Chapter 1

Introduction
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An emerging public health challenge

Following outbreaks of avian influenza (AI) H5N1 virus in birds in China, human cases were first detected in Hong Kong in 1997 (1). Since then, the bird epizootic spread through various parts of Asia and to a number of countries on other continents (2). The periodic detection of human infections, especially clusters of cases, triggered concern amongst the public health community (3). The novel H5N1-virus had demonstrated capacity for human infection, but had not yet acquired the ability for efficient human transmission. Could this virus lead to the next human influenza pandemic?

Indonesia was first affected by the bird epizootic in August 2003. The first human infection was detected in July 2005 (4). By January 2011, the virus remained entrenched in the farmed bird population and periodically “spilled over” into humans (5). Indonesia was recognized as a global hotspot for the epizootic in birds and human infections (6). Public health interventions emphasized disease control at source – in the birds – as well as preventing zoonotic transmission of the virus to humans (7, 8). Yet, despite efforts, control measures have had limited impact (9-12).

Previous studies have shown that live bird markets (LBMs) play an important role in the introduction, entrenchment, emergence and dissemination of AI H5N1 viruses in birds (13-15). LBMs have also been implicated as a source of human AI H5N1 infection (7). In Indonesia, LBMs led to human infection with the H5N1-virus as well as the spread of the virus back into farms through the sale of live birds to consumers (7, 16, 17). LBMs are an integral part of the community as they provide fresh produce and food to consumers. However, since animals are placed in close proximity to consumers and sellers, there is potential for disease transmission in the market setting (18).

In high resource settings, disease control for avian influenza viruses in LBMs is achieved through good infrastructure, hygiene and regulatory practices (19, 20). However, in low resource settings such as Indonesia, there is limited infrastructure and regulatory practice (21). Further, there has been very little research on the control
measures that are feasible to implement and that are effective in LBMs in low resource settings such as Indonesia.

Thus, there was a clear need to build knowledge about AI H5N1 epidemiology in LBMs in low-resource settings and to identify control measures suited to the available capacities. I addressed this research need through a series of studies presented in this thesis.

**Aims and scope**

The aims of this PhD thesis were to examine the epidemiology of human AI H5N1 infection and to inform disease control measures in LBMs. Using outbreak investigation data, I undertook three studies that highlighted that human AI H5N1 infection largely resulted from zoonotic transmission of the virus. This reiterated the importance of disease control at the human-animal interface including in LBMs. I then proceeded to examine the epidemiology of the virus in LBMs, identify control measures suited for limited-resource settings and assess the practical application of implementation of disease control interventions.

A number of cross-sectional surveys and one intervention trial were used to achieve these aims and to answer the specific research questions as outlined in Chapter 3 (Research Design). The different components of the PhD were achieved through a series of studies conducted in Indonesia. All studies were published in international peer-reviewed journals, and are presented as chapters in this thesis.

**Thesis structure**

This thesis is presented as a series of studies that address the research questions on the human epidemiology of AI H5N1 and the control of the virus in LBMs. The thesis begins by providing background about Indonesia, AI H5N1 and LBMs (Chapter 2). An overview of the research design that describes the research questions, data collection and data analysis techniques is then presented in Chapter 3. Each of the six studies is
then presented as chapters in the thesis with an introductory section that describes the publication status and the contribution of the paper to the overall thesis. The last chapter (Chapter 10) then provides an overall discussion about the findings, conclusions and policy recommendations relating to the research questions and PhD aims.

All of the papers have been reproduced with permission of the relevant publishing companies and co-authors. All the papers were prepared during my doctoral candidature.

**Student contribution**

For each of the six papers in this thesis, I was the lead researcher and guarantor of the published work. I took primary responsibility for overall management of the data analysis and drafting process. I also ensured the integrity of the research, and organized all parts of the completed manuscripts, before and after publication. However, as the studies pertain to research conducted in Indonesia, there were a large number of co-authors for some studies. This was important to acknowledge the access provided to me to the data and the support of the government of Indonesia for the research and publication.

Based on the British Medical Journal guidance on contributorship (22), I estimated my specific contribution to each paper as percentages for (a) conception & drafting, (b) analysis and interpretation, and (c) drafting and revising. These are presented in Table 1.1 below.
Table 1.1: Estimate of Gina Samaan’s contribution to each study published and included as part of the PhD thesis.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Article title</th>
<th>Journal</th>
<th>Status</th>
<th>Gina Samaan authorship</th>
<th>Number of co-authors</th>
<th>Conception &amp; designing</th>
<th>Analysis &amp; interpretation</th>
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<td>4</td>
<td>Risk factors for cluster outbreaks of avian influenza A H5N1 infection, Indonesia.</td>
<td>Clinical Infectious Diseases</td>
<td>Published, October 2011</td>
<td>Co-1st author</td>
<td>14</td>
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<td>5</td>
<td>Avian Influenza H5N1 Transmission in Households, Indonesia.</td>
<td>PLOS One</td>
<td>Published, January 2012</td>
<td>Co-1st author</td>
<td>14</td>
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<td>6</td>
<td>Chicken faeces garden fertilizer: possible source of human avian influenza H5N1 infection.</td>
<td>Zoonoses &amp; Public Health</td>
<td>Published, June 2010</td>
<td>2nd author</td>
<td>9</td>
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<td>7</td>
<td>Environmental sampling for avian influenza virus A (H5N1) in live-bird markets, Indonesia.</td>
<td>Emerging Infectious Diseases</td>
<td>Published, December 2010</td>
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<td>8</td>
<td>Critical control points for avian influenza A H5N1 in live bird markets in low resource settings.</td>
<td>Preventive Veterinary Medicine</td>
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<td>9</td>
<td>Application of a healthy food markets guide to two Indonesian markets to reduce transmission of “avian flu.”</td>
<td>Bulletin of the World Health Organization</td>
<td>Published, April 2012</td>
<td>1st author</td>
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For one of the six papers published, I was listed as second author despite major contribution to the study (Table 1.1). This was due to the nature and timing of the work, where as a result of international media attention to Indonesia’s outbreaks of AI H5N1 and human infections, publication of AI H5N1 data became an issue of national sovereignty (23, 24). Indonesia led an international effort to improve the transparency of virus-sharing systems and the receipt of benefits arising from biological materials such as vaccines (23, 25). These issues were discussed over a number of years at the international stage including at the World Health Assembly (26). The main implication for researchers during these negotiations was a limitation in the scope of research published on AI H5N1. Studies that analyzed human surveillance data or reported virological findings were carefully released, limited the involvement of international non-Indonesian scientists and politically limited opportunities for foreign first authorship.

Even though I could not be listed as first author on one of the studies presented in this thesis, it was important to publish the novel findings and add to the overall literature on AI H5N1 globally. Through transparent negotiation, good research practice and commitment between decision-makers at MOH and me, it was feasible for me to be listed as second author on this paper.

References

Introduction


Chapter 2

Background
Indonesia

Indonesia is the largest archipelago country in the world with 17,508 islands of which 6,000 are inhabited (1). It is located in Southeast Asia between the Indian and the Pacific Oceans, and spans 1,919,440 sq km (Figure 2.1). In 2008, the population was estimated at 228.8 million, of which 168.3 million are over 15 years of age. Population density varies widely between the major islands. Nearly 60% of the population lives on Java Island, which constitutes less than 7% of Indonesia’s total land area, whilst the islands of Kalimantan and Papua, which are four to five times larger than Java Island, are inhabited by only 5% and 2% of total population, respectively.

Figure 2.1: Map of Indonesia (courtesy of WHO Indonesia)

In 2007, the poverty rate, defined as the proportion of people living on less than US $1.25 per day, was estimated at 16.6 %, ranging from 12.5% in urban areas to 20.4% in rural areas (2). In 2008, life expectancy at birth was 68 years and infant mortality rate was 26 per 1,000 live births (1). The main religion in Indonesia is Islam (88%), followed by Christian (8%), Hindu (2%), Buddhist (1%) and other (1%) (1).

