies implemented were reviewed (**Chapter 1**). Over 68 countries worldwide since 2003 have reported occurrence of avian influenza H5N1 infection in poultry and wild birds. As of April 2011, 549 human cases of Avian Influenza A (H5N1) from 15 countries were reported to the World Health Organization with a 58% case-fatality rate (320 fatal cases). Apart from H5N1 infections in domestic poultry and humans, the infected host species for H5N1 has expanded to many species of wild birds, canines, felines and swine.

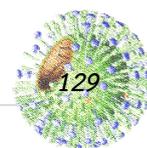
In Chapter 2, *Highly pathogenic avian influenza H5N1, Thailand, 2004*, the outbreak data of HPAI H5N1 in Thailand were analyzed and described for better understanding of the HPAI H5N1 epidemics. Such epidemiological description included most likely introduction route of HPAI virus into Thailand, the temporal and spatial characteristics of the epidemic and the affected species. Effectiveness of the applied control



measures such as outbreak detection, stamping out, pre-emptive slaughtering, poultry movement control, comprehensive surveillance and compartmentalization was discussed.

H5N1 virus was first confirmed in poultry and humans in Thailand in January 2004. A total of 83% of infected flocks confirmed by laboratories were backyard chickens (56%) or ducks (27%). Outbreaks were concentrated in the Central, the southern part of the Northern, and Eastern Regions of Thailand, which are wetlands, water reservoirs, and dense poultry areas. The relative risk for H5N1 infection was high in broilers, layers, quails, geese, and ducks compared to backyard chickens. More than 62 million birds were either killed by H5N1 viruses or culled. A number of domestic cats, dogs, captive tigers, and leopards also died of the H5N1 virus. Control measures, e.g., culling poultry flocks, restricting poultry movement, and improving hygiene, were implemented. Since 2004, a stamping-out policy has been used to control avian influenza outbreaks; vaccination has been prohibited in Thailand. Early detection of all cases was essential to rapidly implement control measures. Meanwhile, comprehensive surveillance and long-term control measures are required to be strengthened. The success of H5N1 elimination, therefore, depends on a collaboration of all stakeholders, including farmers, industries, veterinarians, public health authorities, academic institutions, media, and the government.

In Chapter 3, *Transmission of the highly pathogenic avian influenza H5N1 virus within flocks during the 2004 epidemic in Thailand*, the within-flock transmission analysis was carried out at various types of poultry. The transmission rate parameter (β) and the reproduction ratio (R_0) were estimated from the flock-level daily mortality data. A comparison of the within-flock transmission parameters of H5N1 virus between different flocks of backyard chickens, broilers, fighting cocks, or laying hens was investigated. The point estimates of β varied from 2.26/day (95% confidence interval [CI], 2.01–2.55) for a 1-day infectious period to 0.66/day (95% CI, 0.50–0.87) for a 4-day infectious period, whereas the accompanying R_0 varied from 2.26 (95% CI, 2.01–2.55) to 2.64 (95% CI, 2.02–3.47). Although the estimates of β of backyard chickens and fighting cocks raised together were lower than those of laying hens and broiler chickens, this difference was not statistically significant. To estimate a vaccine coverage, the study showed that at least 80% of the birds in a flock should be effectively vaccinated, on the basis of the fraction of $1-1/R_0$. This quantitative

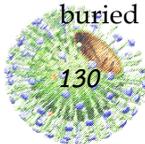




information can be used to plan a future program for control of HPAI and further epidemiological modeling.

In Chapter 4, *Ecologic risk factor investigation of clusters of avian influenza A (H5N1) virus infection in Thailand*, the space and time clusters of HPAI H5N1 outbreaks at the subdistrict level in Thailand during the 2004–2005 outbreaks were investigated. H5N1 clusters were scattered all over the country during January–February 2004. This indicated a long-distance spread of the disease through transportation of poultry and poultry products. After control measures had been implemented (i.e., restrictions on transportation of commercial chickens, free-grazing ducks, and fighting cocks and pretesting for H5N1 infection in poultry flocks before movement), the outbreaks mostly occurred in high-cluster areas in central Thailand. Additionally, risk factors associated with H5N1 infection were identified high-cluster areas with use of a spatial-based case-control study. Risk factors investigated in clustered areas of H5N1 included human and animal demographic characteristics, poultry production systems, and wild birds and their habitats. Six variables remained statistically significant in the final model: flock density of backyard chickens (odds ratio [OR], 0.98), flock density of fighting cocks (OR, 1.02), low and high human density (OR, 0.60), presence of quail flocks (OR, 1.21), free-grazing duck flocks (OR, 2.17), and a poultry slaughterhouse (OR, 1.33). We observed a strong association between subdistricts with H5N1 virus-infected poultry flocks and evidence of prior and concomitant H5N1 infection in wild birds in the same subdistrict.

