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Spatial Risk Assessment of the Zoonotic Influenza A (H7N9) along the Live Meat Chicken Market Chain in Southeast China

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Abstract

Introduction: A new reassortant avian influenza A (H7N9) virus of low pathogenicity to poultry emerged in eastern provinces of China in early 2013. There have been five epidemic waves causing more than 1600 human infections in 29 provinces and municipalities in mainland China till May 2018. Exposure to H7N9 infected live meat chickens at live bird markets (LBMs) within the affected provinces was suggested to be the main risk factor for human infection. Previous studies demonstrated the role of poor biosecurity measures at poultry farms and LBMs and the role of live poultry trade in the dissemination of avian influenza (AI) virus. However, the continued and increasing number of reported human H7N9 cases throughout China indicated that vulnerabilities linked to the live meat chicken market chain remain, that need to be elucidated in order to better design surveillance programmes and health promotion interventions to prevent poultry and human exposure along the live meat chicken market chain.

Aims: The overall aim of the research in this thesis is to define the risks of sustained transmission of H7N9 virus along the live meat chicken market chain in eastern China. To meet the aim, studies were sequentially designed with the following specific objectives: 1) identify the effect of market-level risk factors on avian influenza infections in poultry and humans and generate evidence that will inform avian influenza prevention and control programs at LBMs; 2) to understand the role of live poultry movement and live bird market biosecurity in the epidemiology of H7N9 during the emergency; 3) to understand the level of knowledge, attitudes and practices (KAP) on avian influenza of different actors along the live meat chicken market chain and the risk factors associated with their KAP levels; 4) to develop a spatial risk assessment model of the live chicken market chain within the H7N9 high risk area, to provide essential evidence and recommendations for risk-based AI surveillance programs and appropriate enhancements to current prevention and control policies for H7N9 in the study area.

Methods: To address each of the specific objectives four studies have been designed. Firstly, we performed a systematic literature review and estimated the pooled odds ratios of biosecurity indicators relating to human and poultry AI infections at market level using a quality effects meta-analysis model. Secondly, we identified the biosecurity risk factors associated with the H7N9 presence in the surveyed LBMs in the affected provinces during the H7N9 emergency response. We also used social network analysis and spatial analysis to quantify the connectivity of counties in Eastern china via live poultry movements and identify highly connected areas associated with human cases. Then, to quantify differences in KAP of AI among different actors (chicken farmers, live chicken vendors and consumers at LBMs) along the live chicken market chain operating within the areas in the Eastern provinces (i.e.

Shanghai, Jiangsu, and Anhui) identified in the previous study we designed and implemented a cross-sectional KAP questionnaire survey. Using multivariable generalized least squares random-effects regression models we identified predictors of KAP of AI among different actors. Subsequently, to evaluate the temporal relationship between the onset of human H7N9 infection and poultry serological and virological surveillance results we conducted time-series cross-correlation analysis. Lastly, to predict the geographical risk of H7N9 human infection in selected provinces in Southeast China we built a Bayesian Conditional Autoregressive Model accounting for the presence of poultry virological positives and wholesale LBMs, number of retail markets, network centrality of meat chicken movements, as well as human population and chicken density at county level.

Results: Biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs include smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, ban on overnight storage and sourcing poultry from local areas. Our meta-analysis indicated that higher risk of exposure to AI infection occurred in workers at retail LBMs, female workers and those who contact ducks, conduct cleaning, slaughtering, defeathering or evisceration.

During the H7N9 emergency, chickens were the predominant poultry species traded by affected LBMs. The presence of H7N9 in LBMs was significantly associated with the type of LBMs and with LBMs that sold chickens to other markets. The chicken movements were significantly spatially clustered and was highest in counties from Jiangsu and Anhui provinces.

Our results indicate that KAP scores of chicken farmers were generally higher than for chicken vendors. However, chicken farmers who had worked for more than 15 years had significantly lower total KAP scores than those who had worked for less than six years. In addition, farmers who worked more than 15 hours in a day had significantly lower attitude scores than those who worked less than six hours. For chicken vendors, females and older age groups (>35-year-old) were significantly associated with a lower knowledge scores compared to their counterparts. Our results also indicate that practice scores were significantly higher in female vendors and those vendors who also conducted slaughter compare to their counterparts. Our results for consumers demonstrate that those who bought chicken at least once every month had better risk awareness of AI compared with those who bought chicken at least once every week. In addition, female chicken consumers had significantly better practice scores than male consumers.

Our time-series analyses indicate the peak of poultry H7N9 serological positives is followed by the human H7N9 infections with a two-month lag, and poultry H7N9 virological positives is followed by the human H7N9 infections with a one-month lag. Our results also indicated that the presence of wholesale LBMs, higher retail LBMs density, presence of poultry virological positives, higher degree centrality, high chicken density and lower human population density were significantly associated with human H7N9 incidence in the county. Lastly, a county level risk map demonstrating the relative risks of human H7N9 incidence in Southeast China was produced.

Conclusion: The results of this thesis demonstrate that failures in LBM biosecurity management and live meat chicken movement played an important role in the emergence and spread of H7N9. The most effective strategies to reduce AI market contamination identified in this study should be targeted to the larger size LBMs that are located at non-central city areas, sell and slaughter multi-species of live poultry. LBM workers directly involved in cleaning and poultry processing tasks should participate in occupational health and safety programmes. Our results suggest that risk-based health promotion interventions should be developed and implemented by both animal health (i.e. targeting farmers and vendors) and public health agencies (i.e. targeting consumers) to prevent the continuous incident cases of H7N9 human cases along the live chicken market chain in China. Our results indicate that poultry movement may be an important driver of the onset of human H7N9 infections, and poultry serological positives and virological positives can serve as a predictor for human H7N9 infections. It is recommended that regular monitoring of poultry movement and poultry infections at the counties identified in this Thesis will provide essential evidence for the early warning of H7N9 infections across China.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

1. Xiaoyan Zhou *, Youming Wang, Hualei Liu, Fusheng Guo, Suhail A Doi, Carl Smith, Archie C A Clements, John Edwards, Baoxu Huang, Ricardo J Soares Magalhães; Effectiveness of Market-Level Biosecurity at Reducing Exposure of Poultry and Humans to Avian Influenza: A Systematic Review and Meta-Analysis, The Journal of Infectious Diseases, , jiy400, https://doi.org/10.1093/infdis/jiy400

The concept and design of the methodology was formulated by XZ (80%) with the assistance of RJSM (20%). Protocol and search strategy were developed by XZ (80%) with the assistance of RJSM (20%). XZ was responsible for data management (100%), and data analyses (90%, with assistance from SAD 10%) and the interpretation of results (70%, in consultation with RJSM 20% and all co-authors 10%). XZ was responsible for drafting the manuscript (100%). XZ was responsible for revision of the final version of the manuscript (85%), taking account the comments and suggestions of RJSM (10%) and all co-authors (5%)

2. Xiaoyan Zhou *, Yin Li, Youming Wang, John Edwards, Fusheng Guo b, Archie C.A. Clements, Baoxu Huang, Ricardo J. Soares Magalhaes, The role of live poultry movement and live bird market biosecurity in the epidemiology of influenza A (H7N9): A cross-sectional observational study in four eastern China provinces, Journal of Infection (2015), http://dx.doi.org/10.1016/j.jinf.2015.06.012

The concept and design of the methodology was formulated by XZ (70%) with the assistance of RJSM (20%) and co-authors (10%). XZ was responsible for data management (100%), data analyses (100%) and the interpretation of results (75%) was discussed in consultation with RJSM (15%) and all co-authors (10%). XZ was responsible for drafting the manuscript (100%). XZ was responsible for revision of the final version of the manuscript (90%), taking account the comments and suggestions of RJSM (5%) and all co-supervisors (5%).

3. Xiaoyan Zhou, Yi Zhang, Chaojian Shen, Ailing Liu, Youming Wang, Qi Yu, Fusheng Guo, Archie C.A. Clements, Carl Smith, John Edwards, Baoxu Huang, Ricardo J. Soares Magalhães; Knowledge, attitudes and practices associated with avian influenza along the live chicken market chains in Eastern China: A cross-sectional survey in Shanghai, Anhui and Jiangsu; Accepted by Transboundary and Emerging Diseases.

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The concept and design of the methodology was formulated by XZ (80%) with the assistance of RJSM (20%). XZ was responsible for data management (100%), data analyses (100%) and the interpretation of results (80%) was discussed in consultation with RJSM (15%) and all co-authors (5%). XZ was responsible for drafting the manuscript (100%). XZ was responsible for revision of the final version of the manuscript (80%), taking account the comments and suggestions of RJSM (15%) and all co-supervisors (5%).

Other publications during candidature

1. H. Liu, X. Zhou, Y. Zhao, D. Zheng, J. Wang, X. Wang, D. Castellan, B. Huang, Z. Wang, R. J. Soares Magalhães, Factors Associated with the Emergence of Highly Pathogenic Avian Influenza A (H5N1) Poultry Outbreaks in China: Evidence from an Epidemiological Investigation in Ningxia, 2012, Volume64, Issue3, June 2017, Pages 746-753, https://doi.org/10.1111/tbed.12433

2. Zhijing Xu, Colleen L. Lau, Xiaoyan Zhou, Saipale Fuimaono, Ricardo J. Soares Magalhães & Patricia M. Graves, The extensive networks of frequent population mobility in the Samoan Islands and their implications for infectious disease transmission, Scientific Reports volume 8, Article number: 10136 (2018), http://dx.doi.org/10.1038/s41598-018-28081-x

Contributions by others to the thesis

The concept of my PhD was achieved through discussion with my advisory team. CAHEC provided access to H7N9 investigation data previously collected during the H7N9 emergency (study in Chapter 5) and also assisted with the primary data collection (KAP data in Chapter 6 and poultry movement data in Chapter 6 and Chapter 7) and assisted with the interpretation of the results. Dr Suhail A Doi (Australian National University) provided support and assistance in the development of the methodology of the meta-analysis in Chapter 4.

Statement of parts of the thesis submitted to qualify for the award of another degree

“No works submitted towards another degree have been included in this thesis”.

Research Involving Human or Animal Subjects

The ethical approval for conducting the interviews with chicken farmers, chicken traders and consumers in the markets in the counties in Jiangsu and Anhui provinces was provided by the Behavioural & Social Sciences Ethical Review Committee of the University of Queensland (Approval number: 2014001167, the ethics approval letter is attached in the Appendix E).

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Keywords

China, H7N9, epidemiology, market chain, live bird market, biosecurity, KAP, meta-analysis, spatial modelling, early warning

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FoR code: 0707 Veterinary Sciences, 40%

FoR code: 1117 Public Health and Health Services, 40%

FoR code: 0104 Statistics, 20%

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List of Abbreviations

AI	Avian Influenza
BN	Bayesian Network
EC	European Community
ECTAD	Emergency Centre for Transboundary Animal Diseases
EMPRES-i	Emergency Prevention System
FAO	Food and Agriculture Organization of the United Nations
GLEWS	Global Early Warning System
HA	Haemagglutinin
HPAI	Highly Pathogenic Avian Influenza
LPAI	Low Pathogenic Avian Influenza
LBMs	Live Bird Markets
LPTs	Live Poultry Traders
MARA	Ministry of Agriculture and Rural Affairs of the People’s Republic of China
MoF	Ministry of Finance of the People’s Republic of China
NA	Neuraminidase
NGO	Nongovernmental Organization
NHC	National Health Commission of the People's Republic of China
OFFLU	OIE-FAO Avian Influenza Network
OIE	World Organization for Animal Health
SAIC	State Administration for Industry and Commerce of the People's Republic of China
SAMR	State Administration for Market Regulation of the People's Republic of China
SNA	Social Network Analysis
TADS	Transboundary Animal Diseases
USAID	United States Agency for International Development

WAHID	World Animal Health Information Database
WAHIS	World Animal Health Information System
WB	World Bank
WHO	World Health Organization

Chapter 1 Introduction

1.1 Background

During the last three decades, China's poultry sector has shifted from a predominantly traditional, backyard husbandry system to specialised and commercial intensive systems [1]. There has been an enormous growth of poultry production to meet strong and increasing consumer demand [2]. However, the fast growing concentration of poultry sector provides increasing opportunities for rapid spread of pathogens [3]. One of the major challenges that the poultry sector in China is facing are avian diseases especially avian influenza (AI) which can seriously disrupt the order of the industry and pose the threat of sporadic spillover to human population.

Since late 2003, AI has become one of the most publicized emerging infectious diseases. This followed the detection of highly pathogenic avian influenza (HPAI) caused by the H5N1 subtype in many countries in Asia. These Asian-lineage HPAI viruses produced fatal disease in poultry, wild birds, humans and other mammals, with subsequent spread of disease to some 60 countries across three continents. Affected countries and the international donor community have mobilized hundreds of millions of dollars to assist in controlling this disease, mainly because of concerns about the potential of these viruses to unleash a global pandemic of human influenza [4].

In early 2013, a new reassortant influenza A (H7N9) virus of low pathogenicity to poultry (LPAI) emerged, that caused human infections without preceding or concomitant outbreaks in poultry. There were six epidemic waves until September 2018 causing about 1,600 human infections in 29 provinces and municipalities in mainland China [5]. H7N9 ranked no. 1 in the Influenza Risk Assessment Tool (IRAT) by the CDC Influenza Division in 2017 [6].

Generally occupational exposure to infected live poultry and environment is known to be an important risk factor for AI human infections and exposure to H7N9 infected poultry at LBMs has been implicated as the main risk factor for human infection [7, 8]. Previous studies demonstrated the role of poor biosecurity measures at poultry farms and LBMs and the role of live poultry trade in the dissemination of avian influenza (AI) virus [9, 10]. However, continued and increasing numbers of reported human H7N9 cases throughout China indicated that vulnerabilities linked to the live meat chicken market chain remain which need to be elucidated to better design surveillance programmes and health promotion interventions to prevent poultry and human exposure along the live meat chicken market chain.

Indeed, during late 2016 to early 2017, described in the literature as the fifth epidemic wave, there was a sudden increase of human cases and the geographical distribution of cases was more widespread than in the previous four waves [11, 12]. In February 2017, the LPAI H7N9 virus mutated to become highly pathogenic avian influenza (HPAI) H7N9 virus in poultry and rapidly spread to other provinces of China [13]. As a result, in Feb 2017, the Ministry of Agriculture and Rural Affairs of China (MARA of China) recognising the importance of the live poultry markets in the exposure and dissemination of the virus, established the "1110 policy" on LBMs, i.e. clean once a day, disinfect once a week and close market once a month, and zero overnight storage in market. In July 2017, the "National Immunization Program for HPAI" in the poultry sector began with the adoption of a H5 and H7 bivalent inactivated vaccine. While this vaccine has been effective at controlling the number of H7N9 outbreaks in humans, the virus is still present at lower levels, and in the long-run vaccination alone may not help curb the exposure of humans to these viruses in the live meat chicken market chain. Therefore, a better understanding of the social determinants of exposure is necessary to complement sanitary measures such as vaccination and enhanced LBM biosecurity.

Much has been done in the past to investigate and control the disease in poultry. Most research into H7N9 in China has focused on the human risk factors and poultry-to-human transmission. However, the risk factors causing the bird-human cross-species transmission of influenza A (H7N9) remains unknown. The consensus from the literature is that exposure to H7N9 infected poultry at LBMs is the main risk factor for human infection and live meat chickens are considered to be the species with an important role in the transmission for H7N9 influenza [14-18]. Available evidence indicates that LBMs can serve as potential hubs where AI viruses are transmitted and maintained for prolonged periods of time [19-22]. Therefore, interventions at this stage are the most effective prevention measures. Surveillance and monitoring activities for AI within the poultry market chain (i.e. farms, transport, LBMs and slaughter houses) are important tools to generate epidemiological evidence on affected species, geographical sources of infection and the role of modifiable risk factors on disease transmission [23].

1.2 Research justification

The ongoing sporadic detection of AI viruses such as HPAI H5N1 and H7N9 in China demonstrate that these viruses are now well established within the poultry production and marketing system in specific areas across China. Available evidence indicates that these two viruses have very different epidemiological characteristics in that a) H5N1 is a highly pathogenic virus to both human and poultry [24] whereas H7N9 is of high pathogenicity to humans and low pathogenicity to poultry [16], has recently mutated to be high pathogenicity to humans [25]; b) H5N1 has lower seasonality while H7N9 is more highly seasonal [26]; c) H5N1 mainly affects chicken, duck and geese while H7N9 affects mainly chicken [27]; d) H5N1 viruses have spread globally while H7N9 have not spread in poultry outside China [28]. Despite these key differences available evidence demonstrates that they co-circulate in areas of Eastern China where the risk for human infection has been demonstrated to be highest [29]. Controlling the public and animal health consequences of the disease associated with these infections requires, firstly, understanding of the chicken production systems and how the stakeholders operate and the decisions they make within the chicken systems, secondly, the evaluation of disease risks within the poultry production systems and of control measures to reduce those risks. The first issue involves what in economics is called “value chain analysis”; the second issue entails what in veterinary epidemiology is called “risk analysis” [30].

Previous studies demonstrated the role of poor biosecurity measures at poultry farms and live bird markets [21, 31-38], and the role of live poultry trade in the dissemination of AI viruses [20, 39-42]. However, little is known about live chicken market chains, live meat chicken trade or the extent and frequencies of live meat chicken movements from the farms to markets in areas where AI viruses continue to circulate.

A recent study in China demonstrated that areas of human H7N9 infection overlap with those that reported H5N1 suggesting a common high risk area in an area southeast of Taihu Lake (in the south of Jiangsu Province), bordering the provinces of Anhui and Zhejiang [29]. There is a strong need to design and conduct empirical studies in high risk areas to collect live meat chicken movement data at different points in the poultry marketing chain and integrate that information with data from risk perception and attitudes of actors in the live chicken market chain towards biosecurity.

Besides, to our knowledge there are very few studies that provide a comprehensive account of the Chinese poultry production and marketing system and identify how the different components of this system are associated with AI prevention and control. Therefore, there is a strong need to understand the whole live meat chicken market chain (production, trading, marketing and consumption) and the key points in the chain in south of China where have the highest connectivity in terms of poultry movement, high yellow chicken production and tradition of consuming this type of chicken.

There are very few examples in the literature of field epidemiological investigations that involve primary data collection at different key points along the chicken market chain in areas where H7N9 viruses continue to circulate to answer research questions a) What is the relative importance of biosecurity indicators at market level in China; b) What is role of live poultry movement and live bird market biosecurity in the epidemiology of H7N9 during the emergency?; c) What is the knowledge, attitudes and practices and biosecurity status of different points in the live meat chicken market chain? d) What is the risk distribution of H7N9 infections at county level in the identified high-risk area? These research questions are essential for the development of appropriate and targeted recommendations for active H7N9 surveillance programs in the future.

1.3 Research aims and objectives

The overall aim of the research in this thesis is to identify and quantify the risks of sustained transmission of H7N9 viruses along the live meat chicken market chain in eastern China

To meet the aim, studies were sequentially designed with the following specific objectives:

1) to identify the effect of market-level risk factors on avian influenza infections in poultry and humans and generate evidence that will inform avian influenza prevention and control programs at LBMs;

2) to understand the role of live poultry movement and live bird market biosecurity in the epidemiology of H7N9 during the emergency;

3) to understand the level of knowledge, attitudes and practices (KAP) on avian influenza of different actors along the live meat chicken market chain and the risk factors associated with their KAP levels;

4) to develop a spatial risk assessment model of the live chicken market chain within the H7N9 high risk area, so to provide essential evidence and recommendations for risk-based AI surveillance programs and appropriate enhancements to current prevention and control policies for H7N9 in the study area.

1.4 Significance of the research

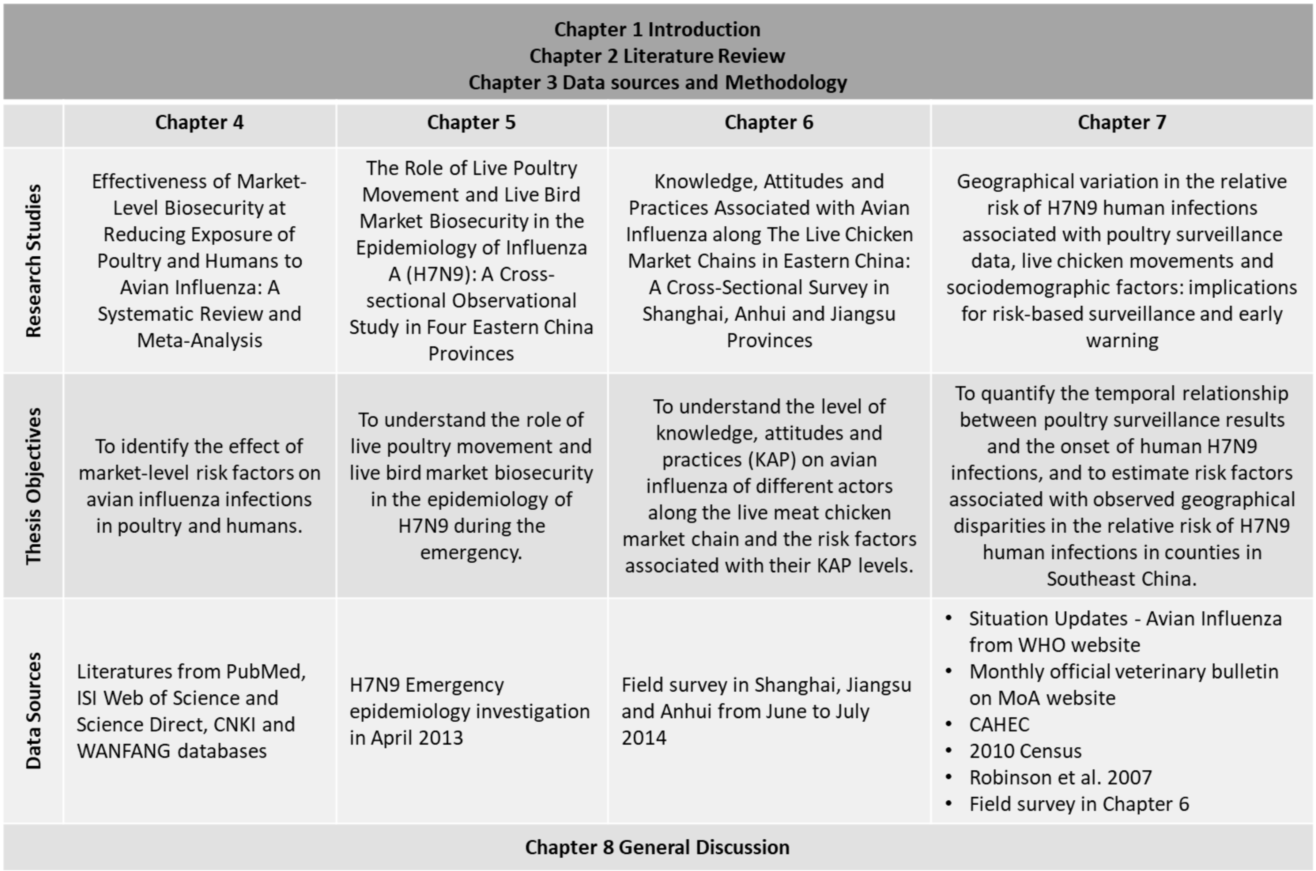
The spatial risk assessment of H7N9 in Southeast China that engages in the understanding of the risk factors that modify the risk profile at different stages of live meat market chain will lead to the development of more robust policies, better resource allocation decisions, more efficient and cost-effective disease control and reduced H7N9 transmission and associated morbidity and mortality in human and poultry populations.

This research will be the first of its kind in several ways: it will be the first to systematically examine biosecurity risk factors associated with AI infections at LBMs level; it will be the first to conduct a comprehensive risk-based survey at all the stages of the live meat chicken market chain in the identified highly connected area of live meat chicken movement in eastern China; and it will be the first time to develop a spatial risk assessment model for the control of H7N9 along the live chicken market chain in Eastern China.