Indonesia is a democratic republic with 33 provinces encompassing 397 districts and 98 cities (1). Indonesia’s system of governance was decentralized to the level of district/city in 2001. The 495 districts and cities are now the key administrative units responsible for providing most government services including health but excluding
Background

defence, national security, foreign affairs, fiscal policy and religion. Decentralisation continues to be consolidated, and local institutions in many districts and cities are still building capacity to fulfil their new mandates comprehensively (3, 4). Development indices, poverty rates and proneness to both natural disasters and man-made crises including conflict vary widely between provinces. With the variation in culture and terrain, these pose challenges to national development and implementation of services (3)

Health care system

There are three main levels in the government’s health care system: the central MOH, the provincial health offices (PHO) and the district (and city) health offices (DHO). At the sub-district level, the Government of Indonesia has long-established health care centres, known as Puskesmas, to provide free primary health services. Puskesmas are staffed by recently graduated doctors and government-employed nurses, midwives and public health professionals including sanitarians. These units are complemented by district-level hospitals to provide tertiary health care. Reports from these facilities form the basis of the notifiable disease surveillance system.

Government decentralization has had a large impact on the health system. Health financing, health information systems, human resources for health and service provision were all affected (5). Under decentralization, district and city governments have the responsibility for health care provision including public and environmental health. The role of the MOH is to establish the national health agenda including disease control strategies, regulations and guidelines. The provincial governments are mandated to adopt national strategies, develop locally-relevant guidance and provide training and monitoring for districts and cities under their authority. In collaboration with the MOH, PHO supervise the implementation of the health agenda and facilitate communication of policy and health information.

In the MOH, the Directorate-General for Disease Control and Environmental Health (DG DC&EH) is responsible for communicable disease control including surveillance,
preparedness and response to emerging infectious diseases (EIDs). DG DC&EH supports and coordinates outbreak response vertically through PHO and DHO, as well as horizontally with other MOH structures such as the Directorate-General of General Medical Services, the Center for Health Promotion, the Directorate-General for Pharmacy and Health Supplies, and the National Institute for Health, Research and Development. In case of national outbreaks or events of international public health concern, DG DC& EH coordinates with other relevant ministries and with the World Health Organization (WHO).

**Animal health system**

The Ministry of Agriculture’s (MOA) Directorate-General of Livestock Services (DGLS) is responsible for animal and plant biosecurity, including animal health and protection of Indonesia against epizootic and enzootic disease threats. The MOA has quarantine services to manage animal movement in and out of the country, as well as between various regions within Indonesia. For animal health, MOA focuses largely on threats to agro-economics to reduce reliance on foreign imports and maximize local livestock outputs (6).

Similar to the structures in the MOH, the MOA has provincial and district level agriculture offices to implement the various national programs. At sub-district level, there are animal health centres known as Puskeswan to support local farmers and to collect disease activity data as part of the national animal health surveillance system (7). There is a large private sector for agriculture initiatives including extension workers who sell feed, vaccination services and veterinary support to farmers. Even though the role of these private stakeholders is recognized as integral to livestock production, they have not been regulated or formally integrated into the MOA’s programs for disease control.

Poultry and poultry product consumption in Indonesia is increasing, where the standing yearly chicken population is estimated at 1.2 billion birds (6, 8). Indonesia has both large and small scale chicken production systems, including farms that supply eggs and
breeding stocks for both meat chickens (broilers) and egg-producing chickens (layers). In addition to the industrialized systems for poultry production, many people in Indonesia also rear chickens, ducks and other poultry such as quails and geese in their backyards. Not only do backyard production systems supplement family nutrition with animal sources of protein, they also generate income through the sale of live poultry and poultry products. Ninety percent of poultry production in Indonesia is marketed and sold to consumers at traditional food markets (9).

Food markets in Indonesia and in many other countries are an integral part of the community – providing foods that reflect the local culture and traditions of the people as well as serving as a commercial and social centre (10). Many markets offer live animals, such as chickens, pigeons and ducks, which are often slaughtered and dressed in the market. Food markets that offer bird carcasses as well as live birds either for sale or for slaughter are collectively referred to as live bird markets (LBMs). Indonesia is estimated to have 13,450 LBMs providing employment to 12.5 million people (9).

**Live bird markets**

LBMs are essential for maintaining the health and nutritional status of both rural and urban populations, especially in developing countries (10). Local governments generally foster the development of LBMs since they are income-generating through the rental of stalls and provision of community services. LBMs are increasingly a tourist attraction offering a window in the traditional way of life and foods of distinct regions and countries. Despite the income generated through LBMs for local government, some LBMs lack investment in infrastructure, practices that promote safe food and environmental sanitation. This may be a result of lack of awareness by the LBM managers, lack of funds available for enhancing the LBM environment or even the lack of minimum standards or regulation for LBM operation. This may lead to loss of business from local consumers and an increased risk of disease transmission (11).

LBMs provide optimal conditions for the zoonotic transfer and evolution of infectious disease pathogens (12). LBMs provide major contact points for people and live animal
mixing, making them important potential sources of infection (13). Live animals are generally enclosed in small cages in numbers exceeding the capacity of cages. In some situations, different species are placed in cages together, providing ample opportunity for disease transmission. For humans, although, direct hand-to-face contact is the most likely path for infection, the flapping by distressed animals handled raises faecal-dust aerosols and exposes sellers, shoppers, and passers-by to various pathogens. LBMs have been associated with major outbreaks of diseases, including cholera, severe-acute respiratory syndrome (SARS) and AI H5N1 (10).

Influenza

Influenza is a respiratory infection caused by an RNA virus spread through droplet and airborne transmission in humans (14). Influenza disease is characterized by the rapid onset of symptoms including fever, myalgia, sore throat and dry cough, but can also lead to more severe symptoms such as pneumonia (15). Studies on seasonal influenza have shown that severe morbidity and mortality are highest in the elderly, young and immuno-compromised (14).

There are three types of influenza viruses: A, B and C. Type A viruses are of greatest public health concern as they can infect both human and various animal species (16). Influenza A viruses are classified and named based on the antigenic nature of two of their surface proteins: hemagglutinin (H) and neuraminidase (N). To date, 16 hemagglutinin (H1-H16) and nine neuraminidase (N1-N9) variants are known. The natural reservoir for Type A viruses is wild aquatic and shore birds, but some subtypes have crossed the species barrier and infect humans, pigs and other mammals. The Type A viruses frequently undergo minor mutations known as antigenic drift and can periodically have major changes known as antigenic shift. Virus re-assortment can also occur when two strains exchange RNA and produce a novel virus. Novel viruses can trigger new pandemics.
Three prerequisites are needed to trigger a new human influenza pandemic: a novel virus to which the population has no prior immunity, the capacity to cause disease in humans and the capacity to transmit efficiently between people (17). Pandemics not only cause excess morbidity and mortality but they also cause social and economic disruption (18).

Records of influenza pandemics are available from the ninth century, and suggest occurrence every few decades (19). The twentieth century experienced three pandemics of varying severity, disease burden and risk groups. In 1918, the pandemic resulting from the H1N1 subtype was considered highly virulent and resulted in a 33% morbidity rate with 2% case fatality rate (20-22). The H2N2 pandemic of 1957 and H3N2 pandemic of 1968 had lower morbidity and case fatality rates than the 1918 pandemic at an estimated 15-25% morbidity rate with 0.2-0.4% case fatality rate (19). Phylogenetic evidence suggests that the viruses for all three pandemics originated from avian influenza A viruses, either unchanged or after reassortment with human influenza A viruses (23).