In Chapter 5, *Risk factors associated with highly pathogenic Avian Influenza A (H5N1) virus infection in Thai poultry flocks, 2004-2005*, the flock-level risk factors were determined. The findings provided insight into mechanism of the occurrence of HPAI H5N1 being useful for improvement of preventive and control measures. Based on data from infected and uninfected poultry flocks from 2004 to 2008, risk factors were quantified to investigate potential risk factors associated with HPAI H5N1 infection in poultry flocks using a matched case-control study. A final multivariate conditional logistic regression model indicated that HPAI H5N1 was more likely to occur in flocks which had fighting cocks on the premise (OR=5.44), shared feed with other flock (OR=3.16), had close proximity to other flocks (OR=11.77), were near a previous outbreak (OR=14.58), or of which farmers had visited suspected flocks (OR=4.71) or vehicles could enter the premise (OR=26.42). In contrast, HPAI H5N1 was less likely to occur in flocks of farmers which used tap water (OR=0.11) or buried dead birds on their premise (OR=0.09). The results showed that



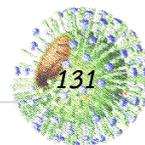
the presence of fighting cocks on the premises had higher infection rates than those without fighting cocks. With fighting cocks, fighting activities within or between villages may cause wider spread of the disease. Our findings showed that flocks in close proximity to outbreaks or other flocks had a greater chance of infection. In addition, poultry flocks of which other vehicles could enter the premise or farmer visited any suspected farm with sick or dead birds increased risk of HPAI infection. Our findings suggest again that keeping poultry isolated from other flocks with high biosecurity level may prevent or minimize infections. In this study, poultry flocks sharing feed sources with other birds were at high risk.

The evidence from this study suggested again that movement of humans and vehicles between flocks contributed significantly to the occurrence of the disease, where disinfection and proper handling of dead birds was protective. Our analysis suggested that commercial poultry production, with an ostensibly more “biosecure” system of production, is not associated with a reduction in risk of HPAI infection at the farm or flock level, compared to subsistence backyard flocks. Biosecurity practice must be revisited because the poultry sector 2 and 3 farms still had high risk for infection compared to backyard poultry. This may indicate that the biosecurity measures might have been breached or incompletely applied somehow resulting in introduction of HPAI H5N1 virus into the flocks of sector 2 and 3.

Finally, in the general discussion (chapter 6) the results of the previous chapters have been integrated and discussed. This thesis described the knowledge obtained from epidemiological analyses of past epidemic of HPAI H5N1. The understanding of HPAI H5N1 outbreaks has led to improvement of preventive and control measures in Thailand including monitoring and surveillance programme, early warning system, poultry movement control, farm biosecurity, compartmentalization, regulation and legislation and public awareness.

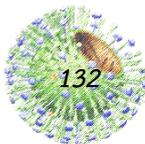
The following main conclusion can be drawn from this thesis:

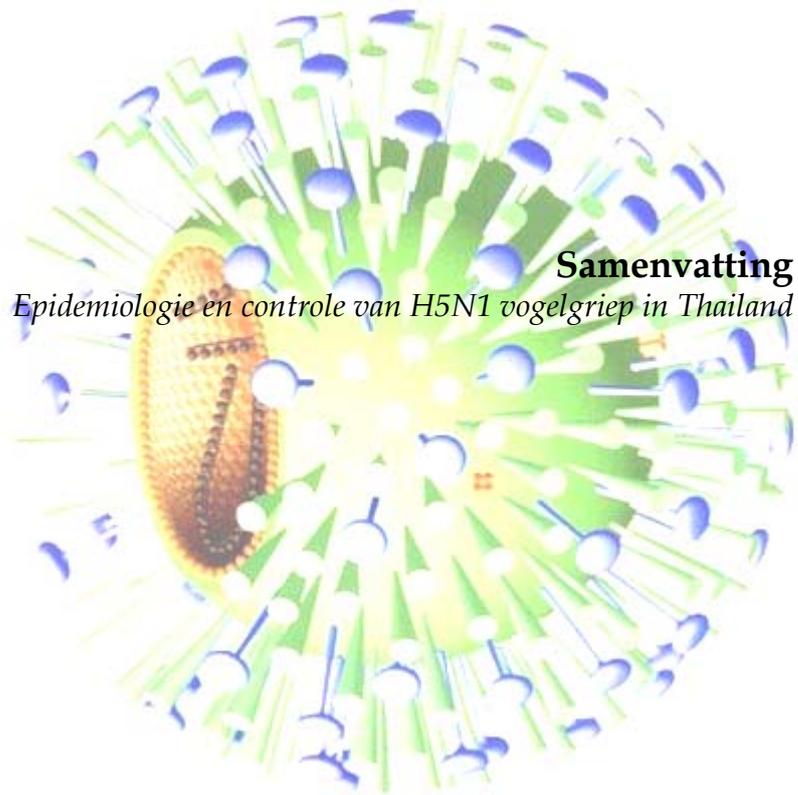
- The risk of HPAI infection in the Central and Eastern Regions was higher than that in other parts of Thailand. The relative risk for HPAI infection was high in broilers, layers, quails, geese, and ducks compared to backyard chickens (**Chapter 2**).
- The within-flock transmission rate of HPAI H5N1 was not different among backyard chickens, fighting cocks, broilers and layer chickens (**Chapter 3**).





- H5N1 clusters were scattered all over the country at the early of outbreaks reflecting a long-distance spread of the disease (**Chapter 4**). The study suggested that efforts to control H5N1 must consider risk factors related to the long distance movement involved with certain poultry activities (i.e., fighting cocks and free-grazing ducks) and also with commercial poultry production (i.e., transportation of poultry, poultry products, equipment, waste, and by-products).
- The higher risk of HPAI was found in commercial operations in contrast to backyard operation even if backyard flocks were the largest single group of case farms. This may indicate that the biosecurity measures might have been breached or incompletely applied somehow resulting in introduction of HPAI H5N1 virus into the flocks of sector 2 and 3 (**Chapter 5**).







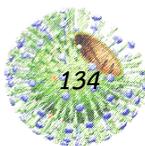
SAMENVATTING

Hoog pathogene aviaire influenza (HPAI) is een aangifteplichtige besmettelijk ziekte die leidt tot ernstige morbiditeit en hoge mortaliteit in pluimvee, tot beperkingen in de nationale en internationale handel in kipproducten, verder heeft het een sterke invloed op bestaan van agrariers en op hun socio-economische omstandigheden. Tot op heden zijn de zeer virulente influenza A virusen die de acute klinische ziekte veroorzaken in kippen, kalkoenen en andere gevogelt van economisch belang, gerelateerd aan de H5 en H7 subtypen.

De HPAI H5N1 uitbraak in Azië heeft in 2003 in diverse landen grote schade veroorzaakt, zowel door massale ziekte en sterfte van pluimvee alsook door humane besmettingen en incidentele sterfte. Thailand behoort tot de zwaarst getroffen landen, waar bovendien in 2004 het virus na aanvankelijke uitroeiing door bestrijding weer opdook en zich wederom verspreidde. Om tot effectievere toekomstige bestrijdingsaanpak te komen is het van belang dat de dynamiek en epidemiologie van HPAI in Thailand gekwantificeerd werd. In ieder land zijn specifieke omstandigheden, zoals het houden van de diverse pluimveesoorten, van invloed op de verspreiding en de effectiviteit van de bestrijding.

Het doel van het dit proefschrift was om met behulp van de beschikbare Thaise gegevens een beter kwantitatief inzicht te krijgen in de verspreiding van HPAI en belangrijke risicofactoren daarbij om zo te kunnen komen tot een betere onderbouwing van voor Thailand optimale bestrijdingsmaatregelen. Dit zou moeten leiden tot verbetering van zowel preventie alsook van bestrijdingsmaatregelen vóór de mogelijk volgende uitbraak.