The project will be significant in that it will lead to improvements in how policy makers in China guide interventions to improve the biosecurity of the market chain for live chickens. This will include improved planning of resource allocation in counties that are most at risk of H7N9 infection and efficient evaluation of the impact of biosecurity measures on H7N9 infection indicators.

1.5 Structure of the Thesis

This Thesis consists of eight Chapters (Figure 1-1). an introductory Chapter (Chapter 1), followed by a literature review (Chapter 2), description of data sources and methods (Chapter 3), and four research Chapters (Chapter 4, Chapter 5, Chapter 6, and Chapter 7), and a general discussion (Chapter 8). All of the eight Chapters of the Thesis start with a brief introduction to the context of the Chapter to explain how it fits with the overall structure of the Thesis. References for all Chapters appear at the end of the Thesis.



Chapter 1 Introduction
Chapter 2 Literature Review
Chapter 3 Data sources and Methodology

	Chapter 4	**Chapter 5**	**Chapter 6**	**Chapter 7**
Research Studies	Effectiveness of Market-Level Biosecurity at Reducing Exposure of Poultry and Humans to Avian Influenza: A Systematic Review and Meta-Analysis	The Role of Live Poultry Movement and Live Bird Market Biosecurity in the Epidemiology of Influenza A (H7N9): A Cross-sectional Observational Study in Four Eastern China Provinces	Knowledge, Attitudes and Practices Associated with Avian Influenza along The Live Chicken Market Chains in Eastern China: A Cross-Sectional Survey in Shanghai, Anhui and Jiangsu Provinces	Geographical variation in the relative risk of H7N9 human infections associated with poultry surveillance data, live chicken movements and sociodemographic factors: implications for risk-based surveillance and early warning
Thesis Objectives	To identify the effect of market-level risk factors on avian influenza infections in poultry and humans.	To understand the role of live poultry movement and live bird market biosecurity in the epidemiology of H7N9 during the emergency.	To understand the level of knowledge, attitudes and practices (KAP) on avian influenza of different actors along the live meat chicken market chain and the risk factors associated with their KAP levels.	To quantify the temporal relationship between poultry surveillance results and the onset of human H7N9 infections, and to estimate risk factors associated with observed geographical disparities in the relative risk of H7N9 human infections in counties in Southeast China.
Data Sources	Literatures from PubMed, ISI Web of Science and Science Direct, CNKI and WANFANG databases	H7N9 Emergency epidemiology investigation in April 2013	Field survey in Shanghai, Jiangsu and Anhui from June to July 2014	• Situation Updates - Avian Influenza from WHO website • Monthly official veterinary bulletin on MoA website • CAHEC • 2010 Census • Robinson et al. 2007 • Field survey in Chapter 6

Chapter 8 General Discussion

Figure 1-1 Thesis structure

Chapter 1 of the Thesis provides a general background on H5N1 and H7N9 in China, the role of biosecurity risk factors along the live chicken market chain and current control measures on H7N9 in China. This chapter also elaborated the justification of the research on H7N9 and objectives of the research. It finishes with the significance of the research.

Chapter 2 is the literature review. It describes the current knowledge on the epidemiology of AI, specifically subtype H7N9 and H5N1, with a focus on the epidemiological risk factors associated with AI infections in both poultry and humans. This Chapter also reviews the current approaches to the prevention and control of AI infections in both poultry and humans. The literature review is divided into three main parts. The first part provides an overview of the Chinese poultry industry, and a review of chicken production and live chicken market chain in China. The second part describes the existing knowledge on the pathophysiology and epidemiology of AI in China, in specific, H5N1, H7N9 and H9N2 subtypes in China, with a focus on including disease history and spatial-temporal pattern in China, this part also reviews the risk factors impacting on AI and the role of live poultry trade in disease circulation.

The third part describes the approaches that have been adopted for the prevention and control of AI in poultry and human population globally and in China.

Chapter 3 describes the data sources and methods used in the research studies. A detailed description of H7N9 infection data, live poultry movement data and other risk factor are described in this Chapter. Analytical methods and models are also described in detail.

Chapter 4 presents a systematic review and meta-analysis of the relative importance of market level biosecurity risk factors (such as cleaning and disinfection, market closures, manure disposal and management practices from different studies) on human (market workers) and poultry AI infection (poultry and environment) at LBMs. I first performed a systematic literature review in both English and Chinese search engines. Then I estimated the pooled odds ratios of biosecurity indicators relating to AI infections at market level using a quality effects (QE) meta-analysis model. I found biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs included smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, ban on overnight storage and sourcing poultry from local areas. The findings indicated that higher risk of exposure to AI infection occurs in workers at retail LBMs, female workers and those who contact ducks, conduct cleaning, slaughtering, defeathering or evisceration. The most effective strategies to reduce AI market contamination identified in this study should target larger LBMs that are located at non-central city areas, sell and slaughter multi-species of live poultry. This Chapter has been published in the Journal of Infectious Diseases as a review paper.

In Chapter 5, I evaluated the biosecurity risk factors associated with H7N9 infections on LBMs during the emergency and identified the role of live poultry movement in the epidemiology of H7N9 human infections. I zoomed in to the originally infected area (Shanghai Municipality, Jiangsu, Zhejiang and Anhui provinces) and obtained a unique dataset collected during the emergency epidemiological investigation on the 24 LBMs within one kilometre of H7N9 human infections and those that marketed large quantities of poultry at the time of the outbreak. Then I used univariable analysis to identify the biosecurity factors associated with the H7N9 presence in LBMs and social network and spatial analysis to quantify the connectivity and geographic variation in the connectivity of poultry movements. This research has extended the knowledge of market-level biosecurity risk factors and enabled the stratification of the risk of H7N9 infection geographically. This Chapter has been published in The Journal of Infection.

In Chapter 6, a primary cross-sectional questionnaire survey was designed and conducted in the hotspot area identified in Chapter 5, from June to July 2014, after the second wave of H7N9 outbreaks in humans in Eastern China. All actors (chicken farmers, vendors and consumers at LBMs) along the live meat chicken market chain were targeted to profile their level of knowledge, attitudes and practices (KAP) towards avian influenza and the risk factors associated with their KAP levels. Multivariable generalized least squares (GLS) random-effects regression models were developed to identify predictors of KAP of AI among different actors along the live chicken market chain. I analysed determinants of KAP within each actor group. The results of Chapter 6 demonstrated that risk-based health promotion interventions should be developed and implemented by both animal health agencies (targeting farmers and vendors) and public health agencies (targeting frequent and male consumers) to prevent transmission of H7N9 along the market chain in China. This Chapter forms a manuscript submitted to the Journal of Transboundary and Emerging Diseases.

In Chapter 7, I assembled the most comprehensive dataset of key risk factors for H7N9 infection in humans, e.g. distribution of LBMs, chicken movement data collected from the study in Chapter 6, and other risk factors based on existing ecological studies (e.g. human population density, chicken density), as well as detailed spatio-temporal data of poultry H7N9 surveillance results. I applied a test of cross-correlation to quantify the temporal relationship between the onset of human H7N9 infections during 2013-2017 and poultry serological and virological surveillance results. I also developed a spatial CAR model that accounted for spatial clustering of incidence to estimate and map the relative risk of H7N9 human incidence in counties in Southeast China by assessing the relationship between human infections as an outcome and poultry surveillance results, live chicken movements and recognized demographic risk factors as predictors. The findings in Chapter 7 revealed the potential for poultry serological and virologic surveillance to anticipate human H7N9 infections and uncovered important geographical variation in the relative risk of human H7N9 incidence at county level in Southeast China. This Chapter is presented as a paper, which has been submitted for publication in Scientific Reports.

My thesis concludes with a discussion of the key findings, the major public health implications of the findings, study limitations and recommendations of possible pathways in future researches.

Chapter 2 Literature Review

2.1 Context

Prior to commencement of the literature review, a theoretical framework was designed to guide the content of the review (Figure 2-1). The specific objectives of this literature review were to a) demonstrate available evidence with respect to chicken production and market chains, with particular emphasis on the yellow meat chicken (going through LBMs) production which represents 50% of the total meat chicken production in China; b) provide a detailed account of the epidemiology and control of AI in China with particular emphasis on H7N9 (predominantly a chicken reservoir); c) identify gaps in the literature in relation to the role of production and marketing of chicken in the epidemiology of H7N9 in China. While focusing on meat chicken production and marketing systems and on the recent H7N9 strain, here we also reviewed evidence on other AI viruses (e.g. H9N2 and H5N1) and associated poultry marketing systems which also need consideration for a more comprehensive understanding of the epidemiology of the AI viruses currently circulating in China.

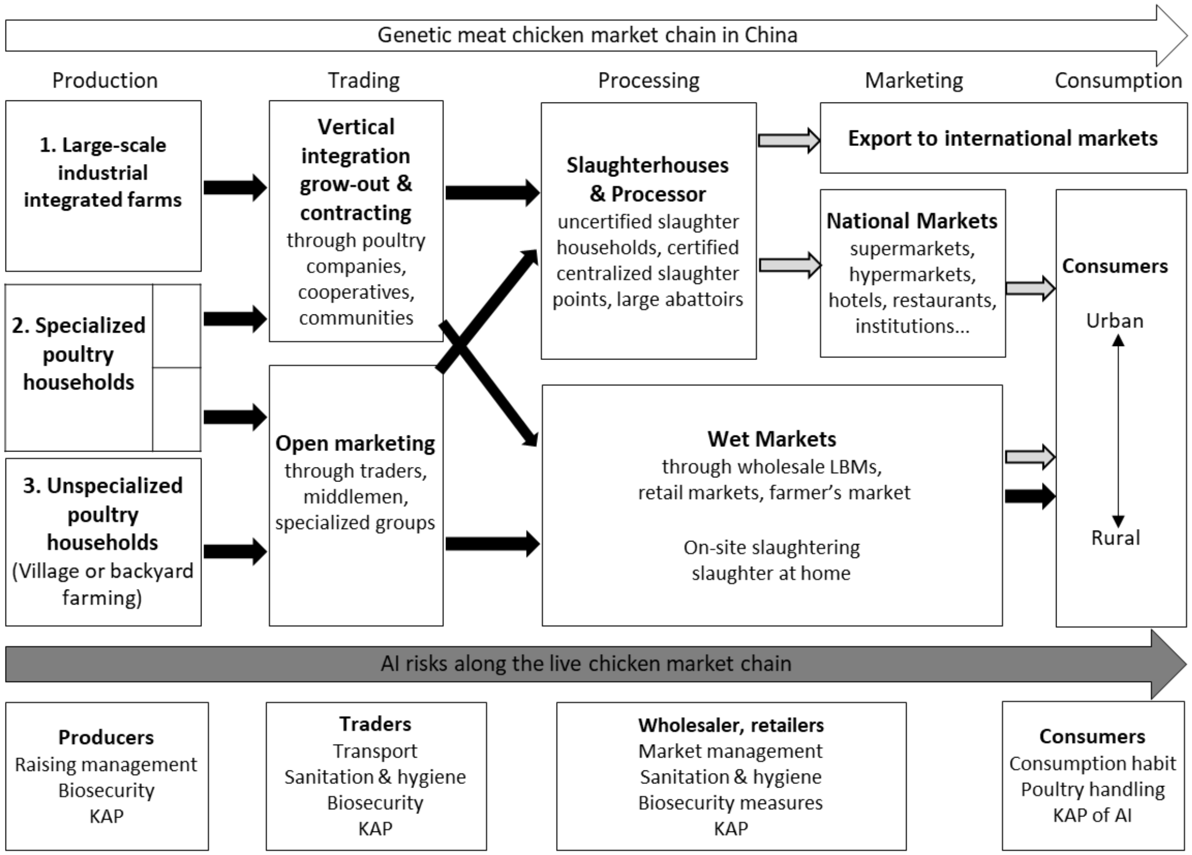


Figure 2-1 The outline of meat chicken industry and the theoretical framework of literature review

The literature review is divided into three main parts. The first part provides an overview of the Chinese poultry industry, and a review of chicken production and live chicken market chain in China (see Chapter 2.3). The second part describes the existing knowledge on the pathophysiology and epidemiology of AI in China, specifically H5N1, H7N9 and H9N2 subtypes in China, with a focus on including disease history and spatial-temporal pattern in China, this part also reviews the risk factors impacting on AI and the role of live poultry trade in disease circulation (see Chapter 2.4, 2.5, 2.6, 2.7). The third part describes the approaches that have been adopted for the prevention and control of AI in poultry and human populations globally and in China (see Chapter 2.8).

The literature review was conducted on published English scientific literature including all relevant articles that were published up until December 2018, identified from PubMED and Web of Knowledge. We supplemented the literature search with Chinese scientific literature using Chinese search engines Wanfang Data and CNKI. The search terms included various combinations from the following categories: poultry production sector, disease of interest, first, search terms on poultry production sector included the following terms: livestock, poultry, meat chicken and industry, production, trade, transport, supply chain, value chain, market chain; terms for the disease of interest included: avian influenza, bird flu, H5N1, H7N9, H9N2; the terms for risk factors included: risk factor or biosecurity, KAP; the terms for disease prevention and control included: prevention and control measures, vaccination, clean and disinfection, market closure, quarantine. Additionally, secondary searches were conducted in reference lists of peer-reviewed studies.

2.2 Overview of the Chinese poultry industry

2.2.1 The general situation of Chinese poultry production

China's poultry sector plays an important role in the national economy, nowadays, poultry meat and eggs are second largest sources of people's protein consumption after pork [43]. However, poultry meat and eggs were not traditionally an important part of the Chinese diet. They were considered luxury goods for consumption on special occasions [2]. Over the past three decades, poultry has been the fastest growing protein sector in China since the 1990s, China's per capita poultry consumption per year has increased from barely 1kg in 1978 to over 9kg in 2009 [44, 45].

Chinese poultry industry developed since 1980's and it experienced rapid growth in the 1990's, till 2000, the poultry production was ranked the largest in Asia, and second largest in the world behind the United States and in front of Brazil. And China is by far the largest egg producer in the world. In 2012, the number of world poultry stocks and output were about 24 billion and 64 billion respectively, the poultry meat production was 105.6 million metric tons. China had about 5.8 billion poultry in stock and 12 billion poultry output, 18.2 million metric tons of poultry meat and 28.6 million metric tons of egg produced by the end of 2012.

The poultry industry is, in some ways, the most vertically integrated and industrialized system of livestock production in China. Industrialization of the poultry sector started in 1984 with the introduction of foreign capital, technology and management expertise. Since then, there has been a rapid growth and concentration of large-scale commercial poultry production operations [1, 2]. In 2012, the output value of poultry production was 689.55 billion RMB, accounted for 25.36% of the China animal husbandry, and the output value of meat chicken and layer chicken was 408 billion RMB and 277.39 billion RMB respectively [46].

By the end of 2012, about 37 percent of China's total poultry population are located in the eastern region (Table 2-1), this region accounts for a much lower proportion (15 to 20 percent) of the number of farms with poultry, but a much higher proportion (over 60 percent) of poultry output. Measured in relation to geographical area, the poultry density in the eastern region is about 30 times as high as that in the western region. The central region has a density half as high as that of the eastern region. The top six poultry producing provinces are Shandong, Guangdong, Henan, Jiangsu, Guangxi and Liaoning (Figure 2-2), accounted for 53 percent of the total poultry production in 2012, while the largest egg producing provinces are Henan, Shandong, Hebei, Liaoning, Jiangsu and Sichuan, accounted for 62 percent of the total egg production in 2012.

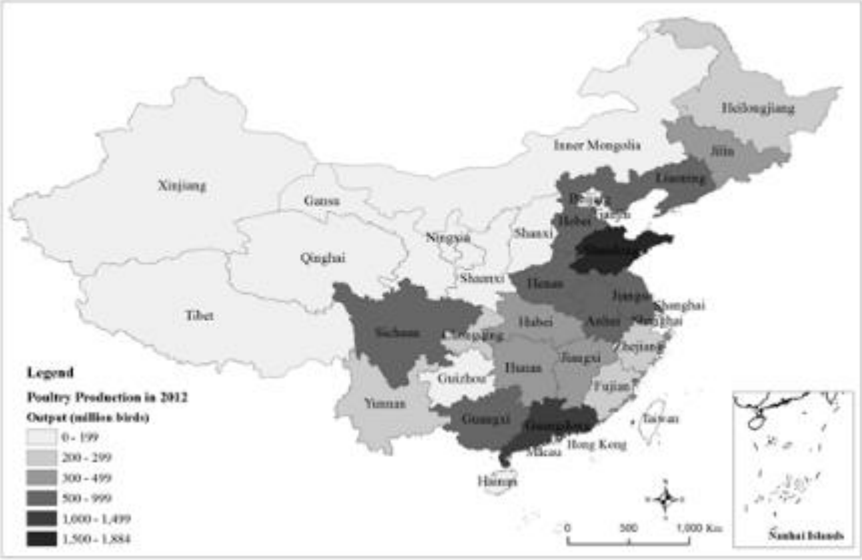


Figure 2-2 Poultry production by province in 2012

Table 2-1 Distribution of poultry production in Mainland China in 2012

Provinces	**Output (million birds)**	**Stock (million birds)**	**Poultry meat production (million tons)**	**Egg production (million tons)**
Northern	949.2 (7.9%)	560.4 (9.7%)	1.4 (7.9%)	5.1 (17.7%)
Beijing	100.9	26.0	0.2	0.2
Tianjin	80.1	25.4	0.1	0.2
Hebei	579.4	385.3	0.9	3.4
Shanxi	70.0	73.0	0.1	0.7
Inner Mongolia	118.9	50.8	0.2	0.5
North-eastern	1,381.9 (11.4%)	712.9 (12.3%)	2.4 (12.9%)	4.9 (17.1%)
Liaoning	766.8	403.5	1.3	2.8
Jilin	412.3	162.8	0.7	1.0
Heilongjiang	202.8	146.6	0.3	1.1
Eastern	4,482 (37.1%)	1,667 (28.7%)	6.8 (37.1%)	8.5 (29.6%)
Shanghai	36.5	11.8	0.1	0.1
Jiangsu	885.8	360.2	1.5	2.0
Zhejiang	251.5	114.5	0.4	0.5
Anhui	709.3	250.4	1.1	1.2
Fujian	284.4	91.9	0.4	0.3
Jiangxi	430.3	200.0	0.6	0.5
Shandong	1,884.4	638.2	2.8	4.0
Southern and Central	3,962.4 (32.8%)	2,017.8 (34.8%)	5.6 (30.8%)	7. (24.3%)
Henan	943.6	682.0	1.2	4.0
Hubei	498.7	325.2	0.7	1.4
Hunan	416.5	290.2	0.6	1.0
Guangdong	1,130.7	356.1	1.5	0.3
Guangxi	826.3	312.0	1.4	0.2
Hainan	146.6	52.3	0.3	0.0
South-western	1,142.6 (9.5%)	693.8 (12%)	1.8 (9.9%)	2.2 (7.8%)
Chongqing	222.2	125.8	0.3	0.4
Sichuan	620.0	360.2	0.9	1.5
Guizhou	96.3	83.6	0.2	0.1
Yunnan	204.1	124.3	0.4	0.2
Tibet	1.6	1.4	0.002	0.004
North-western	158.8 (1.3%)	152.4 (2.6%)	0.3 (1.4%)	1 (3.5%)
Shaanxi	48.7	67.5	0.08	0.5
Gansu	35.6	37.9	0.04	0.15
Qinghai	4.0	2.5	0.01	0.02
Ningxia	11.4	7.8	0.02	0.06
Xinjiang	57.6	35.3	0.10	0.3
Mainland Total	12,077 (100%)	5,804.4 (100%)	18.2 (100%)	28.6 (100%)

Note: data obtained from 2012 Chinese Animal Husbandry Yearbook

With many poultry markets closed in the wake of China’s worst-ever bird flu outbreak, local egg producers are being forced to shell out to feed and water chickens long after they would normally have been killed and sold for meat.

One of the major challenges the poultry sector in China facing is avian diseases high-risk epidemics such as AI, which can seriously disrupt the order of the industry. According to FAO, the economic loss due to HPAI H5N1 during 2003 to 2011 was about 20 billion USD [47]. According to a report from the China Animal Agriculture Association in February 2014, the direct economic loss caused by the two major waves of the H7N9 infections since 2013 was beyond 13 billion US Dollars and more than 40 million farmers were affected. A great number of poultry related companies had to stop business or even broke [48]. Understanding production and marketing systems and improving these systems is vital if gains are to be made in the control and prevention of H5N1 HPAI in endemically infected countries [49].

2.2.2 Main poultry species raised in China

In China there is an extremely large poultry industry and it contains domestic chicken, waterfowl (ducks, geese) and a small proportion of turkey, quail, pigeon and other special species [50]. The stock of the poultry birds, especially the chicken, increased dramatically over the last decade (Fig 3) [51].

Chicken is the always the predominant poultry species in China, accounting for 80% or more of the total poultry stocks, while the proportion of ducks stocks was approximately 14%, geese and others was around 6% of the total stocks in 2010 (Data from FAOSTAT).

China’s chicken meat production went through an enormous growth during the last three decades (1981-2012), increased tenfold from 1.2 million metric tons in 1981 to 13.2 million metric tons in 2012. In China, chicken meat is the second largest protein sector after pork, which accounted for 16% of the total meat production in China in 2011, while the proportion was 9% in 1990 (Pi, Rou et al. 2014). There is a very wide range of chicken production in China, mainly raised in eastern, central and northeast of China.

China’s waterfowl (ducks and geese) production is around 5.5 million metric tons annually during 2000 to 2010, which accounts for 75% of the world’s production [52]. Duck production grew with an annual growth rate of 3% in the past five years (2008–2013), slightly faster than the 2.5% growth of meat chicken during the same period [52]. Industrial duck meat costs less than meat chicken, which has driven increased consumption in factories and school cafeterias. The Chinese also increasingly perceive duck meat as healthier than other meats (with less fat and cholesterol). Duck meat is therefore also experiencing rapid industrialization. Ducks are produced in both extensive semi-intensive and indoor factory farms and many companies have a production capacity of 5 to 10 million ducks per year. Production and processing is rapidly being integrated. Since 2005, the Chinese government has particularly encouraged indoor intensive production of ducks because of the belief that this mitigates the risk of AI (Pi, Rou et al. 2014). Ducks and geese are mainly raised around the Yangtze River basin and provinces at the south of the basin. Domestic duck is considered to have played a key role in the genesis, persistence and spread of Asia-lineage HPAI H5N1 viruses. Ducks are relatively high value animals and are transported over long distances to markets. For example, in China it is known that they travel more than 400 km from inland provinces such as Hunan to coastal markets in Guangdong [53].

In 1984, China surpassed the US in the output volume of poultry eggs and became the largest poultry egg producer in the world (40 percent of the world's production). By 2012, the industry is suggested to have reached output volume of 28.61 million metric tons, growing at an annual growth rate of 1.77% since 1998 [54]. The provinces with the largest poultry egg output are Henan, Shandong, Hebei, Liaoning, Jiangsu, Sichuan, Hubei, Anhui, Heilongjiang and Jilin. The majority of the chicken eggs (over 95 percent) are consumed as table eggs and the remainder are processed [54]. Egg farming is more intensified and integrated than meat chickens, with 70 percent of eggs in 2005 coming from the largest factory farms which comprise nearly 2 percent of all egg producers (Pi, Rou et al. 2014).

2.2.3 Overview of poultry value chains in China

Value chains are groups of people linked by an activity to supply a specific commodity. These chains have inputs that are used to produce and transport a commodity towards a consumer, this is the supply market chain. [30] Value is added to the commodity through the supply market chain, as money is sent from the consumer to the different people in the chains. Value chains also describe the places where each process occurs, and the people involved. They can be a kind of flow chart or process map. The term "market chain" will be used through this review to represent this kind of supply market chain.

