

Towards control of avian influenza H5N1 virus in Indonesia: Human infection, and the role of live bird markets.

by

Gina Samaan, BPsyc (Hons), MAppEpid

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Declaration

I declare that the work contained in this thesis is the result of original research and has not been submitted to any other University or Institution.

The research I conducted and the papers published in this thesis are based on data collected and analysed from a public health surveillance system and three research studies. I was principal researcher in all of the work and played a central role in research design, data collection, analysis and interpretation of findings. For studies pertaining to public health surveillance system data, I facilitated and worked collaboratively with other epidemiologists, statisticians and mathematical modellers to analyze the data and ensure the validity of the findings. For the research studies, I collaborated with laboratory scientists to test collected samples and report microbiological findings. I was solely responsible for overall project management, data management and analysis. I also was responsible for preparation of scientific manuscripts and coordination of co-author input for all papers published as part of this thesis.

The analyses in this thesis are my own work, except where indicated by references or acknowledgements in the text.

Signed:  _____

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Abstract

Background:

Indonesia has been heavily affected by the emerging avian influenza (AI) H5N1 virus, with continued outbreaks in farmed birds and periodic detection of human cases. The epidemiology of human AI H5N1 infection in Indonesia is poorly understood, and control measures at the animal-human interface such as in live bird markets (LBMs) have had limited impact. This thesis had two aims: (a) to examine the epidemiology of human AI H5N1 infection and, (b) to inform disease control measures in LBMs in Indonesia.

Methods:

For the first aim, public health surveillance data from June 2005 till July 2009 were analyzed to assess exposures and risk factors for infection, case clustering and disease transmission patterns in outbreak households. For the second aim, a cross-sectional study was conducted to assess environmental contamination in LBMs and to identify risk factors and critical control points. A non-experimental field intervention trial was conducted to assess the practical application of implementing interventions in two LBMs.

Results:

Multivariable analyses showed that age and type of exposure to virus impact the risk of H5N1 infection and case clustering. First degree relatives to an index case, especially siblings were at most risk of becoming secondary cases in a household. The overall attack rate in households was 18.3% and the secondary attack rate was 5.5%. Secondary attack rate remained stable with household size. The disease transmission models found that the majority of cases resulted from zoonotic transmission of the virus, and most evidence for human-to-human transmission came from one large outlier cluster of eight cases. The reproduction numbers were below the threshold for sustained transmission. The mean interval between onset of illness between cases in a household was 5.6 days. Direct exposure to sources of virus tripled the odds of infection. Contaminated garden fertiliser was found to be a possible source of human infection.

Widespread environmental contamination with the H5N1-virus was found in 47% (39 of 83) LBMs sampled in the cross-sectional study. Slaughter, workflow zoning and sanitation practices impact the risk of environmental contamination. Five critical control points were identified to help control this contamination. The intervention trial found that control measures could be feasibly implemented using a combination of infrastructure and behaviour change interventions. Use of a participatory approach to translate control measures into practice was well received by stakeholders.

Conclusions:

The epidemiological findings can be used to reduce the risk of zoonotic transmission of the virus, prevent secondary cases and provide baseline comparison for the early detection of changes in virus transmissibility. The LBM studies demonstrated that control measures can be introduced in LBMs in a low resource setting such and that the interventions should reflect resources available, stakeholder needs and critical control points.

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Chapter 1

Introduction

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.

Chapter	Article title	Journal	Status	Gina Samaan authorship	Number of co-authors	Conception & designing	Analysis & interpretation	Drafting & revising
4	Risk factors for cluster outbreaks of avian influenza A H5N1 infection, Indonesia.	Clinical Infectious Diseases	Published, October 2011	Co-1 st author	14	80	70	90
5	Avian Influenza H5N1 Transmission in Households, Indonesia.	PLOS One	Published January 2012	Co-1 st author	14	80	70	90
6	Chicken faeces garden fertilizer: possible source of human avian influenza H5N1 infection.	Zoonoses & Public Health	Published, June 2010	2 nd author	9	80	70	90
7	Environmental sampling for avian influenza virus A (H5N1) in live-bird markets, Indonesia.	Emerging Infectious Diseases	Published, December 2010	Co-1 st author	11	70	80	90
8	Critical control points for avian influenza A H5N1 in live bird markets in low resource settings.	Preventive Veterinary Medicine	Published, March 2011	1 st author	4	100	90	90
9	Application of a healthy food markets guide to two Indonesian markets to reduce transmission of "avian flu."	Bulletin of the World Health Organization	Published, April 2012	1 st author	7	80	70	90

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.

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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

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 Puskesmas 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).

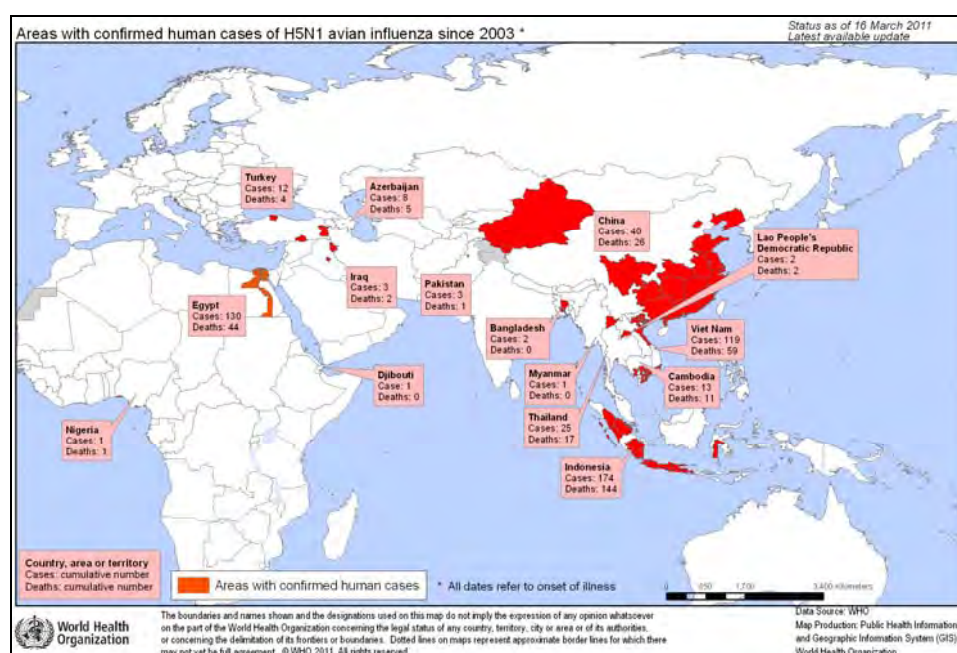
Avian Influenza H5N1 Virus

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

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

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 Subdirectorates maintain a database of all cases of AI H5N1 infection and archive 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

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 (AIC_c) (28). The AIC_c 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.

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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.

Risk Factors for Cluster Outbreaks of Avian Influenza A H5N1 Infection, Indonesia

Tjandra Y. Aditama,^{1,a} Gina Samaan,^{2,a} Rita Kusriastuti,¹ Wilfried H. Purba,¹ Misriyah,¹ Hari Santoso,¹ Arie Bratasena,¹ Anas Maruf,¹ Elvieda Sariwati,¹ Vivi Setiawaty,³ Alex R. Cook,⁴ Mark S. Clements,^{2,5} Kamalini Lokuge,² Paul M. Kelly,^{2,6} and I. Nyoman Kandun¹

¹Directorate-General Disease Control and Environmental Health, Ministry of Health, Jakarta, Indonesia; ²National Centre for Epidemiology and Population Health, College of Medicine, Biology, and Environment, The Australian National University, Canberra; ³National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia; ⁴Department of Statistics and Applied Probability, National University Singapore; ⁵Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; and ⁶Population Health Division, Australian Capital Territory Government Health Directorate, Canberra

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.

Avian influenza A H5N1 virus (AI H5N1) infection is of international public health concern. Although most cases detected have been sporadic cases, there have been a number of case clusters, typically of individuals in the same household [1]. Clusters of AI H5N1 infection may signify an increase in the virus' capacity for human transmissibility, which could then lead to the emergence

of a new influenza pandemic. Clusters are also of interest because they can provide information about the risk factors for infection, the likelihood of genetic susceptibility, and the dynamics of disease transmission within households [2, 3]. A few countries, including Indonesia, have reported on human clusters of AI H5N1 infection. However, previous research has been largely limited to describing the clinical and basic epidemiological features of cases in clusters [1]. Because Indonesia's cumulative AI H5N1 infection case count represents one-third of the world's cases, detailed analysis of the Indonesian outbreaks can add significantly to the body of knowledge on the epidemiology for case clustering. The first human cases of AI H5N1 were detected in July 2005 in Indonesia [4]. By July 2009, 159 human cases were confirmed, and 18 probable cases associated with the

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^aT. Y. A. and G. S. are first coauthors.

Correspondence: Gina Samaan, MAppEpid, National Centre for Epidemiology and Population Health, College of Medicine, Biology, and Environment, The Australian National University, Canberra, ACT 0200, Australia (ginasamaan@yahoo.com).

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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 1 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 semiurban, 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

relations 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

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

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

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

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.

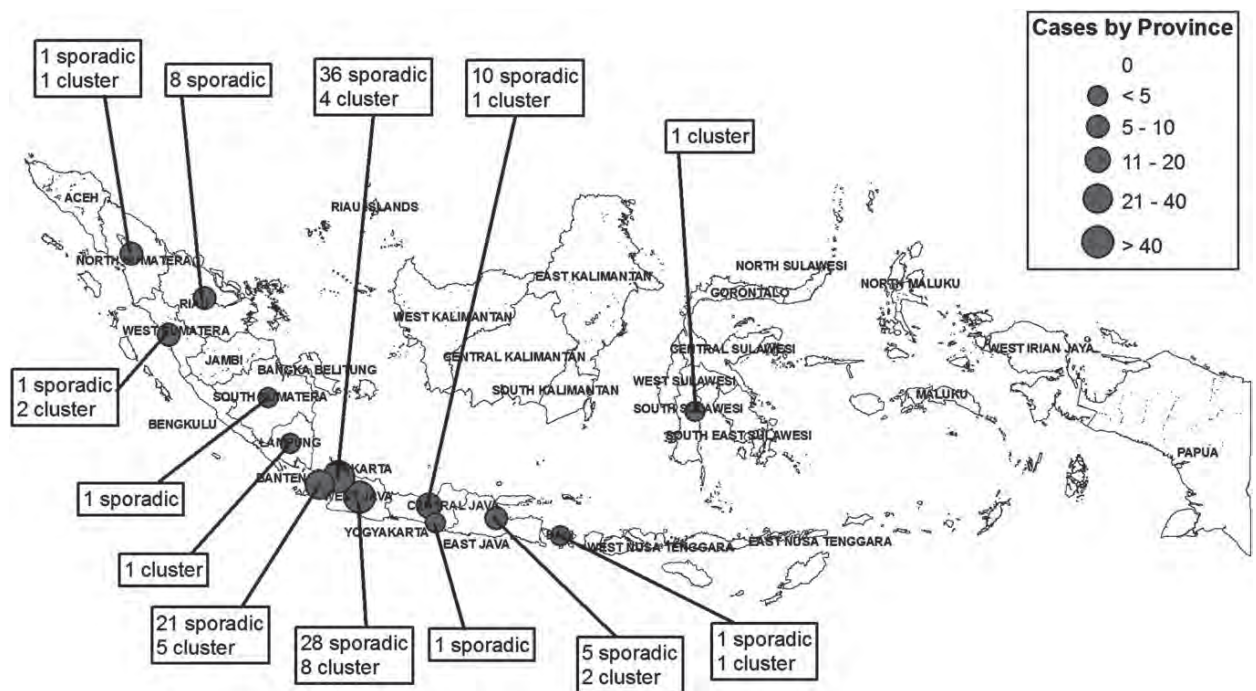
**Figure 1.** Confirmed and probable cases of avian influenza H5N1 infection in Indonesia, by province and outbreak type, July 2005–July 2009.

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

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

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

^a Excluded the 'other' category because data were not available for healthy contacts.

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

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

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

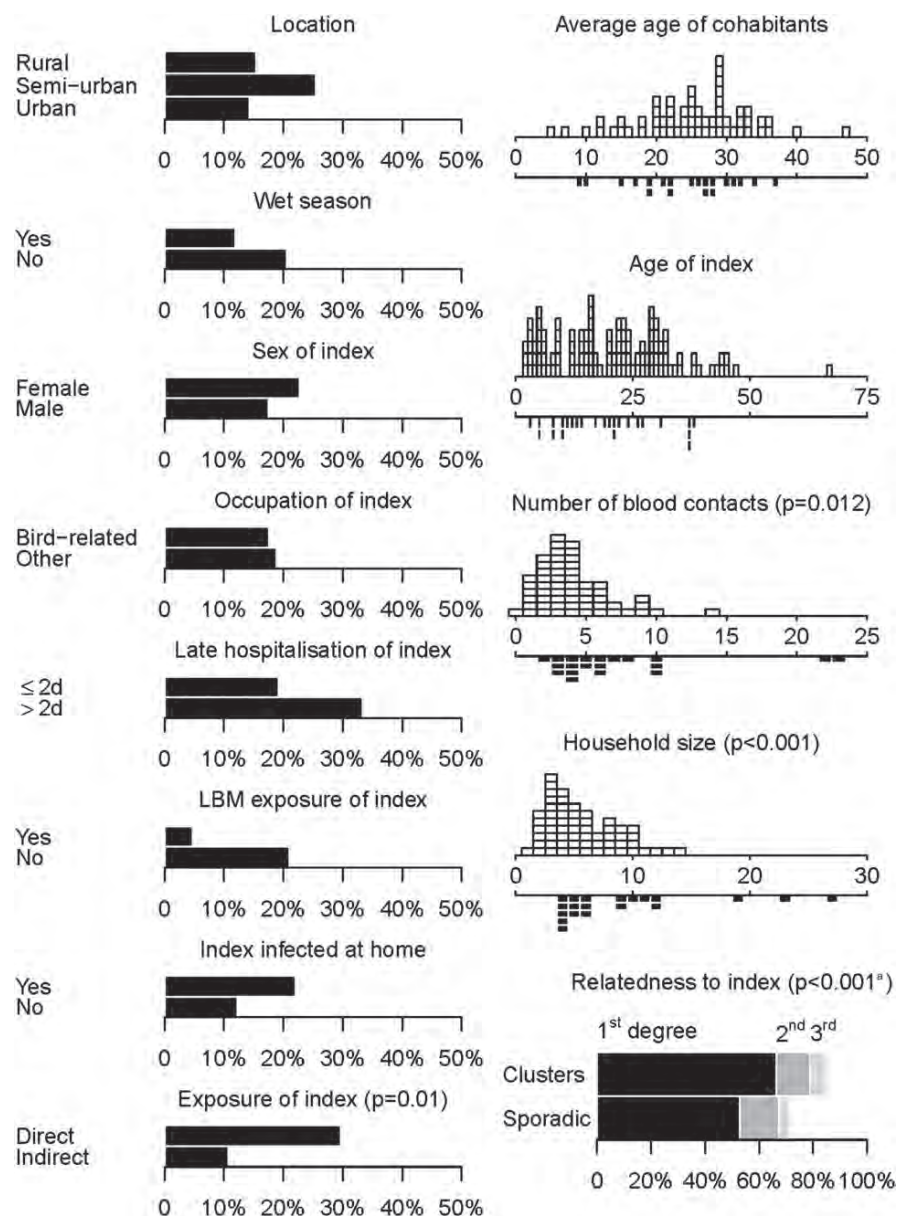


Figure 2. Risk factors for cluster outbreaks of avian influenza A H5N1 infection comparing 113 sporadic and 26 cluster outbreaks. Bars in the left column are empirical proportions of outbreaks that are clusters. White rectangles in the right column are data from sporadic outbreaks and black rectangles 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. ^aFor 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 .27, .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

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

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 le 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 ($P = .003$; 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% CI: 1.72–40.25; $P = .008$) or 18–30 years of age (OR, 6.04, 95% 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, 3.48; 95% CI: 1.28–9.46, $P = .014$) and being a first-degree relatives of an index case (OR, 11.0; 95% 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% 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 genealogical relation 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

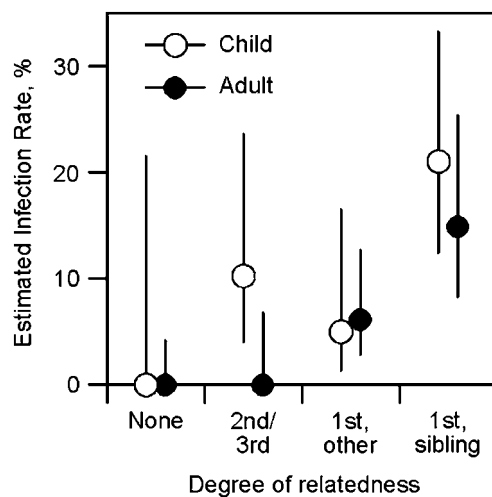


Figure 3. Empirical risk of secondary cases by age group (child <18 years, adult ≥18 years) and degree of blood-relatedness to index case. Bars indicate 95% confidence intervals derived from Wilson score interval.

separated by time and place [2, 22, 23]. This study lends further support to a genetic basis for susceptibility to AI H5N1 infection. The study found that all clusters in Indonesia had cases that were genetically linked [1]. Cases would have been expected among genetically unrelated residents in outbreak households (such as spouses) had infection been influenced by household membership and shared commonalities alone.

The study found that both index and secondary cases in cluster outbreaks were more likely to have direct exposure to sources of AI H5N1 virus. This raises 2 important points. First, this adds evidence that clustering does not necessarily indicate human-to-human transmission but may simply result from common exposures to sources of infection [24, 25]. Second, even if cases in one family were infected from the same source rather than from each other, this does not detract from the hypothesis of genetic susceptibility to infection. It is feasible that family members who are genetically susceptible to AI H5N1 infection are at greater risk of infection from any source.

Young contacts were at greater risk of infection and were also more likely to be directly exposed to sources of AI H5N1 virus. In Indonesia, bird rearing is generally delegated to younger members of the household. This sociobehavioral practice may have increased their level of risk for AI H5N1 infection compared with other age groups, but warrants further investigation.

Household-based studies shed light on risk factors for transmission because household contacts of cases are less likely to be affected by case-ascertainment bias [26]. However, household-based studies have 2 key limitations. First, index cases that are detected through surveillance and that enter the study may have more severe symptoms and exposures that are not representative of typical infections. This may bias the estimates for the risk factors

investigated. Second, outbreaks with more cases are more likely to be detected since healthcare workers are more likely to suspect and report these events to public health authorities. This inflates the secondary attack rate and may also bias the estimates for the risk factors investigated. These limitations could be addressed through prospective community-based studies.

A limitation of the study is that 10% of cases (18 of 177) met only the probable case definition [6]. Some of these cases may not have been AI H5N1 infection. However, we consider risk of misclassification to be low, considering the severity associated with illness, the proximate timeline of illness related to a confirmed case in the same household, and similar exposure patterns.

In conclusion, this is the first study to our knowledge to demonstrate that the interaction between household and individual level variables including genealogical relationships impacts the risk of clusters as well as secondary case infection. The study adds further evidence to the hypothesis that there is a genetic basis for susceptibility to infection.

Notes

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Chapter 5

Paper 2: Avian Influenza H5N1 Transmission in Households, Indonesia.

About this chapter

Building on the findings from Chapter 4, this chapter explored AI H5N1 transmission patterns and risk factors for infection. Using the same data and household-study design as that reported in Chapter 4, this study quantified zoonotic and human transmission as well as the extent to which the virus was transmissible. Other transmission patterns assessed include household attack rates, secondary attack rates and disease intervals between cases in outbreaks.

This study was the first globally to assess transmission patterns for a large number of outbreaks. It found that most H5N1-cases were a result of exposure to zoonotic sources of virus. Strong support for human transmission of the virus was only found when a single large cluster was included in the transmission model. The reproduction number was well below the threshold for sustained transmission.

My role in this study was to design the study research question, extract the relevant data from the MOH existing surveillance system and analyze the data. I worked with a mathematical modeller to develop the disease transmission models. I wrote the paper for publication and obtained feedback from all the co-authors. The study was submitted to PLOS One and was published in January 2012.

Avian Influenza H5N1 Transmission in Households, Indonesia

Tjandra Y. Aditama^{1*}, Gina Samaan^{2,*}, Rita Kusriastuti¹, Ondri Dwi Sampurno³, Wilfried Purba¹, Misriyah¹, Hari Santoso¹, Arie Bratasena¹, Anas Maruf¹, Elvieda Sariwati¹, Vivi Setiawaty³, Kathryn Glass², Kamalini Lokuge², Paul M. Kelly^{2,4}, I. Nyoman Kandun¹

1 Directorate-General Disease Control and Environmental Health, Ministry of Health, Salemba, Jakarta, Indonesia, **2** National Centre for Epidemiology and Population Health, The Australian National University, Canberra, Australian Capital Territory, Australia, **3** National Institute of Health Research and Development, Ministry of Health, Salemba, Jakarta, Indonesia, **4** Population Health Division, Australian Capital Territory Government Health Directorate, Canberra, Australian Capital Territory, Australia

Abstract

Background: Disease transmission patterns are needed to inform public health interventions, but remain largely unknown for avian influenza H5N1 virus infections. A recent study on the 139 outbreaks detected in Indonesia between 2005 and 2009 found that the type of exposure to sources of H5N1 virus for both the index case and their household members impacted the risk of additional cases in the household. This study describes the disease transmission patterns in those outbreak households.

Methodology/Principal Findings: We compared cases ($n=177$) and contacts ($n=496$) in the 113 sporadic and 26 cluster outbreaks detected between July 2005 and July 2009 to estimate attack rates and disease intervals. We used final size household models to fit transmission parameters to data on household size, cases and blood-related household contacts to assess the relative contribution of zoonotic and human-to-human transmission of the virus, as well as the reproduction number for human virus transmission. The overall household attack rate was 18.3% and secondary attack rate was 5.5%. Secondary attack rate remained stable as household size increased. The mean interval between onset of subsequent cases in outbreaks was 5.6 days. The transmission model found that human transmission was very rare, with a reproduction number between 0.1 and 0.25, and the upper confidence bounds below 0.4. Transmission model fit was best when the denominator population was restricted to blood-related household contacts of index cases.