The first influenza pandemic of the twenty-first century was experienced in 2009 – the H1N1 (2009) subtype. Although the overall health impact of this H1N1 (2009) pandemic was lower than that of the three prior pandemics (24), the economic burden was considerable, estimated at 0.7 to 4.8 percent of gross domestic product (GDP) (25). Since the human and socio-economic costs of influenza can be mitigated through control measures including vaccination, global attention to the disease has increased in recent decades (18). Previous pandemics highlighted the need for strong influenza surveillance systems, laboratory capacity for influenza diagnosis, and development of both pharmaceutical and non-pharmaceutical interventions to respond to the threat of emerging and future influenza viruses (24).

**Avian Influenza H5N1 Virus**

Avian influenza viruses cause infections in birds and spread through droplet, airborne and faecal transmission (26). Human infection with avian influenza viruses has been
Background

extremely rare and cases detected usually only suffer mild illness (19). However, in 1997, during an outbreak of the H5N1 subtype in poultry farms in Hong Kong, 18 people were infected with the virus resulting in six deaths (27). These cases were the first recorded transmission of the H5N1 subtype to humans. The clinical presentation of the human cases was severe compared to previously known avian influenza infections, with acute respiratory disease and high case fatality rate. In birds, AI H5N1 virus infection is highly pathogenic and can lead to 90-100% flock mortality within two days (28). However, some birds, especially waterfowl, can be infected with the virus without showing any signs of disease (29).

The outbreak in Hong Kong was controlled by mass slaughter of birds in markets and enhanced biosecurity measures in farms (30). The virus was detected again in Hong Kong in 2001 followed by outbreaks in other parts of Asia in 2003. By May 2011, 60 countries had reported outbreaks of the virus in birds (31). The outbreaks had considerable economic impact and also resulted in human infection (16). By 16 March 2011, 534 human cases of AI H5N1 infection were reported from 15 countries with a 59% case fatality rate (Figure 2.2.).

Figure 2.2: Countries with confirmed cases of H5N1 avian influenza
Clinically, most human cases experience influenza-like illness (ILI) followed by rapid progression to severe respiratory illness including pneumonia and acute respiratory distress (32, 33). In fatal cases, the median time from onset to death was 9 days (34). The incubation period for human AI H5N1 virus infection has been estimated to be up to seven days, but, more commonly 2-5 days after the last known exposure to sick or dead poultry (35, 36). Longer incubation periods have been suggested and possibly reflect the level of exposure, multiple exposures and immunological factors (37). Early diagnosis during AI H5N1 illness is challenging because of the nonspecific signs and symptoms and rarity of AI H5N1 disease (37). Most cases had documented exposure to bird or environmental sources of the virus but some resulted from limited human-to-human transmission of the virus (38).

Even though most countries stamped out the AI H5N1 virus, a small number of countries continue to experience outbreaks and are considered endemically infected. This includes Bangladesh, Cambodia, China, Egypt, Indonesia and Viet Nam (39). Risk factors for outbreaks in birds have been explored in a number of these countries. In Vietnam, the abundance of domestic ducks, especially free-grazing ducks feeding in commercial rice cropping areas, has been identified as a risk factor (40). In China, the virus is known to persist in LBMs despite application of movement control, quarantine and stamping out (41). And, in Bangladesh, high human population density, greater commercial poultry population and an increasing number of roads per subdistrict were found to be significant risk factors for outbreaks (42).

The virus remains of international public health concern due to its potential to trigger a new influenza pandemic. The virus has already satisfied two of the three prerequisites for a new pandemic; a novel strain to which the population has no prior immunity and the ability to infect and cause disease in humans.

To minimize the risk of a new influenza pandemic from the AI H5N1 virus, the global strategy is to control the virus ‘at source’ – in the birds (18). Yet, despite the availability of disease control measures, some countries have faced hindrances. According to the
Food and Agriculture Organization (FAO), there are three main difficulties in eliminating the H5N1 virus from endemically infected countries including Indonesia (39). Firstly, the poultry sector in these countries has undergone rapid and unregulated growth. Secondly, the public and private veterinary and animal production services have limited capacity to impact the poultry production and marketing systems, including tracing back sources of virus spread. Thirdly, there is limited commitment from stakeholders including the poultry industry, the government and the public to intervene, where many do not see AI H5N1 as an animal production or human health threat.

Avian influenza H5N1 in Indonesia

In Indonesia, AI H5N1 outbreaks started in August 2003 in poultry farms on Java Island. By 2006, the virus spread to 31 out of 33 provinces in Indonesia and devastated many industrialized and backyard poultry farms (43). The virus affected many bird species including chicken, duck and quail. The outbreaks affected both national sales of poultry and exports, where the average annual export volume declined from 228,000 tonnes in 2003 to 60 tonnes in 2005. Exports of day-old chicks also stopped in 2004 as there was no demand from neighbouring countries (43). In response, the MOA introduced disease control measures including vaccination of poultry and selective culling in farms. However, these were not applied comprehensively.

Some industrialized production farms responded to AI H5N1 outbreaks by implementing stricter biosecurity measures and introducing poultry vaccines (44). Backyard production farms did not adopt such measures largely due to lack of cost-benefit and inaccessibility (43). Thus, due to the lack of systematic and comprehensive application of disease control measures, the virus became entrenched and continues to circulate between farms and the marketing system including LBMs.

Prior to outbreaks of AI H5N1 in August 2003, influenza was ascribed low priority in the human public health system. There was no specific program for the control and response to influenza in the MOH, and there was a paucity of data on influenza disease
Background

trends and outbreaks (45). Between 1999 and 2003, a small surveillance study was conducted by MOH in collaboration with the United States Centers for Disease Control and Prevention (US CDC) (45). The surveillance study found that in the six sentinel Puskesmas, influenza infection was confirmed in 11% of patients presenting with ILI. However, there have been no disease burden studies on influenza in Indonesia.

Attention to influenza, especially AI H5N1, increased in 2004 upon rumours of bird deaths in poultry farms in western Java. Disease surveillance in humans was commenced through the 44 national referral hospitals, district and city hospitals, as well as in Puskesmas. The national response escalated once the first human cases of AI H5N1 infection were detected in Banten province in western Java in July 2005. Laboratory capacity to test and confirm AI H5N1 virus infection was developed, and currently involves positive Real-Time Reverse Transcriptase Polymerase Chain Reaction (RRT-PCR) test results from two national laboratories.

In December 2005, Indonesia developed the National Strategic Plan (NSP) for controlling AI H5N1 to effectively respond to the problem. The NSP placed emphasis on ‘at source’ disease control activities as well as strengthening underlying capacities for disease detection and outbreak response. Emphasis was also placed on the poultry marketing system, including LBMs, to sever the circulation of virus between the productions systems and points of sale.

In this thesis, I explored the epidemiology of human cases of AI H5N1 infection including the exposures for infection and factors associated with case clustering. Since LBMs have led to human infection with the AI H5N1 virus, the thesis also focused on the interventions required to control the virus in LBMs. I identified risk factors for LBM contamination with the AI H5N1 virus, and critical control points in LBMs for disease control. I then analysed the practical application of the interventions in two LBMs to determine lessons for future practice.
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Chapter 3

Research Design
Research questions

In this thesis, I addressed three research questions:

1. What are the risk factors and transmission patterns for human infection with the AI H5N1 virus in Indonesia?
2. What are the risk factors and critical control points for AI H5N1 virus contamination in LBMs?
3. Is AI H5N1 control feasible in LBMs in Indonesia?

The three questions were addressed through six different studies published in international peer-reviewed journals. All studies were reproduced as chapters in this thesis, as outlined below.

Research Question 1: What are the risk factors and transmission patterns for human infection with the AI H5N1 virus in Indonesia?