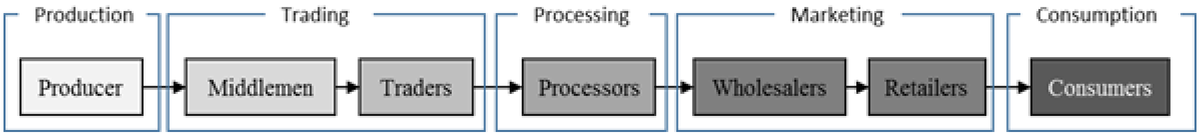


Figure 2-3 A schematic market chain model

A simplified market chain model is provided in Figure 2-3, it describes the key processes of bringing specific products from production, trading, processing and marketing before the commodity reaches the end consumer. Figure 2-1 shows a more extended framework of poultry market chain. Most poultry networks will contain all or most of these elements. It is very important to note that even within the same poultry species there will be many different chains, even for different products.

Networks and linkages in market chains that link production systems, markets and consumers constitute a “contact network” for contagious diseases and provide opportunities for transmission of disease within and between sectors. Therefore, these chains (networks) must be taken into account in planning risk management strategies for disease prevention and control [30].

2.2.4 General classification of the poultry production system in China

The Food and Agriculture Organization of the United Nations (FAO) has classified the poultry production systems into four sectors (Table 2-2). Sector 1 is described as the poultry production system for "industrial and integrated" production system; Sectors 2 and 3 describe "commercial poultry production system" with decreasing levels of biosecurity, respectively; and Sector 4 describes "village or backyard poultry production". [55]

Table 2-2 FAO’s classification of Poultry production sectors

Poultry production system	**Industrial and integrated production**	**Commercial poultry production**		**Village or backyard production**
Sectors	Sector 1	Sector 2	Sector 3	Sector 4
Biosecurity	High	Mod-High	Low	Low
Market outputs	Export and urban	Urban/rural	Live urban/rural	Rural/urban
Dependence on market for inputs	High	High	High	Low
Dependence on goods roads	High	High	High	Low
Location	Near capital and major cities	Near capital and major cities	Smaller towns and rural areas	Everywhere. Dominates in remote areas
Birds kept	Indoors	Indoors	Indoors/Part-time outdoors	Out most of the day
Shed	Closed	Closed	Closed/Open	Open
Contact with other chicken	None	None	Yes	Yes
Contact with other ducks	None	None	Yes	Yes
Contact with other domestic birds	None	None	Yes	Yes
Contact with other wildlife	None	None	Yes	Yes
Veterinary services	Own Veterinarian	Pays for veterinary service	Pays for veterinary service	Irregular, depends on govt vet service
Source of medicine and vaccine	Market	Market	Market	Government and market
Source of technical information	Company and associates	Sellers of inputs	Sellers of inputs	Government extension service
Breed of poultry	Commercial	Commercial	Commercial	Native
Food security of owner	High	OK	OK	From OK to bad

Source: A Strategic Framework for HPAI Prevention and Control in Southeast Asia, Emergency Centre for Transboundary Animal Diseases (ECTAD), FAO, Bangkok, May 2006

Similarly, China poultry production system can be classified into three major categories, namely industrial integrated farms, specialized poultry household and unspecialized poultry households [56].

Poultry raised through different production systems are traded differently. The poultry trading can be generally summarized into two major models, e.g. "vertical integration" (from production to processing to marketing) and open marketing [57, 58], under the two different trading models, the poultry are then marketed through different market chains to reach the end customer. (Figure 2-1)

The Industrial integrated farms are owned and operated by commercial companies (refer to Sector1; Table 2). The company, adopting a "vertical integration" - "grow-out" model [52], owns the majority of the whole market chain (from production to trade to marketing). The raising scale varies from hundreds of thousands to millions of poultry output every year. These farms invest heavily in large scale automated and environment controlled standard production facilities for better management, biosecurity and disease prevention. Employees on these farms are usually well-trained professionals. These farms have the highest level in terms of management, biosecurity, waste disposal and proper construction of supporting facilities, clearly defined and implemented standard operating procedures [56]. Therefore, only poultry raised from these high standard farms may meet the international market [59]. The governments from various levels have put out considerable preferential policies to support this kind of large scale and more efficient animal production systems [2].

Secondly, specialized poultry households usually operated by families, raising thousands or tens of thousands of poultry at one time as their primary business (Sector 2 and Sector 3; Table 2). This type of farm usually operates according to two different modes. One is specialized households which have contracts with poultry companies or cooperatives who represents farmers, known as "contract farming", also belonging to "vertical integration" model [52] (Sector 2; Table 2). This husbandry system remains the most popular business model in China. Under this type of system, companies contract farmers, and supply them with day-old poultry, poultry feed, vaccines and veterinary medicines, and usually deliver trainings on raising management and disease prevention and control regularly. Farmers are responsible for the land, raising facilities and labor. The farmer will be paid based on either the market price or on an agreed margin [52]. As members of commercial poultry companies, these farms receive a unified approach to vaccination, disease prevention and management. However, the biosecurity levels may vary on different farms due to different practices of different farmers. In general, the biosecurity level is considered lower than integrated farms.

Another type of specialized husbandry system are the self-run households (Sector 3; Table 2), these farms usually have their own network regarding supplements of day-old poultry and feeds, and veterinary services. However, due to the limit of financial resources and knowledge, their vaccination and disease prevention and control procedures are not well managed. These types of farms are considered to have a lower biosecurity level compared with those contracting households. [59]

Thirdly, unspecialized poultry households, mainly traditional backyard farms, are mostly located in the rural areas, poultry are raised in small courtyards as a sideline for the family income (Sector 4 in Table 2). In this type of husbandry system poultry are raised in a few dozens or hundreds of poultry. Their raising practices are very flexible in terms of sites, species, feeds, and times of feeding. Labor costs are fully supported by the household. Biosecurity measures, vaccination and a hygienic slaughtering process cannot be guaranteed at these small-scale farming operations. This type of farms is considered to have the lowest biosecurity levels. Poultry raised on these farms are usually consumed locally. These types of poultry are becoming more and more popular due to their flavor, and the unit price is much higher compared to the poultry raised on commercial farms.

It is expected that the intensification process will continue. Unspecialized households and small scale specialized non-contract farmers will continue their exit from the industry. It is possible that the number of poultry farms in China could halve by 2020, particularly for farms in eastern China [2, 59].

Figure 2-4 Backyard farming

(Note: Photo taken in a small village in Fujian province in Nov 2014 by Tao Yang from Fujian Animal CDC)

Figure 2-5 Commercial specialized farms

(Note: Photo taken in a farm Anhui province by Xiaoyan Zhou)

Figure 2-6 Industrialized poultry farms

(Note: Photo taken in a farm in Anhui province by Xiaoyan Zhou)

2.2.5 Identified gaps in knowledge

In the past two decades the poultry industry in China has undergone rapid industrialization and intensification of production. Because of the rapid industrialization there are some vulnerabilities within the three described poultry production systems in China, especially with respect to the biosecurity level and disease prevention and control measures. The standards of poultry production and management vary by poultry species, poultry type, geography, education level of farmers and the quality of local veterinary service. It has been recognized that an understanding of the poultry production systems is vital in the control and prevention of AI in endemically infected countries [30]. However, to our knowledge there are no studies that provide a comprehensive account of the Chinese poultry production and marketing system and identify how the different components of this system are associated with AI prevention and control.

2.3 Overview of the meat chicken industry in China

2.3.1 Evolution of China’s meat chicken industry

China’s meat chicken industry has gone through three development stages [45]. The first stage (1961-1978) is considered “Slow Growth”. Annual production of meat chicken increased from 0.49 million metric tons in 1961 to 1.08 million metric tons in 1978. Backyard farming was the primary production system during this period.

The second stage (1979-1996) is considered “Fast Growth”. The introduction of the household responsibility system in 1978 and the liberalization of livestock market in 1985 facilitated rapid growth of annual production. During this stage, the China meat chicken sector experienced the fastest intensification and phenomenal growth, increasing from 1.08 million metric tons to 4.54 million metric tons with an annual growth rate of 10.15% [45, 60].

The third stage (1997-2009) is considered “Standardization and Scaling-up”. During this period, annual production of meat chicken increased by 5.306 million metric tons with an annual growth rate of 4.91%. In 2005, the share of medium to large scale meat chicken producers (an annual output of 10,000 birds or more) was over 49 percent (Table 2-3) [2].

Table 2-3 Structure of meat chicken production in China in 2005 (by size of farms) (Source: China Animal Industry Yearbook)

Size of farm (annual output of birds)	Number of Farms (million)	Meat chicken production (million birds)	Share of farms (%)	Share of meat chicken production (%)
1-1,999	34.15	1,483	98.6	23.3
2,000-9,999	0.36	1,751	1	27.5
10,000-49,999	0.096	1,687	0.28	26.5
>=50,000	0.008	1,450	0.02	22.7
Total	34.62	6,371	100	100

Since 2010, the meat chicken industry has entered a fourth stage of “Restructuring and Upgrading.” This stage is characterized with a focus on food safety control, the continued push for standardization and scaling-up (Pi, Rou et al. 2014). By the end of 2011, the small meat chicken farms with annual output less than 10,000 birds only had a 32 percent share of the total production, the producers with an annual output of 10,000 birds or more over took up to 68 percent of the total production and the large-scale farms with annual output more than 100,000 birds went up to 21.8 percent share of the total. (Table 2-4)

Table 2-4 Structure of meat chicken production in China in 2011 (by size of farms) (Source: Chinese Animal Industry Yearbook)

Size of farm (annual output of birds)	Number of farms	Meat chicken production (million birds)	Share of farms (%)	Share of meat chicken production (%)
1-1,999	24,834,318	1,368	98.0	14.3
2,000-9,999	330,819	1,707	1.3	17.8
10,000-49,999	157,022	3,325	0.6	34.7
50,000-99,999	17,024	1,096	0.1	11.4
100,000-499,999	4,843	941	0.02	9.8
500,000-999,999	499	326	0.002	3.4
>1,000,000	252	820	0.001	8.6
Total	25,344,777	9,584	100	100

2.3.2 Main meat chicken species raised in China

There are several types of meat chicken (also known as broiler) in China which include white-feathered chicken (below as white chicken), yellow-feathered chicken (below as yellow chicken), mixed (white and yellow) and spent breeding hens. The two major categories are the fast growing white-feathered western type chicken and those very diverse, color-feathered local breeds, a better known one is the three yellow chicken (yellow feather, yellow beak, and yellow shank) [61]. According to the public data from the Poultry Industry Association (Table 2-5), the total annual output of white chicken was approximately 4.7 billion birds in 2012, accounts for about 59% of the total chicken meat production. The number for yellow chicken was about 4.3 billion birds, accounted for 29.5% of the total chicken meat production. There are about 1.3 billion heads of spent hens [62].

Table 2-5 Number of meat chicken output and meat production in China in 2012, source: [62]

Chicken species	Number of output (billion birds)	Meat produced (million tons)	% of total chicken meat production
White chicken	4.7	8	59
Yellow chicken	4.3	4	29.5
Spent hens	1.3	1.55	11.4

White chickens are more commonly produced in northern, northeastern and middle provinces (mainly produced in provinces of Shandong, Liaoning, Henan, Hebei and Jiangsu) where conditions for their production are more favourable and there is greater demand/acceptance for chilled and frozen white chicken meat. White chickens are characterized by a uniform pure white color across all feathers, the species of Arbor Acres (AA+), Ross 308, Avein, Cobb and Hybro are the main species raised in China.[50]. They are noted for having very fast growth rates, a high feed conversion ratio, and low levels of activity. Modern commercial white chicken are bred to reach a slaughter-weight of about 2 kg and above in only 35 to 49 days [63].

Yellow chicken (also known as grass chicken or Chai chicken) are native chickens of China and are mainly present in southeastern and southern areas of China (mainly produced in provinces in Guangdong, Guangxi, Anhui, Jiangsu, Zhejiang, Hunan). By 2014, there are more than 40 hybrid varieties that have been examined and approved by China MARA [64]. There are small variations among yellow chickens in color and confirmation in different parts of the country. The yellow chickens are generally slower growing than white chicken. Based on the speed of growth, yellow chicken is classified into three types, fast growing (45-60 days), medium-speed growing (60-100 days) and slow growing chicken (>100 days) [65], which takes 120 days to grow to market weight, and attract higher prices.

The spent-hens are the layer hens or breeders that have finished their fertility life of producing hatching eggs or commercial eggs, their raising period can reach 500 days. They are mainly produced in northern and western parts of China. The annual output of spent-hens in 2012 was 1.3 billion birds, accounted for about 11.4% of the total chicken meat production.

Chinese companies that churn out eggs for commercial sale typically sell hens at live poultry markets after 400 to 500 days of laying, when they begin to produce less regularly.

Poultry consumption custom in China is specific to each region. Consumer’s preference for meat chicken can be roughly divided between white chicken in north and yellow chicken in the south [52]. Consumers in the north have less preference for bird type than people in the south, therefore white chicken have become the major sources of poultry consumed in the north. As a traditional consumption habit, consumer in the south have a strong preference for high quality yellow chicken, these consumers attach more importance on highly flavoured chicken meat than nutrition or hygiene, therefore they are keen to observe the chicken that they are buying is active and looks healthy. In order to satisfy this special cultural and consumer preferences, the chickens are always freshly slaughtered. In the other hand, driven by the rapid growth of fast food restaurants (KFC and McDonald’s are the most famous ones so far in China), the lower price and ease of cooking of white chicken compared with yellow chicken, the consumption of white chicken will continue to grow. It is estimated over time, the preference for the yellow chicken may gradually decline, the yellow chicken will likely become a niche premium product instead of a product for the mass market [52].

2.3.3 Meat chicken market chain in China

As mentioned in the previous content, different species have different chains making use of different farming systems and supply different consumers through different markets involving different traders. Figure 2-1 can also be used to describe the framework of a meat chicken value chain in China (Black arrows indicate flows of live meat chicken among the market chain, gray arrows indicate the flow of chicken meat or products). This part mainly describes the two different market chains of white meat chicken and yellow meat chicken in China.

2.3.3.1 White chicken market chain

The white chickens are generally produced in highly integrated systems and often under the same ownership along the pathway of breeding, production, slaughter, distribution and marketing in national and international markets. These chickens are rarely seen in live bird markets (usually follow the chain in gray arrows in Figure 2-1). The current breeder flocks of white chicken in China completely rely on import from foreign countries (mainly from the United States, Germany, Canada, England, France etc.). The MARA of China released the plan of the National Poultry Genetic Improvement (2014-2025) emphasizing the importance of recommencing the white chicken breeding program to ensure the stable development of the industry from the long-term strategic perspective.

2.3.3.2 Yellow chicken market chain

The yellow chicken industry sector is less integrated than white chicken production and often uses contract growers. The vast majority are transported alive from the chicken farms to wholesalers, to retailers and finally to consumers [66]. They are generally sold through live bird markets to satisfy cultural and consumer preferences for freshly slaughtered, higher value and more highly-flavored chicken meat (usually follow the chain in black arrows in Figure 6). This type of chicken is usually slaughtered at the live bird markets or taken home for slaughter when convenient.

2.3.3.3 Key players along the live chicken market chain in China

The live poultry movement is deemed to be a very important risk factor for the dissemination of AI viruses [39, 67]. Since yellow chicken is the main species that is transported alive and sold alive in the LBMs, it is of great importance to understand the full range of stakeholders (e.g. farmers, middlemen, traders, wholesalers, and retailers), activities networks and linkages that are required to bring this kind of chicken from production to final consumers.

Traders and middlemen are playing a very important role on moving birds from one place to another. In the vertical integration and contracts systems, the commercial company, acting like a middleman, will be responsible for the coordination between farmers and traders. For large commercial companies who own trading platform (like a wholesale LBM), live birds will be collected and transported to the platform for trade, the transportation of birds may be conducted by either the company or the farmer. In the meantime, the traders will pick up the birds for subsequent trade. These traders mainly own business in the LBMs (in the form of poultry stores or stalls). For those smaller poultry companies who don't have a specific trading venue, the traders may come directly to the farm to collect the birds once the trade has been coordinated by the company.

In the open marketing model, the coordination between the farmers and traders may be conducted by themselves upon an existing network. This network may involve a middleman, a feed provider, a group, or other networks. The traders may come to the farm to collect the birds for subsequent trade, or the farmers will move the birds to the trader (could be a LBM).

Live bird markets (LBMs) is a generally a place for poultry marketing, in which birds can be housed until they are sold. LBMs are common in Asian countries because of a cultural preference to consume freshly slaughtered meat [68]. And the LBMs bring together a mixture of bird species that meet the preferences of their customers and that are commonly produced by multiple suppliers. [69] In some provinces in China, the waterfowl markets are separated from markets with other species (Chicken, pigeon, quail), while in other places multispecies still exist in the same market.

In China, there are several different types of LBMs categorized by the size and type of business, i.e. wholesale LBMs (including trading platforms of commercial poultry companies, or general wholesale LBMs), retail LBMs (can be an agricultural product market with live poultry business in urban or a bazar in a rural area), mixed wholesale/retail LBMs (with both wholesale and retail business). Most of the wholesale markets conduct business from midnight to dawn, some also operate at other times, during which the poultry retailers or other wholesalers will purchase poultry and transport the poultry to the stalls in their LBMs or other market. During the daytime, the birds in the retail markets will be gradually purchased by general consumers or hotel/restaurants.

Figure 2-7 Wenshi poultry trading platform in Anhui

Figure 2-8 Wholesale LBMs in Shanghai and Jiangsu

Figure 2-9 Retail LBMs in Anhui

2.3.3.4 Spent-hens market chain

Spent-hens are usually to be sent to slaughterhouses for further process, and they can also be moved from northern areas during certain seasons as far south as Guangxi province and on to Vietnam to satisfy the demand for soup.

2.3.4 Identified gaps in knowledge

There are very few studies that describe the meat chicken production system in China; this is perhaps surprising given the important role this species has in the overall poultry market in the country and the important role this sector has had in the dissemination of AI. Therefore, there is a strong need to understand the whole live meat chicken market chain (production, trading, marketing and consumption) and the key points in the chain especially in those areas in Jiangsu and Anhui provinces in south of China which have the highest connectivity in terms of poultry movement, high yellow chicken production and tradition of consuming this type of chicken.

2.4 The pathophysiology of AI infection in birds and humans

2.4.1 General characterization of influenza viruses

There are three types of influenza viruses in the *Influenza* genus, e.g. influenza A virus, influenza B virus and influenza C virus, they all belong to the *Orthomyxoviridae* family. The type A viruses are found in avian species and are the most virulent human pathogens among the three influenza types and cause the most severe disease [70]. Type B viruses are found only in humans, it may cause a less severe reaction than type A virus, do not cause pandemics, but occasionally, it can still be extremely harmful. The influenza C virus infects humans and pigs and can cause severe illness and local epidemics. However, influenza C is less common than the other types and usually seems to cause mild disease in children.[71]

2.4.1.1 General characterization of influenza A viruses

The influenza A viruses are categorized by the two proteins on their surface: the haemagglutinin (HA) (18 known subtypes) and the neuraminidase (NA) (11 known subtypes) [72]. Of which, 16 HA (H1-H16) and nine (N1-N9) NA subtypes have been detected in various combinations in wild birds, and for the most part these viruses live harmoniously with their natural hosts, establishing a short-lived subclinical enteric infection [73]. Only three HA and two NA subtypes (H1, H2, H3 and N1, N2) are known to have been widely circulated in humans. [74]

The type A influenza viruses can infect a variety of animals, including wild and domestic birds, but also humans, pigs, horses and sea mammals [70]. The type A influenza viruses occurring in birds are collectively termed avian influenza.

The earliest documented AI outbreak was in 1878 also known as “fowl plague” [75]. It's was a severe, rapidly spreading disease that produced high mortality in chickens [76]. It was only identified and classified as type A influenza virus in 1955 [76, 77].

2.4.1.2 General characterization of high and low pathogenic avian influenza viruses

AI viruses can be classified into low pathogenicity and highly pathogenic forms based on the severity of the illness they cause in poultry [78].

Occasionally, the AI viruses are responsible for severe and acute disease with high mortality in poultry, and are described as highly pathogenic influenza avian influenza (HPAI). To date all isolated HPAI viruses in poultry have been known to contain either H5 or H7 subtypes [13]. These can cross from waterfowl to poultry or mammals and therefore the H5 and H7 subtypes are of great concern to agricultural authorities and international organizations of public health and animal health in the world [73]. For that reason, notification of avian outbreaks involving the H5 and H7 viruses is mandatory, according to the World Animal Health Organization (OIE) *Terrestrial Animal Health Code* [79].

Besides that, low pathogenic avian influenza (LPAI) viruses can contain any HA and NA types, cause a milder disease (primarily respiratory) unless exacerbated [80]. H9, H6 and H3 subtypes that have established (e.g. H9N2 LPAI viruses in chickens is endemic in a large number of countries), or in the process of establishing permanent lineages in chickens can cause severe respiratory disease in poultry if combined with other pathogens [73, 81].

The novel H7N9 virus detected in China in early 2013 is an influenza virus generated through the reassortment of three LPAI viruses. All of these viruses that donated genetic material to make up this novel H7N9 strain are LPAI viruses in birds [82]. This virus caused only mild or no symptoms in birds, and as a consequence stayed undetected in poultry for some time. However, in humans this virus caused severe pneumonia and acute respiratory distress syndrome in a large number of cases [82].

2.4.2 Clinical signs of AI in birds and humans

2.4.2.1 Clinical Signs of AI in birds

Both HPAI and LPAI viruses can spread rapidly through flocks of poultry. Poultry does not generally maintain LPAI viruses, because the virus is not well adapted to poultry as a host species. Wild birds can transmit LPAI viruses to poultry, but the virus usually circulates briefly and dies [83]. Infection of poultry with LPAI viruses may be asymptomatic or mild illness such as ruffled feathers and a drop-in egg production. In very few cases LPAI viruses have been noted to cause severe disease and high mortality [84, 85].

Infection of poultry with HPAI viruses results in severe disease with high mortality. For example, symptoms of HPAI H5N1 in birds range from asymptomatic, mild disease (anorexia, depression, weight loss) to severe neurological symptoms (e.g., tremors, shaking, and lack of coordination, spinning, seizures) and sudden death [86]. HPAI strains (always of the H5 or H7 subtypes) replicate rapidly in the gastrointestinal tract of birds and can systematically spread and replicate in multiple organs often resulting in rapid death [87, 88]. Chickens are more susceptible to influenza A viruses than ducks, geese and swans and therefore are more likely to be diseased and die from infection. Chickens and turkeys with HPAI are typically found dead with few clinical signs other than depression, recumbence and a comatose state [76].

Wild birds are often viewed as reservoirs (hosts) for AI viruses [78]. AI viruses have been isolated from more than 100 different species of wild birds. Most of these viruses have been LPAI viruses. There are two key symptoms noticed, abnormal neurological signs (tremor and opisthotonos) and diarrhea [89]. Most wild ducks, domestic ducks and geese infected with HPAI can be asymptomatic, they may act as silent vectors for transmission and represent a major challenge in controlling the spread of HPAI [90].

2.4.2.2 Clinical Signs of AI in Humans

AI infections with HPAI and LPAI infections in humans can result in a wide range of symptoms, from undetected asymptomatic or sub-clinical to severe disease resulting in death.

HPAI infections of humans have been associated with a wide range of illness. Illness has ranged from conjunctivitis only [91], to influenza-like illness, to severe respiratory illness (e.g. shortness of breath, difficulty breathing, pneumonia, acute respiratory distress, viral pneumonia, respiratory failure) with multi-organ disease, sometimes accompanied by nausea, abdominal pain, diarrhoea, vomiting and sometimes neurologic changes (altered mental status, seizures). Sometimes infection with HPAI leads to death, especially with HPAI H5N1 virus [92, 93].

The incubation period for H5N1 infection may be longer than that for normal seasonal influenza, which is around two to three days. Current knowledge for H5N1 infection indicates an incubation period ranging from two to eight days and ranging up to 17 days [24, 92]. The first HPAI H5N1 patient in 1997 in Hong Kong died from influenza pneumonia, acute respiratory distress syndrome (ARDS), Reye’s syndrome, multiorgan failure, and disseminated intravascular coagulation [94].