Conclusions/Significance: The study only found strong support for human transmission of the virus when a single large cluster was included in the transmission model. The reproduction number was well below the threshold for sustained transmission. This study provides baseline information on the transmission dynamics for the current zoonotic virus and can be used to detect and define signatures of a virus with increasing capacity for human-to-human transmission.

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* E-mail: ginasamaan@yahoo.com

These authors contributed equally to this work.

Introduction

The avian influenza (AI) H5N1 virus remains of international public health concern due to its pandemic potential. Based on analyses of AI H5N1 outbreaks during 2003 to 2009, most cases were sporadic and had documented exposure to zoonotic sources of the virus [1]. For clusters of AI H5N1 infection, the majority occurred in people who were genetically related to each other and most also had exposure to zoonotic (bird to human) sources of virus [1]. Studies suggest that human transmission of the virus occurred in a very limited way in some clusters [2,3]. However, the transmission patterns remain largely unknown.

Quantification of transmission patterns such as the probability of both human and zoonotic transmission of the H5N1-virus, the reproduction number (R_0), secondary attack rates (SAR) and the interval between case onsets are important parameters to inform

preparedness and response measures to outbreaks, especially to signal events that indicate changed virus behavior [4]. It is also crucial that both zoonotic and human infection pathways are considered, and results are interpreted in the context of a zoonotic infection with limited transmission among humans [5,6]. Models that incorporate both the zoonotic and human transmission components are rare [5].

As of July 30, 2009, Indonesia had reported 139 outbreaks of avian influenza (AI) H5N1 infection in humans with a case fatality rate of 85% [7]. The epidemiology of Indonesia's cases has been reported previously [8–10]. A recent study on the 139 outbreaks assessed the risk factors for household clustering of cases and the risk factors for who in the household is likely to become a secondary case of H5N1-infection [11]. The study found that the type of exposure to sources of H5N1 for both the index case and their household members impacted the risk of additional cases in

the household. The study also added evidence that H5N1 infection may be dependent on host genetic susceptibility since first-degree blood relatives to index cases were at greater risk of becoming secondary cases. However, this study did not assess the attack rates (AR), SAR or transmission parameters in those outbreak households.

To date, only one study has estimated the transmission patterns based on case data in Indonesia [4]. Estimates generated were solely based on one outbreak – a cluster of one probable and seven confirmed cases detected in North Sumatra in 2006. The study found statistical evidence of human-to-human transmission and estimated SAR at 29% and R_0 at 1.14 [4]. Since data on the total persons exposed and individual factors such as exposure type were not fully available to that study, the transmission pathways were not investigated in detail. Also, since the model was fitted and transmission estimates generated based only on that one cluster, which is considered atypical due to its large size, the estimates are likely to be an over-estimate for outbreaks in Indonesia.

Since Indonesia's cumulative AI H5N1 infection case count represents one-third of the world's cases, the outbreak transmission patterns are of international importance. Building on previous findings about the epidemiology of H5N1 infection in households [11], we describe infection AR, infection SAR, risk factors for H5N1 infection and intervals between case illness onsets. We then estimate transmission parameters and quantify the relative contribution of zoonotic and human transmission as well as the extent to which the virus was transmissible between people (reproduction number). While international data suggest most transmission is zoonotic, there is also some evidence of human-to-human transmission [2,12]. We fitted household models to the Indonesian data that allow for both zoonotic and human-to-human transmission to assess the extent of transmission from each source and to provide an estimate of the reproduction number in the case that human-to-human transmission occurs.

Results

A total of 139 outbreaks of human AI H5N1 infection were detected in Indonesia in the four year study period. There were 113 sporadic case outbreaks and 26 cluster outbreaks. The total number of cases was 177, with 64 cases in the 26 clusters. Only one cluster had over four cases; the North Sumatran cluster of 2006, which can be considered an outlier based on its large size of seven confirmed and one probable case. There were 535 household contacts to index cases in the study, of which blood

relation was known for 94% ($n = 503$). Most of the 503 contacts were blood relatives ($n = 383$, 76%) and 120 (24%) were non-blood relatives. None of the non-blood related household contacts became secondary cases.

Household Study

For the 80 outbreaks for which household and contact data were available, the proportion of cluster to sporadic outbreaks increased as household size increased (Table 1). To highlight the impact of the outlier cluster on the AR and SAR, findings are presented both including and excluding that cluster. The overall AR was 17.8% (103 cases / 579 exposed) when the outlier cluster was excluded and 18.3% (111 cases / 607 exposed) when included. There was a stable SAR between 3.1–4.5% across household size (Table 1). However, inclusion of the outlier cluster inflated SAR for households with >15 persons to 12.5% (Table 1). These findings are consistent with predominantly zoonotic virus transmission. In the absence of human transmission, and with low levels of zoonotic transmission, the AR would be expected to decline with household size, while the SAR should remain roughly constant.

Cases ($n = 177$) and healthy contacts ($n = 496$) were compared to assess risk factors for infection (Table 2). Young age groups (≤ 30 years) were at increased risk of infection, where individuals between five and 17 years of age had 3.5 times the odds to be infected when compared with those >30 years of age [Adjusted Odds Ratio (aOR) = 3.44, 95% Confidence Interval (CI) 1.86–6.36]. Most cases (87%) and their healthy contacts (69%) had zoonotic exposure. However, direct exposure to zoonotic sources of AI H5N1 virus tripled the odds of infection (aOR = 3.08, 95% CI 1.54–6.13). Lastly, small households (1–5 persons) were significantly more likely to have cases than households with >5 people (Table 2). The final multivariate model with three variables had good fit ($p = 0.17$).

In cluster outbreaks, the median interval between the index case onset and secondary case onset of illness was 8 days (range 1–21 days, Figure 1A). The median interval between the onset of illness of a secondary case and the previous case in the same outbreak was 6 days (range 1–12 days, Figure 1B). Based on the investigation reports, eleven secondary cases had inconclusive exposure to a zoonotic source of virus. All of these had onset of illness at least two days after the index case's onset of illness. For these 11 cases, the median interval between illness onset of serial cases was 8 days (range 2–11 days, Figure 1B).

Table 1. Household size and secondary attack rate for outbreaks of avian influenza H5N1 infection.

Contact data	Household size	Outbreak size (confirmed and probable cases)								Total outbreaks	Proportion cluster	Total contacts	Secondary cases	SAR
		1	2	3	4	5	6	7	8					
Available	1–5	27	4	1	0	0	0	0	0	32	0.16	152	9	0.059
	6–10	25	6	2	0	0	0	0	0	35	0.24	219	8	0.036
	11–15	8	3	0	1	0	0	0	0	12	0.33	85	3	0.035
	>15	0	2	0	0	0	0	0	1	3	1.00	69	9	0.130 ^a
	Sub-total	60	15	3	1	0	0	0	1	80	0.24	525	29	0.055 ^b
Not available		53	5	1	0	0	0	0	0	59	0.10	-	6	-
Total		113	20	4	1	0	0	0	1	139	0.19	-	35	-

^aSAR declines to 0.047 when outlier cluster is excluded.

^bSAR declines to 0.044 when outlier cluster is excluded.

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Table 2. Comparison of cases (n = 177) and healthy contacts (n = 496) in outbreaks of avian influenza H5N1 infection.

			Univariate	Multivariate ^a	
Variable	Cases, n (%)	Healthy contacts, n (%)	OR (P-value)	Adjusted OR (P-value)	95% CI
Age groups (years) ^b					
0–4	18 (10)	41 (9)	2.66 (0.004)	3.18 (0.004)	1.45–6.98
5–17	65 (37)	96 (21)	4.11 (<0.001)	3.44 (<0.001)	1.86–6.36
18–30	61 (35)	125 (28)	2.96 (<0.001)	3.20 (<0.001)	1.81–5.68
>30	31 (18)	188 (42)	Reference group	-	-
Sex					
Male	83(47)	225 (47)	0.99 (0.94)		
Female	94 (53)	258 (53)			
Exposure					
Direct zoonotic	81 (46)	130 (26)	4.02 (0.002)	3.08 (0.001)	1.54–6.13
Indirect zoonotic	72 (41)	211 (43)	2.20 (<0.001)	1.43 (0.29)	0.72–2.81
Inconclusive zoonotic	24 (13)	155 (31)	Reference group	-	-
Household size (persons) ^c					
1–5	51 (46)	143 (29)	Reference group	-	-
6–10	38 (34)	211 (43)	0.51 (0.009)	0.50 (<0.001)	0.34–0.73
11–15	10 (9)	82 (16)	0.35 (0.001)	0.32 (<0.001)	0.18–0.57
>15	12 (11)	60 (12)	0.51 (0.07)	0.40 (0.16)	0.11–1.43

^aObservations = 561, Goodness-of-fit test: P = 0.17, OR denotes odds ratio, CI denotes confidence interval. OR were adjusted for the inclusion of the three variables in the final multivariate model.

^bData missing for two cases and 46 healthy contacts.

^cData missing for 66 cases from the 59 outbreaks for which household data were not available.

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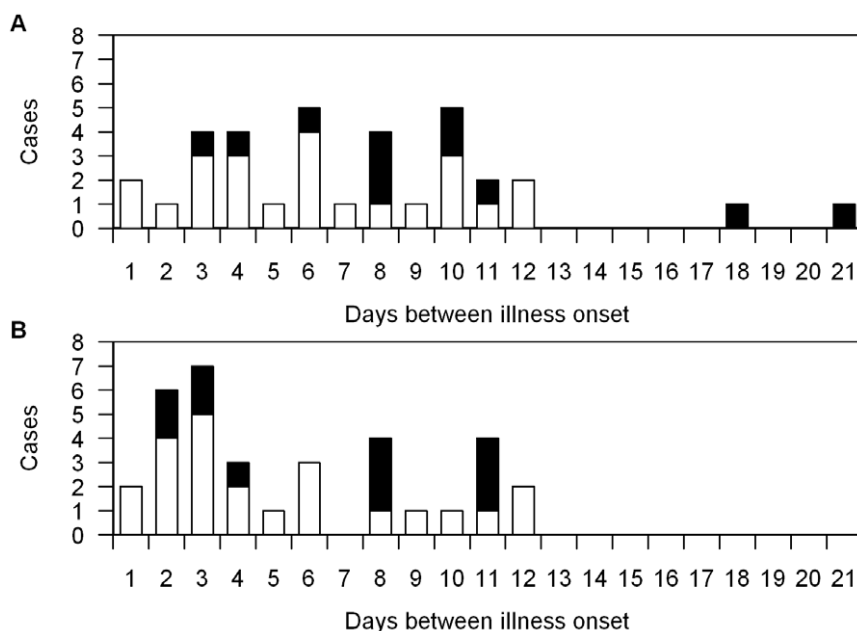


Figure 1. Interval between onset of illness for cases (n = 34) in outbreaks of avian influenza H5N1 infection. Panel A shows the interval between onsets of illness of index and secondary cases in outbreaks. Panel B shows the interval between onsets of illness of serial cases in outbreaks. Black denotes cases not exposed to zoonotic sources of virus and white denotes cases exposed to zoonotic sources of virus.

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Transmission Model

To assess the exposure of secondary cases, Table 3 presents the transmission analysis comparing three model types: all transmission from zoonotic sources (Model A), all transmission was human transmission (Model B) and transmission was from both zoonotic and human sources (Model C). Two denominator populations are presented for comparison; all exposed individuals in outbreaks and all exposed blood-related household members to index cases. The final column of the tables shows the percentage support for the models, which can be interpreted as the probability that the model is the best among those considered. To highlight the impact of the outlier cluster on transmission parameters and model selection, findings for two datasets are presented; one with the outlier cluster included and one with it excluded.

Regardless of the denominator population or the dataset, there was much less support for Model A (zoonotic transmission only) than either Models B (human transmission only) or C (combination of zoonotic and human transmission) (Table 3). This was confirmed by a simulation-based test of model fit, which demonstrated significant differences between Model A and the data ($p < 0.01$ for both). Despite significant evidence that human transmission occurred when the outlier cluster was included in the analysis, estimated human transmission rates were low with the reproduction number lying between 0.1 and 0.25, and the upper confidence bounds all below 0.4 for an exposed population of five individuals. Estimated zoonotic transmission rates ranged from 0 to 0.38 cases in an exposed population of five household members.

When the analysis excluded the outlier cluster (Table 3), similar estimates for the human transmission parameters and the reproduction number were found, but there was no longer significant evidence of human transmission. Indeed, the model with the strongest support was Model A (zoonotic transmission only), with 0.31 zoonotic cases infected in an exposed population of five household members. This suggests that the main evidence

for human transmission comes from the outlier cluster. For all model types, both including and excluding the outlier cluster, use of blood-related household members as the denominator population provided better model fit. A test of the sensitivity of our results to the households in which contact data were missing found very little change to the transmission estimates, with estimates of zoonotic transmission parameters reduced by around 0.05–0.1 cases in an exposed population of size 5, point estimates of human transmission parameters largely unchanged, and a decrease in the upper bound of the human transmission parameter of 0.02–0.08 cases in an exposed population of size 5.

Discussion

This study is the first globally to examine AI H5N1 transmission patterns in households for a large number of outbreaks aimed at quantifying human-to-human transmission of the AI H5N1 virus. The study had three main findings. Firstly, most cases of AI H5N1 infection were a result of exposure to zoonotic sources of virus. In fact, the study only found strong support for human transmission of the virus when a single large cluster was included in the transmission model. Secondly, the overall SAR was 5.5% in the 80 outbreaks for which household contact data were available. This was much lower than previous estimates [4]. Thirdly, the study adds evidence that blood relatives are at greatest risk of becoming secondary cases in outbreak households. This adds support to the hypothesis that there is an element of genetic susceptibility to AI H5N1 infection [3].

The finding that the AI H5N1 virus does not transmit efficiently between humans and that infection remains primarily zoonotic impacts the interpretation of the interval between case onsets and the SAR. These parameters should not be interpreted as human-to-human transmission parameters. Rather, the interval between case onsets (median 6 days, range 1–12 days) represents observed timelines between human cases during an epizootic and indicates

Table 3. Transmission parameters for outbreaks of avian influenza H5N1 infection.

Data	Denominator population	Model description	Mean human transmission cases ^a (95% CI)	Mean zoonotic infected cases ^b (95% CI)	AIC _c percent support ^c
80 outbreaks (North Sumatra cluster included)	All exposed individuals	A) Only zoonotic transmission	-	0.276 (0.126, 0.476)	0.1
		B) Only human transmission	0.172 (0.026, 0.322)	-	6.3
		C) Full model	0.115 (0.009, 0.315)	0.094 (0.000, 0.344)	4.4
	All exposed blood-relatives	A) Only zoonotic transmission	-	0.385 (0.185, 0.635)	2.2
		B) Only human transmission	0.231 (0.082, 0.382)	-	42.9
		C) Full model	0.140 (0.004, 0.390)	0.157 (0.000, 0.452)	44.0
79 outbreaks (North Sumatra cluster excluded)	All exposed individuals	A) Only zoonotic transmission	-	0.221 (0.071, 0.421)	5.1
		B) Only human transmission	0.166 (0.024, 0.316)	-	2.4
		C) Full model	0.052 (0.000, 0.302)	0.158 (0.000, 0.403)	2.5
	All exposed blood-relatives	A) Only zoonotic transmission	-	0.310 (0.110, 0.510)	53.3
		B) Only human transmission	0.227 (0.077, 0.427)	-	14.0
		C) Full model	0.052 (0.000, 0.352)	0.242 (0.000, 0.542)	22.7

^aMean number of secondary cases infected by a single index case in an exposed population of size 5, CI denotes confidence interval.

^bMean number of zoonotic cases in an exposed population of size 5.

^cAIC_c denotes Akaike Information Criterion adjusted for small sample size. This indicates the percent probability that the model is the best amongst those considered. doi:10.1371/journal.pone.0029971.t003

the duration of risk of more cases being detected in association with the epizootic event. This information can guide the length of contact tracing needed to detect and prevent further cases during an outbreak. The findings from this study reinforce the WHO recommendation to trace and monitor case contacts for two weeks after the illness onset of the last case [13].

The SAR results add to the body of knowledge on typical outbreak size associated with the current zoonotic virus, where SAR remained approximately stable with household size. This provides important baseline information for future outbreak investigations and may help in the detection of changes in virus behavior. For a virus on the verge of efficient human spread, the household SAR should be compared to the current findings as well as SAR for other influenza viruses.

Although the SAR remained stable with household size, the proportion of outbreaks with more than one case increased with household size. This highlights an important distinction between individual and household risk for infection with the current zoonotic virus: a person in a large household is less likely to be infected than a person in a small household, but large households are more likely to have a secondary case than small households. Whether SAR was low due to virus and host characteristics or due to public health interventions such as prophylaxis of case contacts or isolation of cases was not explored in this study, but warrants future investigation. Importantly, the SAR could not be calculated for the remaining 59 outbreaks as contact data were not available to determine the household size. The missing data highlight the challenge in standardizing data collection for a new emerging disease. However, as the excluded outbreaks were typically smaller than those with full contact data (90% of excluded outbreaks were sporadic), it seems unlikely that inclusion of those outbreaks would increase the overall SAR or the transmission parameters. Our sensitivity analysis suggested that inclusion of these data would likely result in a slight decrease in the zoonotic transmission parameter, negligible impact on the point estimate of the human transmission parameter, and a slight decrease in the upper bound of the human transmission parameter.

Due to the limited sensitivity of public health surveillance systems, varied health-seeking behavior within the population and the potential for mild infections, it is possible that cases or clusters of H5N1 infection were missed and not included in the analysis. This affects our findings. If sporadic cases of H5N1 infection resulting from zoonotic transmission of the virus were missed, then our study likely over-estimates overall SAR and transmission parameters. If clusters of cases were missed, then our study may under-estimate these parameters. We speculate, based on our H5N1 case investigations, that clusters of disease are less likely to be missed than sporadic cases of infection since families and healthcare workers would raise alarms in the public health system about multiple cases of pneumonia in a single household. For mild cases, it is feasible that cases are missed, which suggests that our results would under-estimate transmission parameters. However, based on studies conducted amongst poultry workers exposed to H5N1 virus in the course of their work, mild and subclinical infections have been limited [14–16]. This is also mirrored in influenza virological surveillance findings conducted by countries affected by H5N1 virus such as Lao PDR, China and Cambodia, whereby these sentinel surveillance systems regularly detect seasonal influenza viruses circulating in the community and in hospital settings, yet they rarely detect cases of H5N1 virus infection [17–19].

The disease transmission model achieved a better fit when the exposed population was restricted to blood-related household members. The study also found that only blood relatives to the

index case developed illness and that none of the 120 non-blood related household members (such as spouses and family-in-law) developed illness. Collectively, these findings add evidence to the hypothesis that there is a host genetic effect on susceptibility to AI H5N1 infection [11]. However, since genetic relationship and household membership are correlated, it is difficult to identify the mechanisms most responsible for household clustering. Thus, further research is needed to explore these findings.

Individuals at most risk of infection were those ≤ 30 years, especially children between five and 17 years. The young age pattern was also observed globally based on analysis of cases from 11 countries [1]. This suggests that young age groups have greater susceptibility to AI H5N1 infection; be it due to social, hygienic or biological factors. Potential reasons include that children are more likely to handle sick and infected birds or to be exposed to contaminated environment through play or through bird rearing. In Indonesia, anecdotal evidence suggests that bird rearing is delegated to young household members. Children are less conscious of hygiene and thus may have had unprotected interaction with sources of virus [20].

Household based studies exploring risk factors for infection are less likely to be affected by case-ascertainment bias [21]. However, since household data were not available for all outbreaks, our analyses and conclusions were based on a restricted dataset and should be interpreted with caution as the missing data limit the power of our study. Nevertheless, as discussed earlier, since 90% outbreaks lacking household data only had one case, our study likely over-estimated the transmission parameters and the SAR, indicating that human transmission rates were very low.

Overall, the study found that AI H5N1 human infection resulting from human transmission of the virus was very limited, and that the reproduction number was well below the threshold for sustained transmission. Case clustering does not always denote human transmission of the virus, but is often the result of household members' shared exposure to zoonotic sources of the virus [22]. The study findings also suggest that there may be a host genetic effect on susceptibility to infection, but this warrants further investigation through epidemiological and immunological studies to untangle the correlation between household membership, shared exposures and genetics.

Materials and Methods

Ethics Statement

All data in this study were obtained from the case-investigation reports and the surveillance database at the Ministry of Health, which were collected as part of an ongoing public-health investigation. Permission to conduct the study and analyze the data was obtained from the data custodian (first author, Director-General for Disease Control and Environmental Health at the Ministry of Health, Republic of Indonesia). Data shared with international study collaborators, who were not involved in the case investigations, were de-identified to protect the confidentiality of the cases and their families, whereby names and addresses were removed. Ethics approval for the study was obtained from the Australian National University's Human Research Ethics Committee.

Setting

The Ministry of Health AI H5N1 case database and detailed case investigation forms were reviewed and analyzed for cases detected in Indonesia between July 2005 and July 2009. The study conformed with the WHO definitions [13], whereby a cluster is a group composed of one confirmed case 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. In households with a cluster of cases, the index case was defined as the one with the earliest symptom onset date amongst all the cases in that household. A sporadic outbreak was defined as one confirmed case of H5N1 virus infection. Case definitions for probable and confirmed cases were based on the WHO definitions described previously [23]. For both sporadic and cluster outbreaks, a household contact was a person who had at least four hours contact with a probable or confirmed case at home within the seven days prior or 14 days after the case's onset of illness.