I undertook three studies to address this research question. As a co-investigator for human cases of AI H5N1 infection between 2005 and 2009 in Indonesia, I participated in the collection of data on disease exposure, clinical findings, healthy contacts and outbreak response measures. To answer the research question, I compared households with a single AI H5N1 case to households with multiple cases to determine the risk factors for cluster outbreaks as well as secondary cases of disease (Chapter 4). This study also assessed cases whose putative source of exposure was LBMs.

In a separate study, I analyzed the data on the 139 outbreaks detected during the study period (July 2005 – July 2009) to determine the risk factors for infection and transmission patterns including quantification of zoonotic and human transmission of the virus (Chapter 5). In a case report, I analyzed data pertaining to a two-person cluster detected in Indonesia in September 2005, and reported on the possibility of garden fertilizer containing chicken faeces as a possible source of infection. This case report was the first to suggest fertilizer as a potential source of human AI H5N1 infection (Chapter 6).
Research Question 2: What are the risk factors and critical control points for AI H5N1 virus contamination in LBMs?
I undertook two studies to answer this question. Through a cross-sectional study utilizing survey data and environmental sampling, I assessed the extent of and risk factors for environmental contamination of LBMs with the AI H5N1 virus (Chapter 7). The study was conducted in 83 LBMs in three provinces in Indonesia. The study provided the basis for and informed the two subsequent chapters in the thesis (Chapters 8 and 9).

Using the information arising from the cross-sectional study described in Chapter 7, I identified a set of critical control points for AI H5N1 that can reduce the risk of virus contamination and transmission in LBMs (Chapter 8). Since identification of critical control points requires thorough knowledge of the poultry workflow, the product and the hazard (AI H5N1 virus) (1), I reviewed the scientific literature, undertook a detailed knowledge, attitudes and practice (KAP) survey in three LBMs and used the logic reasoning approach in the Codex Alimentarius Commission’s decision tree for critical control point determination as part of the study methodology (2).

Research Question 3: Is AI H5N1 control feasible in LBMs in Indonesia?
I conducted one study to answer this research question. I utilized the findings from Chapters 7 and 8 along with recently published guidance from WHO to assess the practical aspects of implementing interventions for the control of AI H5N1 virus in LBMs and to learn lessons for future application (Chapter 9) (3). In a non-experimental field intervention study in two LBMs in Indonesia, I collected pre- and post-intervention data to describe change in knowledge and practice following implementation of interventions, and to assess LBM stakeholder satisfaction with the change process and outcomes.
Research methodology

To address the three research questions, I used different methods to collect and analyze the data. Each published paper in the subsequent chapters provides the detailed methods and statistical analyses for that study. Below, I describe the main methods used in the thesis to highlight the breadth of data sources, data collection tools and data analysis techniques.

1. Analysis of public health surveillance and outbreak investigation data.
2. Collection and analysis of data gathered from cross-sectional surveys.
3. Collection and analysis of data from a non-experimental field intervention study.

Analysis of public health surveillance and outbreak investigation data.
AI H5N1 infection in humans is a nationally notifiable disease in Indonesia, where disease notification is based on the WHO case definitions for suspect, probable and confirmed cases (4). Laboratory-confirmed cases are internationally notifiable to WHO as per the International Health Regulations (5).

MOH investigates every case of AI H5N1 infection to determine source of infection, mode of transmission, risk factors for infection and clinical presentation (6). Outbreak investigations also enable detection of further associated cases through tracing and monitoring of case contacts, and they enable public and veterinary health authorities to commence outbreak control measures such as culling infected flocks of birds or isolating individuals suspected of infection. Epidemiological and clinical data are collected through standard questionnaires developed by the MOH, and samples from suspected and confirmed cases as well as putative sources of infection are collected for laboratory analysis. Since AI H5N1 is a zoonotic disease, outbreak investigations are usually conducted jointly by public and veterinary health authorities.

Surveillance data are usually collected by a variety of staff with differing technical backgrounds and epidemiological expertise, which may result in measurement bias. Fortunately, due to the public health importance of AI H5N1 infection, there is a small number of dedicated outbreak investigation staff at MOH to investigate all confirmed
human AI H5N1 cases. These staff have all received training in outbreak investigation methods and the standardized H5N1 data collection tools. This may help minimize data collection errors and biases commonly associated with public health surveillance systems.

The MOH Zoonosis Subdirectorate maintains a database of all cases of AI H5N1 infection and archives the detailed outbreak reports. The surveillance database is analyzed periodically to monitor the epidemiology and to inform public health action (6-8). This surveillance function is especially important for an EID as it helps build the body of knowledge about the epidemiology, the clinical features of infection and to inform disease prevention programs (9, 10).

In this thesis, I designed the studies’ research questions, extracted the data from the existing surveillance system and analysed the data quantitatively in Chapters 4 and 5. For analyses on household contacts of cases, I extracted the data from the archived outbreak reports and integrated them with data on their respective cases. For the case report in Chapter 6, I described and interpreted the outbreak investigation epidemiological findings, as well as the veterinary and public health laboratory findings. Even though case reports offer the lowest level of evidence for causation, they play an important role in generating hypotheses about disease epidemiology (11). Case reports enrich the body of knowledge on potential associations between factors and disease outcomes, which is critical for EIDs such as AI H5N1.

**Collection and analysis of data gathered from cross-sectional surveys.**

Cross-sectional studies are a type of observational study. In this thesis, I undertook a number of cross-sectional surveys including for Chapters 7, 8 and 9, in which data were collected on both exposure and outcome factors across the study population at a single point in time. The data were then analysed to determine associations between study factors and outcomes.

In Chapter 7, I designed a study to assess point prevalence of AI H5N1 virus contamination in LBMs in three provinces in Indonesia. The survey involved the
collection of questionnaire data from poultry vendors and samples for laboratory analysis from work surfaces. The survey questions were based on sample questionnaires in WHO guidelines for improving biosecurity in LBMs (12).

For Chapters 8 and 9, I designed KAP surveys for poultry vendors in LBMs to assess workflow (Chapter 8) and impact and acceptability of interventions (Chapter 9). KAP surveys are a type of cross-sectional study and are useful to inform policy decisions, especially interventions that need to be tailored for specific stakeholders (13). They enable researchers to explore what people know, how they feel and how they behave about a certain topic. For the KAP surveys reported in Chapters 8 and 9, I designed the questions relating to poultry slaughter, workflow, hygiene and disease control based on WHO guidelines for LBM assessment (3). The questions were close-ended to simplify data collection and to enable the application of quantitative data analysis techniques including descriptive statistics.

In designing the methodology for all of the cross-sectional surveys in this thesis, I considered the purpose of the study, the sample size needed to obtain proper point estimates and the appropriate questionnaire design to maximize the validity of the results. All questions were field tested, translated, back-translated for confirmation and administered by locally trained staff in the local language. I trained all interviewers in questionnaire administration techniques, including seeking informed consent from participants and appropriate documentation of answers during the interview process.

**Collection and analysis of data from a non-experimental field intervention study.**

Field intervention studies measure factors that impact implementation of the intervention (14). They enable researchers to assess whether interventions can be applied and practiced under day-to-day conditions that apply in the real world. Field intervention studies can be experimental or non-experimental depending on whether a control group was included for comparison on the outcome variable (14). For this thesis, I undertook a non-experimental design in Chapter 9 to determine whether proposed measures for the control of AI H5N1 virus in LBMs can be applied and to
Research design

identify lessons for future application. I maximized the validity of the study findings by measuring and comparing the outcome variable at pre- and post-intervention.