The reported signs and symptoms of LPAI virus infections in humans have ranged from conjunctivitis to influenza-like illness (e.g., fever, cough, sore throat, muscle aches) to lower respiratory disease (pneumonia) requiring hospitalization [95, 96]. There have been occasional reports of H7N7-associated conjunctivitis [95-97].

Current knowledge for H7N9 human infection indicates an incubation period ranging from two to eight days, with an average of five days [98]. Currently WHO recommends that an incubation period of seven days to be used for field investigations and the monitoring of patient contacts. H7 viruses occasionally infect humans and usually only cause mild, clinical manifestations, mainly conjunctivitis and/or influenza-like illness (ILI) [99]. However, the first H7N9 patient developed high fever and influenza pneumonia and respiratory failure [100]. Typical symptoms of the H7N9 virus infection in human include fever, cough, even severe pneumonia, and multi-organ dysfunction syndrome [101]. Most patients were hospitalized with severe lower respiratory tract illness, but mild infections have been reported in children and young adults, with an overall case fatality rate of 32% as of 18st Feb, 2014 [102, 103].

2.5 The epidemiology of HPAI H5N1, H7 and LPAI H9N2 outbreaks in birds and human worldwide

2.5.1 Review of HPAI H5N1, H7, and LPAI H9N2 outbreaks in birds

2.5.1.1 Review of HPAI H5N1 outbreaks in birds

Outbreaks of HPAI H5N1 date back to 1959 in Scotland with two flocks of chickens (number not reported) affected and in 1991 in England with one house of about 8,000 turkeys affected [76]. However, these two outbreaks caused little or no spread from the initially infected farms. In 1997, a highly pathogenic strain of H5N1 emerged in Southeast Asia and spread throughout numerous Asian, Middle Eastern, African, and European countries [104]. As of 2014, the epidemic of HPAI H5N1 had spread to about 66 counties (31 Asian, 23 European and 12 African countries) in the world, causing tens of millions of avian death and hundreds of millions more destroyed or slaughtered [105]. Currently, there are at least six countries – Bangladesh, China, Egypt, India, Indonesia and Viet Nam – where the virus is entrenched, and a number of other countries experiencing sporadic outbreaks [49]. The outbreaks of HPAI H5 in poultry since 1959 are listed in Table 2-6 and Table 2-7.

Table 2-6 Reported HPAI H5 isolated from primary outbreaks in poultry since 1959

Year	Location	Subtype	Approximate numbers of poultry affected **
1959	Scotland	H5N1	1 small farm
1966	Ontario, Canada	H5N9	8,000
1983	Pennsylvania, USA	H5N2	17,000,000
1983	Ireland	H5N8	307,000, mostly ducks
1991	England	H5N1	8,000
1994	Mexico	H5N2	Unknown-? millions
1997	Hong Kong SAR, China	H5N1	3,000,000
1997	Italy	H5N2	8,000
2003-	Eurasia and Africa *	H5N1	Unknown-several hundreds of millions
2004	Texas, USA	H5N2	6,600
2004	S. Africa	H5N2	30,000

Note: * 35 Asian, 24 European, 17 African and 2 American countries had reported H5N1 outbreaks up to Dec 2018
** This number includes poultry affected and being slaughtered.
Source: [80, 104, 105].

Table 2-7 Cumulative number of H5N1 outbreaks in poultry, captives and wild birds, summarized by reporting year, 2004-2018

Region	Total	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Africa	4411			667	663	154	179	443	376	99	93	364	706	458	208	1
Americas	3												3			
Asia	14354	4271	1870	480	506	509	1640	1325	1580	469	396	408	276	313	229	76
Europe	1165		239	758	100	25	3	4				2	24	8	2	
Total	19933	4271	2109	1905	1269	688	1822	1772	1956	568	489	774	1009	779	439	77

Source: Data extracted from FAO Empres-i website on Feb 17, 2019. Empres-i (Emergency Prevention System Global Animal Disease Information System) database consolidates disease information from OIE, WHO and National authorities.

2.5.1.2 Review of HPAI and LPAI H7 outbreaks in birds

The H7 subtype HA gene has been found in combination with all nine NA subtype genes. Most exhibit low pathogenicity and only rarely high pathogenicity in poultry. All H7Nx combinations were reported from wild birds, the natural reservoir of the virus. [99] The pathogen, firstly caused fowl plague in chicken in Italy in 1902, is was then identified as a HPAI H7N7 in 1955 [91]. Geographically, the most prevalent subtype is H7N7, which is endemic in wild birds in Europe and was frequently reported in domestic poultry, whereas subtype H7N3 is mostly isolated from the Americas [99]. In recent years, the HPAI H7 and LPAI H7 viruses have caused more than 70 million poultry death [91], and the outbreaks occurred cross Americas (Canada, Mexico and USA), Australia, Europe (Denmark, Germany, Italy, Netherlands, Portugal, Spain, UK and Ireland), Asia (China, DPR Korea, Japan, Macau, Malaysia, Republic of Korea and Vietnam) and South Africa (FAO EMPRES-i). (Table 2-8 and Table 2-9）

Table 2-8 Reported HPAI H7 from primary outbreaks before 2004

Year	Location	Subtype	Approximate numbers of poultry involved *
1902	Brescia, Italy	H7N7	
1963	England	H7N3	29,000
1976	Victoria, Australia	H7N7	58,000
1979	Germany	H7N7	1 chicken farm, 1 goose farm
1979	England	H7N7	9,000
1985	Victoria, Australia	H7N7	240,000
1992	Victoria	H7N3	18,000
1994	Queensland, Australia	H7N3	22,000
1994	Pakistan	H7N3	>6,000,000
1997	NSW, Australia	H7N4	160,000
1999	Italy	H7N1	14,000,000
2002	Chile	H7N3	~700,000
2003	Netherlands	H7N7	>25,000,000
2004	British Columbia, Canada	H7N3	16,000,000

Source: [80, 105]

Note: * This number includes poultry dying and being slaughtered.

Table 2-9 Number of HPAI H7 outbreaks in birds (2004-2018)

Country	2004	2005	2007	2008	2009	2012	2013	2014	2015	2016	2017	2018	Total
Algeria										1			1
Australia						1	2						3
Canada	1		1										2
China											32	5	37
Dem People's Rep of Korea		3											3
Denmark							1						1
Germany									1				1
Italy							6			3			9
Mexico						46	64	1	3	30	1	4	149
South Africa						1							1
Spain					1								1
U.K. of Great Britain and Northern Ireland				1					1				2
United States of America											2		2
Total	1	3	1	1	1	48	73	1	5	34	35	9	212

Source: Data extracted from FAO Empres-i website on Sep 18, 2019.

Since June 2012, two incidents of infections with H7 subtypes were of great concern for animal and human global health organizations. The HPAI H7N3 infection in poultry in Mexico which spilled over to two humans and the most recent H7N9 outbreak in China in 2013 [99].

2.5.1.3 Review of LPAI H9N2 outbreaks in birds

LPAI H9N2 influenza viruses are panzootic in birds worldwide. It has undergone extensive reassortments in different host species, and could lead to the epidemics or pandemics with the potential emergence of novel viruses [106]. For example, the H9N2 viruses found in quail were identified as the contributor of the internal genes of the H5N1 virus that caused human disease in Hong Kong in 1997 [107]. And recent studies found the internal genes of the novel H7N9 and H10N8 human infections are closely related to influenza A(H9N2) viruses [16, 108-112]. Therefore, it is suggested urgent attention should be paid to the control of H9N2 influenza viruses in animals and to the human's influenza pandemic preparedness [106, 113].

Prior to 1990, H9N2 influenza viruses were mainly reported from avian species in North America. H9N2 virus was first isolated in China in 1994, approximately 74 different genotypes have been observed till now and new lineages and genotypes continuously identified throughout China [106]. However, the H9N2 viruses from North America differ from those of Asia [114].

A study of H9N2 subtype based on NCBI database revealed that approximately 60% of all the H9N2 viruses were isolated from chickens, with the remainder from wild birds (16.8%), ducks (8.9%), turkeys (6.7%), and other domestic avian populations (3.7%). The majority (94.2%) of H9N2 influenza viruses were isolated in Asia, with > 65% coming from China (including Hong Kong) [106].

By 1997, the H9N2 viruses had been isolated from northern China, Korea, Pakistan, India, Saudi Arabia, Germany, Italy, Ireland, and South Africa [107]. More recently, H9N2 infections have been reported in the Middle East and Asia causing widespread outbreaks in commercial chickens in Iran, Saudi Arabia, Pakistan, China, Korea, UAE, Israel, Jordan, Kuwait, Lebanon, Libya and Iraq [80].

2.5.2 The epidemiology of HPAI and LPAI H7 outbreaks in humans

2.5.2.1 HPAI H5N1 outbreaks in humans

The isolation of H5NI from a 3-year-old boy in Hong Kong in 1997 was the first known case of H5N1 infecting humans [94]. As of 27 July, 2014, the HPAI H5N1 virus has infected 667 humans in 16 counties, with 393 deaths [115]. Table 2-11 reports the number of cases and fatalities in each country affected by

H5NI in humans. So far, the largest number of human cases has been reported from Indonesia, Egypt and Vietnam each having reported 197, 176 and 127 cases, followed by Cambodia (56) and China (47). No human cases have yet been reported in Western Europe. And there were no new human cases reported in nine countries since 2010, these countries are Thailand, Turkey, Azerbaijan, Iraq, Pakistan, Lao PDR, Djibouti, Myanmar and Nigeria. However, in December 2013, Canada notified a human infection of H5N1, the case had an onset of disease when travelling back to Canada from a three-week trip from China and has died in early January 2014, marking the first human H5N1 case reported in the North America [116].

Table 2-10 Cumulative number of confirmed human cases for H5N1 reported to WHO (2003-2018)

Country	2003-2009		2010-2014		2015		2016		2017		2018		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Egypt	90	27	120	50	136	39	10	3	3	1	0	0	359	120
Indonesia	162	134	35	31	2	2	0	0	1	1	0	0	200	168
Viet Nam	112	57	15	7	0	0	0	0	0	0	0	0	127	64
Cambodia	9	7	47	30	0	0	0	0	0	0	0	0	56	37
China	38	25	9	5	6	1	0	0	0	0	0	0	53	31
Thailand	25	17	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	0	0	12	4
Azerbaijan	8	5	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	0	0	0	0	8	1
Iraq	3	2	0	0	0	0	0	0	0	0	0	0	3	2
Pakistan	3	1	0	0	0	0	0	0	0	0	0	0	3	1
Lao People's Democratic Republic	2	2	0	0	0	0	0	0	0	0	0	0	2	2
Djibouti	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Myanmar	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Nigeria	1	1	0	0	0	0	0	0	0	0	0	0	1	1
Canada	0	0	1	1	0	0	0	0	0	0	0	0	1	1
Total	468	282	233	125	145	42	10	3	4	2	0	0	860	454

Note: Total number of cases includes number of deaths; WHO reports only laboratory cases; all dates refer to onset of illness.
Source: WHO/GIP, data in HQ as of 24 June 2019;
URL: http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

2.5.2.2 HPAI/LPAI H7 outbreaks in humans

Infection of humans with H7 viruses was first recorded for HPAI H7N7 in the USA in 1959, HPAI H7N7 in Australia in 1977, and LPAIV H7N7 from seals to humans in the USA in 1978–1979. Since the 1990s, reports of human infections with H7 viruses have markedly increased [99]. From 2002, H7 subtype AI viruses have caused more than 100 human infection cases in the Netherlands, Italy, Canada, the United

States, and the United Kingdom [91]. The HPAI subtype H7N7 virus was found to be able to transmit to people directly involved in handling infected poultry, and we noted evidence for person-to-person transmission [96, 99]. Since Feburary 2013, the H7N9 viruse was first reported to cause human infection in Eastern China.

Table 2-11 Human cases of subtype H7 influenza A infection

Year	Country	Subtype	Symptoms	Number of cases	Number of Death
1959	USA	HPAI H7N7	unknown	1	0
1977	Australia	HPAI H7N7	conjunctivitis	1	0
1979-1980	USA	HPAI H7N7	conjunctivitis	4	0
1996	USA	LPAI H7N7	conjunctivitis	1	0
2002	USA	LPAI H7N2	flu-like symptoms	1	0
2003	USA	LPAI H7N2	respiratory symptom	1	0
2002-2003	Italy	LPAI H7N3	conjunctivitis, flu-like symptoms	7	0
2003	Holland	HPAI H7N7	conjunctivitis, flu-like symptoms	89	1
2004	Canada	HPAI/LPAI H7N3	conjunctivitis, flu-like symptoms	2	0
2006	England	LPAI H7N3	conjunctivitis	1	0
2007	England	LPAI H7N2	conjunctivitis, flu-like symptoms	4	0
2012	Mexico	HPAI H7N3	conjunctivitis	2	0
2013-2018	China	LPAI H7N9	acute pneumonia, acute respiratory distress syndrome	1567	615

Note: Source: [91], website of WHO (https://www.who.int/csr/don/05-september-2018-ah7n9-china/en/) and other internet sources.

2.5.2.3 LPAI H9N2 outbreaks in humans

Although influenza A H9N2 subtype are now widespread in poultry in Asia, there are occasional reports of human infections H9N2 in Southern China and Hong Kong. Table 2-12 and Table 2-13 show lists of human H9N2 infections [112].

In 1998, H9N2 influenza viruses were isolated from five humans with influenza in Guangdong Province, all the five patients had typical clinical signs of influenza, and all recovered from the disease [117]. In 1999, two human infections (one 4-year-old girl and one 1-year-old girl) were identified in Hong Kong, the illness in both children was mild and self-limited [118]. And another infection in a 5-year-old child with an influenza-like illness were reported in Hong Kong in 2003 [119]. In fact, serologic surveillance revealed that the number of humans infected by H9N2 virus were much higher than that of the confirmed cases. Poultry workers are considered to be at high risk of infection with AI due to their frequent exposure to chickens. About 2.3%–4.6% of poultry workers had antibodies against H9 [120, 121].

Table 2-12 H9N2 human cases in China (1998-2013)

Year	Location/Province	Patient	Clinical signs	Exposure to live poultry
1998	Guangdong	14-year-old male	Acute respiratory infection	lived with chickens in the same house
		75-year-old male	Acute respiratory infection	farmer's market nearby
		4-year-old male	Acute respiratory infection	unknown
		1-year-old female	Acute respiratory infection	unknown
		36-year-old female	Acute respiratory infection	yes
1999	Guangdong	22-month-old female	Fever, cough	no
1999	Hong Kong SAR	13-month-old female	Fever, vomiting, inflamed oropharynx	one was possible exposed
		4-year-old female	Fever, malaise	
2003	Hong Kong SAR	5-year-old male	Mild fever, cough	No
2007	Hong Kong SAR	9-month-old female	Mild upper respiratory	unknown
2008	Guangdong	female	Cough and vomiting	unknown
2013	Guangdong	86-year-old male	Cold and cough	no
2013	Hunan	7-year-old male	Fever and rhinorrhoea	yes

Note: Source: [112].

Table 2-13 H9N2 human cases in China (2014-2018)

Year	Location/Province	Number of cases
2015	Anhui	2
	Guangdong	1
	Hunan	3
	Sichuan	1
2016	Guangdong	4
	Henan	1
	Jiangxi	1
	Sichuan	1
	Yunnan	1
2017	Beijing	1
	Gansu	1
	Guangdong	1
	Henan	1
2018	Guangdong	2
	Guangxi	1

Note: Source: FAO Empres-i website (http://empres-i.fao.org/eipws3g/).

2.6 The epidemiology of H5N1 and H7N9 infections in birds and humans in China

2.6.1 Review of HPAI H5N1 outbreaks in China

In 1996, a HPAI H5N1 outbreak was observed with highly contagious disease among goose flocks in Guangdong, China [122]. Between May and December 1997, in Hong Kong SAR, 18 humans were infected and 6 died in the first known case of H5N1 infecting humans [123]. Before that, a H5N1 outbreak occurred in chickens in Hong Kong in March and May [124]. About 1.3 million chickens were culled by Hong Kong Government in December 1997, its spread was contained in Hong Kong at that point in the region.

In January 2003 the same virus re-emerged for the first time since the first human outbreak in Hong Kong in 1997 [125], suggesting it had never been eradicated and spread relentlessly across East Asia, decimating the poultry population and inflicting heavy losses on poultry industry. From 2003 onwards, infection and disease spread widely to three continents in an unprecedented manner, initially through East and Southeast Asia from 2003 to 2004, and then into Southern Russia, Western and Southern Asia, the Middle East, Europe, Africa and from 2005 to 2006. In May 2005, an outbreak in migratory wild birds in Qinghai Lake, China killed 6000 birds [89, 126]. The virus has since spread across countries in Asia, Europe, the Middle East, as well as some African countries through migration of wild birds. It has been found in chicken and turkey farms and in some wild birds, mainly swans and geese.

As of December 2018, China has reported 130 HPAI H5N1 outbreaks in poultry, 17 outbreaks in wild birds and 52 human infections, which involved 26 provinces and millions of poultry have been culled to control the spread of the disease and caused severe economic damage to the poultry industry in China.

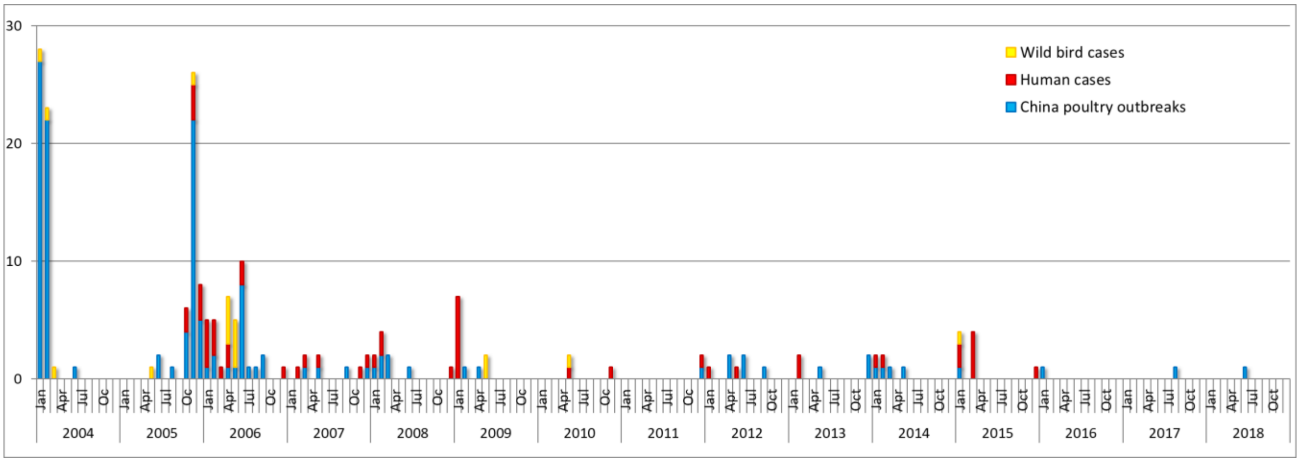


Figure 2-10 China HPAI H5N1 outbreaks in poultry, human, wild birds by year-month since 2004 (As of December 2018)

Note: Source: MARA of China, FAO Empres-i, NHFPC and provincial authorities.

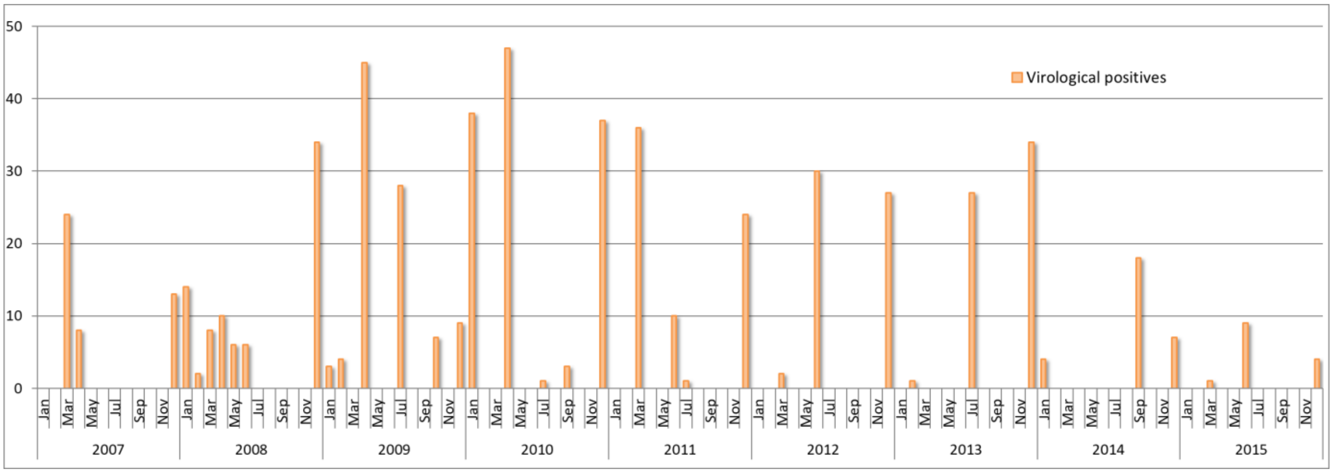


Figure 2-11 China HPAI H5N1 virological surveillance positives by year-month since 2004 (As of December 2015)

Note: The HPAI H5N1 Virological surveillance positives only available since 2007; Source: Monthly veterinary bulletin from MARA website. There were only aggregated reports since 2016 (e.g. 10 positives in first half year of 2016; 1 positive in first half of 2017).

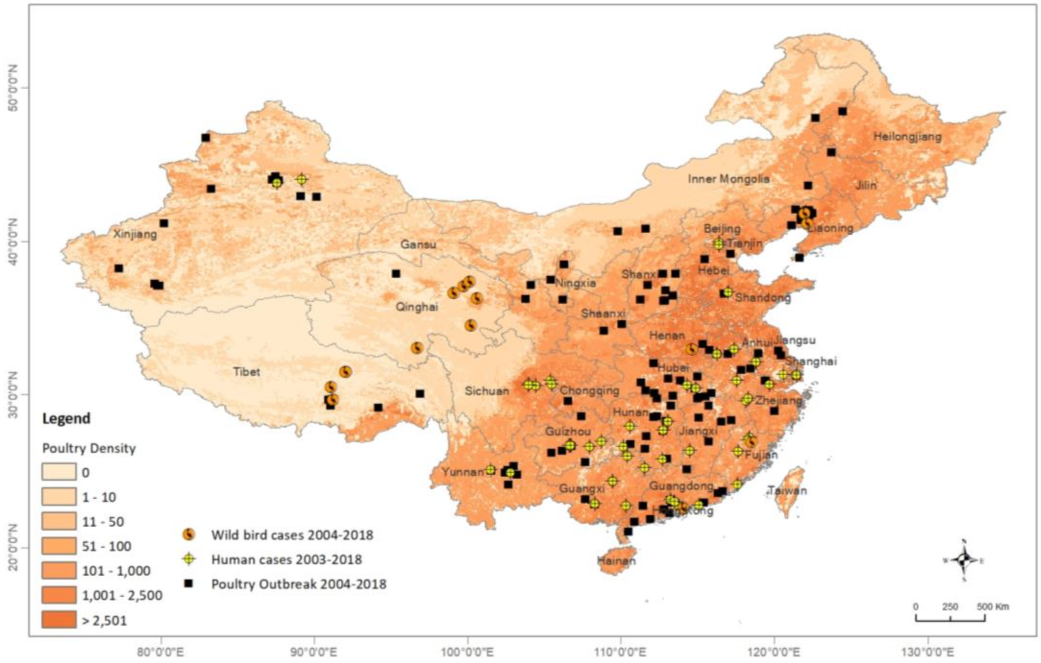


Figure 2-12 Spatial and temporal distribution of HPAI H5N1 outbreaks in poultry in Mainland China, 2004-2014

Source: Data from website of MARA of China and provincial authorities; Poultry density [127]

2.6.1.1 HPAI H5N1 infections in poultry

In China, HPAI H5N1 virus was first detected in a goose in Guangdong Province in 1996. Since 1999, the virus went through series of evolution and diversification, multiple genotypes of H5N1 viruses have been detected from apparently healthy waterfowl, indicating that the virus was still active and widely circulating [122, 128]. Starting from 2003, HPAI H5N1 virus spread across Southeast Asia, causing unprecedented epidemics. In China, poultry were massively infected in 2004 and 2005.