Data Collection

Field investigation teams investigated every outbreak. Teams interviewed cases when possible (since many cases died before investigation teams arrived), family members and key informants such as healthcare workers. As described previously [8,11], data were collected using a standardized H5N1-case questionnaire developed by the Ministry of Health based on WHO guidance [24]. The questionnaire collected data on the case's household, clinical symptoms, healthcare facility attendance and potential zoonotic, human and environmental exposures to sources of H5N1-virus. Medical records from all healthcare facilities visited by cases during the course of their illness were reviewed and extracted to complete the questionnaire.

Contact tracing, clinical examination and testing of household contacts were done during the investigation. Serum samples were collected from all healthy household contacts to assess for H5N1 seroconversion using microneutralization test or haemagglutination inhibition test (with horse red blood cells). For household contacts with symptoms of H5N1 infection, nasal and throat swabs were collected and tested using real-time reverse transcriptase polymerase chain reaction (RT-PCR) test. All tests were conducted according to the WHO guideline on recommended laboratory procedures for H5N1 detection [25]. Healthcare workers from the nearest government primary healthcare centre were instructed to visit the household daily for two weeks to monitor and detect any additional cases.

Household Study

AR, SAR, risk factors for infection and intervals between case onsets were analyzed in a household-based study. Household size was the number of people in the household including cases. A household contact was a person who had at least four hours contact with a case at home within the seven days prior or fourteen days after a case's onset of illness. AR was calculated for the 80 outbreaks (60 sporadic and 20 clusters) out of the 139 for which household data were available. Data on household contacts were missing for 59 outbreaks, of which 90% ($n = 53$) were sporadic case outbreaks and the largest outbreak involved three cases. AR was defined as the proportion of people who met the definition for confirmed or probable AI H5N1 infection in the outbreak (household). SAR was defined as the proportion of household contacts who met the probable or confirmed case definition after the onset date for the index case and within two weeks of the onset of symptoms of a prior household case. Two weeks was selected as the maximum follow up period based on WHO guidance [13]. The intervals (days) between onset of symptoms of index cases and subsequent cases in clusters, and the interval between serial cases in clusters were calculated.

Logistic regression models that accounted for household clustering using a cluster robust standard error for the coefficients were used to evaluate the risk factors for infection. Multivariate

models were constructed using variables significant at $p=0.1$ in the univariate analyses. A final model was achieved by sequentially discarding terms not significant at $P=0.05$ starting with the ones with the highest P-values. 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.* [26–28]. Stata software version 10.0 (StataCorp) was used for this analysis.

Four variables were explored as risk factors for infection: age, sex, exposure type and household size. To simplify interpretation of results, age and household size were analyzed categorically. Categories were based on data spread; four groups for age in years (0–4, 5–17, 18–30 and ≥ 31) and four groups for household size (1–5, 6–10, 11–15 and >15 people). Exposure was defined as whether the individual had direct, indirect or inconclusive zoonotic exposure to a source of AI H5N1 virus. Direct zoonotic 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 zoonotic exposure referred to cases where poultry deaths were reported in the neighborhood, cases where healthy poultry were present in the neighborhood and cases who visited live bird markets. Inconclusive zoonotic exposure refers to cases where no zoonotic source of infection could be found despite investigation.

Transmission Model

To assess the potential for human transmission of the virus, we used final size household models to fit the human and zoonotic transmission parameters to outbreak data (household size, number of cases, blood-related household members to index case) in a manner similar to that described in van Boven *et al.* [29]. This approach allows for both human and zoonotic transmission, and enables comparison of different transmission assumptions. We used Akaike Information Criterion adjusted for small sample size (AIC_C) to select the most appropriate models. The AIC_C percent support gives the probability that the model is the best model of those considered, but does not indicate how well the suite of models fit the data [30]. We used a simulation-based approach to compare the data with each of the model predictions, which allowed us to identify those models that differed significantly ($P<0.05$) from the data. Matlab (version R2010b) was used for this analysis. Our preliminary analysis indicated that density-dependent transmission [31] gave a better fit to the data than frequency-dependent transmission [29], and that assumptions concerning the distribution of the infectious period did not affect our results. Thus, our detailed analysis used a model with a fixed infectious period and density-dependent transmission. Under these assumptions, outbreak sizes will vary according to the exposed population, and we present results for an exposed population of size five (the median household size in the data).

Our estimation methods calculated the best-fit parameters to cluster data consisting of the number of exposed individuals, the number of index cases and the final outbreak size. In our initial analysis, we used all individuals exposed for a period of four hours or more in the household as the exposed population. In light of evidence concerning transmission of H5N1 to blood-related contacts [1,3], we also considered an alternative analysis in which the exposed population was restricted to all blood relatives exposed for a period of four hours or more in the household. Finally, we tested the sensitivity of our results to the inclusion of those households for which contact data were missing, by including the missing households into the data, assuming that they had 5 household members (the median household size in the data) and 4 blood relative contacts (again, the median in the data).

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Author Contributions

Conceived and designed the experiments: TYA GS KG KL PMK INK. Performed the experiments: GS RK WP M HS AB. Analyzed the data: GS AM ES VS KG. Contributed reagents/materials/analysis tools: HS VS ODS. Wrote the paper: GS TYA KG KL PMK.

Chapter 6

Paper 3: Chicken faeces garden fertilizer: possible source of human avian influenza H5N1 infection.

About this chapter

Chapters 4 and 5 explored the epidemiology of AI H5N1 in Indonesia based on the outbreak investigation reports and surveillance dataset at MOH. As part of the data used to explore the disease epidemiology, this chapter describes one specific cluster from the surveillance dataset. The cluster was detected in Indonesia in 2005 and comprises two cases: a 37 year old female and a nine year old male. The cluster is presented as a case report.

This case report is of importance as it was the first report globally to explore poultry faeces in garden fertilizer as a potential source for AI H5N1 human infection. The epidemiological investigation and supporting laboratory findings are presented. Even though the virological evidence does not provide conclusive evidence that the isolate from the H5N1-contaminated garden fertilizer was the source of the index case's infection, it does highlight the importance of environmental investigation and laboratory testing to identify putative sources of infection for this emerging infectious disease.

I participated in the investigation and data collection for the outbreak reported in this chapter. In addition to collecting and analyzing the epidemiological data, I also coordinated with the virologists nationally and at a WHO Collaborating Centre for the virological analyses. I wrote the paper and obtained feedback from all the co-authors pre-publication. The published study has been reproduced here with permission from the publisher, John Wiley and Sons.

ORIGINAL ARTICLE

Chicken Faeces Garden Fertilizer: Possible Source of Human Avian Influenza H5N1 Infection

I. N. Kandun¹, G. Samaan^{2,3}, S. Harun⁴, W. H. Purba¹, E. Sariwati¹, C. Septiawati¹, M. Silitonga², N. P. I. Dharmayanti⁵, P. M. Kelly³ and T. Wandra¹

¹ Directorate General Disease Control & Environmental Health, Ministry of Health, Jakarta, Indonesia

² World Health Organization, Jakarta, Indonesia

³ National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australia

⁴ National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia

⁵ Indonesian Research Center for Veterinary Science, Jakarta, Indonesia

Impacts

- Avian influenza A H5N1 infection in humans is generally associated with close contact with infected birds.
- This paper points to poultry faeces used in garden fertilizer as a potential source for human infection with avian influenza A H5N1 virus.
- Public education is needed to ensure safe handling of poultry faeces, especially in areas affected avian influenza A H5N1.

Keywords:

Avian influenza H5N1; exposure; Indonesia, chicken; fertilizer; transmission

Correspondence:

Toni Wandra. Ministry of Health, Indonesia, Jalan Percetakan Negara No. 29, Salemba, Jakarta Pusat 10560, Indonesia.
Tel.: +62-21-4201255; Fax: 62-21-4201255;
E-mail: tony_wdr2009@yahoo.com

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Summary

Avian influenza H5N1 infection in humans is typically associated with close contact with infected poultry or other infected avian species. We report on human cases of H5N1 infection in Indonesia where exposure to H5N1-infected animals could not be established, but where the investigation found chicken faeces contaminated with viable H5N1 virus in the garden fertilizer. Human cases of avian influenza H5N1 warrant extensive investigations to determine likely sources of illness and to minimize risk to others. Authorities should regulate the sale and transportation of chicken faeces as fertilizer from areas where H5N1 outbreaks are reported.

Introduction

Indonesia detected its first human case of avian influenza H5N1 in June 2005; nearly 2 years after the disease was detected in the local poultry population (World Health Organization, 2005). By end of June 2008, Indonesia had confirmed 135 human cases of H5N1, of which 110 were fatal (case fatality rate = 81%) (World Health Organization, 2008).

Each human case of H5N1 in Indonesia is thoroughly investigated by an epidemiological team to determine the likely source of infection and to determine whether the outbreak led to further human cases. This case report describes a confirmed human case where the investigation found that chicken faeces used as garden fertilizer was contaminated with viable H5N1 virus. We hope that this report will prompt investigators worldwide to consider

this as a possible source of infection for H5N1 human cases and to incorporate testing of poultry products into their investigations.

Case Descriptions

A 37-year-old female from South Jakarta in the western part of Java developed fever and sore throat on 31 August 2005. She consulted a local medical clinic 3 days later because of persistent symptoms. By 6 September, she had also developed cough, shortness of breath and difficulty breathing and was admitted to a private hospital. Based on the clinical presentation, the hospital suspected influenza A H5N1 infection as a differential diagnosis. Throat and nasal swabs, as well as blood samples, were collected from the patient on 6 and 9 September. She developed respiratory distress and died on 10 September 2005.

Samples tested positive for H5N1 by RT-PCR on 11 September at the National Institutes of Health, Research and Development, and the results were confirmed on 16 September by two different WHO H5 reference laboratories (World Health Organization, 2006). A virus was isolated and sequenced, where it was found to be of a purely avian source.

A 9-year-old male, a nephew and blood-relative of the 37-year-old patient, developed headache and fever (40°C) on 4 September; 4 days after the onset of symptoms for the 37-year-old patient. A mild cough began 2 days later. He presented to a doctor on days 6 and 7 of illness due to his persistent symptoms. On 10 September, he was prescribed symptomatic treatments and antibiotics, but no antiviral drugs, as doctors did not suspect H5N1 infection. He did not develop shortness of breath or disabling symptoms. His white cell and platelet counts were 8800 cells/mm³ and 130 000 cells/mm³ respectively on 10 September, and 7100 cells/mm³ and 270 000 cells/mm³ respectively on 11 September.

As part of the contact tracing conducted for the 37-year-old index patient, sera and throat/nasal swabs were collected from the boy on 13 September. As the boy was mildly ill and had contact with a confirmed case of avian influenza, he was referred to hospital to be managed as a suspected H5N1 case. He was administered oseltamivir and remained under observation in the hospital even though his symptoms had subsided. Throat swabs collected on 17 September and 20 September from the boy tested positive by RT-PCR for H5N1, but a virus could not be isolated for sequencing. Samples collected after 20 September were found to be negative for the virus. Acute and convalescent sera were collected from the patient on 13 September and 7 October 2005, respectively. A micro-neutralization titre of 1 : 20 was observed. He survived and was discharged on 26 September.

Epidemiological Investigation

Family members were interviewed to assess exposure history, to cross-check timelines and to determine the mode of disease transmission for the cases, and serum samples were collected from relatives to assess serological evidence of H5N1 infection (below).

The index patient (37-year-old female) managed the family's small printing business. The husband did not identify any occasions in the 2 weeks prior to the onset date where the patient slaughtered chicken or came into direct contact with sick animals. She also had not travelled to areas known to be infected with avian influenza nor was she in close contact with a person with influenza-like illness or acute respiratory illness. The husband reported that the patient never visited wet markets,

preferring to purchase eggs and chicken pieces from the food stalls near her workplace and often purchased ready-to-eat fried chicken. The patient was known to be a keen gardener, and she used to purchase garden fertilizer (chicken faeces) in sealed bags from a gardening shop to use for her potted plants. She did not wear gloves or a mask whilst gardening.

The home and neighbourhood of the index patient were thoroughly inspected to assess the possible source of infection. The 37-year-old patient only kept pet fish and regularly purchased worms for them from a local pet shop. The pet shop owner reported no bird deaths in the 2 weeks preceding the index patient's onset of illness. A bag of chicken faeces garden fertilizer was found at the patient's home. The husband stated that the bag was purchased before the patient's onset, but the exact dates of purchase or manufacture were unknown. The label on the bag indicated that the fertilizer came from a company in East Java (over 1000 km away). Through trace-back, we found out that the company purchased chicken faeces from a variety of collectors for inclusion in their product, and that the collectors sourced the faeces from numerous farms, as far as hundreds of kilometres away. Further trace back was deemed unreliable and was not conducted.

There were many caged birds, chickens and some swans in the index patient's neighbourhood, but no reported deaths. There was also a backyard poultry slaughterhouse approximately 50 metres away from her home. The slaughterhouse was not located on the same road as the patient's home, and could only be reached by entering small side lanes. The slaughterhouse did not keep cages or chicken flocks but received approximately 150 chickens from a neighbouring district to slaughter on a daily basis. Most slaughtered chickens were sold to the wet market 2 km from the patient's home. It is unlikely that the patient had contact with these chickens as they generally arrived and were slaughtered before dawn to be available at the markets by sunrise. The patient did not purchase chicken meat from this slaughterhouse nor the wet market.

The 9-year-old nephew did not report any contact with chicken or birds in the fortnight preceding his illness. Yet, he visited the 37-year-old index-patient in her home 2 days into her illness (1 September). They met again at a large family gathering the next day.

Laboratory Investigation

Serum samples were collected from 119 contacts traced during the investigation to assess for H5N1 seroconversion. Samples were collected from 49 family members, 26 neighbours, 41 healthcare workers and 3 gravediggers. All

samples were collected at least 2 weeks after the index patient's illness onset. The samples were tested by micro-neutralization assay, where none was positive for H5N1 antibodies. Nasal and throat swabs were collected from two individuals who reported recent history of influenza-like illness. None of these contacts was found to be infected with H5N1. All virus isolation, RT-PCR and microneutralization tests were carried out as described previously (Kandun et al., 2006).

Through the course of the investigation, 99 animal and environmental samples were collected from the 37-year-old patient's home, relatives' homes, nearby slaughterhouse and wet market. All of the samples were collected 17 days after the index patient's onset of illness. This included animal samples (chickens, caged birds, swans) and environmental swabs (garden fertilizer from the patient's home and water from the processing tubs at the slaughterhouse). All of the animals sampled appeared healthy at the time of specimen collection. To process environmental samples, 5 g of specimen was dissolved in virus transport medium. The suspension was then centrifuged and the supernatant was inoculated into 9- to 11-old-day specific-pathogen-free embryonated eggs for isolating virus. From the animal and environmental samples, one chicken sampled at a wet market 2 km away from the index patient's home was H5N1 positive by RT-PCR, but the virus could not be isolated for sequencing. The chicken faeces (garden fertilizer) collected from the index patient's home also tested H5N1 positive by RT-PCR. All other samples, including samples from the slaughterhouse, were negative.

For both, patient and fertilizer isolates, RNA extraction, cDNA synthesis and PCR were used as described previously (Guan et al., 2000). Sequencing was performed with the BigDye Terminator v3.1 cycle sequencing kit on an ABI PRISM 3700 DNA analyzer (Applied Biosystems) by following the manufacturer's instructions. Sequence fragments were assembled with Lasergene (version 6.0; DNASTAR) and then aligned by using BioEdit, version 7, and residue analysis was performed with BioEdit, version 7. Phylogenetic trees were generated by neighbour-joining bootstrap analysis (1,000 replicates) by using the Tamura-Nei algorithm in MEGA, version 2.1. The percent nucleotide homology of HA genes of A/Indonesia/6/05 versus A/Indonesia/Environment/05 was calculated using MegAlign v8.0.2 (DNASTAR) and found to be 97.5% (Fig. 1).

Discussion

From the epidemiological aspects of this investigation, we found that the patient had direct and unprotected contact with the H5N1-contaminated fertilizer. Our results also confirmed the prolonged (>2 weeks) environmental

stability of H5N1 virus in bags of chicken faeces garden fertilizer. However, from the virological aspects of this investigation, we could not conclude that the isolate from the H5N1-contaminated garden fertilizer was the source of infection for the index patient.

The phylogenetic tree highlighted that the virus isolated from the index patient was most similar to viruses isolated in the eastern parts of Java, the location of the fertilizer manufacturing company, as opposed to isolates located in the western parts of Java where the index patient resided. It is possible that the fertilizer bag which likely contained poultry faeces from many birds harboured multiple strains of H5N1 viruses, one of which led to her infection. The one we isolated from the fertilizer bag represented a different isolate. Based on information from the fertilizer manufacturer, it is plausible that the same batch of fertilizer may have been contaminated with different strains of H5N1 virus as faeces were sourced from various geographical areas in Java. We also cannot exclude the possibility that the patient was infected from other sources, such as the nearby slaughterhouse or wet market, due to their geographical proximity to the case. However, the interview with the patient's husband suggested that she did not visit these places.

The finding of prolonged environmental stability of H5N1 virus in chicken faeces in this observational study is similar to observations made during the 1983–1985 Pennsylvania outbreak of avian influenza A H5N2, where viable virus was detectable up to 6 weeks in wet faeces (Benedictis et al., 2007). However, our finding contrasts with some studies, where avian influenza viruses in faeces were inactivated within a week at 25–32°C (Beard et al., 1984; Lu et al., 1984; Songserm et al., 2006). These studies suggest that sunlight and viral inactivating factors in the organic material of faeces contribute to more rapid inactivation of the virus. As suggested by Benedicts et al., survival of avian influenza viruses in faeces is likely to be influenced by a number of factors, including the virus strain, temperature, amount of organic material and the host animal type. Further systematic studies need to be conducted to assess factors that explain the conflicting findings.

The source of the 9-year-old male's infection could not be ascertained. Nevertheless, he was considered epidemiologically-linked to the index patient. This is based on the disease timeline, his exposure to the same contaminated environment and close contact with the 37-year-old during her initial phase of illness. A number of hypotheses were considered to determine the source of the 9-year-old patient's infection. Exposure to a discrete source of infection at his home or at another location was possible. However, based on inspection of his home, the surrounding

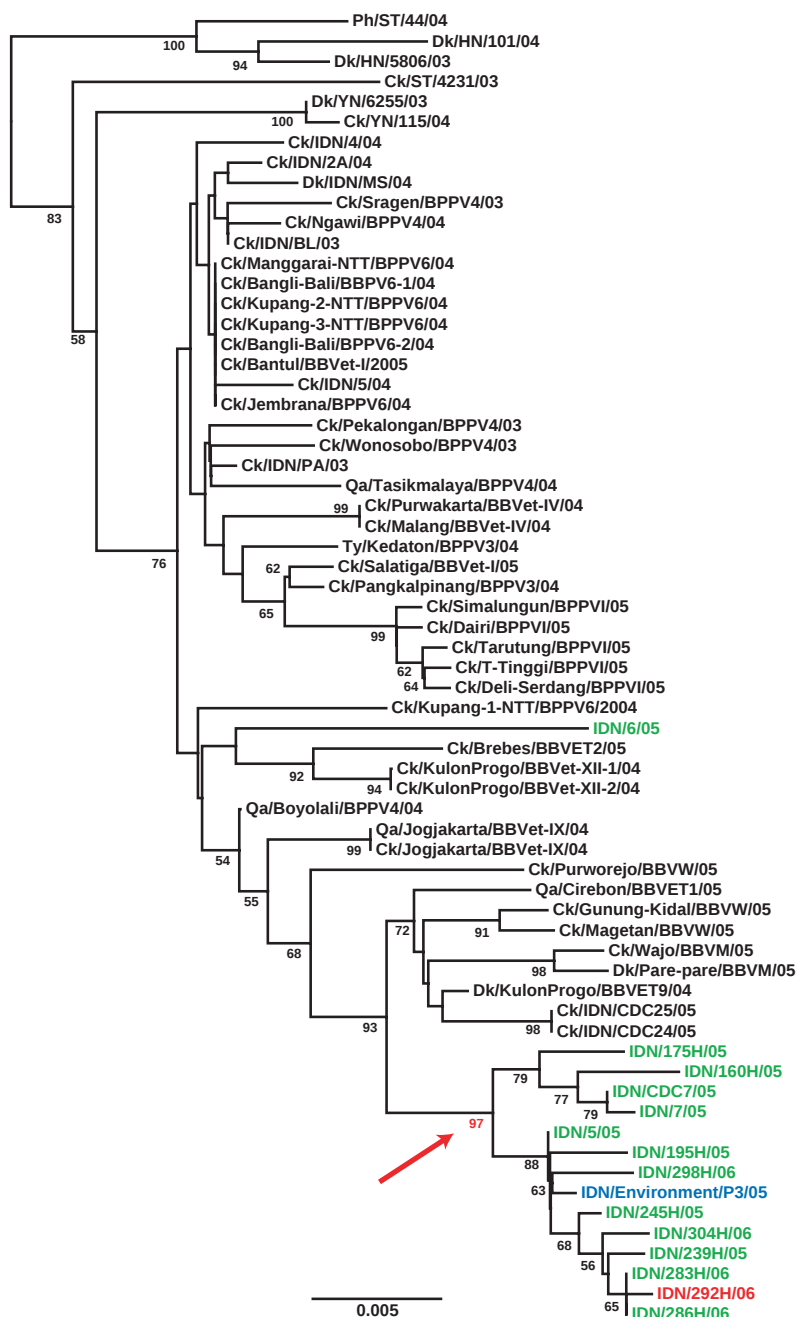


Fig. 1. Phylogenetic tree of the haemagglutinin of the H5N1 viruses isolated from the 37-year-old index patient (IDN/6/05) and the fertilizer sample found at her home (IDN/ENVIRONMENT/P3/05). Trees were generated with MEGA2 by using a Tamura-Nei (gamma) neighbour-joining analysis. The numbers at the nodes indicate bootstrap values from 1000 bootstrap replicates. Accession numbers for patient isolate and fertilizer isolate are EU146624 and EU146642, respectively.

neighbourhood and a general assessment of his activities during the 2 weeks preceding the onset of illness, no independent environmental risk factor for infection could be identified. The second hypothesis was that he acquired infection from a continuing point source at the index patient's home during his visit on 1 September. Details of his activities during that visit, including whether he handled the fertilizer or helped his aunt in gardening,

could not be elucidated. The third hypothesis was that the patient acquired the infection directly from the 37-year-old patient either on 1 or 2 September. It is not possible to either support or discard either the second or third hypotheses, as the patient was exposed to both risk factors in a similar time period, and both are compatible with the incubation period for his illness. Limited human to human transmission could not be excluded in this cluster; how-

ever no further cases were identified in the course of the investigation.