Laboratory methods

For a number of chapters in this thesis, environmental samples were collected and tested to determine presence of AI H5N1 virus. Samples were tested using virus isolation (VI) or real-time reverse transcriptase polymerase chain reaction (RRT-PCR). Even though I did not conduct the laboratory testing myself, I undertook a week-long training in RRT-PCR and VI methods at the Australian Animal Health Laboratory (AAHL) to further my understanding and knowledge of the protocols. This helped me understand the process and limitations of the tests, the interpretation of the laboratory results and contextualizing the results in the overall findings for each study.

General laboratory considerations

To ensure the validity of the laboratory findings, standard protocols and internationally accepted methodologies for sample collection and testing were used (15, 16). Laboratory reagents, primers and reference antisera were purchased. All work was performed using high quality consumables including vials and sterile Dacron® swabs.

For sample collection and storage, quality control was based on the procedures outlined in the WHO guidelines for the collection and preservation of samples for avian influenza H5N1 determination (16). Using sterile collection kits, trained laboratory staff collected samples using disposable gloves, aprons and masks. This was necessary to minimize the risk of sample contamination during the collection process and to protect the laboratory staff from infection or contamination of their clothes and data collection tools. Laboratory staff worked in pairs to collect the samples, label the vials, fill in the specimen collection forms and ensure each vial was properly sealed and handled.

All samples were collected in vials containing the relevant type of transport media (16), and they were transported from the field to the laboratory in cool-boxes to prevent degradation of the sample. In the laboratory, samples for H5N1 virus testing were either
tested immediately using RRT-PCR or VI. If it was not possible to process immediately, samples were stored in -80°C freezers until they were tested. The laboratory where samples were stored in -80°C freezers had back-up generators in case the main power supply failed.

Staff involved in testing the samples were experienced in the techniques used and had all been trained by international scientists. The laboratory testing samples for the studies reported in this PhD participated in international quality assurance programs administered by AAHL.

The two methods used in studies reported in this thesis are described briefly below.

**Virus isolation**

VI was used in the study reported in Chapter 7. VI was conducted according to the WHO manual for animal influenza diagnosis and surveillance (15). VI is considered the gold standard for confirmation of AI H5N1 virus (17). VI is done in embryonated chicken eggs and under strict biosafety measures to avoid transmission to humans. Specific Pathogen Free (SPF) eggs were used in triplicates to maximize the validity of the test findings. As per FAO guidance, two passages four days apart were attempted before a test was declared negative (18).

**Real-time reverse transcriptase polymerase chain reaction**

RRT-PCR was used in the study reported in Chapter 7. RRT-PCR was conducted according to the WHO manual for animal influenza diagnosis and surveillance (15). RRT-PCR amplifies and detects a region of the virus that is specific for the H5N1 virus (19). It is a highly sensitive and specific molecular method that can detect presence of virus rapidly (within three hours) (20). The method has been fully validated for confirmation of H5N1 virus (21). However, since the method is prone to laboratory contamination, it was important to test the samples in duplicate and to use internal controls to validate the RNA extraction procedure and to determine the integrity of the RNA samples. This quality control procedure was done in accordance with the AAHL
protocol, which had been provided to the laboratory prior to the start of the study reported in Chapter 7

**Statistical analysis**

A number of statistical analysis techniques were used in the various thesis chapters. The main statistical methods included logistic regression and disease transmission models. These are described in detail below. For the disease transmission models, I worked with two statisticians (Dr Alex Cook and Dr Mark Clements) and a mathematical modeller (Dr Kathryn Glass) to analyze the findings. I discussed and learnt the various methods used for these analyses including the limitations and interpretation of the outputs. All three biostatisticians were co-authors in the relevant publications.

**Logistic regression**

I performed logistic regression in a number of the studies in this thesis. I received input from Dr Alex Cook and Dr Mark Clements on the appropriate logistic regression techniques to analyze the various datasets, but performed all the analyses myself. In Chapters 4 and 5, logistic regression was used to analyze the risk factors for (a) case infection, (b) secondary cases of disease, and (c) clustering of disease in households. I also used logistic regression in Chapter 7 to assess risk factors for H5N1 virus contamination in LBMs.

In epidemiological studies, logistic regression is widely used to assess associations between exposures and disease outcomes (22). In logistic regression, the log odds ratio of a binary outcome variable such as disease or death is modelled on various explanatory variables. The logistic regression equation results from the selection of explanatory variables that influence the outcome variable. Explanatory variables can be selected for inclusion in the model using various techniques such as forward selection, backward elimination, stepwise regression and various Bayesian methods (23).

An assumption for logistic regression modelling is that the binary response data are independent (24). However, in many studies such as household studies or longitudinal
studies, measurements of the outcome variable are likely to be correlated. It is important to address this correlation to avoid over-dispersion of the data (22). Depending on the study design, various methods are available to account for this correlation, including the use of robust or cluster robust standard errors for the coefficient, generalised linear equations (GLE) or generalised estimating equations (GEE) (24). Since the data analysed in Chapters 4 and 5 were clustered at household level and likely to be correlated, I accounted for this by calculating cluster robust standard errors for the coefficient in the models.

Once explanatory variables are selected for inclusion in the logistic regression model, it is important to assess the adequacy of the model’s fit to the observed data. This can be done using the -2log likelihood estimate, the model Chi-square or the goodness of fit test (23, 25).

**Disease transmission models**

Different models have been posed to detect and quantify disease transmission in households or in community settings (26). These models are useful to inform public health interventions and outbreak control measures, especially for emerging diseases or as part of preparedness planning. For this thesis, final size household models were used to detect and quantify both human and zoonotic transmission of H5N1 virus in outbreak households in Chapter 5.

Final size household models are based on the total number of people infected in a household, as a function of the household size (27). These models estimate the transmission by means of maximum likelihood and calculate the corresponding confidence intervals on the basis of likelihood ratio tests. The strength of these models is that they do not rely on assumptions of the duration or distribution of latent and infection periods (27). This is especially useful for EIDs for which these parameters have not been well established and when there are limited data points (outbreaks). The main limitation of final size household models is that they do not estimate any parameters relating to the time-course of the outbreaks, such as changes in risk of transmission over time.
To select the most appropriate transmission model, the Akaike Information Criterion (AIC) was used and adjusted for small sample size (AICc) (28). The AICc enables comparison between models but does not provide information of the model’s absolute fit to the data. Two transmission models were compared: frequency-dependent models and density-dependent models (27). In frequency-dependent models, the number of contacts per unit of time is fixed and the transmission rate is proportional to the relative frequency of infectious individuals. Thus, in a household of two individuals of which one is infectious, the transmission rate is the same as in a household of four individuals of which two are infectious. In density-dependent models, the transmission rate would be twice as high in the latter household than in the former household. This is because these models assert that the number of contacts per unit of time is proportional to the number of individuals. Density-dependent rather than frequency-dependent transmission was chosen in the analyses as frequency-dependent models did not fit the data adequately (p<0.01).

For Chapter 5, I worked with Dr Kathryn Glass to analyse and compare three different models for different transmission assumptions: only zoonotic transmission, only human transmission, and both zoonotic and human transmission in households. The mean number of cases resulting from each transmission model, along with the confidence interval was generated.

**Ethics**

As the thesis reported on studies conducted and data collected in Indonesia, I obtained ethical clearance from the Human Research Ethics Committee (HREC) at the National Institute of Health Research and Development (NIHRD), MOH in Indonesia. I also obtained ethics approval from the ANU Human Research Ethics Committee (HREC). The reference number for the ANU HREC approval is 2009/599. In accordance with both the NIHRD and ANU HREC requirements, informed consent was sought and obtained from all individuals participating in the studies for which primary data were
collected. Similarly, permission was sought from LBM authorities for the collection of LBM data and environmental samples for laboratory testing.