In January 2004, China reported its first HPAI H5N1 outbreak in poultry (i.e. chickens, ducks and geese) in Guangxi Zhuang Autonomous Region, in South Central China, followed by 49 outbreaks within the next month. These outbreaks involved 16 provinces been infected, multiple species of birds were affected, including chickens, ducks, geese, quails, turkeys and a small amount of wild zoo birds in some areas [128], 140,000 cases were infected, about 89% were dead, and eight million poultry were depopulated in the infection zone to control the endemic of HPAI H5N1 virus. Since June to August 2005, three H5N1 outbreaks in poultry been reported in one small-scale farm and two backyard farms in Qinghai and Xinjiang, about 230,000 birds were depopulated to control the spread of the disease. From October 2005 to the end of the year, a total of 28 outbreaks were detected in domestic poultry in eleven provinces or autonomous regions, successively in Inner Mongolia, Anhui, Hunan, Liaoning, Hubei, Xinjiang, Shanxi, Ningxia, Yunnan, Jiangxi and Sichuan. The delayed disease report in Liaoning of Northern China resulted in the wide spread of the virus, to again control the spread of the disease, 19,958,500 chickens were depopulated. From 2006 until 2014, HPAI H5N1 outbreaks were reported occasionally in Mainland China. (Figure 2-10)

Figure 2-13 shows the geographic distribution of the H5N1 virological surveillance results from the national animal disease surveillance system (Refer to Page 55).

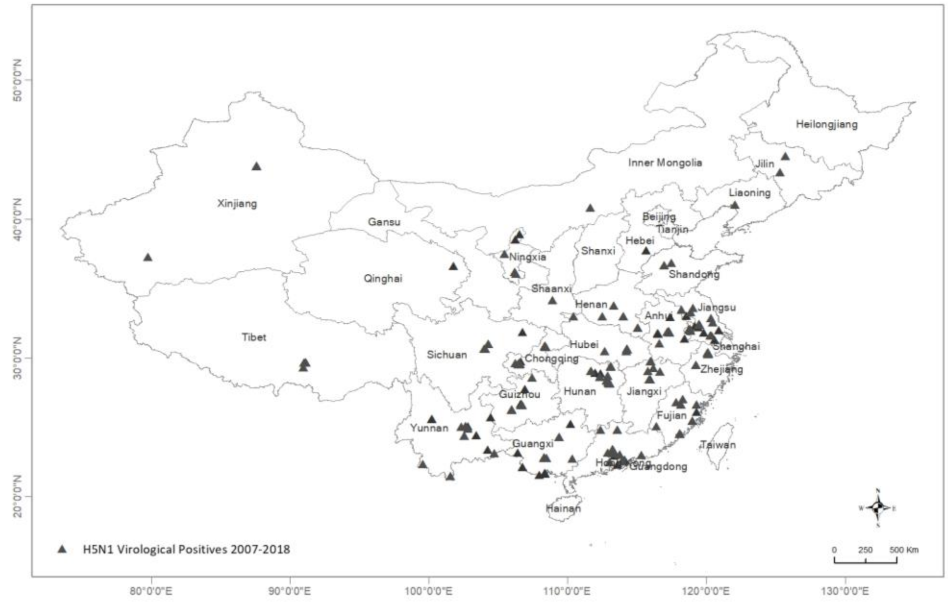


Figure 2-13 H5N1 virological surveillance positives during 2007 to Mar 2013

2.6.1.2 HPAI H5N1 infections in wild birds

HPAI H5N1 viruses were first observed in the wild-bird population in Qinghai Lake in Western China in May 2005. More than a thousand birds were affected by the end of June. Qinghai Lake is one of the most important breeding centers for migrant birds whose flyways extend to Southeast Asia, India, Siberia, Australia, and New Zealand [89]. A previous study discovered that H5N1 viruses isolated in Mongolia, Russia, Inner Mongolia, and the Liaoning Province of China after August 2005 were genetically closely related to one of the genotypes isolated during the Qinghai outbreak in 2005. This finding suggests the dominant nature of this genotype, and the possibility that migratory waterfowl may spread these viruses over a wide range of territories [129]. This same genotype caused the outbreaks in wild birds in Qinghai and Tibet in 2006 and resulted in the deaths of 3461 wild birds. But the origin of the virus responsible for the Qinghai Lake outbreak remains unclear. Since then, one wild case was reported in May 2009 in Qinghai, 121 birds found dead (107 ruddy shelducks, 3 bar-headed geese and 11 brown-headed gulls) and the last outbreak detected in wild birds in China was in Tibet in May 2010, in which about 170 wild birds died (141 brown-headed gull, 27 bar-headed goose, 1 chough and 1 widgeon).

2.6.1.3 HPAI H5N1 infections in humans

The first human cases were reported in 1997 in Hong Kong SAR, from May to December, 18 humans were infected and six died [123]. In mainland China, the first human case of HPAI H5N1 surfaced in November 2003, a 24-year-old man died in Beijing, and was initially thought to be a victim of severe acute respiratory system (SARS); later the case was retrospectively confirmed by laboratory tests to be H5N1.

In Mainland China, humans were mainly infected in 2005 and 2006, with a slight rise in 2009, and today continue to experience sporadic outbreaks across China. As of 2018, Mainland China has reported 52 HPAI H5N1 human infections, and 23 H5N6 human infection [105], involving 17 provinces or autonomous regions, including Anhui, Beijing, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Shandong, Shanghai, Sichuan, Xinjiang, Yunnan and Zhejiang [105].

Among all the 48 human cases, 42 cases (87.5%) were reported in southern China while only 6 cases were reported in northern China. The majority of human H5N1 infections were detected in winter-spring period (December to March), although a few sporadic cases were also occasionally detected in summer time [93]. Most H5N1 human cases are reported during winter-spring period accompanying the increase in poultry outbreaks at that time [130].

2.6.2 Review of H7N9 outbreaks in China

2.6.2.1 Characteristics of the influenza A (H7N9) virus

The novel H7N9 virus is a multiple reassortant of earlier H7N9, H7N3, and H9N2 [16, 82, 109, 131, 132]. Molecular genetics data indicated that the HA gene of the novel H7N9 virus shares 95% identity with the HA genes of isolated from LPAI A (H7N3) viruses isolated in 2011 in Zhejiang province [108]. Surveillance studies in domestic poultry had shown that LPAI A (H7N3) viruses were present in domestic ducks in Zhejiang [133]. Furthermore, the internal genes of the novel H7N9 are closely related to influenza A(H9N2) viruses, which recently circulated in poultry in Shanghai, Zhejiang, Jiangsu and neighbouring provinces [16, 108, 109]. For example, molecular evidence has shown that the NA gene was close to a group of H9N2 viruses circulating in chickens in Jiangsu, whereas the remaining internal genes were closely related to those noted in AI viruses isolated from chickens in Shanghai and the neighbouring provinces [109]. The genetic diversity of H9N2 viruses in chickens in the eastern provinces of China is high and it is postulated that these reassortment events most probably took place in Shanghai or the adjacent provinces such as Zhejiang and Anhui [109]. A study comparing the spatiotemporal distribution of HPAI H5N1 and H7N9 human cases provided compelling supporting evidence in that it demonstrated that H5N1 and H7N9 human cases overlapped in a region bordering the provinces of Anhui and Zhejiang [10].

Adaptive genetic changes of avian influenza viruses in domestic poultry have been shown to enable transmission to humans and in the case of H7N9 there is significant molecular evidence of poultry to human transmission [16, 108]. The H7N9 lineage has diversified since its emergence, which emphasizes the necessity of extensive surveillance of the virus in humans, poultry and wild birds [109].

Figure 2-14 shows the reassortment path of the new H7N9 virus. The earlier H7N9 virus in migratory birds reassorted with H9N2 virus in northern China, so the reassortant virus retained the NA of H7N9 virus and obtained five internal gene fragments (PB2, PB1, PA, NP and M) from H9N2. When birds migrated to Jiangsu, the reassortant virus HXN9 (x =7/9) reassorted with H9N2 (A/chicken/Dawang/1/2011-H9N2) again and gained its NS segments. Meanwhile, the A/duck/Zhejiang/12/2011-H7N3 virus reassorted with some H9N2 strains in Yangtze River Delta and formed H7NX (x = 2/3), which retained H7 of the H7N3 and obtained PB2, PB1, PA, NP and M from the H9N2 viruses; at last, HXN9 reassorted with H7NX, consequently generating this new human-pathogenic H7N9 virus [101].

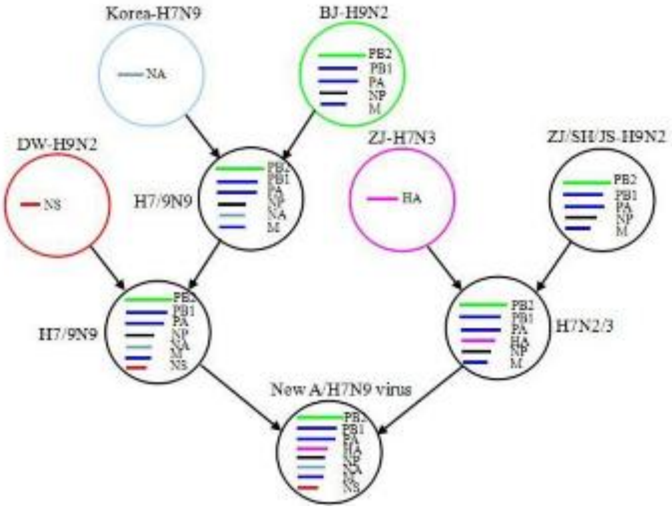


Figure 2-14 The reassortment path of influenza A (H7N9) virus.

Note: BJ, Beijing; DW, Dawang; ZJ, Zhejiang; SH, Shanghai; JS, Jiangsu, Source: [101]

Adaptive genetic changes of AI viruses in domestic poultry have been shown to enable transmission to humans and in the case of H7N9 there is significant molecular evidence of poultry to human transmission [16, 108]. The H7N9 lineage has diversified since its emergence, which emphasizes the necessity of extensive surveillance of the virus in humans, poultry and wild birds [109]. Furthermore, recent laboratory and epidemiological evidence indicates limited non-sustained human-to-human transmission [25, 102, 134, 135].

In February 2017, some strains of the 2013 LPAI H7N9 virus isolated from chickens in Guangdong province mutated to become HPAI H7N9 in poultry and rapidly spread to other provinces in China [13, 136]. The rapid evolution, increased pathogenicity and efficient transmissibility of HPAI H7N9 viruses in mammalian models, together with their extended host range, may have increased the threat to public health and the poultry industry [137, 138].

2.6.2.2 H7N9 infections in human

At the end of March 2013, a new strain of influenza A (H7N9) virus, first identified in a human patient in Shanghai, China, has infected 455 people until 19th Oct, 2014, of whom 176 died [28, 139, 140]. In the first place, this virus caused only mild or no symptoms in birds, and consequently stayed undetected in poultry for some time. However, in humans this virus caused severe pneumonia and acute respiratory distress syndrome in a large number of cases [82]. The notification of human cases of influenza A(H7N9) in China follow a seasonal pattern peaking in the winter months and a few sporadic cases during the summer.

There have been six epidemic waves till Sep 2018 causing more than 1600 human infections in 29 provinces and municipalities in mainland China [5]. The first wave was observed from March to April 2013, starting from Shanghai, Anhui, Jiangsu and Zhejiang provinces, then mainly extended to adjacent provinces around Yangtze River delta: Henan, Shandong, Hunan, Jiangxi and Fujian.

A recent study in China demonstrated that areas of human H7N9 infection overlap with those that reported H5N1 suggesting a common high-risk area in an area southeast of Taihu Lake (south of Jiangsu Province), bordering the provinces of Anhui and Zhejiang [29] (Figure 2-15).

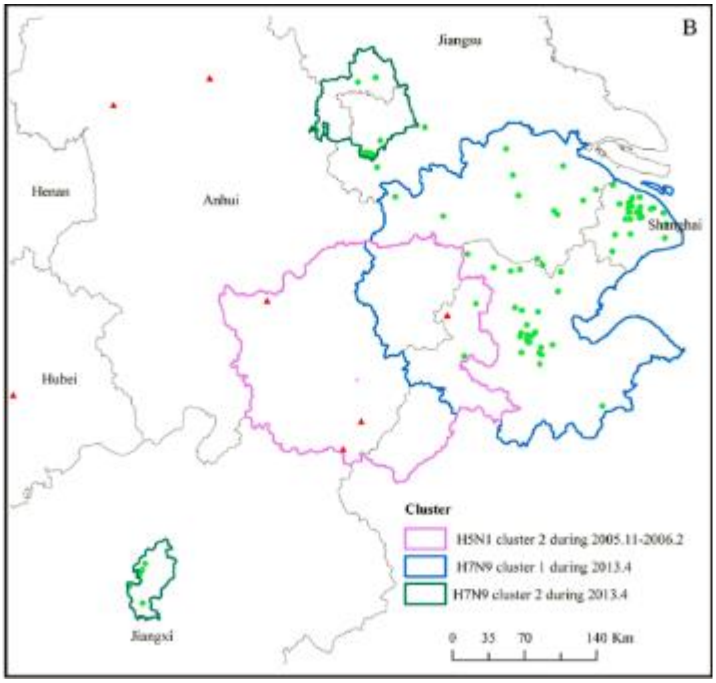


Figure 2-15 Spatial overlap between influenza A (H7N9) and influenza A (H5N1) case clusters in an area bordering the provinces of Anhui, Jiangsu and Zhejiang.

The second wave was observed from January to April 2014, affecting initially the provinces of Zhejiang and Guangdong, then extending to Jiangsu, Anhui, Fujian, Hunan and Guangxi provinces. From May to December 2013 (i.e. the period between the two waves), there were sporadic human cases in Shanghai, Jiangsu, Zhejiang, Jiangxi and Guangdong. From May to September 2014 (i.e. after the second wave), there were occasional reports in Jiangxi, Guangdong, Jilin, Jiangsu, Zhejiang, Hunan and Anhui.

During the fifth epidemic wave from October 2016, the geographic range of H7N9 human cases expanded and more human cases were reported than in any previous wave [12].

Figure 2-16 shows the epidemic curve of the six waves of human H7N9 infections and Figure 2-17 shows the geographic distribution of the six waves of human infections up to May 2018.

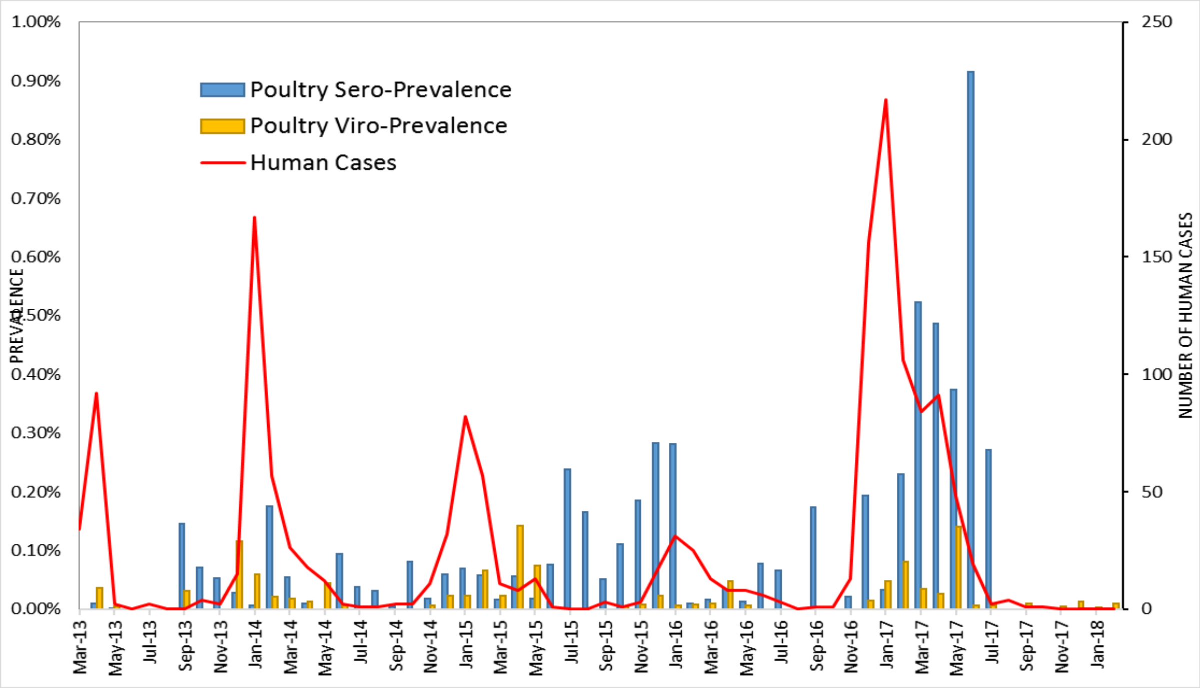
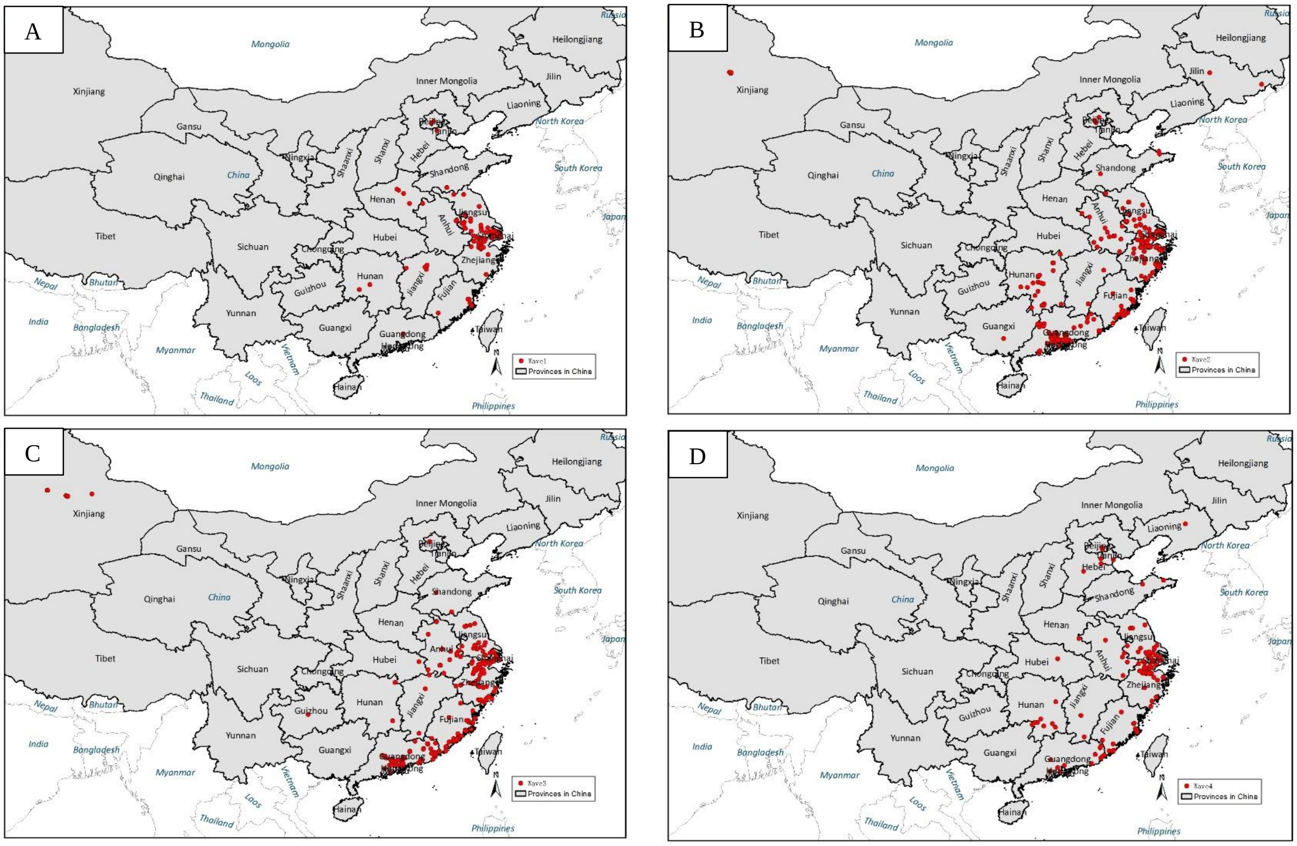


Figure 2-16 Epidemiological curve of avian influenza A(H7N9) human cases, poultry surveillance positives by month of onset, Mar 2013-Feb 2018



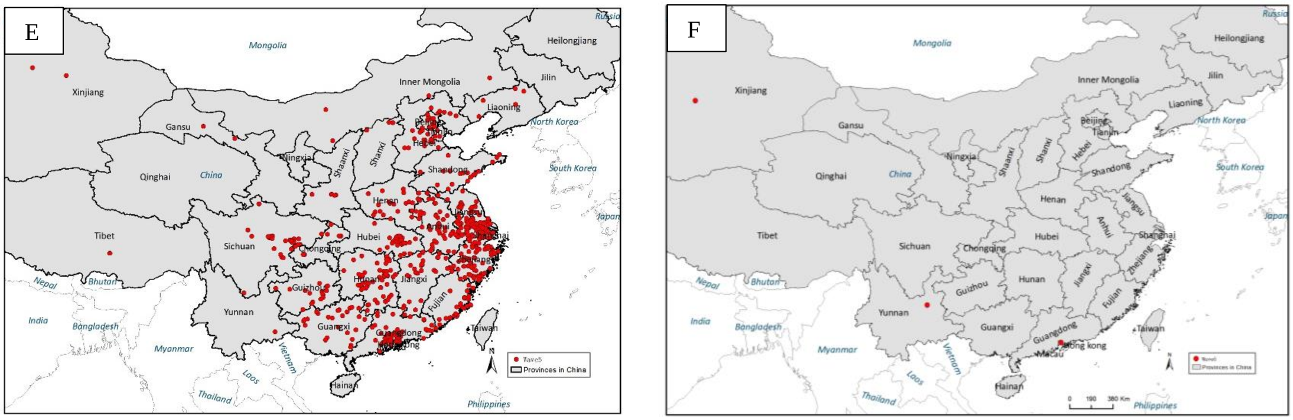


Figure 2-17 Geography distribution of the six epidemic waves of H7N9 human infections up to May 2018

Note: Source: WHO and Chinese authorities; Figure A: 1st Wave (Mar 2013 – Sep 2013) : 134 cases, Figure B: 2nd Wave (Oct 2013 – Sep 2014): 302 cases, Figure C: 3rd Wave (Oct 2014 – Sep 2015): 224 cases, Figure D: 4th Wave (Oct 2015 – Sep 2016): 119 cases, Figure E: 5th Wave (Oct2016 – Sep 2017): 776 cases, Figure F: 6th Wave (Oct2017 – Sep 2018): 4 cases.

2.6.2.3 H7N9 Virological surveillance positives found in birds

According to official reports from the MARA of China, as of May 2018, over 3000 virological samples from chickens, pigeons, ducks, a tree sparrow and the environment tested H7N9 positive; positives were mainly from live bird markets, vendors and farms.

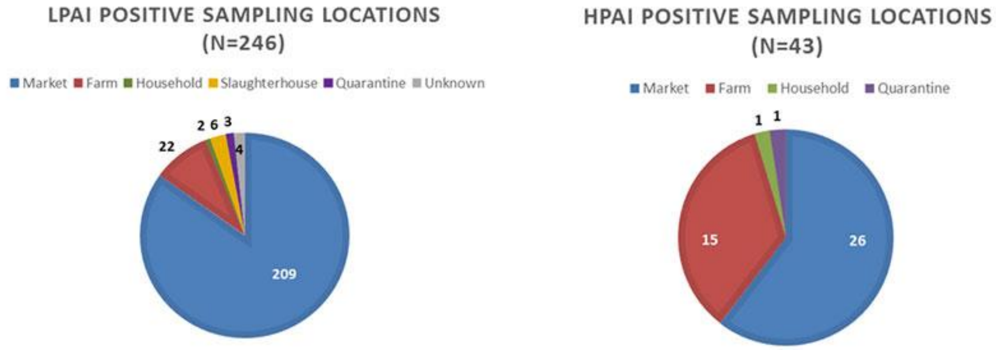


Figure 2-18 Distributions of low and highly pathogenic H7N9 virologically positive samples collected from birds or the environment, by sampling location, between October 2016 and 25 July 2018. Samples from the same location and time are grouped.