Even though the 9-year-old had clinical symptoms, and throat swabs collected up to day 17 of his illness were positive for H5 by RT-PCR, he did not mount a 4-fold rise in antibody titre. A potential reason for the lack of antibody response is the administration of Oseltamivir during his course of illness, however, this finding warrants further investigation and comparison to other surviving cases of avian influenza H5N1 infection.

Our study highlights the importance of timely and comprehensive environmental investigations to identify the likely source of infection for H5N1 cases. Health authorities need to devise sensitive criteria to detect suspect human H5N1 cases, have adequate resources to investigate them in a timely fashion and have functional coordination with the agriculture authorities to share and analyse the microbiological and environmental data. This is especially important when direct contact with infected animals or humans cannot be established, and where environmental contamination appears to be a likely source of infection; perhaps even a continuing source of infection. This study suggests that avian influenza H5N1 may be acquired from contaminated fertilizer or soil, similar to other pathogens like *Legionella longbeachae*, *Chlamydia psittaci* and *Histoplasma capsulatum* (Heymann, 2004).

Further research is needed to learn more about the modes of H5N1 transmission, but our findings suggest that untreated poultry faeces as fertilizer is a possible source of infection. Education efforts should include public education on avoiding direct contact with items that contain poultry sources like fertilizers, and for healthcare providers to recognize possible sources of infection for diagnosis. This is especially important in areas where the virus is endemic and where access to diagnostic facilities is limited. Figure 2 highlights some steps that can be taken to prevent transmission of the virus from contaminated fertilizer to humans. In addition, control measures should be in place to detect such contamination and reduce future spread.

The limitations of this study are common to epidemiological investigations for high case-fatality rate diseases: the lack of primary source data from the case(s) means that definite sources of contact cannot be elucidated. Samples from the fertilizer producer should have been tested for possible viruses that were similar to the cases, but this was not possible, given the lack of batch number on the fertilizer.

We have shown that poultry products such as fertilizer need to be assessed in the course of an investigation, as a possible source of transmission for H5N1. Public and healthcare worker education should warn of the risks in coming into unprotected contact with such products.

1. Authorities should regulate the sale and transportation of chicken feces as fertilizer from areas where H5N1 outbreaks are reported.
2. Chicken feces should be treated before sale for use as fertilizer to inactivate viruses.
3. Promote hand-hygiene and the use of masks for high-risk groups such as gardeners and farm workers handling soil, animal excreta or fertilizer.
4. Encourage primary health-care workers to investigate whether patients presenting with community-acquired pneumonia had contact with soil or fertilizer in the two weeks prior to their illness.
5. Educate the public about dust suppression using water saturation in gardening to prevent the aerosolization of infectious organisms.
6. Educate the public about the early signs and symptoms of avian influenza H5N1 to facilitate early health-seeking behaviour and diagnosis.

Fig. 2. Steps to prevent transmission of H5N1 virus from contaminated fertilizer to humans in H5N1 endemic areas (Smith et al., 2005; Food and Agriculture Organization of the United Nations, 2006).

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Conflicts of Interest

None of the authors has a commercial or other association that might pose a conflict of interest.

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Chapter 7

Paper 4: Environmental sampling for avian influenza virus

A (H5N1) in live-bird markets, Indonesia

About this chapter

Chapters 4 to 6 explored the epidemiology of AI H5N1 in Indonesia based on the outbreak investigation reports and surveillance dataset for 2005-2009 at MOH. One of the main findings of those chapters was that most human cases of AI H5N1 infection resulted from zoonotic, including environmental, transmission of the virus. This highlights the importance of addressing the disease in the birds and the environment that can be contaminated with the virus.

One focus for AI H5N1 disease activity is the LBM setting, where live birds come into the market, are slaughtered and sold to consumers. As was seen in Chapter 4, LBM exposure was the putative source of infection for seven human cases. LBMs are considered a site of disease activity at the interface between birds and humans, thus have the potential for maintaining circulation of virus between birds as well as leading to human infections. Thus, the subsequent chapters in this PhD examine the epidemiology and disease control methods in the LBM setting.

The study reported in Chapter 7 identified environmental sites commonly contaminated by AI H5N1 virus and the risk factors for this contamination. The environmental sites most commonly contaminated are those in the slaughter and subsequent zones in LBMs. Slaughtering birds in LBMs was a risk factor for contamination, whilst daily solid waste removal and clear zoning between work processes such as holding, slaughtering and selling birds were protective.

My role in the study included designing the study, collecting and analysing the data. I was also responsible for writing the manuscript and incorporating co-author feedback. The published study has been reproduced in this chapter with permission from Emerging Infectious Diseases journal.

Environmental Sampling for Avian Influenza Virus A (H5N1) in Live-Bird Markets, Indonesia

Risa Indriani,¹ Gina Samaan,¹ Anita Gultom, Leo Loth, Sri Indryani, Rma Adjid, Ni Luh Putu Indi Dharmayanti, John Weaver, Elizabeth Mumford, Kamalini Lokuge, Paul M. Kelly, and Darminto

To identify environmental sites commonly contaminated by avian influenza virus A (H5N1) in live-bird markets in Indonesia, we investigated 83 markets in 3 provinces in Indonesia. At each market, samples were collected from up to 27 poultry-related sites to assess the extent of contamination. Samples were tested by using real-time reverse transcription-PCR and virus isolation. A questionnaire was used to ascertain types of birds in the market, general infrastructure, and work practices. Thirty-nine (47%) markets showed contamination with avian influenza virus in ≥ 1 of the sites sampled. Risk factors were slaughtering birds in the market and being located in West Java province. Protective factors included daily removal of waste and zoning that segregated poultry-related work flow areas. These results can aid in the design of evidence-based programs concerning environmental sanitation, food safety, and surveillance to reduce the risk for avian influenza virus A (H5N1) transmission in live-bird markets.

Food markets that offer both poultry meat and live birds either for sale or for slaughter are collectively referred to as live-bird markets (LBMs). LBMs are part of the supply chain and are essential for maintaining the health and nutritional status of rural and urban populations, especially in developing countries (1,2). However, LBMs provide op-

timal conditions for the zoonotic transfer and evolution of infectious disease pathogens because they provide major contact points between humans and live animals (3,4).

Studies in Hong Kong Special Administrative Region, People's Republic of China; other areas of China; Indonesia; and the United States have shown that LBMs can harbor avian influenza viruses (AIVs), including highly pathogenic influenza virus A (H5N1), and have been associated with human infection (4–9). Continual movement of birds into, through, and out of markets provides opportunity for the introduction, entrenchment, and dissemination of AIVs. Most studies have focused on testing live birds rather than environmental sites in the LBMs (6,7,10). However, a study in New York, NY, that tested environmental sites for AIV (H7N2) found that virus could be isolated from samples from floors, walls, and drains from the poultry areas of LBMs (8). The study also found that despite the ongoing influx of infected birds into LBMs, the level of environmental contamination decreased with routine cleaning and disinfection. Another study in Hong Kong LBMs showed that AIV (H9N2) could be isolated at higher rates from poultry drinking water than from samples of bird fecal droppings (11). Environmental aspects of LBMs are needed for an avian influenza control program for 2 reasons. First, a contaminated environment can provide a continuing source of virus transmission, in which healthy birds coming into the market may become infected and persons working in or visiting the market may also be exposed. Second, ongoing surveillance programs in LBMs based on environmental sampling are more likely than those based on invasive bird testing to be acceptable to traders and stall vendors. Environmental sampling is also safer for public health officers and veterinary health officers than handling and sampling live birds that may be infected with AIV.

¹These authors contributed equally to this article.

Author affiliations: Indonesian Research Center for Veterinary Science, Bogor, Indonesia (R. Indriani, R. Adjid, N.L.P.I. Dharmayanti, Darminto); The Australian National University, Canberra, Australian Capital Territory, Australia (G. Samaan, K. Lokuge, P.M. Kelly); World Health Organization, Jakarta, Indonesia (G. Samaan); Ministry of Health, Jakarta (A. Gultom, S. Indryani); Food and Agriculture Organization, Dhaka, Bangladesh (L. Loth); Food and Agriculture Organization, Hanoi, Vietnam (J. Weaver); and World Health Organization, Geneva, Switzerland (E. Mumford)

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In this study, we aimed to identify the environmental sites commonly contaminated by AIV (H5N1) in LBMs in Indonesia. Identifying these sites is the first step in the design of evidence-based environmental sanitation, food safety, and surveillance programs to reduce the risk for virus transmission and to develop environmental surveillance programs to monitor LBM contamination status.

Methods

Three provinces in the western part of Java Island in Indonesia participated in the study: Jakarta, Banten, and West Java (Figure). Eighteen districts in these provinces were selected on the basis of their proximity to the laboratory, high levels of avian influenza activity in farmed birds (Ministry of Agriculture, unpub. data), and high number of LBMs available for study ($n = 300$). The required sample size was 73 markets based on an estimated disease prevalence of 50% and a maximum error of 10% at 95% confidence. We based our assumption that 50% of LBMs would be contaminated with AIV (H5N1) on results from a previous study in US LBMs in 2001 (12). This study found that 60% of markets tested positive for AIV (H7N2) virus in areas in which the virus was endemic. To account for nonresponse, we increased the total sample size to 83 LBMs. We selected markets for inclusion in the study using systematic sampling. On the basis of a sampling frame of 300 markets, every fourth market (the sampling interval) was selected from a list of all the markets. A random numbers table was used to determine the starting point for selection of the 83 markets from the list. Diagnostic specimens and data were collected during October 2007–March 2008. These months have high rainfall and high AIV transmission according to data gathered during 2005–2007 about AIV (H5N1) outbreaks in farmed birds (Ministry of Agriculture, unpub. data).

A structured questionnaire containing 42 questions to assess risk factors for AIV (H5N1) contamination was developed. Responses to questions were obtained through visual inspection of each LBM and through an interview with the manager of the participating LBM. The questions sought information about volume of poultry in the LBM and the infrastructure in the delivery, holding, slaughter, sale, and waste-disposal zones of the market. These 5 zones reflect general demarcation of work flow and activities relating to poultry in LBMs (13). Questions about the sanitation and slaughtering practices were also included.

Questionnaire validation was conducted by members of a study advisory team. The team comprised 2 food safety/environmental health officers from the Ministry of Health, a communicable disease epidemiologist from the World Health Organization, a veterinary epidemiologist from the Food and Agriculture Organization, and 2 virologists from the Ministry of Agriculture in Indonesia. The questionnaire was tested in 3 LBMs in West Java province

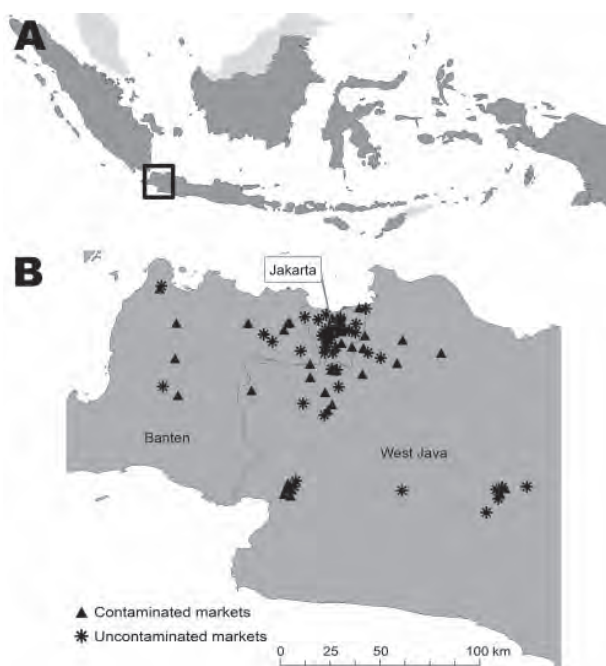


Figure. A) Area of study of avian influenza virus A (H5N1) contamination in live-bird markets (black box), western Java, Indonesia, 2007–2008. B) Distribution of contaminated and uncontaminated markets in the study area.

to ensure coherence, appropriate use of terminology, and high face validity. The same markets were also inspected to ensure that the questionnaire addressed all aspects of the poultry-related work flow in the 5 poultry zones and relevant infrastructure. Members of the study advisory team trained 3 study data collection teams in questionnaire administration and sample collection procedures.

To select the environmental sites to be sampled in each LBM, the study advisory team visually inspected 3 markets and reviewed the literature to identify LBM sites commonly contaminated with AIVs or similar pathogens. Sites sampled in previous studies for AIV included floors, drains, and water troughs (8,11,12). In this study, 27 sites were selected for environmental sampling (Table 1). The sites represented different poultry-related work activities: 3 sites related to delivery of birds into LBMs, 7 in the bird-holding zone, 9 in the slaughter zone, 6 in the sale zone, and 2 in the waste-disposal zone. Because of variation in LBM infrastructure and processes, each LBM did not necessarily have all 27 sites. Samples were collected from as many of the 27 sites as were available in each LBM.

For each of the 27 sites, 6 swab specimens were collected and pooled. Each pool (vial) consisted of a maximum of 3 swabs. The data collection teams were instructed to increase the representativeness of the samples by swabbing different locations for each environmental site. For

Table 1. Environmental sites in LBMs contaminated by influenza virus A (H5N1) as detected by RT-PCR and virus isolation, Indonesia, 2007–2008*

Poultry zone	Site no.	Environmental site	RT-PCR–positive/markets tested (%), N = 1,862	VI–positive/RT-PCR positive, n = 280	LBMs positive for zone
Delivery	1	Inside cages on truck	6/45 (13.3)	1/6	11
	2	Floor in delivery area	6/49 (12.2)	0/6	
	3	Water run-off in delivery area	4/38 (10.5)	0/4	
Holding	4	Poultry cage floors	6/79 (7.6)	0/6	24
	5	Holding area floor	8/80 (10)	1/8	
	6	Water run-off	11/72 (15.3)	0/11	
	7	Poultry feeding bottle water	8/67 (11.9)	0/8	
	8	Poultry feeding basket food	6/72 (8.3)	0/6	
	9	Handles to poultry cages	9/79 (11.4)	0/9	
	10	Inside of waste bins	10/59 (16.9)	0/10	
Slaughter	11	Handles of knives used for slaughtering	8/75 (10.7)	1/8	29
	12	Basket holding dying chickens	8/71 (11.3)	2/8	
	13	Floor in slaughter area	10/77 (13)	0/10	
	14	Chopping or slaughtering board	14/71 (19.7)	2/14	
	15	Processing table after de-feathering	15/70 (21.4)	0/15	
	16	Baskets holding poultry meat	14/70 (20)	1/14	
	17	Drain path	12/75 (16)	0/12	
	18	Tap handles in slaughter area	7/65 (10.8)	0/7	
	19	Waste bin	13/71 (18.3)	1/13	
Sale	20	Chopping boards	15/80 (18.8)	1/15	30
	21	Scales	12/57 (21.1)	0/12	
	22	Knife handles	12/78 (15.4)	1/12	
	23	Waste bins	10/60 (16.7)	1/10	
	24	Wet cloths for cleaning surfaces	14/78 (17.9)	0/14	
	25	Tables for poultry display	19/80 (23.8)	0/19	
Waste disposal	26	Area waste-disposal bin	15/78 (19.2)	1/15	9
	27	Wet cleaning mops	8/66 (12.1)	0/8	
Total positive			280 (15)	13 (4.6)	

*LBM, live-bird market; RT-PCR, reverse transcription–PCR; VI, virus isolation.

example, if the market had 6 poultry stalls, each with its own scale for weighing poultry, then teams collected 1 swab from each scale and pooled them into 2 pools of 3 swabs each. Swab specimens were pooled in the market, and swabs remained inside the vials until testing. The data collection teams were instructed to focus on visibly dirty, moist, or difficult-to-clean surfaces in an effort to increase the sensitivity of the sampling.

Sample collection, pooling, transportation, and storage were based on techniques used in previous studies (10,12). Each data collection team comprised 3 persons, 2 of whom collected samples and 1 administered the questionnaire. To reduce the risk for cross-contamination during sample collection, teams changed disposable gloves and shoe covers between each of the 5 LBM poultry zones. Sterile cotton-tipped swabs were used to collect all samples, and samples were placed in viral transport media and transported immediately back to the laboratory on frozen gel packs. The viral transport media consisted of Dulbecco modified Eagle medium (Sigma-Aldrich, St. Louis, MO, USA) with 1,000 IU penicillin and gentamicin, and 1% fetal buffer serum (14). Samples were stored in the laboratory at -70°C until tested.

RNA extraction, cDNA synthesis, and real-time reverse transcription–PCR (RT-PCR) were used as described (15). Virus isolation methods have also been described (16) but in general involved supernatants from a 1,000- μL sample homogenized by vortex and centrifuged at 2,500–3,000 rpm into 9- to 10-day-old specific pathogen-free eggs. Those positive in the hemagglutination assay were tested by hemagglutination-inhibition test with reference antiserum (A/chicken/West Java/Hamd/2006).

The degree of association between AIV (H5N1) positivity in the 5 LBM poultry zones was determined by using Spearman rank correlation. To assess risk factors for environmental virus (H5N1) contamination, we estimated odds ratios (ORs) using multivariable logistic regression analyses, where variables with $p \leq 0.1$ from the univariate analyses were included in the initial model. A backward stepwise variable-selection strategy was used to construct a final model with a significance level of $p < 0.05$. The Hosmer and Lemeshow test and the residual χ^2 goodness-of-fit test were used to assess model stability. Microsoft Excel (Microsoft, Redmond, WA, USA), Epi Info (Centers for Disease Control and Prevention, Atlanta, GA, USA), and

Stata version 10.0 (StataCorp, College Station, TX, USA) were used for the descriptive and statistical analyses.

Approval for the study was obtained from the Health Research Ethics Committee at the Indonesian Ministry of Health and the Australian National University Human Research Ethics Committee. Permission was obtained from LBM managers before participation in the study.

Results

LBM Demographics and Practices

All 83 LBMs selected participated in the study; 62 (75%) were located in urban and 21 in rural areas. LBMs were from 16 districts in 3 provinces: 31 (38%) from Jakarta province, 11 (13%) from Banten province, and 41 (49%) from West Java province (Figure). Most (49 [59%]) LBMs were retail markets, 10 (12%) were wholesale only, and 24 (29%) were a combination of retail and wholesale. Most (82 [99%]) LBMs operated daily, with the same vendors operating in the same stalls.

Most LBMs received their poultry from commercial farms (71 [86%]), and some also sourced poultry from small-scale holders (36 [43%]). Most (42 [51%]) LBMs had medium-sized poultry areas (11–50 poultry cages), and 21 (25%) had large poultry areas (>50 cages). LBMs had village free-ranging chickens (69 [83%]), fighting cocks (13 [16%]), broilers (67 [81%]), spent hens (24 [29%]), Muscovy ducks (48 [58%]), ducks other than Muscovy (32 [39%]), and pigeons (16 [19%]). Most (71 [86%]) LBMs generally kept live poultry in the market for a few days until sold, housing them overnight in cages.

Forty-eight (58%) LBMs reported monthly or more frequent visits from animal/human health personnel to inspect the poultry zones. Eight (10%) LBMs reported that live birds were tested periodically (less frequently than weekly) for AIV infection. For cleaning and sanitation, 80 (96%) LBMs reported washing poultry zones daily, and 55 (66%) applied detergent or disinfectant daily.

Laboratory Findings

Thirty-nine (47%) LBMs had evidence of contamination. For 17 (44%) of these, ≤ 5 environmental sites were positive for AIV (H5N1) by real-time RT-PCR. For each of 22 (56%) LBMs, ≥ 6 environmental sites were positive.

The environmental sites most heavily contaminated were in the slaughter and sale zones (Table 1). In the slaughter zone, the most contaminated sites were the poultry-processing tables (21%), baskets holding poultry meat (20%), and chopping boards (20%). In the sale zone, the most contaminated sites were the tables for carcass display (24%) and scales (21%). Another commonly contaminated site was the waste-disposal bin in the waste-disposal zone (19%). In most cases, this bin is not an enclosed bin but

rather was a dedicated uncovered floor space where remnants are dumped daily and collected weekly by the local government rubbish collection team.

Thirteen viruses were isolated from LBMs, most frequently from the slaughter zone (7 of 13 viruses isolated, Table 1). All isolated viruses came from 6 LBMs, from which 1–4 viruses were isolated per LBM.

From the zones contaminated in each LBM (Table 1), we calculated correlations between different zones. Contamination in preceding LBM poultry zones correlated with contamination in the subsequent zones (Table 2). Correlations were high between holding and slaughter zones, slaughter and sale zones, and sale and waste-disposal zones.

Risk Factors for Contamination

We assessed risk factors for AIV (H5N1) contamination in LBMs. We compared exposures in 39 LBMs with a minimum of 1 contaminated environmental site to 44 LBMs with no contamination. From the univariate analyses, several exposures predicted AIV (H5N1) contamination in LBMs (Table 3). LBMs with wooden tables, Muscovy ducks, or ≥ 200 ducks other than Muscovy were at greater risk for AIV (H5N1) contamination, as were LBMs in West Java province.