As this PhD reported on data collected in Indonesia and since AI H5N1 was of national and international media attention, political attention from both the MOH and MOA to the research was very high. Even though this attention was welcome in maximizing the feedback and potential utilization of the research findings, it had an important impact on authorship of some of the publications. Publication of one out of the six studies in this PhD was contingent on Indonesian first authorship, and for another three studies it was contingent on first co-authorship. However, in recognition of my central role to the conduct of the various studies and where journals permitted, I was granted permission to be listed as corresponding author for the publications.

**Funding**

Two sources of funding enabled the conduct of the studies reported in this thesis. The ANU National Centre for Epidemiology and Population Health (NCEPH) provided research funds that enabled the collection and laboratory testing of environmental samples for H5N1 virus. I also obtained research funds as part of the Australian Prime Minister’s Australia-Asia Endeavour Award scholarship. This facilitated further laboratory testing and logistics including travel to field sites. Other aspects of the studies were funded external to the PhD and permission was obtained from the Indonesian authorities including the Market Authority, MOA and MOH for research access.

**References**


Research design
Chapter 4

Paper 1: Risk factors for cluster outbreaks of avian influenza A H5N1 infection, Indonesia
About this chapter

This chapter explored the risk factors for cluster outbreaks of AI H5N1 infection as well as risk factors for secondary cases of disease in Indonesia. This was the first study to address these questions globally.

Using a household-based study, a number of variables were assessed including those never reported previously in the literature such as household size and genealogical relationships between index cases and their contacts. The study identified two risk factors for cluster outbreaks: index case direct exposure to sources of AI H5N1 virus and an increasing number of first degree relatives (parents, offspring and siblings) to index cases. For secondary cases of disease, the study identified three risk factors: young age, direct exposure to sources of AI H5N1 virus and being a first degree relative, especially a sibling, to the index case in an outbreak.

In this study, I was involved in the outbreak investigation and data collection for some of the outbreaks. I designed the research question for this study and conducted the analysis using data from the MOH routine surveillance system. For data analysis, I worked with two statisticians to determine the best statistical models to account for the household clustering of the data and to develop the multivariate logistic regression models. I wrote the paper and obtained input from all the co-authors. The published study has been reproduced here with permission from the publisher, Oxford University Press.
Background. By 30 July 2009, Indonesia had reported 139 outbreaks of avian influenza (AI) H5N1 infection in humans. Risk factors for case clustering remain largely unknown. This study assesses risk factors for cluster outbreaks and for secondary case infection.

Methods. The 113 sporadic and 26 cluster outbreaks were compared on household and individual level variables. Variables assessed include those never reported previously, including household size and genealogical relationships between cases and their contacts.

Results. Cluster outbreaks had larger households and more blood-related contacts, especially first-degree relatives, compared with sporadic case outbreaks. Risk factors for cluster outbreaks were the number of first-degree blood-relatives to the index case (adjusted odds ratio [aOR], 1.50; 95% confidence interval [CI]: 1.20–1.86) and index cases having direct exposure to sources of AI H5N1 virus (aOR, 3.20; 95% CI: 1.15–8.90). Risk factors for secondary case infection were being aged between 5 and 17 years (aOR, 8.32; 95% CI: 1.72–40.25), or 18 and 30 years (aOR, 6.04; 95% CI: 1.21–30.08), having direct exposure to sources of AI H5N1 virus (aOR, 3.48; 95% CI: 1.28–9.46), and being a first-degree relative to an index case (aOR, 11.0; 95% CI: 1.43–84.66). Siblings to index cases were 5 times more likely to become secondary cases (OR, 4.72; 95% CI: 1.67–13.35).

Conclusions. The type of exposure and the genealogical relationship between index cases and their contacts impacts the risk of clustering. The study adds evidence that AI H5N1 infection is influenced by, and may even depend on, host genetic susceptibility.
confirmed cases were identified from 139 households [5]. The Indonesian Ministry of Health investigates all human cases of AI H5N1 to determine the source of illness and disease exposure [4]. This is to our knowledge the first study globally to compare outbreaks involving a single case with those involving >1 case to identify risk factors for cluster outbreaks. There are 2 tiers of analyses reported in the study: (1) risk factors for cluster outbreaks and (2) risk factors for secondary case infection. Both tiers explore household and individual level variables including those never to our knowledge reported previously, such as household size and genealogical relationships between cases and their contacts.

METHODS

Definitions

The Ministry of Health AI H5N1 case database and detailed case investigation forms were reviewed and analyzed for outbreaks detected between July 2005 and July 2009. The study conformed with the World Health Organization (WHO) case definitions of probable and confirmed categories of human AI H5N1 infection [6], and definitions of cluster and sporadic outbreaks [7]. A cluster is a group composed of ≥1 confirmed cases of H5N1 virus infection and additional confirmed or probable cases associated with a specific setting, with the onset of cases occurring within 2 weeks of each other. A sporadic outbreak was defined as a confirmed case of H5N1 virus infection. For both sporadic and cluster outbreaks, a household contact was a person who had at least 4 hours contact with a case at home within the 7 days prior or 14 days after a case’s onset of illness.

Other data used include household setting, for which cities or towns were defined as urban, fringes of cities as semirural, and villages as rural. Seasonal outbreaks were defined as outbreaks occurring in the wet season from December to March versus those occurring in the dry season (April until November). Three categories were used to describe disease exposure: direct, indirect and other: “Direct” exposure referred to cases who handled sick or dead poultry, handled poultry products such as fertilizers, or who had poultry deaths in the home; “indirect” exposure to cases where poultry deaths were reported in the case’s neighborhood, cases where healthy poultry were present in the neighborhood, and cases who visited live bird markets; and “other” to cases whose exposure was inconclusive despite investigation or who apparently were only exposed to a prior case but not to direct or indirect exposure types.

Household size was the number of people in the household including all cases. Household size was analyzed as both a continuous and categorical variable. Contacts were classified in their genealogical relationship to the index case. First-degree relatives comprised parents, offspring, and siblings; second-degree relatives were aunts, uncles, grandchildren, grandparents, nephews, and nieces; and third-degree relatives were cousins. Non-blood relatives to the index case comprised spouses, family-in-law, and household help.

Data Collection

Field investigation teams were deployed to investigate and instigate disease control measures for every outbreak [4, 8–10]. District level teams were deployed on the same day of outbreak detection to initiate the investigation. A provincial/national team rapidly followed to systematically collect data and to cross-check the district level team findings and activities. Most provincial/national teams also comprised a WHO epidemiologist to support data collection. Teams interviewed cases when possible (because many cases died before investigation teams arrived), family members (especially those who could report on the case), and key informants (including village leaders). Data collection forms, developed based on WHO guidelines [7, 11, 12], were used to obtain demographic, clinical, and epidemiological data. Household contacts were traced, and healthcare workers from the nearest government primary healthcare center were instructed to visit the household daily for 2 weeks to monitor and detect any additional cases in the household. The definition of household contact and their monitoring for 2 weeks was uniformly and systematically applied in both sporadic and cluster outbreaks.

Statistical Methods and Ethics

We used logistic regression to assess risk factors for cluster outbreaks, starting with univariate analyses and subsequently constructing multivariate models using variables significant at $\alpha = .1$ in univariate analysis and sequentially discarding terms not significant at $\alpha = .05$ starting with the one with the highest $P$ value. We used the le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares goodness-of-fit test to assess model validity, as advocated by Hosmer et al [13–15]. Independent predictors of cluster outbreaks were explored further using descriptive statistics (Table 1). We used Wilson score interval method to derive confidence intervals for proportions [16].

To assess risk factors for secondary case infection, logistic regression was used with adjustments for clustering (Tables 2 and 3) by computing a cluster robust standard error for the coefficient. Stata software, version 10-0 (StataCorp) and the R statistical environment were used for the descriptive and statistical analyses [17]. This study was part of an ongoing public-health investigation and is therefore exempt from review by an institutional review board.