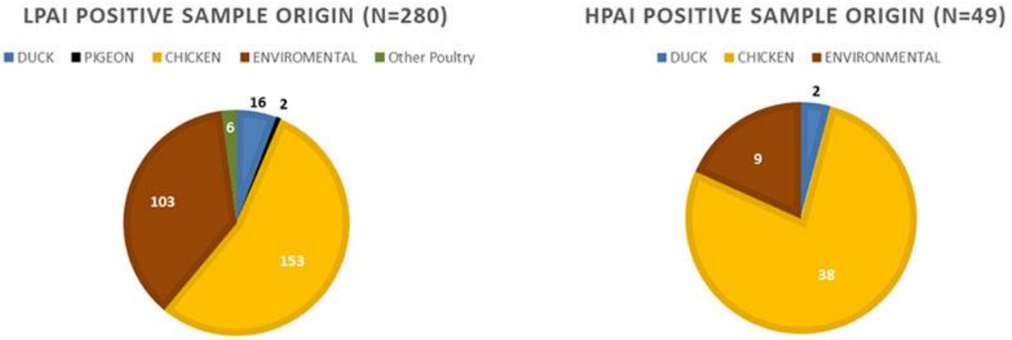


Figure 2-19 Distributions of low and highly pathogenic H7N9 virologically positive samples collected from birds or the environment, by sample origin between October 2016 and 25 July 2018. Samples from the same origin, location and time are grouped. (Source: FAO)

2.6.3 Review of influenza A (H9N2) outbreaks in China

The influenza A (H9N2) viruses were identified as the contributor of the internal genes of the H5N1 [107] and H7N9 in China [109]. In China, the H9N2 virus has been detected in several avian species, including chicken, duck, quail, pheasant, partridge, pigeon, silky chicken, chukar, and egret [141, 142]. The first outbreak of the H9N2 influenza virus in China occurred in Guangdong province of Southern China during November 1992 to May 1994, the outbreak affected, seventeen chicken farms and two rare bird farms. After this outbreak, the H9N2 infection sporadically occurred in chickens, ducks, and geese and spread to Northern China. The H9N2 influenza virus spread to most provinces of China within two months in 1998, and is now the most prevalent subtype of influenza viruses in chickens in China [112].

H9N2 infections occur throughout the whole year, with lower morbidity during summer time. The isolation rate of H9N2 virus in apparently healthy chickens, ducks, and other minor poultry species in LBMs were 2.5%, 0.18%, and 4.7%, respectively between 2000 and 2005 [142]. The H9N2 virus isolation rate in poultry in LBMs in Shanghai was 2.6% between 2008 and 2010 [143]. The patterns of human infection in China is described in the front text. (Refer to LPAI H9N2 outbreaks in humans)

2.6.4 Identified gaps in knowledge

Many studies have been conducted to understand the biological characteristics of HPAI H5N1 and LPAI H7N9 viruses and the population health effects of associated outbreaks in China. However, epidemiological information on the husbandry conditions associated with the emergence of these viruses in China is largely lacking. Evidence suggests that areas where human infection with H5N1 and H7N9 have been observed overlap in border area of the provinces of Anhui, Jiangsu and Zhejiang. More research is necessary to understand the local conditions of the poultry systems in these high-risk areas which could account for the exposure of humans to the H7N9 virus.

2.7 Risk factors for AI infection in poultry and human populations along the market chain

The transmission of H5NI can occur via direct or indirect contact with an infected bird. The major sources of risk for HPAI introduction and transmission in poultry are well known – the introduction of infected birds into flocks; contact with infected wild birds; movement of contaminated materials particularly containers, vehicles and personal clothing. Direct airborne transmission of virus can occur, but generally over very short distances. Live bird markets have played a major role in spreading disease from unit to unit because they mix birds from many sources. Trade in poultry and the movement of contaminated materials remain key transmission pathways [49].

The drivers of AI infections tend to be disparate at different points along the poultry market chain. For example, farm's environmental conditions are important at poultry farms, while human behavioural and socioeconomic factors are more important at the poultry trade and transport stage, and cleaning and disinfection and manure/waste disposal are more important at LBMs.

"Biosecurity" has been used widely in the debate on HPAI control. In this manner, biosecurity refers to those measures that should be taken to minimise the risk of incursion of HPAI into individual production units (bio-exclusion) and the risk of outward transmission (bio-containment) and onward transmission through the market chain [144].

2.7.1 Farm level biosecurity risk factors for AI in birds and humans

The use of biosecurity in poultry rearing according to FAO varies from high (closed, controlled heating and cooling system), to medium (open system, netting to prevent entrance of outside birds), to low (fences around poultry areas, poultry roam free in specified areas) to non-existent (free ranging animals) (FAO 2006). In many developing countries or areas where AI is endemic, little or no biosecurity is employed in poultry farming.

2.7.1.1 Risk factors on large-scale industrial integrated farms

Large-scale industrial integrated farms are physically isolated and can practise effective barrier control. However, if not managed appropriately, large production units face a high risk of disease introduction and onward transmission. The inward movement of people, poultry [145] and commodities is high in large production enterprises with different production components (day-old-chicks, nurseries, pullets, broilers, layers), providing many possible sources of disease introduction. When infected, they may run a higher risk of virus spread through higher levels of virus shedding and movement (of persons, animals, vehicles, equipment, feed, manure, etc.) on and off the production units [144].

2.7.1.2 Risk factors on specialized households or small-scale farms

Lack of bio-exclusion measures is very frequently found in this sector. Introduction of infected birds to flocks, contact with wild birds, housing multiple species and fomite transmission are the key risk factors [32, 42]. The level of risk in small-scale units may also be increased by sociocultural practices or lack of information (for example, the practice of throwing the carcasses of dead birds onto the street for dogs;

workers and the owners of poultry farms rearing scavenging birds at home; and the practice of giving live birds as gifts to visitors and the obligation for visitors to accept) [144].

Study in Bangladesh also found “footbath at entry to farm/shed” are risk factors for H5N1 infection in commercial farms (FAO poultry production sector 2 and 3) [32]. Study in Vietnam found the risk of an outbreak of HPAI H5N1 was increased in flocks that had received no vaccination or only one vaccination of flocks compared to flocks received vaccinations twice a year, and in flocks on farms that had family and friends visiting and geese present. And sharing of scavenging areas with flocks from other farms was associated with increased risk of an outbreak [34]. Research in Japan identified the introduction of end-of-lay chickens, sharing of farm equipment among farms, incomplete hygiene measures of farm visitors on shoes, clothes and hands and direct distance to the nearest case farm were risk factors associated with the introduction of AIV [33]. Study in the U.S. suggests the disposal of dead birds via rendering off-farm is an important factor contributing to rapid early spread of AI infection among commercial poultry farms [146]. Study in Bangladesh identified that the numbers of staff, frequency of veterinary visits, presence of village chickens roaming on the farm and staff trading birds are risk factors of AI infection on commercial farms [147].

2.7.1.3 Risk factors on backyard farms

Backyard poultry is characterized by small flock with low biosecurity measures. And backyard poultry have also been found to be an important source of spread and persistence of HPAI H5N1 in South East Asia [148].

Offering slaughter remnants of purchased chickens to backyard chickens, having a nearby water body and having contact with pigeons are identified as risk factors of AI infections on backyard farms in Bangladesh [149]. A study in Netherlands showed that raising of multiple species in backyard poultry and living in close proximity to infected commercial farm premises increases the risk of infection [150]. Study in south Africa also found out that large population of birds within farm, poor biosecurity and presence of outside birds were associated with increasing the risk of infection of ostriches with the H5 influenza virus[151]. A study in Nigeria indicated that receiving visitors on farm, purchasing live poultry/products, and farm workers living outside the premises were identified as risk factors for HPAI in poultry farms [152]. A study in Thailand show that farms where owners bought live chickens from another backyard farm had a higher risk of HPAI H5N1 infection, while those where owners used a disinfectant to clean poultry areas were exposed to lower risk [153]. A study in Vietnam found that increased density of ponds and streams, commonly used for waterfowl production, and greater number of duck flocks in the village increased the risk of AI infection [154].

Retail marketing of live poultry in LBMs was implicated as important source of exposure to infection on chicken farms in Hong Kong and the US [145, 155].

2.7.2 The role of live poultry trade

Live birds are presumed to constitute the highest risk because of virus replication, virus shed into the environment and movement over long distances [69]. The transport systems that carry birds from farms to markets or slaughterhouses presents risks of disease spread. The transportation they use is often dirty and not disinfected before entering farms or villages or markets, indeed there may be no facilities available for cleaning or instructions that it should be done – and birds are transported in cages or baskets that cannot be cleaned easily [144].

Understanding poultry movement is essential to develop appropriate and targeted surveillance recommendations for active HPAI H5N1 surveillance programs [41]. Traditional wet markets were village operations. Now, poultry can move from a mega-farm to a wet market and the cages, trucks and humans can return to the mega-farm (with viruses) in sufficient time to ensure occasional transmission [73]. Previous studies have shown evidence of the role of live poultry trade, traders, middlemen or catching teams in the dissemination of AI virus between farms, between farm and markets, farm to slaughterhouse or multiple slaughterhouses, and personnel [20, 41, 42, 156, 157].

A study in Vietnam also identified the role of trader's trading experience, those traders who have a trading year less than a year and operating at retail markets are more likely to source poultry from flocks located in communes with a past history of HPAI H5N1 than those trading longer than a year and operating at wholesale markets [39]. Large-scale movement of poultry is now commonplace and provides increasing opportunities for rapid spread of pathogens [3]. Designated vehicle for sending eggs to a vendor or market appeared to be a protective factor [32]. Besides, the cross-border trade via traders and middlemen may promote transboundary virus circulation [41, 158]. It is true in all situations that the greatest risk of spread of disease lies in the movement of live animals and contaminated materials, so biosecurity measures to reduce risk are heavily dependent on movement management [144].

2.7.3 Market level biosecurity risk factors for AI in poultry

LBMs are recognized as a reservoir of AI viruses and a possible source of infection for domestic poultry [69, 159-161]. Many markets, whether large or small, urban or roadside, have a low standard of sanitary conditions. Most LBMs tend to connect to one another. Markets that operate day in day out pose a higher risk than those that have closing days when premises can be disinfected [21, 35, 162, 163]. Those where birds of different ages and different species from different locations are mixed at the market, and then returned to their farm of origin or sold on to another farm, creating the potential for disease spread over a wide area [144]. Continual movement of birds into, though and out of markets provides opportunity for the introduction, entrenchment and dissemination of AIVs [164].

Previous studies identify the number of times the market was cleaned and disinfected [21], slaughtering at LBMs and trash disposal of dead birds [35] are risk factors for AI infection, while clear zoning at LBMs, daily removal of waste [36] and cage disinfection are protective factors [31].

A more detailed systematic review and meta-analysis was conducted to identify the effect of different biosecurity risk factors in the epidemiology of avian influenza at LBMs. This analysis was explored in Chapter 4 and has been published in the Journal of Infectious Diseases.

2.7.4 Risk factors associated with AI infections in humans

For human H5N1 infections, evidence is consistent with bird-to-human, possibly environment-to-human, and limited, non-sustained human-to-human transmission to date [24, 102, 165].

Probable risk factors for human infection include direct contact with sick or dead poultry, indirect exposure to sick or dead poultry, in the form of handling poultry, slaughtering, de-feathering, butchering, close contact with wild birds, visiting a wet poultry market, ingesting undercooked poultry products, direct contact with contaminated surfaces, close contact with infected humans [67, 166].

A study in China found the genetic sequences of the environmental and corresponding human H5N1 isolates were highly similar, demonstrating a link between human infection and LBMs [167]. Exposure to live poultry (by visiting either a retail poultry stall or a market selling live poultry) in the week before illness began was significantly associated with H5N1 disease [168].

The age of the patients ranged from 2 to 91 years with an average of 61.5 years. Over 69% of the patients were male with regional differences probably due to variable contact with poultry and about 84% were urban residents [99].

Exposure to market poultry is well acknowledged [169-172], however, exposure to farm poultry is also a cause for concern, especially in rural China [15]. As of February 2017, 887 of 1220 (about 73%) of human cases of laboratory-confirmed A H7N9 virus infection reported exposure to poultry within the 10 days before the onset of symptoms [173].

Case-control study in Shanghai suggests that chronic disease and frequency of visiting a live poultry market (>10 times, or 1-9 times during the 2 weeks before illness onset) were likely to be significantly associated with H7N9 infection [174]. Another case-control study in Jiangsu province found that direct contact with poultry or birds in the two weeks before illness onset, chronic medical conditions (hypertension excluded), and environment-related exposures were significantly associated with A (H7N9) infection [175]. A case-control study in Zhejiang province revealed that buying live or freshly slaughtered poultry from a market is a risk factor for both urban and rural residents. Tending to home-raised poultry and existence of a poultry farm in the vicinity of the residence are risk factors unique for rural residents [176].

A study of H7N9 also suggests that increased risk among older men is not due to greater exposure time at live bird markets, other factors may be contributing [177]. Elderly men, especially those with chronic diseases were at high risk of human infection with H7N9 [178, 179]. Study in Cambodia shows that males had a higher exposure risk potential than females across all age groups, males between the ages of 26-40 reported practices of contact with poultry that give rise to the highest H5N1 transmission risk potential, followed closely by males between the ages of 16-25 [180]. The live poultry purchasing habits, poultry handling, and living conditions are also found to be important factors that increase the risk of exposure to H7N9 virus contaminated environments in China [181].

2.7.5 Knowledge, Attitudes and Practices (KAP) of poultry workers and consumers towards AI

Existing literature indicates that age, sex/gender, education/knowledge and religion can influence the health behaviours, hygiene practices and utilization of advice in humans [182]. Many of these risk factors can easily be reduced through simple and inexpensive procedures, however lack of knowledge often leads to bad application and unsafe behaviour (for example, working in a dirty area and then moving to a clean area rather than the reverse; failure to quarantine new birds; and generally poor hygiene) [144].

Previous study found there was a higher level of biosecurity practices adopted in poultry farms compared with those adopted in LBM. Most poultry workers who were aware of AI had high knowledge regarding measures of prevention, but there was a poor correlation with actual practice [183]. People transporting live birds usually have little or no information on the health status of the birds they are carrying. Bird handlers with secondary level of education were more likely to be involved in open site slaughter of poultry than their counterparts without formal education. Comparatively, bird handlers in urban area were less likely to share poultry equipment than rural resident handlers [182].

A KAP study of H7N9 risk among live poultry traders in Guangzhou province of China was carried out after the emergency of H7N9 in China, found that only 46.1% of the LPTs recognized risks associated with contacts with bird secretions or droppings, and only 22.9% perceived personally “likely/very likely” to contract H7N9 infection. Besides, around 60% of the respondents complied with hand-washing and wearing gloves, and only 20% reported wearing face masks. Only 16.3% of the respondents agreed on introducing central slaughtering of poultry [184].

A KAP survey of poultry handling behavior among villagers in rural Cambodia revealed that despite high awareness and widespread knowledge about AI and personal protection measures, most rural Cambodians still often practice at-risk poultry handling. Intervention programs must include feasible options for resource-poor settings that have limited materials for personal protection (water, soap, rubber gloves, and masks) and must offer farmers alternative methods to safely work with poultry on a daily basis [185, 186].

A study of AI risk perception among householders in Hong Kong, which was carried out after the first H5N1 epidemic with human deaths, found that 11% of the participants touched live chicken when buying; 36% perceived this behaviour as risky, 9% estimated the likelihood of resultant sickness is over 50%, whereas 46% (43%–49%) said friends worried about such sickness [187].

A study of knowledge and risk perception of AI in Taiwan found those more knowledgeable of AI with relatively high levels of risk perceptions would be likely to stay away from birds and the crowd. Respondents with relatively low levels of AI knowledge were likely to prefer not eating chicken at all under a possible threat of AI outbreaks. Respondents with low risk perception levels would be more likely to maintain usual chicken consumption than those with high risk perception levels if outbreaks of AI occurred [188].

Study in Guangdong province of China found generally high support for regular market rest days among the general public and live poultry traders (LPTs), but only limited support for permanent central slaughtering of poultry. LPTs' support for relevant control measures declined after the citywide wet market closure [189].

A study on employees of food production and operation in Guangzhou found about 70% worried about being infected with the A/H7N9. Nearly one-third (32.35%) did not believe that the government could control the A/H7N9 epidemic. Most participants (80.76%) reported washing hands more frequently than before, while over one-third (37.17%) stated no longer buying poultry. A total of 84 % indicated a willingness to receive an A/H7N9 vaccine, and the primary reason for not being willing was concern about safety (58.19%). A history of influenza vaccination and worry about being infected with the A/H7N9 were significantly associated with intention to receive an A/H7N9 vaccine [190].

A novel educational program conducted in Vietnam found improved awareness of H5N1 resulted in more people seeking early access to healthcare, and also resulted in earlier medical intervention for patients with H5N1 infection in Vietnam [191]. The internet (76.92%), television (67.56%), and newspapers (56.26%) were the main sources for food company employees obtaining information, and varied by demographic variables [190]. Radio, leaflets/booklets, school students and village health volunteers were found to be the most effective sources in increasing knowledge of rural dwellers [186].

2.7.6 Identified gaps in knowledge

There are dozens of researches on identifying the risk factors associated with AI infection on poultry farm level and LBMs, it is still unclear what role LBM has played in the circulation of AI in many Asian countries where LBM are prevalent. And there is already one meta-analysis of the association of production, management, environment and biological factors on poultry farm level [9]. However, systematic review or meta-analysis at LBMs hasn’t been done so far, it is very necessary to conduct a formal systematic review and meta-analysis to quantify the relative importance of market level biosecurity risk factors such as cleaning and disinfection, market closures, manure disposal and management practices of all-in-all-out from different studies, so to better understand how each risk factor influences the AI infection at market level.

Planning effective changes in health-related behaviour requires a deeper understanding of the perceptions of risks, biases, causal attributions, and both the facilitators and barriers to health behaviour change. Studies on the knowledge, attitudes and practices of the general public and poultry workers (including farmers, sellers and traders) associated with AI have been previously reported. These studies have been conducted on discrete groups of different poultry networks which poses a problem of generalizability and risk attribution across the whole network. More studies are need that consider the continuous of knowledge, attitudes and health beliefs of all actors across the entire poultry system.

2.8 Approaches to the prevention and control of AI globally and in China

2.8.1 Approaches to the prevention and control of AI in poultry globally

Biosecurity is the first line of defence in the prevention and control of all AI viruses [192]. But when the AI viruses have been introduced into poultry populations, control is largely dependent on a range of instruments, vaccination, surveillance, stamping out, and quarantine and movement control besides biosecurity. To date, control of HPAI H5N1 in endemic countries has basically relied on poultry vaccination and massive culling. And none of them can defend against the virus on its own [144, 193]. OIE affirms the most effective strategies for dealing directly with AI are early detection and early warning, rapid confirmation of suspects, rapid and transparent notification, rapid response (including containment, management of poultry movement, zoning and compartmentalization, humane stamping out and vaccination where appropriate) [194]. These measures are widely adopted in different countries.

Enhanced biosecurity by cleaning and disinfecting of infected premises, restricting domestic and wild bird mixing, separating poultry areas from other domestic animal areas and separating poultry and human areas greatly reduces the likelihood of transmission between animals [96, 148, 155]. Control of AI in Hong Kong has been effected through a combination of quarantine, tightening of biosecurity measures, and depopulation of infected and contact farms [195].

Since the late 1990s, due to the continuing and widespread outbreaks of LPAIV, the use of vaccines for the control of H5 and H7 infections has been approved to control the disease in poultry and to prevent possible mutations to HPAI viruses [99]. Vaccination against HPAI H5N1 is currently allowed and used in some countries, Hong Kong, Vietnam, Indonesia, China, India, Russia, Egypt, and Pakistan [196].

Measures such as culling, stamping-out plus cleaning and disinfection that have been effective in Europe have not been successful in eradicating the disease in Asia [197]. However, massive culling whenever HPAI/LPAI H5 or H7 is detected has largely been successful at preventing transmission among poultry farms during HPAI outbreaks in Netherlands, Italy, Canada and Hong Kong SAR of China [87, 96, 128, 148, 155, 156, 198, 199]. Since July 2017, after a sudden increase of human cases and more widespread geographical distribution of H7N9 in China as well the mutation of LPAI H7N9 to HPAI H7N9, a H5 and H7 bivalent inactivated vaccine [138] was adopted by Chinese government in the poultry sector through the “National Immunization Program for HPAI”.

Kingdom of Saudi Arabia (KSA) was declared free of HPAI in April 2008 by the WHO. During the H5N1 outbreak from 2007 to 2008, the KSA government made immediate decisions to depopulate all

H5N1-affected and non-affected flocks within a 5-km radius area and applied quarantine zones to prevent the virus from spreading to other areas. Other control measures, such as closure of live bird markets and intensive surveillance tests on all poultry species within quarantine zones, were in place during the outbreak [200].

Strict quarantine and controls on movement of poultry have been largely used in Europe, Asia and Africa after the detection of H5N1 [55, 201]. Quarantine, tracing and screening of suspected flocks are also undertaken by endemic countries whenever HPAI/LPAI H5 OR H7 is identified [148, 155, 156].

However, there are also some issues raised during the implementation of these measures, for example, difficulty in implementing biosecurity measures in settings with backyard farms, as well as vaccination and movement restrictions; the massive culling causes huge losses for commercial farmers; the poor quality of AI vaccines and the insufficient vaccination coverage could only partially reduce virus shedding and the bird or some flock might still spread the virus; the possibility of mutation in the circulating virus that AI vaccines may promote may perpetuate the risk of infection in the original species or in another; and the major difficulty of differentiation between infected and vaccinated animals [196]. Culling without compensation policies as in Southeast Asia can prove counterproductive i.e. economic losses that discourage reporting [202].

Currently no H5 nor H7 vaccines are currently commercially available for humans. Suspected H5N1 patients who are confirmed by diagnostic testing usually receive a neuraminidase inhibitor (oseltamivir or zanamivir) despite the uncertainty regarding the optimal dose and duration of treatment. For health workers and close contacts in a non-pandemic setting, precautions like isolation, high-efficiency masks (N-95 masks or multiple surgical masks) and restricted visiting have often been used. For persons who have had a possible unprotected exposure to H5N1 virus, chemoprophylaxis with 75 mg of oseltamivir once daily for seven to ten days is warranted [24]. In China, for H7N9 confirmed patients, the neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) are used for clinical treatment [101]. Measures of publicized preventive knowledge through various kinds of media are also widely adopted.

Since late 2003 and early 2004, after the intensive report of HPAI H5N1 outbreaks in Southeast Asian countries, the international organizations, particularly FAO, OIE, WHO, WB and EC, often actively work together to support the affected and at-risk countries. Their continuous assistances cover broad aspects, including strengthening capabilities for field surveillance, laboratory, early warning and emergency preparedness; coordinating information sharing and networking, researches, provision of experts; and support of broad awareness creation and risk communication, analysis of and advice on the social and economic consequences of both the disease and its control, implementing emergency and mid- to long-term national control strategies and policies. In addition, several networks and platforms are built for continuous supportive of disease prevention and control in both animal and human health, including Global Early Warning System (GLEWS), OIE-FAO Avian Influenza Network (OFFLU), the Emergency Centre for Transboundary Animal Diseases (ECTAD), FAO’s EMPRES Global Animal Disease Information System (EMPRES-i), OIE’s WAHID Interface provides access to all data held within OIE's new World Animal Health Information System (WAHIS) (former named Handistatus II System). OIE and FAO also set up their Reference Centres for different diseases in different countries.