Six other exposures approached significance, either as protective factors or as risk factors. LBMs that disposed and removed solid waste daily (OR 0.41, 95% confidence interval [CI] 0.16–1.09); had zoning that clearly segregated poultry delivery, holding, slaughter, sale, and waste-disposal areas (OR 0.28, 95% CI 0.07–1.11); or stacked poultry cages vertically rather than side by side (OR 0.38, 95% CI 0.13–1.10) had less risk for avian influenza virus (H5N1) contamination. LBMs with pigeons (OR 3.06, 95% CI 0.96–9.81), mixed bird species in the same cages (OR 2.92, 95% CI 0.98–8.70), or slaughtered birds in the market (OR 3.53, 95% CI 0.89–13.93) were more likely to be contaminated.

None of the 9 other variables considered in the study were associated with AIV (H5N1) contamination in LBMs (data not shown). These included the LBM trading category (wholesale, retail, or combination), days operational per week, chicken population in LBM, source of chickens (small-scale backyard farmers, commercial farms, or com-

Table 2. Correlation coefficient of influenza virus A (H5N1) positivity between 5 poultry zones in live-bird markets, Indonesia, 2007–2008

Site	Delivery	Holding	Slaughter	Sale	Waste disposal
Delivery	1				
Holding	0.84	1			
Slaughter	0.82	0.89	1		
Sale	0.63	0.84	0.87	1	
Waste disposal	0.50	0.26	0.52	1	1

Table 3. Comparison of exposures in LBMs with AIV (H5N1) environmental contamination and in LBMs with no environmental AI (H5N1) contamination, Indonesia, 2007–2008*

Exposure	No. positive markets, n = 39	No. negative markets, n = 44	OR (95% CI)	p value
No. ducks other than Muscovy in LBM				
<11	8	11	Reference group	
11–100	12	16	1.03 (0.32–3.35)	0.959
101–200	2	2	4.13 (0.16–11.95)	0.773
>200	10	2	6.88 (1.17–40.38)	0.033
Muscovy ducks	28	20	3.05 (1.22–7.63)	0.017
Pigeons	11	5	3.06 (0.96–9.81)	0.059
Clear zoning in LBM	3	10	0.28 (0.07–1.11)	0.072
Wooden tables	23	12	3.83 (1.53–9.62)	0.004
Slaughtering in LBM	36	34	3.53 (0.89–13.93)	0.072
Daily solid waste disposal	24	35	0.41 (0.16–1.09)	0.075
Mixing of species in same cage	13	6	2.92 (0.98–8.70)	0.055
Cages stacked vertically	25	33	0.38 (0.13–1.10)	0.069
Province				
Jakarta	23	8	Reference group	
West Java	25	16	4.49 (1.62–12.46)	0.004
Banten	6	5	3.45 (0.82–14.47)	0.090
Multivariable analysis†				
Clear zoning in LBM			0.16 (0.03–0.86)‡	0.030
Slaughtering in LBM			6.43 (1.01–40.82)‡	0.048
Daily solid waste disposal			0.20 (0.06–0.69)‡	0.010
Province				
Jakarta			Reference group	
West Java			6.83 (2.01–23.19)‡	0.002
Banten			2.94 (0.59–14.69)‡	0.190

*LBM, live-bird market; AI, avian influenza; OR, odds ratio; CI, confidence interval.

†Final model with 4 variables, no. observations = 83, goodness-of-fit tests: residual χ^2 , $p = 0.38$; Hosmer and Lemeshow test, $p = 0.45$.

‡Adjusted OR.

bination), inspection from authorities, use of detergent during cleaning, mixing poultry arriving on different days in the same cages, average length of poultry stay in LBM, and whether poultry were removed from stalls before cleaning.

From the univariate analyses, 10 variables were significant at $p \leq 0.1$. However, the ducks other than Muscovy variable was removed from the multivariate analyses because of its collinearity with another variable (presence of Muscovy ducks, $r > 0.4$). Nine variables were considered for the multivariate analyses. The final multivariable logistic regression model had 4 variables, of which 2 were independent risk factors for subtype H5N1 contamination in LBMs (Table 3). They were location in West Java province (adjusted OR [aOR] 6.83, 95% CI 2.01–23.19) and bird slaughtering in the LBM (aOR 6.43, 95% CI 1.01–40.82). Two variables were independent protective factors: zoning of poultry activities in LBMs (aOR 0.16, 95% CI 0.03–0.86) and daily disposal of solid waste (aOR 0.2, CI 95% 0.06–0.69).

Discussion

We have demonstrated extensive environmental contamination in LBMs with the AIV (H5N1) in Indonesia. Nearly 50% of LBMs in AIV (H5N1)–endemic districts

were positive, with all 5 poultry zones affected. The study identified environmental points of contamination and protective and risk factors for contamination. This study provides baseline information for 2 aspects that can aid in control of AIV (H5N1) in LBMs: 1) development of routine monitoring and surveillance programs and 2) structural interventions and work flow modifications to minimize risk for contamination.

Our findings provide further evidence that environmental contamination with AIVs is not uncommon (8,14). Poultry water, drains, tabletops, cages, tablecloths, utensils, bins, and floors were all contaminated. Environmental sites most commonly contaminated were located in slaughter zones and zones where carcasses were taken after slaughtering, such as the sale and waste-disposal zones. This contamination can be expected because slaughtering generates droplets that may contain viral particles and exposes internal organs with potentially high viral loads. Even if slaughtering is conducted in a separate zone, contamination can spread to the sale and waste-disposal zone through the carcasses and through the process of evisceration usually conducted in both slaughter and sale stalls.

We found rates of contamination in water from poultry feeding bottles similar to those from the study in Hong

Kong on AIV (H9N2) (11% and 7% markets with contamination respectively, $p = 0.12$) (11). Even though AIVs were detected from poultry drinking water, our study suggests that other environmental sites are more efficient for monitoring AIV (H5N1) in markets. Processing tables and baskets holding freshly cut poultry meat in the slaughter area, as well as display tables and scales in the sale area, were positive in 20 (24%) LBMs surveyed.

The risk and protective factors we identified complement findings from previous studies. Daily disposal and removal of waste from the market is part of routine environmental cleaning and sanitation and eliminates AIV reservoirs (8). Segregating poultry-related activities into zones limits virus spread (17). Vertical stacking of cages can limit transmission because trays between layers of birds prevent the scatter of fecal matter. These results add evidence to the World Health Organization current recommendation that waste trays should be used to segregate stacked cages in markets to prevent cross-contamination (13).

LBMs in West Java province had a higher risk for contamination than did other provinces. This risk probably is due to greater AIV (H5N1) disease activity in the province. Surveillance activities during 2006–2008 showed that West Java had a 4.7% outbreak detection rate compared with rates in Banten (4%) and Jakarta (0.2%) (18). Furthermore, in West Java province chicken density is high: 14,000 birds/km² compared with densities in the neighboring provinces Banten and Jakarta (3,900 birds/km² and 400 birds/km², respectively) (19). Poultry density data are commonly used as a proxy for disease activity where areas of high poultry density have the highest risk for an outbreak (20,21).

Several issues need to be considered regarding our finding of low virus isolation rates compared with real-time RT-PCR–positive rates. Virus isolation detects viable virus, whereas real-time RT-PCR detects small stretches of nucleic acid, even if the larger genomic RNA is inactivated. This makes real-time RT-PCR a more sensitive detection tool but does not provide information about virus viability. Samples obtained from the environment may be less suitable than animal samples for virus isolation techniques. Organic matter, duration and temperature of exposure, and humidity can all affect virus survival outside the animal host (22). Three studies conducted in LBMs tested environmental samples and bird samples by using virus isolation (8,10,23). Only 1 of these studies stratified the avian influenza detection rates by type of sample (bird vs. environment) (8); that study found that from 12 LBMs, 11 were positive for avian influenza in bird samples compared with only 5 positive in environmental samples. These results were based on a small sample of LBMs, and real-time RT-PCR was not conducted. Therefore, to determine the suitability of virus isolation for environmental samples,

we recommend that future studies compare real-time RT-PCR–positive rates to virus isolation rates in both environmental swab and bird samples.

Risk and protective factors identified in this study, together with findings from other studies, can assist in developing environmental or behavioral interventions to reduce AIV transmission in LBMs. Previous studies have shown that regular cleaning with detergents, including free chlorine concentrations typically used in drinking water treatment, can rapidly decontaminate surfaces from AIVs (8,24). Previous studies also have shown that periodic market rest days coupled with thorough cleaning can minimize the reservoir of AIV in LBMs (4,12,25). These messages have been disseminated to LBMs throughout Indonesia and formed the basis of the Ministry of Health Decree in 2008 on building healthy food markets (26).

For a more systematic food safety monitoring system, this study will be used to develop a risk-based approach for AIV risk reduction in LBMs in Indonesia (27). The contamination sites and risk factors will be used to determine critical control points and critical limits for intervention. LBM operators, stall vendors, and other stakeholders (e.g., sanitarians and public health officers) will need to be provided with simple monitoring plans to reduce the risk for contamination. Such monitoring plans are expected to have an impact not only on AIV (H5N1) but also on other viruses and bacteria commonly associated with food safety for poultry products.

In addition to tools for disease control, the study findings can aid AIV (H5N1) surveillance activities in LBMs. Commonly contaminated environmental sites in LBMs can form the basis of an environmental sampling strategy for detection of AIV (H5N1) in LBMs. Environmental sampling is more beneficial than live-bird sampling because it is less time and labor intensive and eliminates the need to handle and restrain live birds. Environmental sampling reduces the potential for virus aerosolization and the risk for infection for persons collecting the samples or standing nearby. Further work is needed to assess the adequacy of environmental sampling for surveillance in LBMs under different conditions, especially because detection sensitivity will vary by AIV (H5N1) prevalence in farms supplying the birds.

A limitation of this study is that the observation of environmental contamination was based on a cross-sectional survey in which LBMs were sampled only once. We recommend that future studies observe persistence of the virus over time in the various environmental sites. Reports from market managers and vendors about inspection and cleaning practices in the LBMs were not verified during the course of the study. These activities may have been overreported because respondents may have wanted to report what they perceived interviewers wanted to hear. Be-

cause of the high cost associated with the field and laboratory work for such studies, studies should focus on a small number of markets and collect in-depth information about contamination trends and associated risk factors, as well as data on other indicator organisms, such as *Escherichia coli* or *Enterobacteriaceae*, that provide information about general market hygiene. Future work also should evaluate the effects of interventions in markets especially in low-resource settings because this would be of most benefit to low-income and middle-income countries.

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Dr Indriani is a researcher in veterinary public health at the Indonesian Research Center for Veterinary Science. Her primary research interest is avian influenza.

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Address for correspondence: Gina Samaan, National Centre for Epidemiology and Population Health, College of Medicine, Biology and Environment, The Australian National University, Canberra, ACT, 0200, Australia; email: ginasamaan@yahoo.com

Chapter 8

**Paper 5: Critical control points for avian influenza A H5N1
in live bird markets in low resource settings.**

About this chapter

The epidemiological findings from Chapter 7 were important to inform disease control activities in LBMs in Indonesia. This chapter builds on the findings from Chapter 7 by identifying a set of critical control points to reduce the risk of virus contamination in the LBM environment. Three sources of information were used in this chapter: (a) Chapter 7 findings, (b) Other published literature on avian influenza viruses in LBMs, (c) Flowchart decision tree for critical control point determination. The study found five points in the poultry workflow that qualify as critical control points.

My role in this study was to design the study methodology, collate the data, review the literature, identify the critical control points and write the manuscript. The study was published in Preventive Veterinary Medicine and has been reproduced in this chapter with permission from the publisher, Elsevier.



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Critical control points for avian influenza A H5N1 in live bird markets in low resource settings

Gina Samaan^{a,*}, Anita Gultom^b, Risa Indriani^c, Kamalini Lokuge^a, Paul M. Kelly^a

^a National Centre for Epidemiology and Population Health, College of Medicine, Biology & Environment, The Australian National University, Canberra, ACT, 0200, Australia

^b Level 2, Building D, Directorate-General Disease Control & Environmental Health, Ministry of Health, Jalan Percetakan Negara No. 29, Salemba, Jakarta, Indonesia

^c Indonesian Research Center for Veterinary Science, Agency for Agricultural Research and Development, Ministry of Agriculture, Jalan. RE. Martadinata 30, Bogor, Indonesia

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ABSTRACT

Live bird markets can become contaminated with and become a source of transmission for avian influenza viruses including the highly pathogenic H5N1 strain. Many countries affected by the H5N1-virus have limited resources for programs in environmental health, sanitation and disease control in live bird markets. This study proposes five critical control points (CCPs) to reduce the risk of H5N1-virus contamination in markets in low resource settings. The CCPs were developed based on three surveys conducted in Indonesia: a cross-sectional survey in 119 markets, a knowledge, attitudes and practice survey in 3 markets and a microbiological survey in 83 markets. These surveys assessed poultry workflow, market infrastructure, hygiene and regulatory practices and microbiological contamination with the H5N1-virus. The five CCPs identified were (1) reducing risk of receiving infected birds into the market, (2) reducing the risk of virus spread between different bird flocks in holding cages, (3) reducing surface contamination by isolating slaughter processes from other poultry-related processes, (4) minimizing the potential for contamination during evisceration of carcasses and (5) reducing the risk of surface contamination in the sale zone of the market. To be relevant for low resource settings, the CCPs do not necessitate large infrastructure changes. The CCPs are suited for markets that slaughter poultry and have capacity for daily disposal and removal of solid waste from the market. However, it is envisaged that the CCPs can be adapted for the development of risk-based programs in various settings.

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1. Introduction

In many countries, food markets 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. 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) (World Health Organization, 2006b).

LBMs provide major contact points between people and animals, creating optimal conditions for the zoonotic transfer and evolution of infectious disease pathogens. LBMs are known to amplify and disseminate the highly pathogenic avian influenza A H5N1 (AI H5N1) virus (Kung et al., 2007). Studies have shown that AI H5N1 can be found in birds as well as on work surfaces in LBMs (Desvaux et al., 2006; Santhia et al., 2009; Wang et al., 2006). There is also evidence that humans have been infected with AI H5N1 in LBMs (World Health Organization, 2006a; Zhou et al., 2009). AI H5N1 virus infection in humans is of public health concern; the zoonotic disease has a high case fatality rate, and the virus has pandemic potential if it mutates into

* Corresponding author. Tel.: +62 813 1753 3978.
E-mail address: ginasamaan@yahoo.com (G. Samaan).

a form that transmits rapidly between humans (Kandun et al., 2006; Webster et al., 2005).

Most guidance on AI H5N1 recommends controlling the disease “at source” – that is, in the birds (Food and Agriculture Organization, 2009). Controlling AI H5N1 virus in LBMs is critical to prevent the dissemination of the virus back into farms and backyard bird holdings which can occur through movement of live birds, infective poultry by-products and fomites (Santhia et al., 2009). However, complete elimination of the virus from LBMs is not realistic if the farms supplying the LBMs continue to have outbreaks of AI H5N1 (Mullaney, 2003). Therefore, the goal in LBMs in areas endemic for the virus is to reduce the risk of contamination that may lead to human infection and continued circulation of the virus in birds.

Risk-based programs relate hazards to public health outcomes (Simjee et al., 2007). Risk-based programs have been used to control avian influenza viruses in LBMs (Kung et al., 2003; Mullaney, 2003). LBMs implementing such programs have been in high resource settings where there is good public health regulation and pre-requisite programs (PRPs) for general hygiene. PRPs are practices and conditions needed prior to and during implementation of risk-based programs such as good management, training and equipment (World Health Organization, 1999). In addition to PRPs, risk-based programs usually involve critical control points (CCPs) for managing hazards. CCPs are steps at which control can be applied and are essential to reduce, prevent or eliminate a hazard to an acceptable level (Codex Committee on Food Hygiene, 1997).

In high resource setting, risk-based programs in LBMs involve cleaning and disinfection activities as well as periodic rest days, for which impact is monitored through microbial surveillance (Kung et al., 2003; Mullaney, 2003; Trock et al., 2008). Risk-based programs would be difficult to implement in many countries affected by AI H5N1 due to limitations in resources and capacity, especially the availability of PRPs and an environment operating to good standards of hygiene. In such settings, disease control may be feasible if CCPs are not dependent on good infrastructure and capacity for PRPs.

Taking into account the limited capacity for PRPs, limited disease surveillance activities and low levels of public health regulation, this study aimed to identify CCPs for H5N1-virus control in LBMs in low-resource settings. The study was conducted in Indonesia – a low resource country that has been heavily affected by AI H5N1 (World Health Organization, 2010; World Organization for Animal Health, 2010).

1.1. Setting

There are an estimated 13,000 LBMs across the 17,000 islands of Indonesia (Raharjo, 2010). Many LBMs in Indonesia offer a variety of birds for slaughter and sale, including broilers, layers, village chicken, Muscovy ducks and geese. Birds coming into LBMs may be sourced from a variety of farms or backyard holdings, and may have travelled hundreds of kilometers from neighboring districts or provinces.

The bird area of an LBM in Indonesia generally has five zones: (1) bird delivery zone where trucks unload live birds, (2) bird holding zone which comprises cages or pens to house live birds, (3) bird slaughter zone where birds are killed, defeathered, eviscerated and dressed, (4) bird sale zone with display tables, and lastly, (5) waste management zone where remnants such as innards and feathers are discarded. In some LBMs, bird zones are combined. For example, small markets may have two or three bird vendors, where each operates a self-sufficient stall. In these stalls, live birds are held in cages placed under the work table, birds are killed on the floor or on the work table, a hot water barrel and a defeathering machine are used to scald and defeather the carcass and the internal organs are removed on a work table. The final products – whole bird carcasses or pieces of meat – are then displayed at the front of the stall on display tables made either of ceramic tiles, steel or wood covered with a plastic sheet.

2. Materials and methods

Since CCP identification relies on thorough knowledge of the workflow, the product and the hazard itself, three surveys were conducted. The three surveys assessed poultry workflow, infrastructure, regulatory practices and LBM contamination with the H5N1 virus. The findings from the surveys were then used to quantitatively summarize and synthesize the existing capacity for PRPs. The findings from the surveys and synthesis of PRP capacity in LBMs formed the basis of CCP identification. This multi-step process was critical to reduce the subjectivity associated with CCP selection. Methods for the (a) three surveys, (b) synthesis of PRP capacity, and, (c) CCP identification, are described in more detail below. Approval for the study was obtained from the Health Research Ethics Committee at the Indonesian Ministry of Health as well as the Australian National University Human Research Ethics Committee.

2.1. Cross sectional survey

A cross-sectional survey was conducted in 119 LBMs to assess infrastructure and hygiene practices. The 119 LBMs were selected from 17 districts in four provinces in Indonesia: Jakarta, West Java, Banten and South Sulawesi. Methods for selecting the 31 LBMs in Jakarta, 41 in West Java and 11 in Banten province have been described previously (Indriani et al., 2010). In South Sulawesi, all 36 LBMs in the provincial capital Makassar were included. The survey was conducted using a structured questionnaire with 33 close-ended questions. The questions were divided in five sections: (a) seven questions about general conditions in the market, (b) five questions about market infrastructure, (c) nine questions about poultry in markets, (d) five questions about poultry processing, and (e) seven questions about cleaning and disinfection. The interviews were conducted in Bahasa Indonesia by three interviewer teams who received one-day training in survey administration and documentation. Data were entered and descriptive statistics were calculated in Microsoft Excel® (Microsoft Corp., Redmond, WA, USA).

2.2. Knowledge, attitude and practice survey

A survey on knowledge, attitudes and practices (KAP) was undertaken in three LBMs in South Sulawesi province to assess in detail the poultry workflow, hygiene practices, regulation and knowledge about AI H5N1. The KAP survey was conducted amongst three market managers and 37 bird vendors. For market managers, the survey had open-ended questions regarding the LBM management structure and staffing, market rules, utilities and hygiene practices, and relationships with government authorities such as the district veterinary and health offices. For the poultry vendors, the survey questions focused on documenting the poultry workflow steps, equipment used including personal protective equipment, knowledge and attitudes on avian influenza, and hygiene practices. The interviews were conducted in Bahasa Indonesia by three interviewers who received one-day training in survey administration and documentation. During the interviews, responses were paraphrased and repeated to the respondent to ensure accurate interpretation of answers. The qualitative data arising from the interviews with the market managers were analyzed by themes where one perspective was compared to responses in the same category. Descriptive statistics were calculated for responses from bird vendors in Microsoft Excel® (Microsoft Corp., Redmond, WA, USA).

2.3. Microbiological survey

Contamination with the H5N1-virus was assessed through a survey in 83 LBMs in three provinces in Indonesia. The methods for this survey have been described in a related study that focused on identifying risk factors for LBM contamination (Indriani et al., 2010). For the current study, the microbiological findings are assessed in context of CCP identification. In brief, the survey method involved swabbing up to 27 different environmental sites in 83 LBMs. The sites represented different poultry-related work activities; three sites related to the delivery of birds into LBMs, seven for holding birds in cages/pens, nine sites related to bird slaughter, six for sale and two sites for waste disposal. The samples collected from these sites were tested for AI H5N1 using real time reverse transcriptase polymerase chain reaction (rRT-PCR) (Indriani et al., 2010).

2.4. Capacity for PRP

A table was constructed with the 14 standard PRPs for food production as defined by the Codex Alimentarius Commission (Codex Committee on Food Hygiene, 1997). Definitions for each standard PRP were operationalized for the context of poultry workflow in LBMs. Based on the findings from the three surveys, LBM PRPs capacity was summarized by qualitatively assessing existing capacity against the 14 standard PRPs.

2.5. CCP identification

The three surveys, general capacity for PRPs in LBMs and relevant scientific literature were used to identify CCPs. A diagram outlining poultry workflow in an LBM

was constructed to identify potential CCPs. Confirmation or exclusion of each potential CCP was guided by a logic reasoning approach as per the Codex decision tree for CCP determination (Codex Committee on Food Hygiene, 1997). Using the decision tree, a CCP denotes a step at which the hazard can be controlled using available measures and where it is necessary to achieve hazard control. For steps where the hazard exists but no control measure exist, then the process should be modified at one or more steps to include a control measure that ensures the ultimate safety of the product (Lu, 1970).