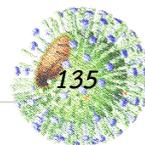
Om de recente situatie met betrekking tot HPAI en H5N1 in kaart te brengen, wordt aandacht geschonken aan het beloop van HPAI sinds de ontdekking van de Aziatische stam H5N1 in 1996, manieren van besmetting en de strategieën voor controle en preventie (hoofdstuk 1). Meer dan 68 landen wereldwijd hebben sinds 2003 het voorkomen van vogelgriep H5N1 in pluimvee en vogels in het wild gerapporteerd. Tot april 2011 zijn 549 gevallen van mensen met vogelgriep (H5N1) door 15 landen gemeld aan de Wereldgezondheidsorganisatie met een dodelijke afloop bij 58% (320 dodelijke gevallen). Afgezien van de H5N1 besmettingen in pluimvee en de mens, heeft H5N1 zich ook verspreid in vele soorten vogels in het wild, honden, katten en varkens.



In Hoofdstuk 2 worden gegevens over de uitbraak van HPAI H5N1 beschreven en geanalyseerd om een beter begrip te krijgen van de HPAI en H5N1 epidemieën. Dit omvat de meeste waarschijnlijke route zoals het HPAI virus Thailand is binnengekomen, de karakteristieken in tijd en plaats van de epidemie en de besmette diersoorten. De effectiviteit van de toegepaste maatregelen ter inperking, zoals vaststelling van de uitbraak, indamming, preventief ruimen, beperking in transport van pluimvee, toegepaste controle-maatregelen en compartimentalisatie worden besproken.

Het bestaan van het HPAI H5N1 virus kon voor het eerst bevestigd worden in pluimvee en de mens in Thailand in januari 2003. Een totaal van 83% van geïnfecteerde tomen kippen met bevestiging van de ziekte in het laboratorium, waren kippen gehouden op het erf (56%) of eenden (27%). De uitbraken waren geconcentreerd in centraal Thailand, het zuidelijk deel van Noord-Thailand en de oostelijke delen van Thailand, hetgeen waterrijke gebieden, met water-reservoirs en een dichte concentraties van pluimvee zijn. Het relative risico voor HPAI infectie was hoog in kuikens, legkippen, kwartels, ganzen en eenden in vergelijking met kippen op het erf. Meer dan 62 miljoen vogels stierven door het HPAI virus of werden geruimd. Een aantal huisdieren (katten, honden) en tijgers en luipaarden in gevangenschap stierven eveneens door het H5N1 virus. Maatregelen ter indamming zoals het ruimen van tomen kippen, vervoersbeperkingen van pluimvee en een betere hygiëne werden eveneens toegepast. Vanaf 2004 is een beleid van eradicatie toegepast om uitbraken van vogelgriep onder controle te houden; vaccinatie is verboden in Thailand. Vroege ontdekking van alle zich voordoende gevallen was essentieel om de zaak onder controle te houden. Intussen dienen betere methoden ter vaststelling en lange termijn maatregelen voor een betere controle verbeterd worden. Succes van uitroeiing van HPAI is afhankelijk van de samenwerking van allen die er belang bij hebben, zoals boeren, de industrie, veterinair, academische instellingen, de media en de overheid.

In hoofdstuk 3 wordt het patroon van besmetting geanalyseerd binnen tomen van verschillende soorten pluimvee. De besmettingsgraad-parameter (β) en de reproductie ratio (R_0) worden geschat op basis van de mortaliteits-data op het niveau van een toom. De parameters van de besmetting binnen één en dezelfde toom van verschillende tomen van kippen op het erf, kuikens, hanen voor hanengevechten of legkippen werden met elkaar vergeleken. De op één tijdstip gemaakte schattingen van β varied van 2.26/dag (95% confidence interval [CI], 2.01–2.55) voor een

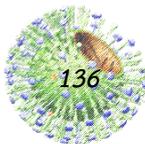




1-dag infectieuze periode tot 0.66/dag (95% CI, 0.50–0.87) voor een 4-dagen infectieuze periode, terwijl de daarmee gepaarde R_0 varieerde van 2.26 (95% CI, 2.01–2.55) tot 2.64 (95% CI, 2.02–3.47). Alhoewel de schattingen van β van kippen op het erf en hanen voor hanengevechten die samen gehouden werden, lager waren dan die van legkippen en kuikens, kon een statistisch significant verschil niet aangetoond worden. In een schatting wat vaccinatie hier zou uithalen, liet het onderzoek zien dat minstens 80% van de vogels binnen een toom effectief gevaccineerd zou moeten zijn op basis van de fractie $1-1/R_0$. Deze kwantitatieve informatie kan gebruikt worden voor toekomstige planning van het onder controle houden van HPAI en voor verdere epidemiologische modeling.