2.8.2 Approaches to the prevention and control of AI in China

Control of AI in poultry in China is led by the Veterinary Bureau (VB), a Department/Bureau within the MARA of China, which is responsible for livestock health and disease control and prevention in China, implemented by provincial and local veterinary service departments and animal health supervision and inspection departments. The disease control and prevention in wild life populations fall under the responsibility of the State Forestry Administration (SFA, formerly belongs to MARA of China). The human infection with AI is the responsibility of the National Health Commission (NHC, former Ministry of Health), implemented by provincial and local human health departments.

2.8.2.1 Overview of poultry AI surveillance system in China

Every year, a large amount of surveillance testing is being undertaken by national and provincial veterinary services. The primary target of national surveillance are chickens, ducks, geese and other poultry or wild birds. These are sampled from poultry farms, commercial poultry farms, and backyard poultry raising households, poultry trading markets and slaughter houses as well as from main habitats of migratory birds for wild birds. The surveillance design and sampling frame follow the national guidelines established by the VB of MARA of China. Generally, there are two types of surveillance schemes, routine surveillance and centralized surveillance program.

The routine surveillance program consists of a combination of active surveillance and passive surveillance. Passive surveillance targets poultry and wild birds which are found dead of sickness or unknown apparent reasons. The active surveillance focuses on establishing fixed sentinels and randomly sampling the sentinels to monitor. The active surveillance, consisting of serological and virological surveillance, is conducted monthly at provincial level by provincial animal CDC, and the implementation may be subject to the actual situation of each province.

The centralized surveillance is conducted twice a year, usually during spring and autumn, to complement the routine surveillance performed by provincial animal CDCs on a monthly basis. The centralized surveillance also consists of serological and virological surveillance.

The virological surveillance is designed to detect the silent circulation of HPAI, H5N1 virus, the emergence of new strains and identify potential vaccination failure. The serological surveillance is designed to test the vaccination coverage and proficiency of the vaccines used.

Since the emergence of H7N9 outbreaks in China, the MARA OF CHINA issued an Emergency Surveillance Scheme on H7N9 on 7th August 2013. The surveillance targets were specified chickens (especially layer, yellow feather broilers and other breeds which have long raising cycle), waterfowl (ducks, geese), domestic pigeons and quail, wild birds and environment in high risk areas. The scope of surveillance was specified to be all poultry trading markets in China, stalls selling live poultry in farmers markets, poultry with certain size, backyard poultry raising farmers, poultry slaughter houses, and habitats of migratory birds [203]. All the results are then compiled at national level by MARA of China that publishes the monthly Veterinary Official Bulletin which aggregates all the results of post-vaccination surveillance, active virological surveillance and outbreak reports for several notifiable diseases [204]. Since July 2013 and onwards, the surveillance results of H7N9 are also reported in the Veterinary Official Bulletin.

In February 2014, in order to better respond to H7N9 human infection, MARA of China released the National H7N9 Elimination Plan to ensure timely detection and remove of H7N9 influenza virus from poultry farming and market circulation and to enhance AI surveillance capacity of veterinary laboratories at provincial, prefectural and county levels. The plan aims to ensure proper disposal of confirmed poultry cases, and to make efforts to reduce the risk of transmission to human as well as the risk of virus spreading from the live bird markets to poultry farms. By these means to ensure the safety of poultry production, the quality of poultry products and public health [64].

2.8.2.2 Prevention and control of AI in poultry in China

MARA of China has established regulations of biosecurity management on farms. (E.g. local animal health supervision institution shall not issue the Animal Quarantine Certification to farms which failed to meet the standards in the censorship; disinfection shall be carried out for vehicles, loading tools and relative personnel; prevent the poultry of contacting with wild birds and other wild animals; encourage the partition on the farms to improve the biosecurity levels.) Most of the large-scale farms conform to the regulations, however, the majority of domestic poultry is bred in small-scale farms and backyard that lack biosecurity practices. Therefore, in China it is a great challenge to completely control or eradicate AI relying on biosecurity measures [128].

Beginning from 2004, a culling plus vaccination strategy has been implemented for the control of HPAI epidemics. Since then by 2008, over 35 million poultry have been depopulated, and over 55 billion doses of the different vaccines have been used to control the outbreaks [128]. After the final confirmation of a highly pathogenic H5N1 avian influenza infection, all of the poultry within a 3-kilometre radius should be depopulated and compensated with 10 Chinese Yuan for each poultry (subject to the poultry species and the ages in days). Disinfection and movement control are implemented for 21 days after the poultry depopulation. Any existing live bird market within a 10 kilometre radius will be shut down for at least 21 days [128, 205].

Mass vaccination against HPAI H5N1 has been implemented since November 2005. The vaccination of HPAI H5N1 in poultry (mainly chickens, ducks and geese) in China is compulsory. Vaccine is freely provided by government, the vaccine coverage of the nominated species is requested to reach 100%, and the positive rate of the antibody is supposed to reach 70% [206]. Massive vaccination has been effective and played an important role in reducing the incidence of H5N1 infection in poultry and in markedly reducing the number of cases of human infection [207]. An inactivated vaccine has been used in chickens to control the H9N2 infection since 1998. It is voluntary, some poultry farms may apply, and some may not. From July 2017, Chinese government implemented National Vaccination Program for H7N9 in the poultry sector through the adoption of a H5 and H7 bivalent inactivated vaccine, currently this vaccine seems to be effective at controlling the virus circulation and consequently decreased the number of human cases [136, 138, 208].

However, the vaccination strategy against H5N1and H7N9 still faces different challenges in different avian species. The H5 vaccines are relatively easy to apply in chickens on large farms but are difficult to administer to chickens raised in backyard and small-scale farms which accounts for over 70% of the total birds [207]. The H5 vaccination rates in duck and geese are relatively low and could still serve as a reservoir for the virus [207]. The continued circulation of H5N1 viruses in southern China may then be a result of the low vaccination coverage [207, 209]. Similarly, although the adoption of vaccination against H7N9 seems to be effective, the fact that the virus is still present at low levels in farms and LBMs means that there is still a chance of resurgence in future, particularly in areas where vaccination has not been used [136].

The China MARA, MoH (current NHC) and SAIC (current SAMR) jointly issued the ‘Regulations of prevention and control of HPAI in LBMs” in 2006 [210]. The regulations emphasized the strict implementation of the regular market rest and disinfection day policy. And all live poultry that enter wholesale LBM should present the animal quarantine confirmative certificate. Unsold live poultry are prohibited from being shipped back to the farm. Once positive results are found, animal husbandry and veterinary departments advise the local government to close the LBMs. After thorough cleaning and disinfection and 21 days later, after joint analysis and evaluation and approval from the provincial animal husbandry and veterinary authorities and relevant departments, then the market can be re-opened. After the emergence of H7N9, MARA of China revised the regulations that the retention of live poultry overnight in the LBMs (live poultry booth/stall) is not allowed. Sold live birds should be slaughtered before they are taken out of the market [64]. Recognizing the importance of the LBM in the exposure and dissemination of H7N9 viruses, in Feb 2017, the MARA of China established the “1110 policy” on LBMs, i.e. clean once a day, disinfect once a week and close market once a month, and zero overnight storage in market.

The supervision of live poultry movement was enhanced with detailed pre-movement testing specifications as a requirement for the issue of the animal quarantine confirmative certificate in the No. 2516 Announcement of MARA of China released in April 2017 [211]. This announcement specified that poultry farms who are going to undertake inter-provincial live poultry transport should take the initiative to entrust the local veterinarians to collect serological and virological samples to test for H7N9 virus within 21 days before the transport and the samples should be sent to local animal disease prevention and control institutions for laboratory testing within 48 hours after sampling. Meanwhile, the sampling should cover all the sheds on the farm. The sampling quantity should not be less than 30 feathers per sample and records shall be kept for future reference. The animal quarantine confirmative certificate shall not be issued if there is any positive detected or the testing report is issued 21 days before the day of sampling.

Additionally, there are many foreign aid projects in China delivered through the VB of MARA of China, the most influential ones are the USAID project on HPAI (CFETPV program, LBM market investigation and social economic analysis) and the World Bank project on HPAI (phase 1, 2, 3).

2.8.2.3 Prevention and control of AI in humans in China

The Chinese government has been focused on enhancing surveillance and laboratory testing for human AI. Besides that, enhanced epidemiology investigation is conducted immediately after report of the human infection, observation of all close contacts and health workers related to the human cases. The

China CDC also publishes interim guidelines to limit the possibility of human infections during the outbreaks of AI in poultry or wild birds [212].

In China, all suspected H5N1 cases are reported to the Chinese Centre for Disease Control and Prevention (China CDC, Beijing, China) through a national surveillance system, which is based upon two reporting mechanisms, one is hospitalized cases of pneumonia of unknown origin, and the other one is enhanced 1-month surveillance for cases of influenza-like illness at all health-care facilities within a 3-km radius after the occurrence of a suspected or confirmed H5N1 poultry outbreak with high bird mortality [213, 214]. The specimens from the suspected H5N1 human case are initially tested by influenza laboratories at provincial level, as part of the nationwide influenza surveillance network. The network consists of 200 sentinel hospitals and 84 influenza diagnosis laboratories [93].

After the emergence of H7N9, the NHFPC established the Joint Prevention and Control Mechanism (JPCM) to lead the national response to H7N9, issued the first national H7N9 technical guideline, and implemented enhanced surveillance for H7N9 virus infection among persons with influenza-like illness in the existing sentinel surveillance system. Local JPCM was established in several provinces as well. [102].

2.8.3 Identified gaps in knowledge

Many prevention and control measures have been identified to be effective to contain AI infection by many countries. Despite existing control programmes there are still infections of AI being reported and AI viruses isolated from surveillance programs from time to time. There is a strong need to assess the vulnerabilities of the system for effective prevention and control of AI. And there is a need to consider the whole poultry market chain system approach to integrate prevention and control practices at all levels, i.e. farm level, trader/intermediary, market level and consumer. Only researches into understanding of the biosecurity behaviour and risk perceptions of different actors along the poultry market chain can provide the necessary evidence to address these control and prevention issues.

Chapter 3 Data Sources and Methodology

This Chapter contains a detailed description of the data sources and methods used in each research Chapter.

3.1 H7N9 infections in human and poultry dataset and risk factors

The data on the presence of H7N9 in the 24 LBMs (analysed in Chapter 5)was available from the official national emergency surveillance activities conducted during 31 March to 19 April 2013; data on H7N9 infection in poultry and humans used for mapping was up to the end of May 2013, both infection data were extracted from the official website of MARA of China [215] and WHO [216], and geocoded based on the reported geographical source of infection.

We obtained all laboratory-confirmed H7N9 human cases reported during 2013-2017 (analysed in Chapter 7) from "Situation Updates - Avian Influenza" of WHO [216] and "Avian Influenza Report" from Centre for Health Protection of Department of Health of Hong Kong SAR [217]. Case definitions and laboratory testing have been described previously [218, 219]. For each human H7N9 case, the information of county of residence and date of onset symptoms was extracted. Data on poultry serological and virological surveillance were obtained from the monthly official Veterinary Bulletin released by the Veterinary Bureau of the MARA of China [204], from which we extracted data on positive identification of H7N9 from the national H7N9 surveillance program between 2013 and 2017. All samples were tested at provincial level and confirmation of H7N9 virus was based on polymerase chain reaction (PCR). All AI positive samples were sent to the Harbin National Veterinary Research Institute for confirmation, subtyping and virus isolation. All reported H7N9 human cases and poultry virological surveillance positives were then geo-referenced and linked to county level map of China. The georeferencing was based on the geographic locations of the H7N9 infections or county centroids when detailed location was not available.

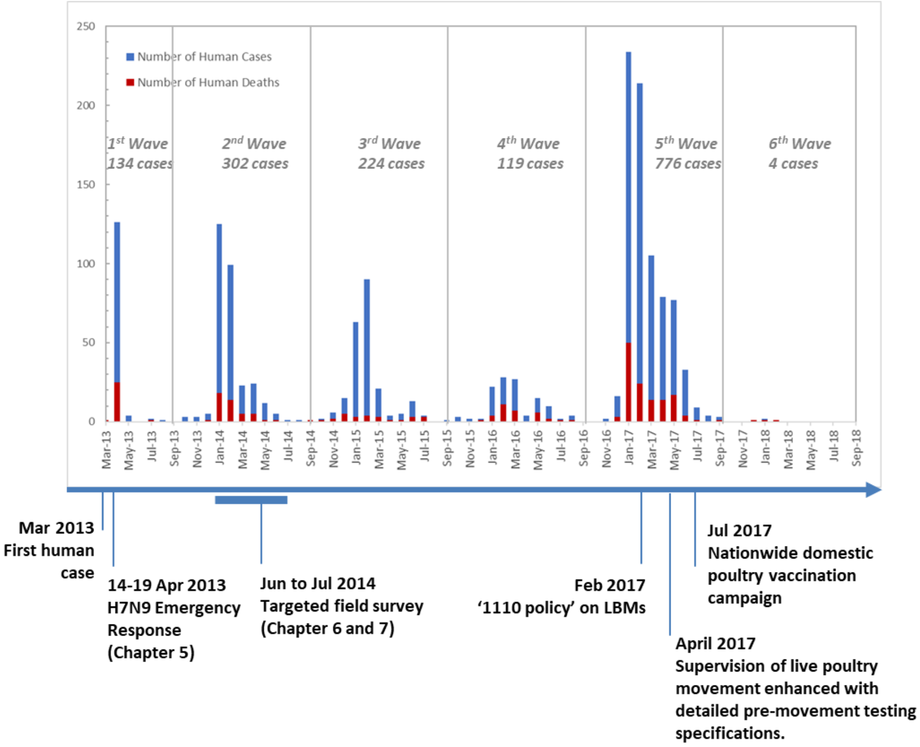


Figure 3-1 Timeline of the six H7N9 epidemic waves and some implemented control policies, as well as the conducted studies in this Thesis.

3.2 Epidemiological field surveys

3.2.1 Epidemiological investigation during the H7N9 emergency response in April 2013

During the emergency response to influenza A (H7N9) infection in humans, a cross-sectional survey was conducted in 24 LBMs (15 wholesale markets and 9 retail markets) in the Shanghai provincial-level municipality, Jiangsu, Zhejiang and Anhui provinces from 14 to 19 April 2013. The 24 LBMs included in the study were selected purposively based on two criteria: first, we focused on all LBMs within 1 kilometre in the adjacency of areas with reported H7N9 human infections (

-A), and second, we selected all LBMs that marketed more than 20,000 heads of poultry per day at the time of the outbreak. Therefore, the LBMs selected best represented existing production and LBM marketing systems.

This investigation included data collection on live poultry movement and market biosecurity. The managers of the 24 LBMs were interviewed using a standardized, validated questionnaire to capture information relative to trade and general market biosecurity measures. The questionnaires had been used in previous studies by our team [31, 40]. About 90% of live poultry are thought to come through wholesale markets on the way to retail markets. Some wholesale markets that also sell live poultry directly to the consumer have been defined as mixed markets. Information on poultry movements was obtained from poultry movement certificates available at nine of the 24 surveyed markets (four wholesale LBMs and five mixed LBMs), and was recorded for up to three months before the reporting of the first human case in the province, i.e. Shanghai (1 January to 5 April), Jiangsu (1 January to 4 April), Anhui (1 January to 15 April) and Zhejiang (1 January to 11 April). The nine markets were from Shanghai (n=3), Nanjing (n=2), Hangzhou (n=1), Huzhou (n=1), Hefei (n=1) and Chuzhou (n=1). The poultry movement certificates recorded the name of the county of poultry sources, poultry species (e.g. chickens, pigeons, ducks and other types) and the numbers of poultry transported.

3.2.2 Epidemiological field survey in Anhui, Jiangsu and Shanghai during June - July 2014

3.2.2.1 Study design

A cross-sectional questionnaire survey was conducted from June to July in 2014 targeting meat chicken farmers, live chicken vendors and chicken consumers in LBMs in six counties located in Jiangsu (Lishui, Jintan, Jiangyan) and Anhui (Feixi, Quanjiao and Chaohu) provinces. Selection of counties was based on findings from the program of research in Chapter 5 (conducted during the H7N9 emergency response), which demonstrated that the chicken movements were highest in those six counties from Jiangsu and Anhui provinces [10]. One county in Shanghai municipality (Fengxian) was also included in this survey because Shanghai was the origin of the first H7N9 human cases reported in 2013 and it is adjacent to Jiangsu province where there was a geographic co-distribution of H7N9 and H5N1 in humans [29]. An initial sample size of 10 commercial meat chicken farms, one wholesale LBMs and two to three retail LBMs (subject to actual numbers of LBMs) in the seven high risk counties were included. Within each LBM 10 chicken vendors and 10 consumers were interviewed. Maps of the study area and the locations of participants are presented in Figure 3-2 and Figure 3-3. The surveyed farms are located in rural area in each county while the surveyed LBMs are located in urban area.

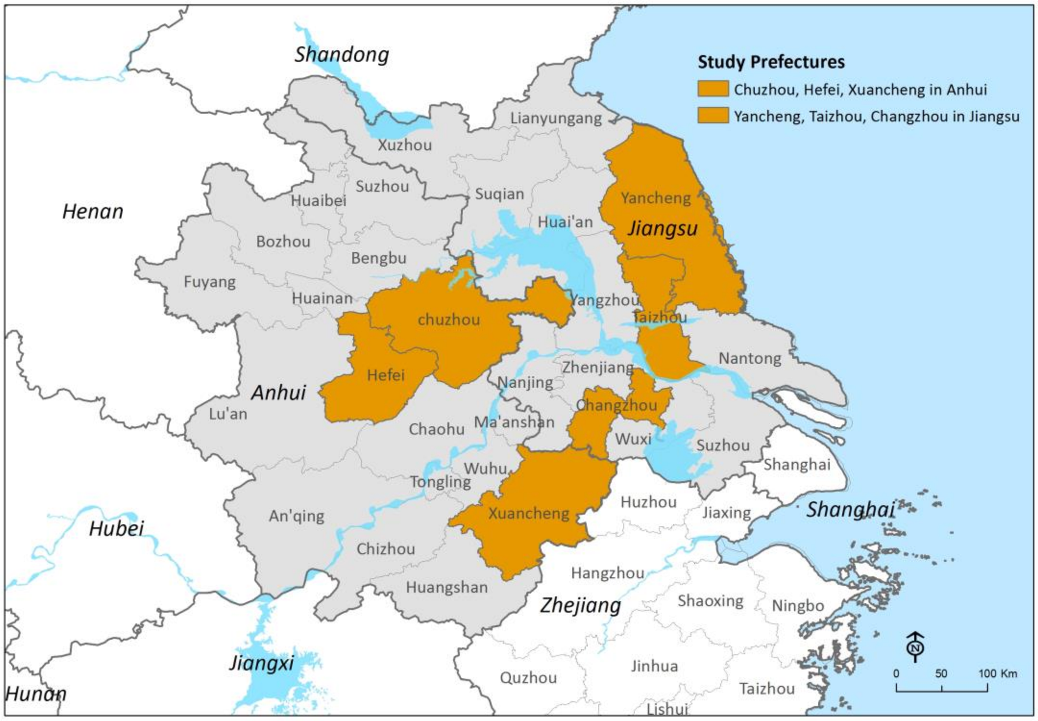


Figure 3-2 Field survey area

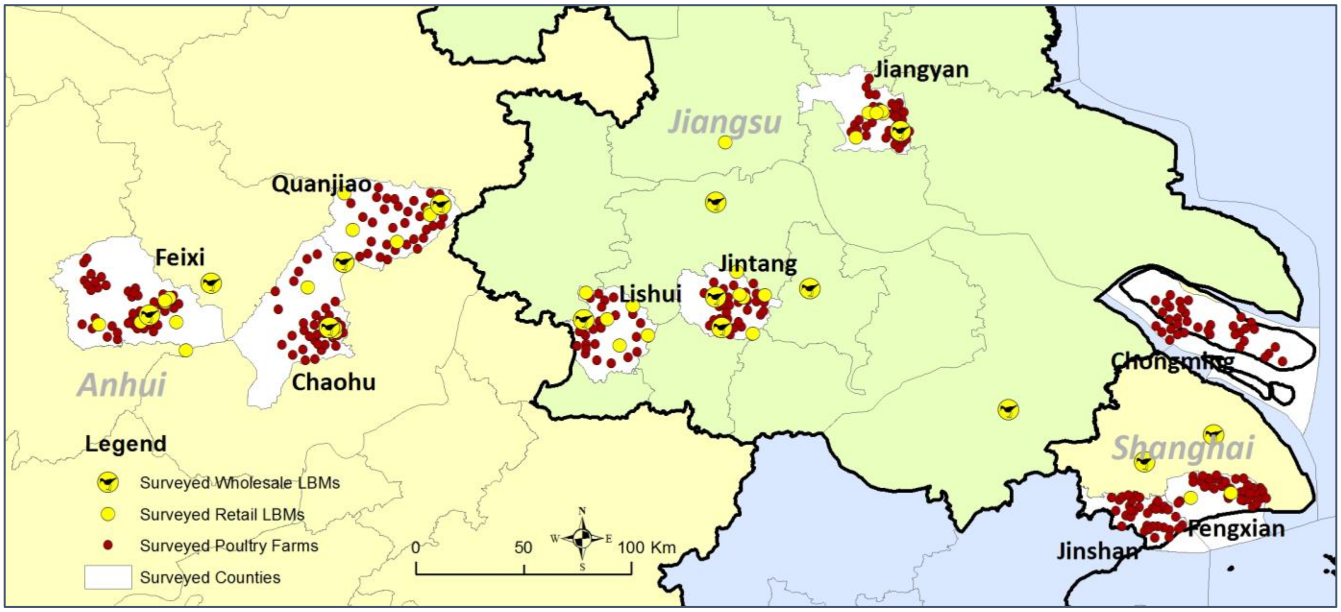


Figure 3-3 Map of survey locations, the yellow dots are the surveyed live bird markets, the big ones with a bird inside represent the wholesale LBMs, the small ones are retail LBMs, the red dots are chicken farms.

3.2.2.2 Knowledge, attitudes and practice data of farmers, traders and consumers

Face-to-face interviews were used to elicit knowledge, attitudes and practices of chicken farmers, live chicken vendors and chicken consumers at LBMs in the target counties associated with avian influenza. Structured questionnaires included questions to capture data on characteristics of participants (e.g. gender, age, education level, employment status, years of working with chicken, hours of contacting chicken in a day), knowledge about AI (e.g. "Is AI an infectious disease?" "what types of animals can be infected with AI" etc.), attitudes towards AI (e.g. "Do you think AI is a severe disease for humans?", "What do you think is the likelihood for you to get AI?" etc.), and practices regarding AI prevention (e.g. What do you do when you suspect that you have flu symptoms?) (See Appendix C for full set of questions).