3. Results and discussion

3.1. Cross sectional survey

Most LBMs surveyed were open daily ($n = 117$, 98.3%). Most LBMs ($n = 82$, 68.9%) had pests such as scavenging cats, dogs, rats and insect infestation. For infrastructure, stalls in 13 (10.9%) LBMs had wooden work surfaces, 68 (57.1%) had ceramic surfaces and 38 (31.9%) had a combination. The majority ($n = 82$, 68.9%) had water piped to each stall, but the others ($n = 37$, 31.1%) had to source water using buckets from wells inside or outside the LBM. For waste management, the majority of LBMs ($n = 83$, 69.7%) removed solid waste daily, whilst some removed waste less frequently than daily ($n = 29$, 24.4%) and 7 (5.9%) LBMs had no solid waste management system. For liquid waste, the majority of LBMs had open drainage systems ($n = 73$, 61.3%), 16 (13.4%) had covered drains and 29 (24.3%) LBMs had no liquid waste management system.

In terms of bird management, the majority of the LBMs surveyed ($n = 93$, 78.2%) kept unsold birds in the LBM for more than one night. Additional bird flocks coming into the LBM on different days were mixed with flocks already inside the LBM ($n = 46$, 38.7%). Some LBMs ($n = 30$, 25.2%) mixed different species of birds in the same cages/pens. Vendors in six LBMs admitted to selling sick birds to consumers. For poultry processing, 20 (16.8%) LBMs surveyed did not slaughter birds in the LBM. Vendors in the majority of LBMs slaughtered birds in the individual stalls ($n = 68$, 57.1%), 4 (3.4%) LBMs had a separate common slaughter and 27 (22.7%) LBMs had slaughtering in both stalls and a common area.

For cleaning, most LBMs cleaned their stalls and slaughter areas daily ($n = 116$, 97.5%). However, only 72 LBMs (60.5%) used soap during cleaning. Based on visual inspection, vendors in only 25 (21%) LBMs had cleaning equipment like brushes, brooms and buckets. A minority of LBMs mandated that birds be removed before cleaning ($n = 44$, 37%). At the time of the survey, 59 LBMs (49.6%) appeared clean defined as no visible waste, blood or remnants on tables or floors.

3.2. Knowledge, attitude and practice survey

Two LBMs in the KAP survey had 17 personnel and the third had 28 personnel. The KAP survey in 3 LBMs revealed a lack of food safety awareness amongst market managers and bird vendors. No training was provided for vendors in disease prevention and food safety practices. Use of

personal protective equipment was limited with only 11 (29.7%) workers wearing boots and 11 (29.7%) wearing aprons. Cages in each stall were overcrowded with birds and they were placed in close proximity to work surfaces. Study teams observed feathers and feces transfer from inside the cage to work surfaces when birds flapped around inside cages. None of the workers reported using soap or detergents when cleaning work surfaces and only 7 (18.9%) used soap to clean knives and defeathering equipment. The majority of vendors ($n = 32$, 86.5%) reported cleaning chopping boards several times per day and the others cleaned the boards once at the end of trade ($n = 5$, 13.5%). One-third ($n = 11$, 29.7%) of vendors did not know or gave incorrect symptoms of AI infection in birds, and only one wrapped birds that died naturally in the market before disposing of it in the waste disposal area.

LBM did not have specific regulations for hygiene, zoning of activities or poultry workflow. Birds were allowed to be sold live in all LBMs. Due to limited budgets provided by local government, the LBMs could not guarantee water or electricity supply to vendors even though stall fees were paid by vendors. This resulted in vendors seeking private sources for electricity and water supply. For water, this included establishing private wells inside or near the LBM that were not monitored or tested routinely. None of the three LBM managers had standard procedures to deal with mass bird deaths. Further, they did not understand the regulatory and inspection roles of government agencies, nor the support that these authorities can provide to assist LBMs.

The majority ($n = 26$ 70.3%) of vendors and all LBM managers mentioned the need to improve infrastructure such as roofing, drainage, flooring and supply to utilities such as water and electricity. All managers also wanted training in healthy market concepts as well as business training to improve market income.

3.3. Microbiological survey

Thirty-nine out of the 83 LBMs had evidence of H5N1-virus contamination. Most LBMs had contamination in the sale ($n = 30$, 36.1%), slaughter ($n = 29$, 34.9%) and bird holding ($n = 24$, 28.9%) zones (Table 1). However, a few LBMs also had contamination in the delivery zone ($n = 11$, 13.2%) and waste disposal zone ($n = 9$, 10.8%). Surfaces in the slaughter zone, such as tables chopping boards and baskets, were found to be amongst the most contaminated (Table 1). In the sale area, display tables, weigh scales and chopping boards were most contaminated (Table 1).

3.4. Capacity for PRPs

Table 2 outlines the standard Codex PRPs (Codex Committee on Food Hygiene, 1997). In the context of LBMs, PRPs mandate a number of activities including cleaning and disinfection, monitoring systems, segregation of processes that have potential for cross-contamination and training of workers/managers in hygiene and sanitation. Based on the three surveys, many LBMs do not comply with the PRPs required for good hygiene (Table 2). Capacity for safe packaging (PRP 12) is deemed to be high and availability of

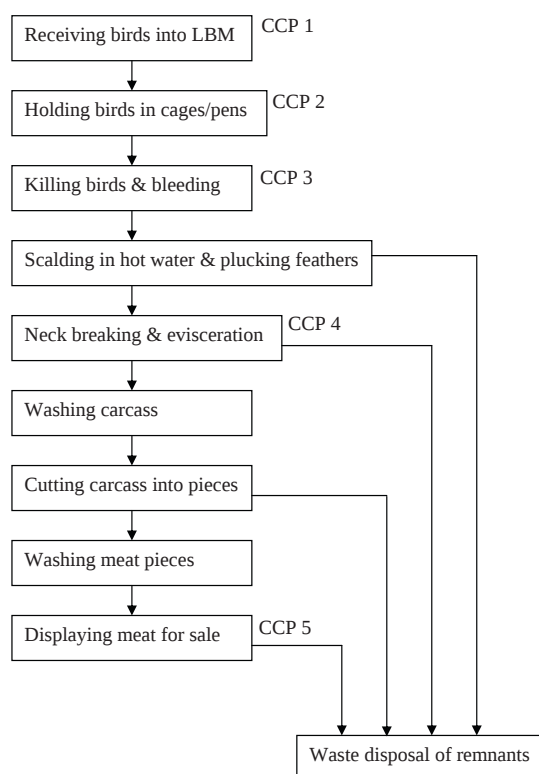


Fig. 1. Critical control points for avian influenza A H5N1 in the workflow of live bird markets.

durable and easy to clean equipment (PRP 4) is moderate. However, LBMs surveyed had low capacity for all other 12 PRPs including waste management, prevention of cross-contamination and maintenance, cleaning and sanitation.

3.5. CCP identification

Based on the LBMs surveyed, the poultry workflow is presented in Fig. 1. The workflow represents processes in LBMs that either slaughter birds at stalls or in a common slaughter location, and that, at minimum, have a waste management system that removes rubbish and remnants daily. Fig. 1 also presents the five CCPs identified in the study. The workflow and rationale for each CCPs is described below. Mechanisms to manage CCPs in the context of LBMs in a low resource setting are suggested.

Birds come into an LBM from a variety of sources. There is no documentation of bird sources nor is there routine health inspection of birds entering the LBM. However, as a number of studies have shown, birds can enter an LBM infected with AI H5N1 virus (Santhia et al., 2009; Webster, 2004). Some species such as chicken will become symptomatic generally within two days of infection and the majority of the flock will die, but other species such as ducks and geese may remain asymptomatic (Nguyen et al., 2005). Since chicken is the most common species sold in LBMs, there is an opportunity to reduce the number of infected birds entering the LBM by excluding sick incoming

Table 1
Environmental contamination with avian influenza A H5N1 virus in 83 live bird markets.

No.	Poultry zone	Environmental site	RT-PCR positive LBMs/LBMs tested (%)	LBMs +ve for zone, N=83 (%)
1	Delivery	Inside cages on truck	6/45 (13.3)	11 (13.2)
2	Delivery	Floor in delivery area	6/49 (12.2)	
3	Delivery	Water run-off in delivery area	4/38 (10.5)	
4	Holding	Poultry cage floors	6/79 (7.6)	24 (28.9)
5	Holding	Holding area floor	8/80 (10)	
6	Holding	Water run-off	11/72 (15.3)	
7	Holding	Poultry feeding bottle water	8/67 (11.9)	
8	Holding	Poultry feeding basket food	6/72 (8.3)	
9	Holding	Handles to poultry cages	9/79 (11.7)	
10	Holding	Inside of waste bins	10/59 (16.7)	29 (34.9)
11	Slaughter	Knife handles used for slaughtering	8/75 (10.7)	
12	Slaughter	Basket holding dying chickens	8/71 (11.3)	
13	Slaughter	Floor in slaughter area	10/77 (13)	
14	Slaughter	Chopping or slaughtering board	14/71 (19.7)	
15	Slaughter	Processing table after de-feathering	15/70 (20)	
16	Slaughter	Baskets holding poultry meat	14/70 (20)	
17	Slaughter	Drain path	12/75 (16)	
18	Slaughter	Tap handles in slaughter area	7/65 (10.7)	
19	Slaughter	Waste bin	13/71 (18.3)	30 (36.1)
20	Sale	Chopping boards	15/80 (18.8)	
21	Sale	Weigh scales	12/57 (21.1)	
22	Sale	Knife handles	12/78 (15.4)	
23	Sale	Waste bins	10/60 (16.7)	
24	Sale	Wet cloths for cleaning surfaces	14/78 (17.9)	
25	Sale	Tables for poultry display	19/80 (23.8)	9 (10.8)
26	Waste disposal	Area waste disposal bin	15/78 (19.2)	
27	Waste disposal	Wet cleaning mops	8/66 (12.1)	

flocks of chickens. Hence, CCP 1 is at receipt of birds into the LBM, where vendors must reject sick flocks of chicken. One mechanism would be for vendors to inspect and check flocks for signs of AI H5N1 infection using a clinical case definition. This involves searching for symptoms such as reduced bird movement, discharge from the mouth, eyes or cloacae, bluish comb or reduced movement of head. If the inspection suggests there is illness amongst the flock, vendors must reject the flock and inform the LBM manager so that district agriculture authorities can initiate outbreak control measures.

Once flocks of birds come into the LBM holding zone, there is an opportunity for the virus to spread between birds held in cages or pens. This is CCP 2. Flock separation is needed to reduce virus spread within the LBM especially for LBMs that keep birds for a number of days or sell live birds to consumers. Specifically, vendors must separate flocks coming into the LBM from different sources. This may require adding panels between stacked cages to prevent bird contact, increasing the number of feeding bottles and having a daily cleaning regimen to remove dirt and excreta from cages before the next flock of incoming birds.

The next step in the workflow is to kill and bleed the birds. During this process, birds flap around which can generate droplets carrying viral particles. Since large droplets settle within one meter of the source (World Health Organization, 2007), it is important to keep consumers and other birds at a safe distance from the slaughter process. Therefore, CCP 3 is to isolate the slaughter step. Slaughtering should be conducted at least two meters away

from the bird holding and sale zones. If distancing is not possible due to the layout or size of the LBM, another option is to install an easy-to-clean physical barrier such as plastic that prevents splashing.

After the birds are killed and the blood drained, the vendors scald the carcass in hot water to pluck the feathers. Dipping the carcass in water reduces the dust expelled when the feathers are plucked. This is practiced routinely as part of the workflow and inherently minimizes the risk of environmental contamination (World Health Organization, 2004). Therefore, it is not considered a CCP. The plucked feathers are then gathered by the vendor and are disposed off at the end of the trading day along with remnants arising from the slaughter process.

Vendors break the bird neck and eviscerate the carcass. Evisceration is a critical step in the slaughter process because it may rupture the bird's internal organs and result in spillage of fecal material, viruses and bacteria onto the meat and on environmental surfaces. From the microbiological survey, surfaces associated with slaughter such as work tables and chopping boards were amongst the most contaminated. Thus, reducing contamination in the evisceration step is CCP 4. Mechanisms to address this CCP are to provide technical training in appropriate slaughtering techniques to slaughterers and supplying them with simple but appropriate evisceration tools. Another mechanism is through regular cleaning of surfaces using detergents after the evisceration process. Previous studies have shown that simple soap products, detergents and alkali destroy H5N1-infectivity within 5 min at 0.1%, 0.2% and 0.3% dilution (Shahid et al., 2009).

Table 2
Capacity for prerequisite programs in live bird markets surveyed in Indonesia.

Standard PRPs ^a	Standard PRPs in context of LBMs	Current PRP capacity in LBMs	PRP capacity examples in LBMs surveyed ^b
1. Management and supervision	Local health and agriculture authorities and market managers trained in hygiene principles and have monitoring plans	Low	- No training provided to managers in hygiene - Roles of different local agencies unclear 84.9% LBMs are not zoned 57.1% LBMs have easy-to-clean work surfaces - No documentation of source of incoming birds in LBMs
2. Facilities	- Zoning to prevent cross-contamination - Surfaces easy-to-clean to prevent pathogen build-up	Low	
3. Supplier control	Only healthy birds enter LBMs	Low	
4. Equipment	- Mechanism to trace back bird origin - Cages, knives, chopping boards and tools need to be easy-to-clean	Moderate	- Chopping boards and knives suitable, but 75.9% used difficult-to-clean bird cages 47.9% LBMs had visible dirt on cages and in sale area 35.3% LBMs never used detergents to clean surfaces or equipment - No protocols for monitoring hygiene
5. Maintenance, cleaning and sanitation	- Surfaces and equipment need to be easy-to-clean with detergents - Cleaning program monitored	Low	68.9% LBMs had evidence of pest infestation 24.4% LBMs had no drainage system for liquid waste 30.3% LBMs did not remove solid waste daily - Limited pay-by-use toilets
6. Pest control	All zones clean to prevent pest infestations	Low	
7. Waste management	- Drainage for liquid waste such as bird blood and wastewater - Solid waste such as innards and feathers removed daily	Low	
8. Personal hygiene and health status	- Bird workers excluded if sick - Hand-washing facilities and toilets available	Low	- No soap for hand-hygiene - No protocols for exclusion of sick staff - Customers can handle meat and live birds in selection process
9. Visitors	Prevent consumer access to slaughter zone or handling raw meat	Low	75.9% LBMs use difficult-to-clean cages - Meat frozen overnight and thawed daily
10. Receiving, storage and shipping	- Live birds held in appropriate caging - Unsold meat refrigerated or disposed	Low	40% LBMs keep birds in LBMs during cleaning 38% never use detergent for cleaning
11. Cross-contamination	- Separation of slaughter zone from other bird zones - Regular cleaning with detergent	Low	- Available at LBMs - No hygiene or slaughter training provided 70% personnel in 3 LBMs do not think bird slaughter is a human health risk
12. Packaging	Clean plastic bags for meat	High	
13. Staff training	Staff trained in general hygiene, food safety and sanitary slaughter techniques	Low	No documentation of source of birds
14. Traceability and recall	Trace back possible if sick birds detected.	Low	

^a According to Codex.

^b Findings quoting percentages are from the survey conducted in 119 LBMs. All other findings are based on the KAP survey in three LBMs.

After evisceration, the carcass is washed in water and cut into pieces. This may be followed with a second wash in water. These three steps are not considered CCPs as they usually take place at the same location as evisceration. Therefore, by applying the controls in the evisceration step (CCP 4), especially regular cleaning of surfaces, then contamination will be reduced.

The meat is then displayed on tables for sale. Since the microbiological survey showed that the sale zone was frequently contaminated with H5N1-virus, CCP 5 is to reduce contamination at the sale point. Previous studies have shown that cleaning is effective at eliminating influenza

viruses from environmental surfaces in LBMs (Bulaga et al., 2003; Trock et al., 2008). Therefore, CCP 5 can be managed through daily cleaning with detergents of these surfaces. All remnants and visible dirt must be removed and disposed off in the waste disposal zone to reduce the potential for environmental contamination.

4. Conclusions

Control of AI H5N1 in LBMs is critical as part of the strategy to control the disease in birds and to reduce the risk of human infection. Risk-based programs focus on preven-

tion rather than inspection, take a bottom-up approach that acknowledge the variability in context and process, and are generally simple to implement (Herrera, 2004). This is the first study to consider H5N1 risk management in LBMs in low resource settings by proposing control points based on the scientific data presented. Five CCPs were proposed in this study based on three surveys that assessed infrastructure, hygiene practices, poultry work flow and H5N1-virus contamination in LBMs in Indonesia. The CCPs identified are relevant for LBMs that slaughter birds and that, at minimum, remove rubbish/solid waste daily.

The three surveys revealed limited infrastructure, hygiene and regulatory practices in LBMs. The findings were aligned with previous work that explored high risk practices in LBMs (World Health Organization, 2006b). Vendors and LBM managers had limited food safety knowledge and information about avian influenza. Further, the microbiological survey showed alarming frequency of contamination in some of the LBM work surfaces surveyed, including areas accessible to consumers such as sale tables and weigh scales. This increases the risk of infection and spread of virus through fomites. Despite these challenges, the KAP survey showed a general willingness amongst vendors and LBM managers to enhance conditions in the markets.

Based on the synthesis of PRP capacity, some PRPs may potentially be attainable in the context of the low-resource LBMs surveyed. Two examples are staff training and management and supervision, where the existing human resources and organizational structure overseeing LBMs has the potential to be utilized for enhanced application of disease control principles. Each LBM has a manager with a number of deputies and cleaning staff. These human resources can eventually be trained and mobilized to manage disease risk. Further, most district agriculture authorities have part-time representatives in LBMs for meat inspection and revenue collection. These staff may be mobilized to provide training in hygiene, slaughtering techniques and safe work practices.

Since each LBM has its unique characteristics for which control activities will need to be specifically tailored, a full risk-based program for the control of H5N1-virus was not proposed in this study. However, it is expected that the CCPs identified and the mechanisms suggested for the control of each CCP are adaptable to various types of LBMs, especially those that slaughter live birds and those with limited zoning of poultry workflow. Further, LBMs in Indonesia are similar to LBMs in many other low-resource countries with limited infrastructure, facility maintenance and public health regulation (World Health Organization, 2006b). Therefore, the CCPs identified in this study may be applicable to other countries with minor modification. This should make the task of developing a comprehensive risk-based control program relatively easy for market, veterinary and public health authorities in different settings.

The CCPs identified in this study do not only address the safety of the final product (meat), but also the risks associated with the entire poultry workflow which can place humans and other birds at risk of AI H5N1 infection. This is an atypical approach. Traditionally, CCPs address processes that contaminate the final product whilst PRPs

address risk associated with the process (Bas et al., 2007). However, since PRPs in the LBMs surveyed were found to be sub-optimal, the risks associated with the entire poultry workflow had to be addressed through CCPs. This was considered critical for LBMs in low resource settings since enhancement of PRP capacity will likely involve expensive infrastructure changes such as piping water, supplying electricity and upgrading physical infrastructure such as tabletops, floors and roofing.

The main limitation of this study is that the CCPs identified were not validated. However, this can be seen as a next step in a few trial LBMs, where it will necessitate microbiological surveillance to determine if a reduction in AI H5N1 contamination was achieved. Even though the objective of this study was to aid in the control of AI H5N1 in LBMs, it is anticipated that the recommendations could also assist in the control of other pathogens, such as *Salmonella* and *Campylobacter*, which are associated with bird slaughter (McCrea et al., 2006). Such benefits should be explored in the validation of the CCPs, as they may appeal to national decision-makers as an opportunity for integrated disease control activities.

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Chapter 9

**Paper 6: Application of a healthy food markets
guide to two Indonesian markets to reduce
transmission of “avian flu.”**

About this chapter

The previous two chapters provided some of the epidemiological information needed to establish and operationalize disease control programs for AI H5N1 in LBMs in low resource settings such as Indonesia. This chapter describes a non-experimental field intervention trial where disease control measures were implemented to determine the practical aspects of implementation and to learn lessons for future application. Such research is needed in developing countries to highlight that international guidance can be implemented in low resource settings. The control measures were selected based on the WHO recommendations for developing healthy LBMs but also arise from the findings of the previous studies in this PhD, including recommendations for daily solid waste removal, zoning poultry workflow to reduce cross contamination and instituting daily cleaning routines.

The study described in this chapter found that disease control measures could be established for each of the WHO-recommended control measures. The participatory approach to operationalising the control measures involving LBM managers and vendors was well received by these stakeholders.

This study is of international importance as it is the first to demonstrate that AI H5N1 control measures can be instigated in LBMs in low resource settings in virus endemic countries. The study provides a proof-of-concept for future trials, including experimental designs and application in different settings.

My role in this study was to provide technical input into the interventions applied in the two trial LBMs, to determine the outcome measures and to collect the data to assess implementation and to identify lessons learnt. I wrote the manuscript and coordinated all co-author inputs pre-submission. The study was submitted to the Bulletin of the World Health Organization and was published in April 2012.

Application of a healthy food markets guide to two Indonesian markets to reduce transmission of “avian flu”

Gina Samaan,^a Ferra Hendrawati,^b Trevor Taylor,^c Tangguh Pitona,^b Dini Marmansari,^b Ratna Rahman,^b Kamalini Lokuge^a & Paul M Kelly^a

Problem The World Health Organization (WHO) developed a guideline with 10 control measures to reduce transmission of A(H5N1) avian influenza virus in markets in low-resource settings. The practical aspects of guide implementation have never been described.

Approach WHO's guideline was implemented in two Indonesian markets in the city of Makassar to try to reduce transmission of the A(H5N1) virus. The guideline was operationalized using a participatory approach to introduce a combination of infrastructural and behavioural changes.

Local setting Avian influenza is endemic in birds in Makassar. Two of the city's 22 dilapidated, poorly-run bird markets were chosen for the study. Before the intervention, neither market was following any of WHO's 10 recommended control measures except for batch processing.