RESULTS

In the 4-year study period, 139 outbreaks of human AI H5N1 infection were detected, of which 113 were sporadic case outbreaks and 26, clusters. There were 177 cases (159 laboratory-confirmed and 18 probable). For the 113 sporadic case outbreaks, only 1 case
was detected in each of those outbreaks despite investigation and monitoring of their household contacts. In the 26 cluster outbreaks, there were 64 confirmed and probable cases, where the average cluster size was 2.5 (median, 2; range, 2–8). Only 1 cluster had 4 cases—a cluster from North Sumatra province that had 7 confirmed and 1 probable case [18]. Case fatality rate was 85%. A map of cases and outbreak type by province can be seen in Figure 1.

### Risk Factors for Cluster Outbreaks

Thirteen variables at household and individual level were explored as potential risk factors for cluster outbreaks. Four variables had $P$ values $<.1$ on univariate analyses: household size, number of blood contacts to the index case, the index case’s main exposure type, and the degree of blood relation to the index case (Figure 2). The risk factors and the multivariate model are presented below.

#### Table 1. Main Putative Exposure for Sporadic Cases ($n = 113$), Cluster Index Cases ($n = 29$), and Cluster Secondary Cases ($n = 35$)

<table>
<thead>
<tr>
<th>Exposure type</th>
<th>Sporadic case (%)</th>
<th>Cluster index case (%)</th>
<th>Cluster secondary cases (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bird deaths or H5N1 confirmed in birds in the home</td>
<td>13 (12)</td>
<td>9 (31)</td>
<td>9 (26)</td>
<td>31</td>
</tr>
<tr>
<td>Handled bird products</td>
<td>7 (6)</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td>10</td>
</tr>
<tr>
<td>Handled sick/dead birds</td>
<td>25 (22)</td>
<td>9 (31)</td>
<td>6 (17)</td>
<td>40</td>
</tr>
<tr>
<td>Subtotal</td>
<td>45 (40)</td>
<td>19 (66)</td>
<td>17 (49)</td>
<td>81</td>
</tr>
<tr>
<td>Indirect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy birds in neighborhood</td>
<td>18 (16)</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td>21</td>
</tr>
<tr>
<td>Bird deaths in neighborhood</td>
<td>33 (29)</td>
<td>6 (21)</td>
<td>5 (14)</td>
<td>44</td>
</tr>
<tr>
<td>Visited live bird market</td>
<td>7 (6)</td>
<td>0*</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Sub-total</td>
<td>58 (51)</td>
<td>7 (24)</td>
<td>7 (20)</td>
<td>72</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive but exposed to prior case</td>
<td>...</td>
<td>...</td>
<td>11 (31)</td>
<td>11</td>
</tr>
<tr>
<td>Inconclusive despite investigation</td>
<td>10 (9)</td>
<td>3 (10)</td>
<td>...</td>
<td>13</td>
</tr>
<tr>
<td>Sub-total</td>
<td>10 (9)</td>
<td>3 (10)</td>
<td>11 (31)</td>
<td>24</td>
</tr>
<tr>
<td>Totala</td>
<td>113</td>
<td>29</td>
<td>35</td>
<td>177</td>
</tr>
</tbody>
</table>

* Even though 1 cluster index case had exposure to live bird markets, the case’s main exposure was classified as handling sick/dead birds.

#### Figure 1. Confirmed and probable cases of avian influenza H5N1 infection in Indonesia, by province and outbreak type, July 2005–July 2009.
Household-Level Risk Factors

Information about household contacts was available for 80 of the 139 outbreaks (60 sporadic and 20 cluster outbreaks). For these 80 outbreaks, 607 individuals were investigated, of whom 111 developed illness (82 index cases and 29 secondary cases) and 496 remained healthy. Since 1 cluster outbreak only had coindex cases but no secondary cases, the 29 secondary cases came from 19 cluster outbreaks. The overall attack rate in the 80 outbreaks was 18%. Thirty-three households had 6–10 persons (41%), 32 had 1–5 persons (40%), 12 had 11–15 persons (15%), and 3 had 15 persons (4%). The mean size of households was 6 persons for sporadic outbreaks and 9 for cluster outbreaks. Each additional household member increased the odds for developing a cluster by 20% (odds ratio [OR], 1.20; 95% confidence interval [CI]: 1.09–1.33, \( P < .001 \)). The increased risk was marginally stronger for each additional blood relative contact (OR 1.25; 95% CI: 1.05–1.48, \( P = .012 \)). The majority of household contacts for both sporadic and cluster outbreak types were first-degree relatives (Figure 2), but this proportion was significantly higher in cluster outbreaks (\( P = .008 \)) and the risk of an infection developing into a cluster increased markedly with the number of first-degree relatives (OR, 1.51; 95% CI: 1.24–1.84, \( P < .001 \)). In contrast, these outbreaks provide no evidence that the risk increased with more second-degree (\( P = .27 \)), or third-degree (\( P = .08 \)) relatives, nor for unrelated cohabitants (\( P = .69 \)), suggesting that the increased risk attributable to large household sizes is likely due to the increased number of first-degree blood relatives such as siblings, parents, and offspring.

Among households, 28% were urban, 36% semiurban, and 36% rural. Both sporadic and cluster outbreaks occurred in all 3 settings (Figure 2). There was no evidence that other household level variables such as household location, time of year, or mean age of cohabitants were risk factors for cluster outbreaks (Figure 2).

### Table 2. Risk Factors for Secondary Case Infection Comparing 35 Secondary Cases and 496 Healthy Contacts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Secondary cases (%)</th>
<th>Healthy contacts (%)</th>
<th>Univariate OR (P value)</th>
<th>Multivariate Adjusted OR (P value)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>6 (18)</td>
<td>41 (9)</td>
<td>6.88 (.04)</td>
<td>5.77 (.07)</td>
<td>.88–37.68</td>
</tr>
<tr>
<td>5–17</td>
<td>12 (35)</td>
<td>96 (21)</td>
<td>5.86 (.003)</td>
<td>8.32 (.008)</td>
<td>1.72–40.25</td>
</tr>
<tr>
<td>18–30</td>
<td>12 (35)</td>
<td>125 (28)</td>
<td>4.51 (.01)</td>
<td>6.04 (.03)</td>
<td>1.21–30.08</td>
</tr>
<tr>
<td>&gt;30</td>
<td>4 (12)</td>
<td>188 (42)</td>
<td>Reference group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (57)</td>
<td>225 (47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15 (43)</td>
<td>258 (53)</td>
<td>0.65 (.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure type*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>17 (71)</td>
<td>130 (38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect</td>
<td>7 (29)</td>
<td>211 (62)</td>
<td>3.94 (.003)</td>
<td>3.48 (.01)</td>
<td>1.28–9.46</td>
</tr>
<tr>
<td>First degree relative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31 (89)</td>
<td>257 (55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4 (11)</td>
<td>211 (45)</td>
<td>6.36 (.001)</td>
<td>11.0 (.02)</td>
<td>1.43–84.66</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval.

* Excluded the ‘other’ category because data were not available for healthy contacts.