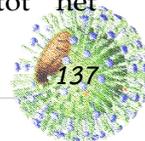
In hoofdstuk 4 worden de plaats en tijd van de clusters van de uitbraken van HPAI en H5N1 op het niveau van subdistricten in Thailand gedurende de uitbraken in de periode 2004-2005 onderzocht. H5N1 clusters kwamen verspreid over het hele land voor in januari–februari 2004. Dit was een aanwijzing dat de ziekte zich over grote afstanden had kunnen verspreiden door vervoer van pluimvee en zijn produkten. Nadat maatregelen tegen verspreiding waren genomen (vervoers-beperkingen van kippen in de agrarische sector, van eenden niet gebonden aan één plaats en hanen voor hanengevechten en testen op H5N1 besmetting vóór vervoer, kwamen de uitbraken voornamelijk voor in geclustede gebieden in Centraal Thailand. Daarenboven konden risico-factoren voor H5N1 infectie worden vastgesteld in gebieden met een hoge clustering door middel van ‘case-control’ studies gebaseerd op de ruimtelijke verspreiding. Risico-factoren die onderzocht werden in gebieden met clusters van H5N1 omvatten demografische karakteristieken van mens en dier, van de productie-methoden in de pluimvee-teelt en van de leefomgeving van in het wild voorkomende vogels. Uiteindelijk waren er zes variabelen statistisch significant in het ziekte-model: dichtheid van tomen kippen op het erf, (odds ratio [OR], 0.98), dichtheid van tomen van hanen voor hanengevechten, (OR, 1.02), hoge of lage dichtheid van bevolking (OR, 0.60), aanwezigheid van tomen kwartels (OR, 1.21), vrij en loslopende tomen van eenden (OR, 2.17), en een slachthuis voor pluimvee (OR, 1.33). Er werd een sterk verband aangetroffen tussen subdistricten met tomen gevogelte besmet met H5N1 en aanwijzingen dat er eerdere of gelijktijdige besmetting bestond met H5N1 van in het wild levende vogels in één en hetzelfde subdistrict.

In hoofdstuk 5 worden de risico factoren op het niveau van tomen pluimvee besproken. De bevindingen geven inzicht in de mechanismen



van optreden van HPAI H5N1 besmettingen hetgeen nuttig is voor preventie en het onder controle houden van uitbraken. Op basis van de data van geïnfecteerde en niet geïnfecteerde tomen pluimvee tussen 2004 en 2008, konden, met gebruikmaking van 'matched case control studies', potentiële risico-factoren voor HPAI H5N1 in tomen pluimvee gekwantificeerd worden. Een statistisch model (final multivariate conditional logistic regression model) gaf aan dat HPAI H5N1 een grotere kans van optreden had wanneer er hanen voor hanengevechten op het terrein aanwezig waren, wanneer voer gedeeld werd met een andere toom, of tomen zich in dichte nabijheid van andere tomen bevonden, of dichtbij een plaats van een eerdere uitbraak, of waar boeren verdachte tomen bezocht hadden, of voertuigen het terrein van het bedrijf opkonden. Daarentegen was er een geringere kans op HPAI H5N1 infectie als boeren leidingwater gebruikten of dode dieren begroeven op eigen terrein. De resultaten lieten ook zien dat hanen deelnemend aan hanengevechten in het eigen dorp of in verschillende dorpen tot een grotere verspreiding van de ziekte leidden. Onze bevindingen lieten zien dat tomen in dichte nabijheid van uitbraken bij andere tomen, een grotere kans op infectie hadden. Dat gold ook als andere dan eigen voertuigen de boerderij bezochten of wanneer de boer boerderijen bezocht met zieke of dode vogels. Onze bevindingen bevestigen andermaals dat het gescheiden houden van pluimvee van andere tomen met een hoog niveau van bioveiligheid, infecties kan voorkomen of de kans daarop sterk verminderen. In dit onderzoek liepen tomen die voer deelden met andere vogels duidelijk een groter risico.