Relevant changes Market stakeholders' knowledge about the avian influenza A(H5N1) virus improved after the interventions. WHO guideline recommendations for visual inspection, cleaning and poultry-holding practices, as well as infrastructural requirements for zoning and for water supply and utilities, began to conform to the WHO guideline. Low-maintenance solutions such as installation of wastewater treatment systems and economic incentives such as composting were well received and appropriate for the low-resource setting.

Lessons learnt Combining infrastructural changes with behaviour change interventions was critical to guideline implementation. Despite initial resistance to behaviour change, the participatory approach involving monthly consultations and educational sessions facilitated the adoption of safe food-handling practices and sanitation. Market authorities assumed important leadership roles during the interventions and this helped shift attitudes towards regulation and market maintenance needs. This shift may enhance the sustainability of the interventions.

Abstracts in **عربي**, **中文**, **Français**, **Русский** and **Español** at the end of each article.

Introduction

Live bird markets have been implicated in the circulation of avian influenza A(H5N1) virus¹ and are a potential source of viral transmission among humans and animals.^{2,3} In 2006 the World Health Organization (WHO) developed a guideline – *A guide to healthy food markets* – to reduce contamination with and transmission of A(H5N1) virus in live bird markets.⁴ The guideline lists 10 control measures for the poultry area of markets, the main aims of which are to improve the environment and ensure safe food-handling practices. The 10 control measures involve education and awareness of how avian influenza is transmitted; monitoring of conditions and food-handling practices; visual inspection of fowl to look for signs of infection; use of personal protective equipment (masks, gloves, disposable aprons, rubber boots, etc.); market zoning to prevent public access to potentially contaminated areas; use of potable water for cleaning and hand-washing; appropriate cage design and holding practices; appropriate cleaning practices; properly designed utilities, such as drainage systems, and batch processing. This study reports on the lessons learnt from implementing the guideline in two live bird markets in Indonesia, a low-resource country with areas where avian influenza A(H5N1) virus infection is endemic in fowl.

Problem and local setting

The site of the study was the city of Makassar (population 1.6 million), where 80 000 birds are slaughtered daily and where avian influenza A(H5N1) virus infection is endemic in birds.⁵ Makassar has 22 live bird markets under the purview of

the municipal market authority. All of them have dilapidated infrastructure, no health services and an inadequate operational environment. Two markets were selected for this study based on the management teams' readiness to undergo the interventions. Market A had 186 kiosks, 17 management and sanitation staff, and 5 poultry kiosks that received and slaughtered a daily total of 500 birds; Market B had 247 kiosks, 17 management and sanitation staff, and 13 poultry kiosks that received and slaughtered a daily total of 2700 birds.

Before the intervention, an assessment was conducted to determine the extent to which WHO's 10 control measures were being practised.⁶ The assessment showed that only one of the measures – batch processing – was being followed as recommended in the WHO guideline, which calls for separating poultry batches, cleaning between batches and at the end of the trading day, and having the capacity to trace back poultry through the use of regular suppliers. The other nine control measures were not met. For example, each poultry kiosk held, slaughtered and sold birds without separate zoning for these different processes; drainage, bins, electricity and water supplies were limited; work surfaces, cages and floors were hard to clean; and no regulated inspection or sanitation programmes were in place.

Approach

A municipal-level taskforce was established. It was composed of the finance and operations staff of the municipal market authority, general managers and sanitation teams of the live bird market, and members of the nongovernmental organization (NGO), CHF International, that was funded to implement the

^a National Centre for Epidemiology and Population Health, College of Medicine, Biology & Environment, Australian National University, Canberra, ACT, 0200, Australia.

^b Disease Investigation Center, Ministry of Agriculture, Maros, South Sulawesi Province, Indonesia.

^c Australian Animal Health Laboratory, East Geelong, Australia.

Correspondence to Gina Samaan (e-mail: ginasamaan@yahoo.com).

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Table 1. **Comparison of poultry vendor knowledge, attitudes and practices before and after intervention to reduce transmission of A(H5N1) influenza virus in two poultry markets, Makassar, Indonesia, 2008–2009**

Control measure ^a and related knowledge, attitude or practice	Before (<i>n</i> = 34) No. (%)	After (<i>n</i> = 29) No. (%)	<i>P</i>
Education and awareness			
Aware that people can get sick from working with poultry	8 (24)	18 (62)	0.002
Practising slaughter of sick birds and sale of sick or dead birds	5 (15)	6 (21)	0.533
Education and awareness, monitoring, visual inspection			
Able to identify three symptoms of avian influenza infection in chickens	26 (76)	27 (93)	0.092
Personal protective equipment			
Wearing rubber boots ^a	22 (65)	16 (55)	0.441
Wearing plastic aprons ^a	5 (15)	16 (55)	0.001
Cages and holding practices, cleaning			
Cleaning cages daily	28 (82)	29 (100)	0.027
Cleaning			
Using soap when cleaning chopping boards, knives and defeathering machines	13 (38)	18 (62)	0.059

^a Based on observation of poultry vendors.

project to improve the two markets according to the WHO guideline. The task-force oversaw the change process and monitored the interventions monthly.

Interventions promoting infra-structural and behavioural changes were introduced over an 18-month period (January 2008–June 2009). The interventions were specifically aimed at achieving compliance with the nine recommended measures not being practised at the markets (batch processing was excluded since it was already being practised). A participatory approach involving market managers, sanitation teams and poultry vendors was applied to put the measures into operation. Under this approach, problems were posed and potential solutions discussed at monthly consultation meetings held at the markets until acceptable options emerged.⁷

Interventions that required construction or introduction of new goods were designed to ensure sustainability, low ongoing costs and easy maintenance. Education sessions lasting two hours were held monthly to improve market managers', sanitation teams' and poultry vendors' knowledge and practices in the area of sanitation and food handling. These 18 sessions were held at canteens near the markets and addressed waste management, food safety, recognition of signs of infection with avian influenza A(H5N1) virus and notification of affected fowl. The staff of CHF International developed key messages based on the WHO guideline

and provided the training.⁴ Information was discussed and monitoring protocols and logs were developed during these 18 sessions.

Progress in implementing the intervention was evaluated through a post-intervention inspection, interviews with market managers, sanitation teams and poultry vendor surveys. These activities were conducted by a two-person team composed of one external evaluator (GS) experienced in avian influenza control in live bird markets^{2,8} and one NGO officer responsible for overseeing guideline implementation at both markets. GS developed the necessary evaluation tools based on the WHO guideline and provided one day of training to the NGO officer on questionnaire administration and data collection and recording.

An unannounced one-day inspection was conducted at each market by the team one month after the intervention. The team used a checklist to confirm that the necessary goods had been installed and that the protocols and logs developed were in use. Interviews with market managers and sanitation teams were conducted using semi-structured questionnaires developed with guidance from WHO and field tested locally.⁶ The questions explored the presence of any roadblocks to guideline implementation and the adequacy of the change process and the interventions. Answers to each question were summarized and differences in perspectives identified.

Changes in vendor knowledge, attitudes and behaviour before and

after the intervention were measured using a field-tested, structured survey instrument containing 38 close-ended questions. The survey was conducted verbally in the local dialect. The NGO officer conducted the pre- and post-intervention surveys among 34 and 29 poultry vendors, respectively (Table 1). These numbers represent all vendors present in the market on the days the interviews were conducted. Changes in vendors' knowledge, attitudes and behaviours before and after the intervention were analysed using χ^2 or Fisher's exact tests, as required.

Ethics approval for the study was obtained from the Health Research Ethics Committee at the Indonesian Ministry of Health and from the Australian National University Human Research Ethics Committee.

Findings

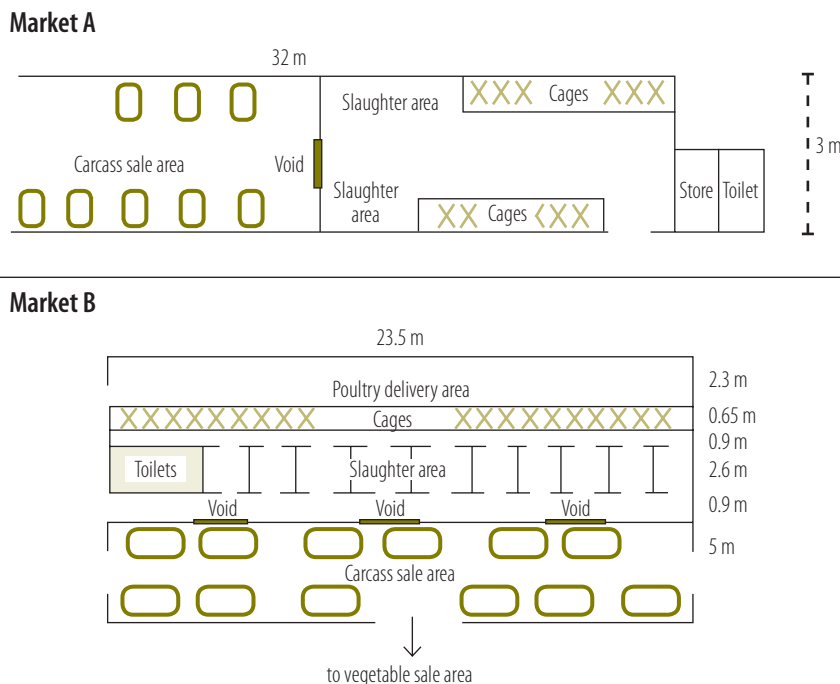
Education and awareness

Poultry vendors' knowledge and attitudes surrounding avian influenza A(H5N1) virus transmission improved after the intervention. Six vendors from both markets continued to slaughter sick chickens and to sell sick or dead chickens (Table 1). They stated that they sold these chickens as feed for fish farms, but no follow-up was conducted to verify this information.

Monitoring

With the aid of municipal agriculture officers, both markets developed disease-

Fig. 1. Layout of the poultry areas in markets A and B after interventions in both poultry markets, Makassar, Indonesia, 2008–2009



Note: Pre-intervention layout not shown because each poultry kiosk operated all aspects of the workflow inside the kiosk without zoning.

monitoring protocols. These protocols provided for simple visual inspection of incoming birds, a cost-free intervention. Monitoring logs were filled daily by market managers in both markets and kept in the communal poultry holding zone.

Visual inspection

Posters and protocols for poultry inspection and disease notification were developed and placed in a visible location in the poultry area of each market. More poultry vendors could correctly identify signs of avian influenza A(H5N1) virus infection in birds after the intervention than before it ($P=0.09$) (Table 1).

Personal protective equipment

Poultry vendors rejected face masks and goggles because they made them feel too hot when worn during poultry slaughter. However, the use of plastic aprons increased after the intervention ($P=0.001$).

Market zoning

The poultry area was completely reconstructed within a four-month period in both markets. The new structures adhered to zoning and unidirectional workflow, as per the WHO guideline

(Fig. 1).⁴ Of the 29 poultry vendors surveyed after the intervention, 25 (86%) expressed satisfaction with the changes. The remaining vendors indicated that they had fewer buyers because the area where poultry is sold to the public had been isolated. Eleven vendors (38%) mentioned a dip in sales after the interventions, but seven of these vendors (64%) still felt satisfied with the changes.

Water

In both markets, existing water wells were closed and city water was piped to the poultry areas. Toilets with hand-washing facilities were installed, with easy access for all workers and customers.

Cages and holding practices

After the intervention, poultry species were placed in separate holding zones and kept in clean cages. More vendors reported cleaning cages and trays daily ($P=0.027$; Table 1). Market A vendors expressed concern that the poultry holding zone was too hot because of limited airflow. Additional fans were installed to overcome this design problem, but management still faced difficulty in getting vendors to hold poultry in that zone.

Cleaning

Market sanitation teams were provided with high-pressure hoses. Easy to clean stainless-steel work surfaces were installed. Cleaning logs were filled daily by the market sanitation teams. Cleaning practices by poultry vendors improved after the intervention (Table 1).

Utilities

The poultry areas were provided with electricity, and an anaerobic wastewater treatment system that decreases organic matter was installed in them. Composting bins and rubbish bins with lids were provided and placed in visible locations, and drains were covered and sloped. One vendor who was unhappy with the intervention claimed that drainage was slow. On verification, market managers suggested that this vendor was unhappy with his corner location in the sale area as he felt that it was isolated. No other vendor complained about the drainage.

Conclusion

Behavioural change was critical to the adoption of hygienic practices and the implementation of the WHO guideline. Since people tend to resist changes in their work routines,^{9,10} we achieved success in this respect by applying the participatory approach consisting of regular consultations, educational sessions and by making infrastructural changes that facilitated and provided an incentive for behaviour change.¹¹

Market managers and the municipal market authority assumed important leadership roles in overseeing adoption of the guideline. All stakeholders recognized the need to regulate market sanitation practices and utilities to maintain consumer interest and sustain live bird markets as points of municipal revenue. This resulted in a commitment by the municipal market authority to use funds already allocated by the local government to provide maintenance and uninterrupted supplies of electricity and water, without additional cost to vendors in the two markets. We believe this commitment will ensure the intervention's sustainability. It may also provide impetus for the municipal market authority to roll out the intervention in Makassar's other 20 live bird markets over the next 5 years using municipal funds.

Box 1. Summary of main lessons learnt

- The interventions outlined in the World Health Organization's guide to healthy food markets can be implemented in low-resource settings endemic for avian influenza A(H5N1) virus.
- To implement the interventions and maximize potential for sustainability, various stakeholders had to be involved in the change process, including the government market authority, market managers, sanitation teams and poultry vendors.
- Regular consultation and education sessions, as well as infrastructural changes with financial incentives, facilitated behaviour change and the adoption of hygienic practices by market stakeholders.

Anaerobic wastewater treatment systems and composting reduce the risk of contamination with the A(H5N1) virus and are cheap and easy to maintain.¹² Composting also enables sanitation staff to supplement their income by turning poultry waste into a marketable commodity. Such economic incentives increase compliance with interventions, especially in low-resource settings.¹³

The intervention did not result in any increase in kiosk fees, since it was funded through CHF International. Although cost-sharing would have been favourable, initial buy-in from authori-

ties and vendors was limited by the fact that WHO's guideline had never before been applied in Indonesia. Therefore, this experience was treated as a proof-of-concept. Future applications of the guideline in Indonesia should explore other funding models (e.g. public-private co-contributions).

The fact that the two bird markets were chosen because their management teams showed readiness to implement the interventions may have increased the likelihood of success. However, the intervention should yield similar results in other low-resource settings, since the

workflow in markets is generic. Furthermore, managers of other live bird markets may be motivated by the lessons learnt from this experience (Box 1).⁶ Since the WHO guideline prioritizes certain interventions more than others, managers of markets with limited resources may choose to implement the interventions having higher priority. ■

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Competing interests: None declared.

ملخص

تطبيق دليل أسواق الأطعمة الصحية على سوقين إندونيسيين للحد من انتقال "أنفلونزا الطيور"

توصيات المبادئ التوجيهية لمنظمة الصحة العالمية حول الفحص البصري وممارسات تنظيف وحمل الدجاج، بالإضافة إلى متطلبات البنية التحتية للتقسيم إلى مناطق وتوفير المياه والمرافق في التوافق مع المبادئ التوجيهية لمنظمة الصحة العالمية. ولاقت الحلول التي لا تتطلب سوى القليل من الصيانة مثل تركيب أنظمة معالجة مياه الصرف والحوافز الاقتصادية مثل التسميد ترحابًا ووجدت ملائمة للمواقع منخفضة الموارد.

الدروس المستفادة كان دمج تغييرات البنية التحتية في تدخلات تغيير السلوك مهماً لتنفيذ المبادئ التوجيهية. وعلى الرغم من المقاومة الأولية لتغيير السلوك، يسر النهج التشاركي الذي تضمن استشارات شهرية وجلسات تعليمية تبني ممارسات التداول الآمن للغذاء وخدمات الإصحاح. وتولت سلطات السوق أدوارًا قيادية هامة أثناء التدخلات، وساعد هذا في تحول الاتجاهات نحو التنظيم واحتياجات صيانة السوق. وقد يعزز هذا التحول من استدامة التدخلات.

المشكلة وضعت منظمة الصحة العالمية (WHO) مبادئ توجيهية تحتوي على 10 تدابير مكافحة تهدف إلى الحد من انتقال فيروس أنفلونزا الطيور A(H5N1) في الأسواق في المواقع منخفضة الموارد. ولم يتم تناول الجوانب العملية لتنفيذ الدليل بالوصف على الإطلاق.

الأسلوب تم تنفيذ المبادئ التوجيهية الخاصة بمنظمة الصحة العالمية في سوقين إندونيسيين في مدينة ماكاسار لمحاولة الحد من انتقال فيروس A(H5N1). وأصبحت المبادئ التوجيهية نافذة باستخدام نهج تشاركي لطرح مجموعة من التغييرات في البنية التحتية والسلوك. المواقع المحلية تعتبر أنفلونزا الطيور متوطنة في الطيور في ماكاسار. وتم اختيار سوقين من أسواق الطيور المهملة ذات الإدارة السيئة بالمدينة البالغ عدد أسواقها 22 سوقًا لإجراء الدراسة. وقبل التدخل، لم يكن أي من السوقين يتبع أيًا من تدابير مكافحة العشرة الموصى بها من قبل منظمة الصحة العالمية باستثناء التجهيز على دفعات. التغييرات ذات الصلة تحسنت معرفة أصحاب المصالح في السوق بفيروس أنفلونزا الطيور A(H5N1) بعد التدخلات. وبدأت

摘要

印度尼西亚两个市场应用健康食品市场指导方针以减少“禽流感”传播

问题 世界卫生组织 (WHO) 制定了含有 10 种控制方法的指导方针，用于减少低资源配置市场中的 A(H5N1) 禽流感病毒的传播。指导方针实施的实际方面尚未描述。

方法 在印度尼西亚城市孟加锡的两个市场内实施 WHO 的指导方针，用以减少 A(H5N1) 病毒的传播。此指导方针

采用参与式教学法实施，结合执行基础架构和行为变更。
当地状况 孟加锡禽类的禽流感较为常见。在该城市 22 个岌岌可危、管理不善的禽类市场中选择 2 个市场进行研究。在干预之前，两个市场都没有遵循 WHO 的 10 个建议控制方法（分批处理除外）。

相关变化 经过干预，市场利益相关者对禽流感 A(H5N1) 病毒的认识提高了。对目视检查、清洁和家禽保持实践以及分区和水源及设施的基础架构要求方面的 WHO 指导方针建议逐步符合 WHO 指导方针的要求。低维护解决方案如安装废水处理系统和经济刺激如堆制肥料均受到好评，适用于低资源配置。

经验教训 将基础架构变更和行动变更结合起来，对实施指导方针至关重要。尽管行动变更开始时遇到阻力，但涉及每月协商和教育讲习会的参与式教学方法还是促进了安全食品处理实践和卫生设施的采用。市场管理部门在干预中担任重要的领导角色，这有助于转变对管理和市场维护需求的态度。这种转变可以强化干预的可持续性。

Résumé

Application d'un guide des marchés d'alimentation saine à deux marchés indonésiens afin de réduire la transmission de la «grippe aviaire»

Problème L'Organisation mondiale de la Santé (OMS) a conçu un guide avec 10 mesures de contrôle permettant de réduire la transmission du virus de la grippe aviaire A(H5N1) sur les marchés à faibles ressources. Les aspects pratiques de l'application du guide n'ont jamais été décrits.

Approche Le guide de l'OMS a été appliqué à deux marchés indonésiens dans la ville de Makassar afin de tenter de réduire la transmission du virus A(H5N1). Le guide a été utilisé à l'aide d'une approche participative pour présenter une combinaison de changements infrastructurels et comportementaux.

Environnement local La grippe aviaire est endémique chez les oiseaux à Makassar. Deux des 22 marchés à oiseaux délabrés et pauvres de la ville ont été choisis pour l'étude. Avant l'intervention, aucun des deux marchés ne suivait les 10 mesures de contrôle recommandées par l'OMS, à l'exception du traitement des lots.

Changements significatifs Les connaissances des parties prenantes des marchés sur le virus de la grippe aviaire A(H5N1) se sont améliorées après les interventions. Les recommandations du guide de l'OMS en matière d'inspection visuelle, de nettoyage et de pratiques de

conservation de la volaille, ainsi que les exigences infrastructurelles pour le zonage et pour les équipements et l'alimentation en eau ont commencé à être conformes au guide de l'OMS. Des solutions nécessitant peu de maintenance, comme l'installation de systèmes de traitement des eaux usées, ainsi que des incitations économiques comme le compostage ont été bien accueillies et s'adaptaient parfaitement au système à faibles ressources.

Leçons tirées Combiner les changements infrastructurels aux interventions de changements des comportements était essentiel à l'application du guide. Malgré une première résistance au changement comportemental, l'approche participative impliquant des consultations mensuelles et des sessions de formation ont facilité l'adoption d'une hygiène publique et de pratiques de gestion d'une alimentation saine. Les autorités des marchés ont joué un rôle de leader important lors des interventions, ce qui a aidé à modifier les attitudes envers la réglementation et les besoins en maintenance des marchés. Ce changement peut améliorer la durabilité des interventions.

Резюме

Реализация указаний по охране здоровья на продовольственных рынках на двух индонезийских рынках в целях снижения передачи «птичьего гриппа»

Проблема Всемирная организация здравоохранения (ВОЗ) разработала указания, содержащие 10 мер, направленных на снижение передачи вируса птичьего гриппа A(H5N1) на рынках в условиях с ограниченными ресурсами. Практические аспекты реализации инструкции ранее никогда не были описаны.

Подход Указания ВОЗ были реализованы на двух индонезийских рынках в городе Макаassar в целях уменьшения передачи вируса A(H5N1). Реализация указаний была осуществлена с использованием активного подхода и была нацелена на совместное внедрение инфраструктурных и поведенческих изменений.

Местные условия Птичий грипп является эндемическим для птиц в Макаассаре. Для исследования были выбраны два из 22 ветхих и плохо организованных птичьих рынков. До реализации мероприятий ни один из рынков не выполнял ни одной из 10 рекомендуемых ВОЗ мер контроля, за исключением контроля партий товара.