### Table 3. Genealogical Relationships Associated With Secondary Cases, Comparing 35 Secondary Cases and 348 Healthy Contacts

<table>
<thead>
<tr>
<th>Relation</th>
<th>Secondary Cases, (%)</th>
<th>Healthy Contacts, (%)</th>
<th>OR (P value)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibling</td>
<td>22 (62)</td>
<td>106 (21)</td>
<td>4.72 (.003)</td>
<td>1.67–13.35</td>
</tr>
<tr>
<td>Other first degree</td>
<td>9 (26)</td>
<td>151 (30)</td>
<td>1.36 (.56)</td>
<td>.49–3.79</td>
</tr>
<tr>
<td>Father</td>
<td>1 (3)</td>
<td>47 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>3 (9)</td>
<td>55 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring</td>
<td>5 (14)</td>
<td>49 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second and third degree</td>
<td>4 (11)</td>
<td>91 (18)</td>
<td>Reference group</td>
<td></td>
</tr>
<tr>
<td>Grandchild</td>
<td>0</td>
<td>2 (0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grandparent</td>
<td>0</td>
<td>15 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (aunt/uncles, cousins, nephews/nieces)</td>
<td>4 (11)</td>
<td>74 (15)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval.
Individual Level Risk Factors

There were 113 sporadic outbreak cases and 26 cluster outbreaks with 29 index cases (3 cluster outbreaks had 2 index cases with the same illness onset date). Similar proportions of sporadic cases and cluster index cases worked in bird-related occupations (12%) were likely to be infected in the home (51% sporadic and 68% cluster index) and had timely hospitalization (7% sporadic and 11% cluster index). These variables, along with age and sex, were not associated with sporadic or cluster outbreaks (Figure 2).

Index case exposure was significantly associated with outbreak type, where index cases with direct exposure to sources of AI H5N1 virus were more likely to lead to clusters (OR, 3.50; 95% CI: 1.35–9.10, P = .01, Figure 2). Table 1 presents the main

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**Figure 2.** Risk factors for cluster outbreaks of avian influenza A H5N1 infection comparing 113 sporadic and 26 cluster outbreaks. Bars in the left column are empirical proportions of outbreaks that are clusters. White rectangles in the right column are data from sporadic outbreaks and black rectangles represent cluster outbreaks. The relationship between relatedness and cluster formation is presented by the proportion of all household contacts by degree of relatedness for cluster and sporadic outbreaks (right, bottom). Variables significant at the 5% level are indicated with P values next to the title. *For relatedness to case, P value presented for the number of first-degree relatives. For second- or third-degree relatives or unrelated cohabitants, the P values were .21, .08, and .69, respectively. The 13 index cases whose exposure could not be determined despite investigation are omitted from the exposure of index panel. Analyses for index case level variables included 29 index cases for clusters (as 3 clusters had coindex cases).**

**Individual Level Risk Factors**

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putative exposures for cases in sporadic and cluster outbreaks. Compared to sporadic cases, a greater proportion of cluster index cases had bird deaths in the home (31% vs 12%) or handled sick/dead birds (31% vs 22%). A greater proportion of sporadic cases had indirect exposures as their main putative exposure, where a greater proportion was exposed to poultry deaths in their neighborhood (29% vs 21%), had healthy poultry in their neighborhood (16% vs 3%), or visited a live bird market (6% vs 0).

**Multivariate Model**

Variables significant at \( \alpha = .1 \) on univariate analysis—exposure of index, household size, number of blood contacts, and number of first-degree relatives—were considered for a multivariate logistic regression model. Because risk associated with household size and relatedness were attributable to the number of first-degree relatives, and given the correlation between these 3 factors, we dropped household size and number of blood contacts. Both remaining variables were statistically significant as independent risk factors for cluster outbreaks: the number of first-degree relatives of the index case(s) (adjusted odds ratio [aOR], 1.50 per first-degree relative; 95% CI: 1.20–1.86, \( P < .001 \)) and index case direct exposure to sources of AI H5N1 virus (aOR, 3.20; 95% CI: 1.15–8.90, \( P = .026 \)). The final multivariate model passed the Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares goodness-of-fit test \( (P = .62) \). No other household or individual level variables were associated with sporadic outbreaks (Figure 2 and Table 1).

**Risk Factors for Secondary Case Infection**

Three variables were found significant at \( \alpha = .1 \) on univariate analyses as risk factors for secondary case infection (Table 2): age, exposure, and first-degree blood-relatives. There was no statistical difference in sex between secondary cases and healthy contacts.

Contacts under 30 years of age were at greater risk of becoming secondary cases (Table 2). Further analysis found that the age distribution of secondary cases (mean, 17.1; range, 1–39 years) was similar to cluster index cases (mean, 17.3; range, 3–38 years). Overall, the age of all cluster cases (mean, 17.5; range, 1–39 years) did not differ substantially from that of sporadic cases (mean, 20.6; range 2–67, \( P = .054 \)).

Contacts who had direct exposure to sources of AI H5N1 virus were also at greater risk of infection than contacts who had indirect exposure \( (P = .003; \text{Table 2}) \). Secondary case exposures were similar to cluster index cases, where most had bird deaths in the home (26%) or handled sick/dead birds (17%) (Table 1). Being a first-degree relative to an index case was a risk factor for becoming a secondary case \( (P = .001) \). The majority of secondary cases (89%) were first-degree relatives (Table 2). No non-blood relatives of index cases were infected in any of the outbreaks.

**Multivariate Model**

The 3 variables significant on univariate analyses were considered in the multivariate logistic regression model. The final model had 3 risk factors for secondary case infection (Table 2). These were age where individuals between 5 and 17 years of age \( (OR, 8.32; 95\% \text{ CI}: 1.72–40.25; \( P = .008 \)) \) or 18–30 years of age \( (OR, 6.04, 95\% \text{ CI}: 1.21–30.08, \( P = .028 \)) \) were more likely to be infected compared with contacts >30 years old, having direct exposure to sources of AI virus \( (OR, 348; 95\% \text{ CI}: 1.28–9.46, \( P = .014 \)) \) and being a first-degree relatives of an index case \( (OR, 11.0; 95\% \text{ CI}: 1.43–84.66, \( P = .02 \)) \). The final multivariate model had good fit \( (P = .21) \).

The finding that first-degree relatives were at greater risk of secondary infection was explored further. Restricting analysis to only blood-relative contacts of index cases, we found that siblings were nearly five times more likely to become secondary cases compared with second- or third-degree blood-relatives \( (OR, 4.72; 95\% \text{ CI}: 1.67–13.35, \( P = .003 \); Table 3) \). Other first-degree relatives (parents or offspring) were statistically at no greater risk of infection than second- or third-degree relatives. The empirical infection rates for children and adults as a function of genetic relatedness to index cases highlights that in addition to relatedness, age is an important determinant of infection (Figure 3).

**DISCUSSION**

Understanding risk factors for outbreaks, especially clustering, have important implications for disease control and prevention [2, 19, 20]. The major conclusion of this study is that an interplay of exposure type and genetic susceptibility predisposes the formation of AI H5N1 cluster outbreaks. Households with many blood-related contacts to the index case were more likely to develop secondary cases, and those who became secondary cases were more likely to be first-degree relatives of the index case. To minimize the risk of clustering, the public health implications are 2-fold: (1) household contacts, especially first-degree relatives, need to be traced and monitored for infection, and (2) household contacts should be educated about appropriate methods for handling birds, especially sick and dead birds, to minimize direct exposure to sources of virus.

Identifying the mechanisms most responsible for household clustering is difficult because genetic relationship and household membership are correlated. Even for diseases for which a genetic mechanism for infection has been identified through whole-genome research, such as for leprosy, the extent to which genetic versus household exposure factors explain clustering has been difficult to determine [21]. For AI H5N1, arguments for genetic susceptibility include the preponderance of familial clustering of cases, with 50 of the 54 clusters detected globally (as of March 2009) having cases that were all genetically linked [18]. Further arguments include the paucity of cases in highly exposed groups such as poultry workers and the occurrence of familial cases.
Figure 3. Empirical risk of secondary cases by age group (child <18 years, adult ≥18 years) and degree of blood-relatedness to index case. Bars indicate 95% confidence intervals derived from Wilson score interval.

Risk Factors for Avian Influenza A H5N1 Clusters • CID • 7