Het materiaal in deze studie suggereerde wederom dat verkeer van mensen en voertuigen tussen verschillende tomen een significante bijdrage leverden aan het optreden van de ziekte, terwijl desinfectie en een adequaat omgaan met dode dieren een beschermend effect hadden. Onze analyse laat zien dat commerciële aanpak van productie van pluimvee, met een op het oog betere bioveilige manier van produceren, niet noodzakelijkerwijs gepaard gaat met een vermindering van het risico op HPAI infectie op niveau van bedrijf of toom in vergelijking met tomen simpelweg gehouden op het erf. De praktijk van bioveiligheid moet opnieuw bekeken worden omdat pluimveebedrijven sector 2 en 3 nog steeds een hoog infectie-risico hadden in vergelijking met kippen rondlopend op het erf. Dit zou kunnen betekenen dat maatregelen voor bioveiligheid niet goed of incompleet uitgevoerd en aldus op een of andere manier geleid hebben tot het binnenkomen van de infectie bij tomen in sector 2 en 3.

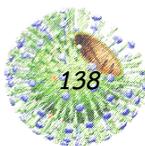




Tenslotte, in de algemene discussie in hoofdstuk 6 worden de bevindingen van de vorige hoofdstukken geïntegreerd en en becommentarieerd. Dit proefschrift beschrijft de kennis opgedaan met epidemiologische analyses van voorbije epidemieën van HPAI en H5N1. De kennis omtrent uitbraken van HPAI H5N1 heeft geleid tot verbetering van maatregelen voor preventieve en het onder controle houden in Thailand, inhoudende monitoring en bewakings-programma's, een systeem voor vroegtijdige waarschuwing, regulering van vervoer van pluimvee, bioveiligheid op de boerderij, compartimentalisatie, regel- en wetgeving en een bewustzijn bij het grote publiek.

De hoofdconclusies uit dit proefschrift zijn als volgt:

- Het risico op HPAI infecties in centraal en oost Thailand was groter dan in andere delen van Thailand. Het relatieve risico op HPAI infectie was hoger in kuikens, leghennen, kwartels, ganzen en eenden in vergelijking met kippen op het erf verblijvend (hoofdstuk 2).
- De besmettingsgraad met HPAI H5N1 binnen een toom was niet verschillend tussen kippen rondlopend op het erf, hanen voor hanengevechten, kuikens en leghennen (hoofdstuk 3)
- De clusters van H5N1 waren reeds verspreid over het hele land bij de vroege uitbraken, wijzend op een verspreiding over grote afstanden (hoofdstuk 4). Het onderzoek bood aanwijzingen dat pogingen om de H5N1 infectie onder controle te houden de volgende risico-factoren in aanmerking moeten nemen: factoren verband houdend met het vervoer over lange afstand voorkomend bij zekere activiteiten, zoals hanengevechten en eenden niet gebonden aan een plaats en ten aanzien van de commerciële productie van pluimvee, het transport van pluimvee, hun produkten, gereedschap, afval en andere bijprodukten.
- Er was een hogere risico op HPAI infecties optredend in commercieel werkende bedrijven in tegenstelling tot kippen gehouden op het erf, zelfs wanneer de laatste de grootste groep uitmaakten van boerderijen betrokken in het onderzoek. Dat kan een aanwijzing zijn dat maatregelen voor bioveiligheid niet goed opgevolgd zijn of incompleet zijn toegepast hetgeen heeft geresulteerd in het binnendringen van het HPAI H5N1 virus in tomen van sector 2 en 3 (hoofdstuk 5).