Осуществленные перемены Осведомленность заинтересованных лиц, участвующих в работе рынка, о птичьем гриппе A(H5N1) после реализации мероприятий улучшилась. Рекомендации ВОЗ, касающиеся визуального осмотра, чистки

и содержания птиц, а также инфраструктурные требования к зонированию, водоснабжению и системам коммунального обслуживания стали соответствовать указаниям ВОЗ. Такие решения с низкими эксплуатационными расходами, как установка систем очистки сточных вод, а также такие экономические стимулы, как, например, компостирование, были хорошо приняты и подходят для условий с ограниченными ресурсами.

Выводы Сочетание инфраструктурных изменений с мерами, направленными на изменение поведения, имеет решающее значение для реализации указаний. Несмотря на первоначальное сопротивление изменению поведения, подход, предполагающий активное участие и включающий ежемесячные консультации и учебные занятия, способствовал принятию практик безопасного обращения с пищевыми продуктами и санитарии. Руководство рынка в течение проведения мероприятий приняли на себя важную направляющую роль, что помогло склонить заинтересованных лиц в пользу осознания необходимости регулирования и содержания рынка в надлежащем состоянии. Такое изменение отношения может повысить устойчивость результатов от реализации мероприятий.

Resumen

Aplicación de la guía para mercados de alimentos saludables en dos mercados indonesios con el fin de reducir la transmisión de la «gripe aviar»

Situación La Organización Mundial de la Salud (OMS) desarrolló una guía con 10 medidas de control para reducir la transmisión del virus de la gripe aviar A(H5N1) en mercados en entornos con escasez de recursos. Nunca se describieron los aspectos prácticos de la aplicación de dicha guía.

Enfoque La guía de la OMS se aplicó en dos mercados indonesios de la ciudad de Makassar con el fin de intentar reducir la transmisión del virus A (H5N1). La guía se hizo más funcional a través un enfoque participativo para introducir una combinación de cambios tanto en las infraestructuras como en los comportamientos.

Marco regional La gripe aviar es endémica en las aves de Makassar. Para este estudio se eligieron dos de los 22 mercados de aves deteriorados y mal gestionados de la ciudad. Antes de la intervención, ninguno de los dos mercados seguía ninguna de las 10 medidas de control recomendadas por la OMS, exceptuando la de procesamiento en lotes.

Cambios importantes Tras la intervención, se observó una mejora considerable de los conocimientos de los participantes en el mercado sobre el virus de la gripe aviar A (H5N1). Empezaron a aplicarse las

recomendaciones de la guía de la OMS en cuanto a inspección visual, limpieza y prácticas de explotación avícola. Del mismo modo, los requisitos infraestructurales de distribución en zonas, suministro de agua y servicios públicos empezaron a adherirse a la guía de la OMS. Las soluciones de bajo mantenimiento como la instalación de sistemas de tratamiento de aguas residuales y los incentivos económicos como el del compostaje fueron bien recibidos y adecuados para este entorno con escasez de recursos.

Lecciones aprendidas La combinación de intervenciones para realizar cambios en las infraestructuras y en el comportamiento resultó fundamental en la puesta en práctica de la guía. A pesar de la resistencia inicial a los cambios de comportamiento, el enfoque participativo con consultas mensuales y sesiones educativas facilitó la adopción de unas prácticas seguras de manipulación de alimentos y de saneamiento. Las autoridades competentes asumieron un importante rol de liderazgo durante las intervenciones, lo que ayudó a cambiar actitudes respecto a las necesidades de regulación y de mantenimiento de los mercados. Este cambio podría potenciar la sostenibilidad de las intervenciones.

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Chapter 10

Discussion and Conclusions

The H5N1 virus is of international public health concern as it has the potential to trigger a pandemic if it acquires the capacity for efficient transmission between people (1). The current virus has caused the death of millions of birds and economic loss for farmers, and it has a high case fatality rate in humans. Despite these concerns and although many countries have been affected by outbreaks of the virus, gaps remain in the knowledge about the epidemiology of human infection and effective disease control measures. This is especially the case for low-resource countries such as Indonesia which are considered likely sites for pandemic emergence due to limitations in disease control, surveillance and public health management (1-3). Therefore, research is much needed to better inform and operationalize public health action.

This thesis presented six studies conducted in Indonesia that addressed two key aims:

- (a) To examine the epidemiology of human AI H5N1 infection, and
- (b) To inform disease control measures in LBMs in low resource settings.

Both aims address gaps in the literature and have important policy and future research implications.

The first aim was addressed by the three studies presented in Chapters 4-6. These studies added new information to the body of knowledge on human AI H5N1 infection. Chapter 4 found that an interplay of genetic susceptibility, age and types of exposure impact the risk of infection and clustering of cases. Chapter 5 was the first globally to quantify household attack rates, disease intervals and reproduction numbers for a large number of outbreaks. Chapter 6 was the first globally to report on contaminated fertilizer as a potential source of human AI H5N1 exposure.

The second aim was addressed by four studies presented in Chapters 7-9. These studies filled gaps in knowledge about LBM environmental contamination with the AI H5N1 virus and risk factors for this contamination (Chapter 7). The studies also highlighted the potential to control AI H5N1 viruses through the application of interventions designed specifically for low resource settings (Chapters 8 and 9), where they built on existing WHO guidelines and trialled the recommended interventions. This aspect of

the thesis was novel as the studies were the first globally to highlight the practical potential for AI H5N1 virus control in LBMs low resource settings.

The key conclusions arising from the various studies in this thesis are presented below. These are discussed in the wider context of AI H5N1 epidemiology and disease control, as well as the recommendations for policy and research.

Human AI H5N1 infection

Understanding the epidemiological characteristics of human H5N1 infection improves capacity to predict and respond to outbreaks (4). This thesis carefully assessed the epidemiology associated with cluster outbreaks. Clusters represent 36% of all cases detected in Indonesia between June 2005 and July 2009. Clusters are critical to the overall epidemiology of H5N1 since they can be an early warning signal for changes in virus behaviour. This is important to assess the virus' capacity for transmission between people and its pandemic potential. Overall, the thesis added evidence that the dynamics and risk factors for human AI H5N1 infection vary depending on the setting (1). A number of key conclusions were drawn regarding the risk factors for infection, clustering and transmission dynamics. These are discussed below.

Genetic susceptibility to AI H5N1 infection

The role of host genetics in AI H5N1 and other infectious diseases is increasingly being explored (5). The studies reported in Chapters 4 and 5 added evidence that there may be genetic predisposition to infection. Chapter 4 quantified the risk of infection for different genealogical relations to index cases, and found that first degree relatives, especially siblings, were at highest risk of infection. This does not imply that human-to-human transmission occurred in these outbreaks since common point source exposures could not be ruled out. However, it does suggest that there is a host genetic effect on susceptibility to infection from any source.

There is a need to acknowledge the potential for both bias and confounding in these findings. Since the findings relate to surveillance outbreak investigation data, there may

be case ascertainment bias, where cases in one household were more likely to be detected by the health system than cases from different households. As noted in previous studies (5, 6), this bias is likely to be occurring to some degree. However, in Indonesia, both family and other exposed populations such as neighbours, co-workers, students and healthcare workers were laboratory-tested for H5N1 infection during outbreak investigations. Despite casting a wide net to detect additional outbreak-associated cases, no additional cases were identified amongst non-blood relatives in any of the 139 outbreaks reported. This effect was also observed in other countries (4). Thus, bias alone does not seem to fully explain familial clustering.

Another potential explanation for these findings is confounding. Since family members share food, activities and the household, it is feasible that they share the high-risk exposures for infection. However, this also does not seem to offer a full explanation for the observed clustering amongst blood-related household members. For example, if household members share high-risk exposures, why were no clusters of genetically-unrelated spouses detected? The explanation that household members share high-risk exposures can be disputed. As presented in Chapter 4, not all cases had high risk exposures. Further, there is likely to be an ecological fallacy since many individuals with high risk exposures such as slaughtering infected birds did not develop infection. Thus, high-risk exposures alone do not seem to offer a full explanation for human infection with the current AI H5N1 virus.

Future studies including genome-wide linkage studies in affected families may be informative and further our collective understanding on the role of host genetics in AI H5N1.

Transmission dynamics for AI H5N1 outbreaks

Elucidating outbreak transmission dynamics is essential to enable pandemic risk assessment and to prompt rapid response to a virus capable of sustained human-to-human transmission (1, 7). The study reported in Chapter 5 was the first globally to quantify the transmission dynamics for a large number of AI H5N1 outbreaks (n=80). The findings demonstrated the value of using detailed household epidemiological data

to determine reproduction numbers, disease intervals and attack rates within households.

In the Indonesian dataset, one outbreak was considered an outlier based on its large size; eight cases compared to four or less cases in all other 138 outbreaks. To show the impact of the outlier on the transmission parameters, results were presented and compared for both (a) the outlier included, and (b) the outlier excluded (8). The findings were also compared with the previous Yang et al study (9) that assessed the transmission parameters for the outlier cluster alone, and helped place those findings in the larger context of all Indonesia outbreaks. The analyses found that most evidence for human to human transmission of the H5N1 virus came from this outlier cluster. This underscored the importance of checking for and handling outliers in the analysis process since they have the potential to skew findings and conclusions made (8). Specifically, the secondary attack rate and human transmission reproduction number found in Chapter 5 were much lower compared to the Yang et al study (9) that assessed these parameters for the outlier cluster.

One limitation of the study reported in Chapter 5 was that the number of secondary cases was small ($n=35$). This limited the type of models that could be used to explore transmission dynamics and it limited the parameters that could be quantified. Future studies utilizing global or cross-country datasets should be conducted to enable quantification of other parameters. Future research should also explore whether reproduction numbers changed over time to determine whether there have been any increases or decreases in transmission (10). This is necessary due to the virus' pandemic potential.

Exposures to AI H5N1 virus

Based on the analysis of the public health surveillance data in Chapters 4-6, a major conclusion of this thesis was that most human AI H5N1 cases were due to exposure to sick poultry or contaminated environments such as households, neighbourhoods or LBMs. Very few cases were likely due to human-to-human transmission of the virus. However, it is important to note that there is likely to be an under-reporting of cases

both in Indonesia and in other countries, and that the range and types of exposures may differ in reported cases from unreported cases. This case ascertainment bias may potentially limit knowledge of risk factor and epidemiological characteristics of the virus. Thus, prospective cohort studies in outbreak areas and studies that extensively assess potential exposures such as that reported in Chapter 6 are required to address this limitation. Chapter 6 involved extensive laboratory testing of samples collected from various environmental sites and from contacts to explore potential exposures and the magnitude of the outbreak. The study found that contaminated garden fertilizer may be a possible source for human AI virus infection but that the outbreak was limited to two cases.

According to a systematic review published in 2011 on H5N1 exposures for human infection, the relative risk of different exposures remains unknown (11). In that review, exposures were categorized into two groups as summarized in Table 10.1 below. In this thesis, exposures were categorized into three groups that represent hypothesized differences in risk (Table 10.1). An important difference was in the direct exposure group. In the thesis, direct exposure denoted all direct contact and handling of birds as per the systematic review, but the category also included cases that had sick/dead birds in the household. This was done to denote the likely higher risk of exposure in one's home compared to other settings such as the neighbourhood or LBM.

Table 10.1: Categories of exposures for human AI H5N1 infection

	Systematic Review, 2011	Thesis
Category 1	Bird (direct contact) • Food preparation (e.g. chopping carcass, defeathering) • Bird care (e.g. feeding) • Bird slaughter (e.g. culling)	Direct • Handled sick/dead birds • Handled bird products (e.g. restaurant worker) • Sick/dead birds in home
Category 2	Other (no direct contact) • Environmental contamination (e.g. home, village, LBM) • Fomites (e.g. farm vehicle) • Intermediate host (e.g. cat)	Indirect • Bird deaths in neighbourhood • LBM visit • Healthy birds in neighbourhood
Category 3	-	Other • Inconclusive but exposed to prior case • Inconclusive despite investigation

The findings from Chapter 4 supported this approach to exposure categorization, where direct exposure to sources of virus was more likely to result in case clustering. This suggests that the relative risk of exposures classified as direct is higher than exposures classified as indirect. This hypothesis warrants further investigation and can lead to quantification of the relative risks for the different exposure categories. It can also help determine what constitutes meaningful exposure and the denominators for estimating the risk of infection in outbreaks (1).

Effect of age on AI H5N1 infection

This thesis found that young people (5-30 years) were at greater risk of infection (Chapters 4 and 5). This supports findings from other countries (4). Reasons for this age-related risk were not explored but may be a result of (a) socio-cultural practices for bird-rearing, (b) heightened immunological susceptibility to infection or (c) hygiene and

sanitary behaviours. These warrant further investigation as they may help in the design of age-relevant risk communication messages.

Control of AI H5N1 in live bird markets

Chapters 4-6 highlighted that the majority of human AI H5N1 infection result from zoonotic transmission of the virus. These findings reiterate the importance of controlling the virus in birds and at the human-animal interface to minimize the risk of human infection (12). Since LBMs have been shown to be a source of human infection and can propagate outbreaks in birds, they are an important site for intervention (13-19).

Even though LBMs are commonplace in both high and low resource countries, much of what is known about the control of avian influenza viruses in LBMs comes from high resource countries such as the United States (18, 20-22). Prior to this thesis, published studies on LBMs from low resource countries such as Indonesia, China, Viet Nam and Egypt reported virological surveillance findings and presented case reports for human infections with LBM exposure (19, 23-27). This thesis was the first to explore implementation of disease control measures in LBMs in a low resource setting. Three major conclusions arise from the studies conducted in this thesis.

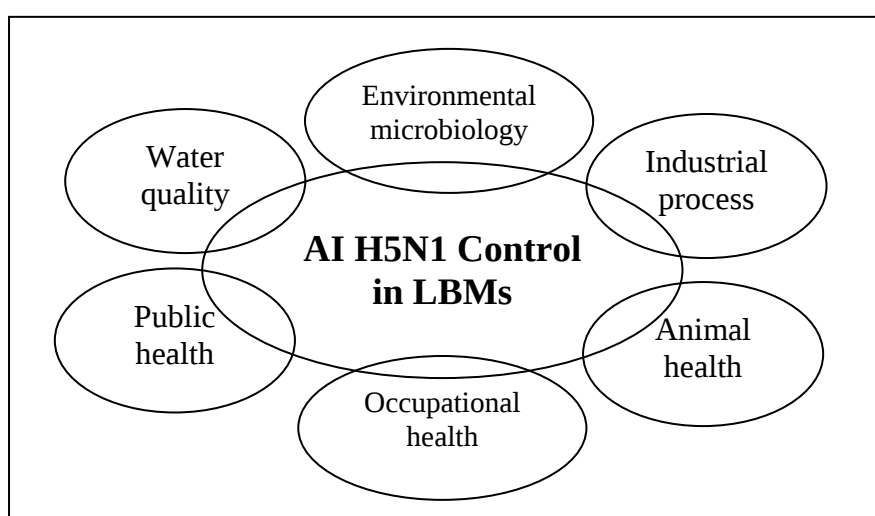
Potential for AI H5N1 virus control in LBMs in low resource settings.

The identification of CCPs (Chapter 8) and the field intervention trial (Chapter 9) added evidence and tools that controls for AI H5N1 virus can be applied in LBMs in low resource settings. Even though the virus cannot be eliminated from the LBM environment as long as outbreaks occur in farms supplying birds (12), the thesis showed that control measures can be tailored and applied in LBMs in Indonesia.

The field intervention trial was the first globally to demonstrate that comprehensive control measures can be practically implemented in LBMs in a low resource setting. Recognizing that there are many ways in which contamination can occur, a holistic approach was applied combining both infrastructure and behaviour change interventions. This holistic approach, operationalised in a participatory manner, was

aligned with the WHO guideline applied in the trial as well as the CCPs identified in Chapter 8. As can be seen in Figure 10.1, the interventions were multi-faceted reflecting the connection between the various disciplines in pathogen control. This approach is commonly used to address food safety issues in both high and low resource countries, where hazard control is predicated on various interventions including water quality assurance, improvement in workflow and better management of live animals (28).

Figure 10.1: Interrelationship between various disciplines in control of AI H5N1 virus in LBMs



There were two main limitations to the field intervention trial that impact the conclusions made in this thesis. First, the trial was not conducted using an experimental research design which would have offered more robust evidence. There may have been selection bias since LBMs were selected based on market manager willingness to participate. Thus, these LBMs might have had a greater likelihood of a successful outcome. Further, since the study was limited to two LBMs, the findings are not necessarily representative of all LBMs. Recognizing these potential biases, it was not possible to apply an experimental design as it was outside the financial scope of this PhD, but it is recommended as a future research step.

Second, the trial did not assess the impact of the control measures on AI H5N1 virus contamination in the LBMs. The study reported in Chapter 9 focused solely on the practical application of the interventions to identify lessons learnt for future application.

Thus, future research is needed to assess the direct impact of interventions on virus presence in LBMs.

Cross-benefits of interventions for AI H5N1 in LBMs.

Interventions for AI H5N1 virus in LBMs have the potential to control other pathogens. As discussed in Chapter 8, the H5N1 control measures may yield benefit for foodborne pathogens such as *Salmonella* and *Campylobacter*. Previous research published on avian influenza control in LBMs did not consider cross-cutting benefits of the interventions. Since most intervention research was conducted in high resource countries where control for other pathogens may already exist, it is possible that the linkages did not warrant investigation. However, for low resource countries where food safety, regulation capacity and infrastructure are less developed (12), demonstrating the cross-cutting benefits of interventions in LBMs may play an important role in advocating for change. As countries like Indonesia develop, they move towards targeted interventions for specific hazards and gain greater capacity for disease surveillance, risk assessment and regulatory practice (28). Thus, studies that show interventions can carry cross-cutting benefits for public health provide stronger rationale for government investment.

AI H5N1 surveillance in LBMs

A recent systematic review on exposure pathways at the human-animal interface found gaps in knowledge on potential routes of AI H5N1 virus transmission from contaminated environments to humans (11). The Chapter 7 findings add to this body of knowledge by identifying environmental sites that can be contaminated in LBMs.

Chapter 7 showed that AI H5N1 virus can be found throughout LBMs especially in the slaughter and sale zones. The sites and risk factors for contamination support findings from previous research (29-31). Widespread contamination in LBMs raises two key issues. First, it emphasizes the need for disease control to minimize the risk to human health for both workers and consumers in LBMs.

Second, the findings should be explored to determine sites most efficient for virus surveillance programs. Previous research conducted in Hong Kong suggests poultry feeding water as a potential surveillance site (31). However, this thesis found sites more commonly contaminated including tables for meat display, weigh scales and carcass processing tables. Further, since publication of the study reported in Chapter 7, the MOA in Indonesia has used the five most commonly contaminated sites in the routine surveillance conducted in LBMs in the wider-Jakarta region (personal communication). The surveillance at these sites continues to yield high rates of positivity of up to 60% of samples tested. Thus, the development of an international standard protocol for avian influenza virus surveillance in LBMs based on such findings would be beneficial for authorities monitoring virus prevalence and for researchers monitoring virus evolution.

Recommendations for policy

The findings from the various studies in the thesis are directly relevant to policy and policy implementation. The findings about the epidemiology of human AI H5N1 infection inform future outbreak investigations and warrant updates in Indonesia's curricula for training rapid response teams for AI H5N1 outbreaks. The findings can also be used to revise the outbreak investigation protocols to include the collection of more detailed data on household contacts and microbiological investigations of possible environmental sources of infection.

For LBMs, the studies reported in this thesis warrant updates to the MOH's guidelines for improving the health of food markets. The guideline can reflect the various findings for prioritizing LBMs for intervention, types of intervention and the process of change. These should be disseminated to district level officials who have the mandate and direct responsibility for LBMs.

Recommendations for future research

Reflecting back at the various studies undertaken in this thesis, I would have liked to explore a number of topics both more broadly and in greater detail. For example, for my

studies on the epidemiology of human AI H5N1 infection, I would have also liked to evaluate the MOH surveillance system for human AI H5N1 infections. The studies in Chapters 4 and 5 showed that information on household contacts of cases was only available for 80 of the total 139 outbreaks. Reasons for the missing data could have been explored and remedied.

However, due to both time and financial limitations, such endeavours were not feasible. Nevertheless, all the studies reported in this thesis were shared with stakeholders at the MOH and MOA. This may help authorities identify and follow up future research priorities pertaining to the human disease epidemiology and control of the AI H5N1 virus in LBMs.

In this chapter, I have highlighted specific areas for future research in the area of AI H5N1 infection in humans and in disease control efforts in LBMs. These specific research needs are summarized below:

1. Role of host genetics in AI H5N1 infection – to better understand the mechanisms for infection and risk groups for the zoonotic infection.
2. Transmission dynamics – exploration of the global dataset to enable quantification of various parameters and comparison between countries.
3. AI H5N1 exposures to infection – to assess the relative risk of various exposures and establish denominators for estimating risk for infection during outbreaks to better support contact tracing and additional case detection efforts.
4. Age – to explore the reasons for age-related risk and determine their impact on disease control programs including risk communication messaging.
5. LBM intervention trials – conduct trials to assess impact of interventions on AI H5N1 virus control in LBMs as well as cross-benefits for other pathogens such as foodborne pathogens associated with bird slaughter.
6. AI H5N1 surveillance in LBMs – further exploration of environmental sites efficient for routine surveillance programs, and the development of a standard protocol for virological surveillance in LBMs.

Outcomes and conclusions

This thesis explored host-pathogen interaction, disease dynamics and distribution, and risk factors for pathogen spread and human infection. The thesis applied a variety of research methods and emphasized operational and implementation research. The thesis added evidence and tools that support the development and implementation of disease control programs for AI H5N1 virus in Indonesia and potentially other virus-endemic countries. Collectively, the findings and conclusions made will help public and veterinary health authorities in addressing the current risk posed by AI H5N1 and in the early detection of a virus with the capacity for human transmission.

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