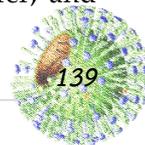


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Accomplishing this dissertation entitled “Epidemiology and Control of Avian Influenza H5N1 Virus in Thailand” would not have been possible without the contributions of numerous individuals, the greatest of which have been from my colleagues who performed meticulous works during the outbreaks of HPAI H5N1 in Thailand. The research findings in this dissertation were made possible by the support and collaboration of several organizations and institutes including Thailand’s Department of Livestock Development, National Institute of Animal Health, Regional Veterinary Research and Development Centers, Faculties of Veterinary Medicine, Chiang Mai University, Chulalongkorn University, Kasetsart University, Mahidol University, Geo-Informatics and Space Technology Development Agency, Department of National Parks, Wildlife and Plant Conservation and the World’s Poultry Science Association, Thailand Branch.

I have a large thank you to my supervisors, Arjan Stegeman and Mirjam Nielen and my co-supervisors, Thaweesak Songserm and Wantanee Kalpravidh, who have been extremely supportive through this whole process, all the way from initiating contact, getting the project proposal approved, breaking down barriers of tradition, inspiring new thoughts and ideas and to completing the formalities of this thesis. I could not have finished this thesis alongside my other work and assignment obligations in other countries as international consultant without support from all of them.

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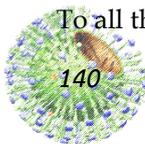
In support of M.D. Salman and Jerome Freier, I had an opportunity to gain more experience on modeling of infectious diseases and spatial epidemiology at Colorado State University and the Centers for Epidemiology and Animal Health (CEAH), APHIS, USDA. I also wish to thank all persons who provided support during my visit in Fort Collins, Colorado, USA.

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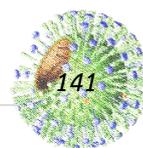
Finally, I would also like to thank the Royal Thai Government for providing financial support and to the Department of Livestock Development, Ministry of Agriculture and Cooperatives for allowing me to leave my work during my Ph.D. study.

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Curriculum Vitae

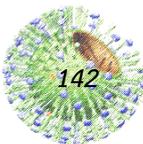
Born on 11 September 1972 in Yala Province, southernmost Thailand. In 1996, he received a degree of Doctor of Veterinary Medicine (D.V.M.) from Faculty of Veterinary Medicine, Kasetsart University, Thailand. Then, he started his career at the Regional Livestock Office in Suratthani Province, southern Thailand and practised as a field veterinarian for 2 years. In 1998-1999, he joined the Food and Agriculture Organization of the United Nations (FAO), Regional Office for Asia and the Pacific in Young Professional Programme where he had a chance to work on the Global Rinderpest Eradication Programme in Sri Lanka and India. During 2000-2002, he worked at the Office of Director General (CVO) and the Division of Veterinary Epidemiology, Department of Livestock Development, Bangkok, Thailand. In January-July 2001, he was awarded to participate in the international training course on Globalization of Trade and International Quality Standard in Infectious Disease Control at the German Foundation for International Development (DSE) and Freie Universiteit für Berlin, Germany. During 2002-2003, he worked at the Office of Agricultural Affairs to the European Union, Royal Thai Embassy in Brussels, Belgium being responsible for the international trade and food safety related to SPS agreement and veterinary issues. In September 2003, he was granted scholarships from the Royal Thai Government and the World Bank (JJ/WBGSP scholarship programme) to do his postgraduate studies at Utrecht University. He obtained a master degree (M.Sc.) on Veterinary Epidemiology and Economics in 2005. Following his M.Sc. programme, he continued to pursue his PhD degree at Faculty of Veterinary Medicine, Utrecht University under supervision of Prof. Dr. J.A. Stegeman and Dr. M. Nielen. His Ph.D. research project focused on "Epidemiology and Control of Avian Influenza H5N1 Virus in Thailand". Whilst he had been doing on his Ph.D., he also worked as an international consultant on disease surveillance and livestock emergency response for FAO in North Korea, Myanmar, and Nepal. In addition, he has contributed as a reviewer for scientific journals. i.e. Emerging Infectious Diseases, Vaccine, BMC Infectious Diseases, Virology Journal, International Journal of Infectious Diseases, PLoS ONE, and etc. Currently, he works at the Center for Veterinary Epidemiology Development, Bureau of Disease Control and Veterinary Services, Department of Livestock Development, Thailand. Outside of work, his main interests are family, gardening, swimming, running, hiking, salsa dance, cycling, badminton, tennis, golf, yoga, art modeling, travelling, and outdoor activities.





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- Survival Analysis (6-8 August 2009, ISVEE pre-conference workshop, Durban, South Africa)
- Surveillance of Aquatic Animal Diseases (15-16 August 2009, ISVEE post-conference workshop, Durban, South Africa)

2008:

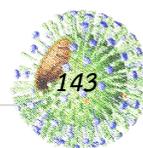
- Epidemiologic Simulation Modeling Course (4-8 August 2008, Animal Population Health Institute, Colorado State University and USDA Centers for Epidemiology and Animal Health, Fort Collins, Colorado, USA)
- Foreign Animal Diseases Course (14-18 July 2008, Animal Population Health Institute, Colorado State University and USDA APHIS, Fort Collins, Colorado, USA)

2007:

- Course on Epidemiology and Control of Infectious Diseases: introduction to mathematical models of global and emerging infection (2-14 September 2007, Imperial College, London, UK)
- Course on Decision support and decision making in highly contagious live-stock diseases (24-27 April 2004, Budapest, Hungary)
- The 2007 Annual Conference of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) (28-30 March 2007, Helsinki/Espoo, Finland)
- Pre-conference SVEPM-NOSOVE course on "Surveys and monitoring systems - design questions, analysis and presentation of results" (25-26 March 2007, Helsinki/Espoo, Finland)
- Writing for Academic Publication (January - March 2007, Faculty of Veterinary Medicine, Utrecht University, The Netherlands)

2006:

- Spatial Analysis with GIS (GEO4-4412) (November 2006 - January 2007, Department of Physical Geography, Faculty of Geosciences, Utrecht University, The Netherlands)
- ISVEE post-workshop on Methods on Risk Assessment of Animal Health and Food Safety (13-15 August 2006, Cairns, Australia)

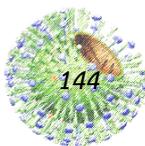




- ISVEE pre-workshop on Risk-based National Surveillance (2-4 August 2006, Cairns, Australia)
- Hand-on GIS (April - June 2006, Department of Human Geography and Planning, Faculty of Geosciences, Utrecht University, The Netherlands)
- Introduction of GIS - Cartography (GEO2-3031) (February - April 2006, Department of Human Geography and Planning, Faculty of Geosciences, Utrecht University, The Netherlands)

2005:

- FAO Advanced Workshop on Epidemiology Techniques for Surveillance and Control of Avian Influenza (26-29 July 2005, Bangkok, Thailand)
- FAO Regional Training Course on Epidemiology and Data Analysis for HPAI (27 June-1 July 2005, Bangkok, Thailand)
- Presentation Techniques (May 2005, Utrecht University, Utrecht, the Netherlands)



List of publications and contributions

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- Tiensin, T.**, Ahmed, S.S.U., Rojanasthien, S., Songserm, T., Ratanakorn, P., Chaichoun, K., Kalpravidh, K., Wongkasemjit, S., Patchimasiri, T., Chanachai, K., Thanapongtham, W., Chotinan[†], S., Stegeman, A., Nielen, M. 2009. Ecologic risk factor investigation of clusters of avian influenza (H5N1) virus infection in Thailand. *The Journal of Infectious Diseases*, 199: 1735-1743.
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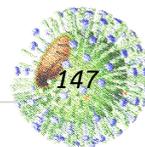
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- Tiensin, T.** 2010. Animal-Human Interface and Infectious Disease in Industrial Food and Poultry Production: Rethinking Biosecurity and Biocontainment. In the Annual Conference of the World Poultry Science Association (Thailand Branch), Bangkok, Thailand, October 28, 2010.
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- Tiensin, T.** 2006. Epidemiology and implications for control of H5N1 avian influenza in Thailand. In the Series Current Themes in Ecology: Influenza Ecology and Pandemics, Wageningen University, Wageningen, The Netherlands, April 19, 2006.

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- Tiensin, T.**, Chaitaweesub, P., Songserm, T., Chaisingh, A., Hoonsuwan, W., Buranathai, C., Parakamawongsa, T., Premashthira, S., Amonsin, A., Gilbert, M., Nielen, M. and Stegeman, A. 2005. Descriptive analysis of the highly pathogenic avian influenza virus (H5N1) epidemic in Thailand in 2004. In the Proceedings of the OIE/FAO International Conference on Avian Influenza. Paris, France, April 7-8, 2005. p. 54.

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