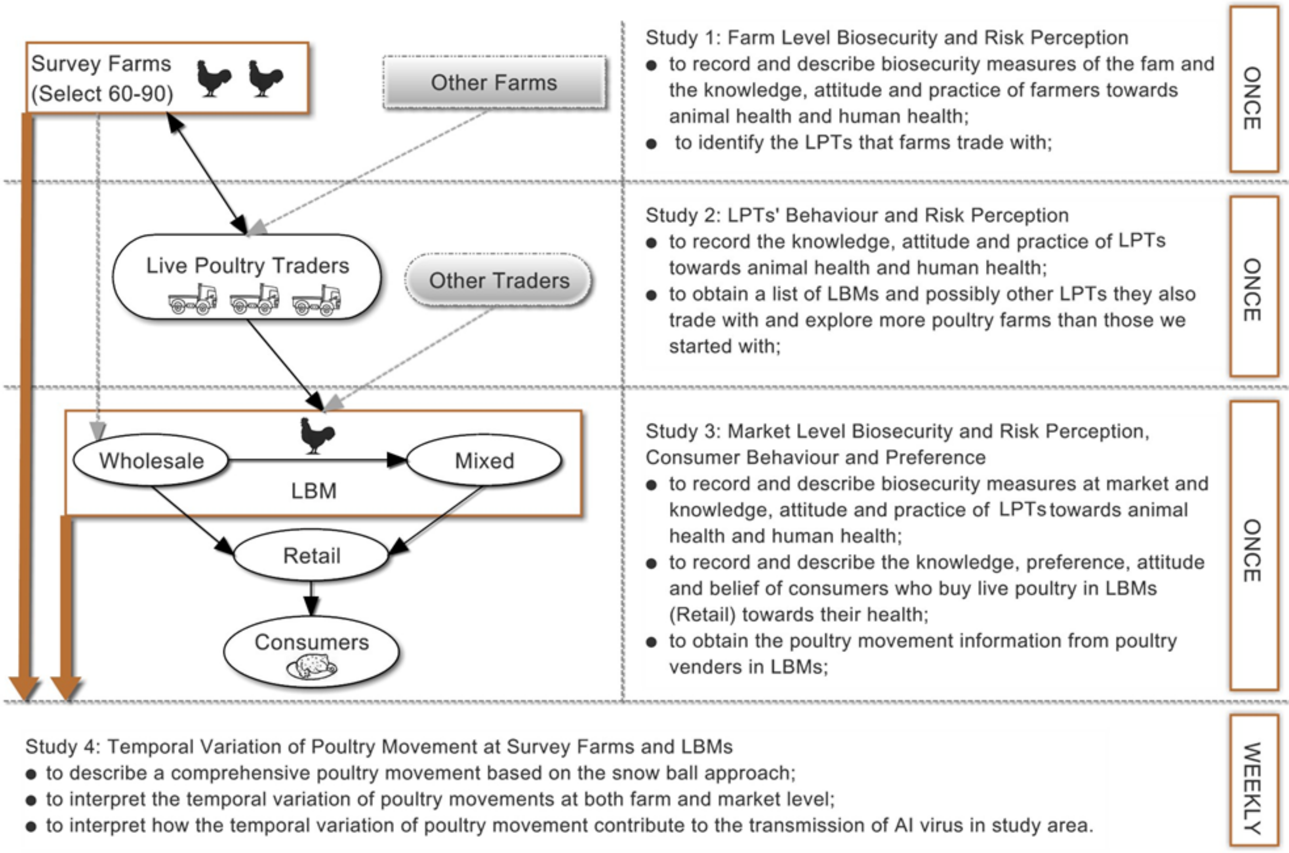


Figure 3-4 Field survey plan

3.2.2.3 Live chicken movement datasets

Information on live poultry movements was obtained from poultry movement certificates available at wholesale LBMs and live poultry trading platforms (similar to a wholesale LBM) in the selected counties. All available chicken movement certificates in the trading locations surveyed were recorded from January 2014 to July 2014. This dataset records half-year chicken movements covering the time period of Chinese New Year festivals, during which period the temporal variation of live poultry trade in Southern China was found to be associated with higher HPAIV H5N1 infection risk in humans and poultry [40]. This dataset is representative of the extent of live chicken movement within and beyond that region.

3.2.3 Ecological risk factors

The analysis in the program of research in Chapter 7 focused on a limited set of risk factors, including the density of live-poultry markets, in addition to a set of other factors that have been proven in the past to show consistent geographical correlation with avian influenza. The set of risk factors included the presence of wholesale LBMs, number of retail LBMs (markets per county), presence of poultry virological positives, human population density (people per km2), chicken density (birds per km2) and network degree centrality estimated based on the chicken movement data collected in the field survey. All risk factors were compiled at county level where the policy is most likely to be made. These risk factors are described in Table 7-1.

3.3 Systematic review and meta-analysis

3.3.1 Search strategy

Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, we performed a systematic literature search using PubMed, ISI Web of Science and Science Direct, CNKI (the China Academic Journals full-text database) and WANFANG database (includes most comprehensive online full-text Chinese medical journals) with no starting time limits, up to 10 Jun 2018. The search strategy used four PICO (participants, interventions, comparators, and outcomes) components (Table A-1).

3.3.2 Eligibility criteria

Epidemiological studies were included if they evaluated biosecurity risk factors for AI infection in LBMs in poultry, the environment or human populations. Studies were excluded if: 1) they were laboratory studies, descriptive studies, case reports, and vaccine efficacy studies; 2) the outcome recorded was not AI infections; 3) they were not LBM based studies (i.e. ecological studies, studies at local, regional or national levels, studies at farm level); 4) there was no effect size for LBM biosecurity risk factors reported.

3.3.3 Data abstraction for market-level biosecurity indicators

For each of the papers that met the inclusion criteria, we recorded information on subject title, first author, year of publication, country, language, subtype of AIV and its pathogenicity, total sample size, number of AI positives and negatives, risk factors, infection type, study type and analysis methods. Data on biosecurity indicators were extracted by two independent reviewers (XZ, RSM), and a dataset based on these characteristics was created in MS Excel. In this study, we analysed biosecurity indicators for poultry and market environment infection and market workers' infection (i.e. non-symptomatic seropositive) separately. A total of 34 biosecurity risk factors were explored in our study (Table A-2). For market infection, the following groups of biosecurity indicators were considered: A) market characteristics: market type, market size, market location (rural or urban area), market location (central city or non-central city areas), and presence of multiple species, presence of ducks and presence of rabbits ; B) market biosecurity management: conduct cleaning and disinfection, before and after cleaning and disinfection, conduct waste disposal, conduct market closure, before and after rest day, ban on overnight storage, poultry sources, separate different species and conduct slaughter in market; C) seasonality, temperature. For market workers' infection: D) socio-demographic characteristics: sex, age, years working in LBMs, type of market (wholesale or retail), vaccination history and occupation; E) activities involving exposure to poultry: conduct cleaning, conduct feeding, contact poultry, conduct slaughtering, defeathering and evisceration.

3.3.4 Study quality and bias assessment

Two authors (XZ, RJSM) independently reviewed and assessed the quality of each English paper using a structured approach. Papers in Chinese were translated by XZ and evaluated by XZ and RJSM. The quality of each study was scored on seven quality assessment criteria (**Error! Reference source not found.**Table A-3). Studies that recorded a higher overall score were considered of superior quality. The scores from quality assessment were then rescaled into quality ranks between 0 and 1 by making them relative to the highest scoring study in the group; then the best study was ranked 1 and those with lower scores were ranked lower. These ranks were then utilized by the quality effects model to adjust estimates of effect [11].

3.3.5 Meta-analysis

The odds ratio (OR) and 95% confidential interval of each biosecurity factor were extracted from each study, or if the odds ratio was not reported, we calculated it using Epi Info TM 7.1.5.2 based on the raw data reported. When a factor was tested in both a univariable and multivariable model, the effect size of the factor in the multivariable model was used. The odds ratios of each factor was modelled by applying a quality effects (QE) meta-analysis model that assumed heterogeneity across the included studies [11]. The results of the analyses were statistically significant if the 95% confidence interval did not include the value one.

The QE model redistribution of weights due to the rescaled quality rank (called Qi in the MetaXL software described below) helps reduce estimator variance as well as allowing for proper error estimation through the confidence interval thus generated. Nevertheless, the random effects results are in Appendix Figure A-1, Figure A-2, Figure A-3, Figure A-4 and Figure A-5 for comparison. All results are presented as a forest plot that shows individual OR estimates for each group of biosecurity indicators and overall for the category. We assessed study heterogeneity by the Cochran Q Chi-square test, and this is also used by the I2 index statistic to estimate the proportion of total variation due to heterogeneity. An I2 value of <25% indicated low heterogeneity, 25-75% indicated moderate, and a score of >=75% suggested high degree of heterogeneity. Publication bias was assessed using Doi and funnel plots. All analyses were conducted using MetaXL, version 5, Epigear International, Sunrise Beach, QLD, Australia (www.epigear.com).

3.4 Analysis of spatial autocorrelation

To assess whether there was spatial autocorrelation in the observed pattern of human H7N9 infections in the study area we used the Global Moran's Index (Moran’s I), a measure of spatial autocorrelation for spatially aggregated data. We used the incidence rate of human H7N9 infections per 1,000 (i.e. estimated by dividing the observed number of human cases by the total human population in the county and multiplied by 1,000) for estimation of Moran’s I. Moran's I is positive when nearby areas tend to be similar, negative when they tend to be dissimilar, and approximately zero when attribute values are arranged randomly and independently in space [220]. The Moran's I value and a Z-score (evaluating the significance of the index) were estimated using ArcGIS 10.1.

3.5 Social Network Analysis (SNA)

Social network analysis (SNA) methods are used to characterize the network of live chicken movements in the study areas. The networks were illustrated using NetDraw and ArcGIS. ArcGIS will also be used to calculate the extent of the network, and geographic distribution of the network over time.

SNA was used to characterize the network of live poultry movements in the study areas [41]. To describe the connectivity pattern within the network dataset consisting of records of paired trading events between a particular LBM and the county of origin of the purchased chickens (termed as "chicken source"), SNA will be used. A trade event is defined with a threshold of at least one movement between the LBM and chicken sources. Network connectivity of all networks examined in the study will be summarised by using the number of links (indicating the frequency of movement), movement length (representing the catchment area of LBMs), degree centrality, *k*-core and the components of the network. The degree represents the absolute number of unique links of a given node to another one. The *k*-core represents the maximal group of nodes, all of whom are connected to a number (*k*) of other nodes; it describes the connectivity of different groups in a network. These two network indicators are important for describing the levels of connectivity and centrality that exist between the different elements of a network and play a key role while identifying the most influential spreaders within a network. The components of the network include a maximal connected sub-graph where all nodes (i.e. chicken sources) are connected through paths.

3.6 Generalized Linear Square (GLS) regression model

Questions of knowledge, attitudes and practices were scored individually. Scores of all the KAP questions were then summed accordingly to represent the levels of each participant's overall knowledge, overall attitudes and overall practices towards AI. Details of the scoring method is included in Table C-1. For categorical variables, frequencies for different groups were compared using chi-square tests. To identify the predictors of KAP scores of AI among different actors along the live chicken market chain, e.g. chicken farmers, vendors and consumers, we built four multivariable generalized linear regression models by using the generalized linear square (GLS) random-effects models. In case of chicken farmers, we included gender, age, educational level, years of working and hours of contacting. In case of chicken vendors, we included gender, age, educational level, type of stalls, type of vendors, years of working with chicken and hours of contacting chicken. In case of market consumers, we included gender, age, education level, employment status, the frequency of buying chicken, type of chicken bought. Model 1 integrates all participants, model 2-4 are built for chicken farmers, chicken vendors and chicken consumers respectively. For all the four models, pairwise deletion was employed, thus participants with missing values (the missing rate is around 0.56%) were excluded on a test-by-test basis. Two-tailed test were utilized with a p-value<0.05 considered statistically significant. Data were analysed with STATA (version 12.0; SPSS Inc., Chicago, IL, USA).

3.7 Bayesian Conditional Autoregressive (CAR) Model

A Bayesian framework was used to construct a Poisson regression model of the observed incidence data of human H7N9 infections in each county using the OpenBUGS software 3.2.3 rev 1012 [221]. The model included the explanatory variables and a spatially structured random effect. The mathematical notation for the model is provided in Appendix D.2. It assumed that the observed counts of H7N9 human infections in the county (from 1 to 1181) followed a Poisson distribution.

The spatially structured random effect was modelled using a conditional autoregressive (CAR) prior structure [222]. This approach uses an adjacency weights matrix to determine spatial relationships between counties. If two counties share a border, it was assumed the weight = 1 and if they do not the weight = 0. The adjacency matrix was constructed using the "Adjacency for WinBUGS tools" in ArcGIS software [221]. A flat prior distribution was specified for the intercept, whereas a normal non-informative prior distribution was used for the coefficients (with a mean = 0 and a precision = 0.001). The priors for the precision of spatially structured random effects were specified using non-informative gamma distributions (0.5, 0.0005). (The OpenBugs code is in Appendix D.3)

The first 1,000 iterations were run as a burn-in period and discarded. Subsequent sets of 20,000 iterations were run and examined for convergence. Convergence was determined by visual inspection of posterior density and history plots and by examining autocorrelation plots of model parameters. Convergence occurred at approximately 100,000 iterations for each model. Another 20,000 values from the posterior distributions of the model parameters were stored and summarized for the analysis. Statistical significance was indicated by 95% credible intervals (95% CrI), a variable was considered significant if CrI excluded 0.

Choropleth maps were created using the ArcGIS software to visualize the geographical distribution of crude incidence for the 1181 counties in the study area. The posterior means of the CAR random effects obtained from the models were also mapped.

Chapter 4 Effectiveness of Market-Level Biosecurity at Reducing Exposure of Poultry and Humans to Avian Influenza: A Systematic Review and Meta-Analysis

4.1 Context

The Literature Review in Chapter 2 demonstrated a large number of studies which attempted to identify risk factors associated with AI infection at LBMs and that some had identified exposure to H7N9 infected poultry or their environment at LBMs as the main risk factor for human infections [21, 69, 159-161, 163, 223-233]. However, results from those studies were inconsistent and even contradictory and the relative efficacy of different LBM biosecurity risk factors at reducing the transmission of AI to both poultry and humans in the LBM setting was still unclear. At the time when this Chapter was initiated there was one meta-analysis looking at poultry farm level the association of production, management, environment and biological factors on [9]. Therefore, there was a need to conduct a systematic review and meta-analysis to quantify the relative importance of market level biosecurity risk factors (such as cleaning and disinfection, market closures, manure disposal and management practices from different studies) on human (market workers) and poultry AI infection at LBMs.

In Chapter 4, I set out to conduct a systematic review and meta-analysis following the PRISMA guidelines and a PICO searching strategy. A total of 79 epidemiological studies were identified by fitting the inclusion/exclusion criteria and were ranked based on their research quality. Then, the effect size of biosecurity indicators from those included studies were extracted and meta-analysed using a quality effects (QE) meta-analysis model using MetaXL software.

The results from Chapter 4 demonstrated that biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs included smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, ban on overnight storage. The results also revealed that markets located at non-central city areas and markets that source poultry from other areas, i.e. possible cross-regional and long distant poultry movements, were associated with a higher risk of poultry AI infection at LBMs. Our meta-analysis also suggested that poultry slaughter operations at LBMs increase the risk of AI transmission within the LBM environment. The results of our meta-analysis also suggested that Spring and Winter seasons have posed significantly higher risk of AI infection in the LBM environment compared to Summer and Autumn. In addition, our results also demonstrated that human AI infection at LBMs is dependent on important demographic and occupational hazards; these include, higher risk of exposure in workers at retail LBMs, female workers and those who contact ducks, conduct cleaning, slaughtering, defeathering or evisceration.

The findings in Chapter 4 suggests the most effective strategies to reduce AI market contamination identified in this study should target larger LBMs that are located at non-central city areas, sell and slaughter multi-species of live poultry. LBM workers directly involved in cleaning and poultry processing tasks should participate in occupational health and safety programmes. The finding also has important implication for the temporal targeting of surveillance at LBMs in Spring and Winter seasons. The findings in Chapter 4 will allow AI prevention and control program officers or market managers to make informed decisions on targeted risk-reduction strategies at LBMs and in this way to protect poultry, poultry workers and consumers visiting LBMs.

To the best of my knowledge, the systematic review and meta-analysis presented in Chapter 4 is the first of its kind to report on the combined effectiveness of market-level biosecurity indicators for both poultry and human AI infections at LBMs.

This Chapter has been published in the Journal of Infectious Diseases as a review paper, and supporting technical information is presented in Appendix A of this Thesis.

4.2 Abstract

In this study, we aimed to identify the effect of market-level risk factors on avian influenza (AI) infection in poultry and humans and generate evidence that will inform AI prevention and control programs at live bird markets (LBMs). We performed a systematic literature review in both English and Chinese search engines. We estimated the pooled odds ratios of biosecurity indicators relating to AI infections at market level using a quality effects (QE) meta-analysis model. We found biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs included smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, ban on overnight storage and sourcing poultry from local areas. Our meta-analysis indicates that higher risk of exposure to AI infection occurs in workers at retail LBMs, female workers and those who contact ducks, conduct cleaning, slaughtering, defeathering or evisceration. The most effective strategies to reduce AI market contamination identified in this study should target larger LBMs that are located at non-central city areas, sell and slaughter multi-species of live poultry. LBM workers directly involved in cleaning and poultry processing tasks should participate in occupational health and safety programmes.

Keywords: Avian influenza; biosecurity; live bird markets; meta-analysis; risk factors.

4.3 Introduction

In the past ten years, several Asian-lineage HPAI viruses caused fatal disease in poultry, wild birds, humans and other mammals, and some have spread across three continents [234]. Affected countries and the international community have mobilized funds to assist in the control of the disease because of the potential of these viruses to develop into a global influenza pandemic [235, 236].

Available evidence indicates that live bird markets (LBMs) can serve as potential hubs where AI viruses are maintained and transmitted for long periods of time. After the emergence of HPAI H5N1 influenza in 2003, several studies have documented that LBMs could be sources of human AI infections [237]. The importance of LBMs in the transmission of AI to humans was also highlighted by the emergence of influenza A (H7N9) viruses of low pathogenicity to poultry in early 2013, causing human infections without preceding or concomitant outbreaks in poultry. Exposure to H7N9 infected poultry at LBMs has been implicated as the main risk factor for human infection [7]. During the fifth wave of influenza A (H7N9) from October 2016 to April 2017, an increasing proportion of human cases were related to poultry exposures in rural farms and backyard flocks [238].

In the context of animal health, biosecurity is the application of management practices that aim to reduce the risk of the introduction and spread of disease agents within and between animal populations. At LBMs, these practices can include introducing rest days, limiting the number of poultry species sold at a market, the use of cleanable cages and the deployment of adequate cleaning and disinfection procedures. While some studies have demonstrated that good biosecurity practices at LBM-level are associated with reduced risk of AI infection, the relative efficacy of different LBM biosecurity practices at reducing the transmission of AI to both humans and poultry in the LBM setting is still unclear.

The role of farm-level biosecurity indicators, such as production, management, environment and biological factors in AI infection in poultry have been quantified in a recent study [239]. A recent systematic review and meta-analysis evaluated risk factors for clinical outcomes in H5N1 patients [240]. There were also systematic reviews of pathways of AI exposure at the animal-human interface and meta-analyses estimated the prevalence of AI infection among humans and birds [27]. A previous systematic review assessed the impact of different interventions implemented in LBMs to control the infection of zoonotic influenza [241]. There is a need for similar studies to quantify the impact of relative efficacy of biosecurity measures on human (market workers) and poultry infection at LBMs. This information will allow national AI control program managers to make informed decisions on targeted risk-reduction strategies at LBMs and in this way to protect poultry, poultry workers and consumers visiting LBMs.

In this study, we systematically reviewed and meta-analysed the overall effect of different biosecurity indicators on AI infection from different studies, to understand more about how each risk factor influences AI infection at market level and to generate evidence that will inform AI prevention and control programs at LBMs.

4.4 Methods

4.4.1 Search strategy

Following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, we performed a systematic literature search using PubMed, ISI Web of Science and Science Direct, CNKI (the China Academic Journals full-text database) and WANFANG database (includes most comprehensive online full-text Chinese medical journals) with no starting time limits, up to 10 Jun 2018. The search strategy used four PICO (participants, interventions, comparators, and outcomes) components (Table A-1).

4.4.2 Eligibility criteria

Epidemiological studies were included if they evaluated biosecurity risk factors for AI infection in LBMs in poultry, the environment or human populations. Studies were excluded if: 1) they were laboratory studies, descriptive studies, case reports, and vaccine efficacy studies; 2) the outcome recorded was not AI infections; 3) they were not LBM based studies (i.e. ecological studies, studies at local, regional or national levels, studies at farm level); 4) there was no effect size for LBM biosecurity risk factors reported.

4.4.3 Data abstraction for market-level biosecurity indicators

For each of the papers that met the inclusion criteria, we recorded information on subject title, first author, year of publication, country, language, subtype of AIV and its pathogenicity, total sample size, number of AI positives and negatives, risk factors, infection type, study type and analysis methods. Data on biosecurity indicators were extracted by two independent reviewers (XZ, RSM), and a dataset based on these characteristics was created in MS Excel. In this study, we analysed biosecurity indicators for poultry and market environment infection and market workers' infection (i.e. non-symptomatic seropositive) separately. A total of 34 biosecurity risk factors were explored in our study (Table A-2). For market infection, the following five groups of biosecurity indicators were considered: A) market characteristics: market type, market size, market location (rural or urban area), market location (central city or non-central city areas), and presence of multiple species, presence of ducks and presence of rabbits ; B) market biosecurity management: conduct cleaning and disinfection, before and after cleaning and disinfection, conduct waste disposal, conduct market closure, before and after rest day, ban on overnight storage, poultry sources, separate different species and conduct slaughter in market; C) seasonality, temperature. For market workers' infection: D) socio-demographic characteristics: sex, age, years working in LBMs, type of market (wholesale or retail), vaccination history and occupation; E) activities involving exposure to poultry: conduct cleaning, conduct feeding, contact poultry, conduct slaughtering, defeathering and evisceration.

4.4.4 Study quality and bias assessment

Two authors (XZ, RJSM) independently reviewed and assessed the quality of each English paper using a structured approach. Papers in Chinese were translated by XZ and evaluated by XZ and RJSM. The quality of each study was scored on seven quality assessment criteria (Table A-3). Studies that recorded a higher overall score were considered to superior quality. The scores from quality assessment were then rescaled into quality ranks between 0 and 1 by making them relative to the highest scoring study in the group; then the best study was ranked 1 and those with lower scores were ranked lower. These ranks were then utilized by the quality effects model to adjust estimates of effect [242].

4.4.5 Statistical Analyses

The odds ratio (OR) and 95% confidential interval of each biosecurity factor were extracted from each study, or if the odds ratio was not reported, we calculated it using Epi Info TM 7.1.5.2 based on the raw data reported. When a factor was tested in both a univariable and multivariable model, the effect size of the factor in the multivariable model was used. The odds ratios of each factor were modelled by applying a quality effects (QE) meta-analysis model that assumed heterogeneity across the included studies [242]. The results of the analyses were statistically significant if the 95% confidence interval did not include the value one.

The QE model redistribution of weights due to the rescaled quality rank (called Qi in the MetaXL software described below) helps reduce estimator variance as well as allowing for proper error estimation through the confidence interval thus generated. Nevertheless, the random effects results are in Table A-5 for comparison. All results are presented as a forest plot that shows individual OR estimates for each group of biosecurity indicators and overall for the category. We assessed study heterogeneity by the Cochran Q Chi-square test, and this is also used by the I^2 index statistic to estimate the proportion of total variation due to heterogeneity. An I^2 value of <25% indicated low heterogeneity, 25-75% indicated moderate, and a score of >=75% suggested high degree of heterogeneity. Publication bias was assessed using Doi and funnel plots. All analyses were conducted using MetaXL, version 5, Epigear International, Sunrise Beach, QLD, Australia (www.epigear.com).

4.5 Results

4.5.1 Search results and study characteristics

Our literature search strategy yielded a total of 249 citations by searching PubMed, 554 articles from Web of Science (Web of Science^{TM} Core Collection and MEDLINE) and 111 articles from Science Direct; we also found 269 articles from CNKI and 266 articles from the WANFANG database (Figure 4-1). After removing duplicates, and applying inclusion and exclusion criteria, we finally included 79 articles for the systematic review and meta-analysis.

The 79 studies included in the analysis were published between 2003 and 2018. The studies were from seven countries or regions including Mainland China (65), Hong Kong SAR China (2), Vietnam (4), Bangladesh (4), the USA (3), Egypt (1) and Indonesia (1). Of the included 79 studies, 25 were in English and 54 were in Chinese; 69 studies investigated biosecurity indicators associated with market infection, 12 studies investigated biosecurity indicators associated with market workers' infection at LBMs, out of that, two studies investigated on both poultry and poultry worker infections at LBMs (please see Table 4-1).

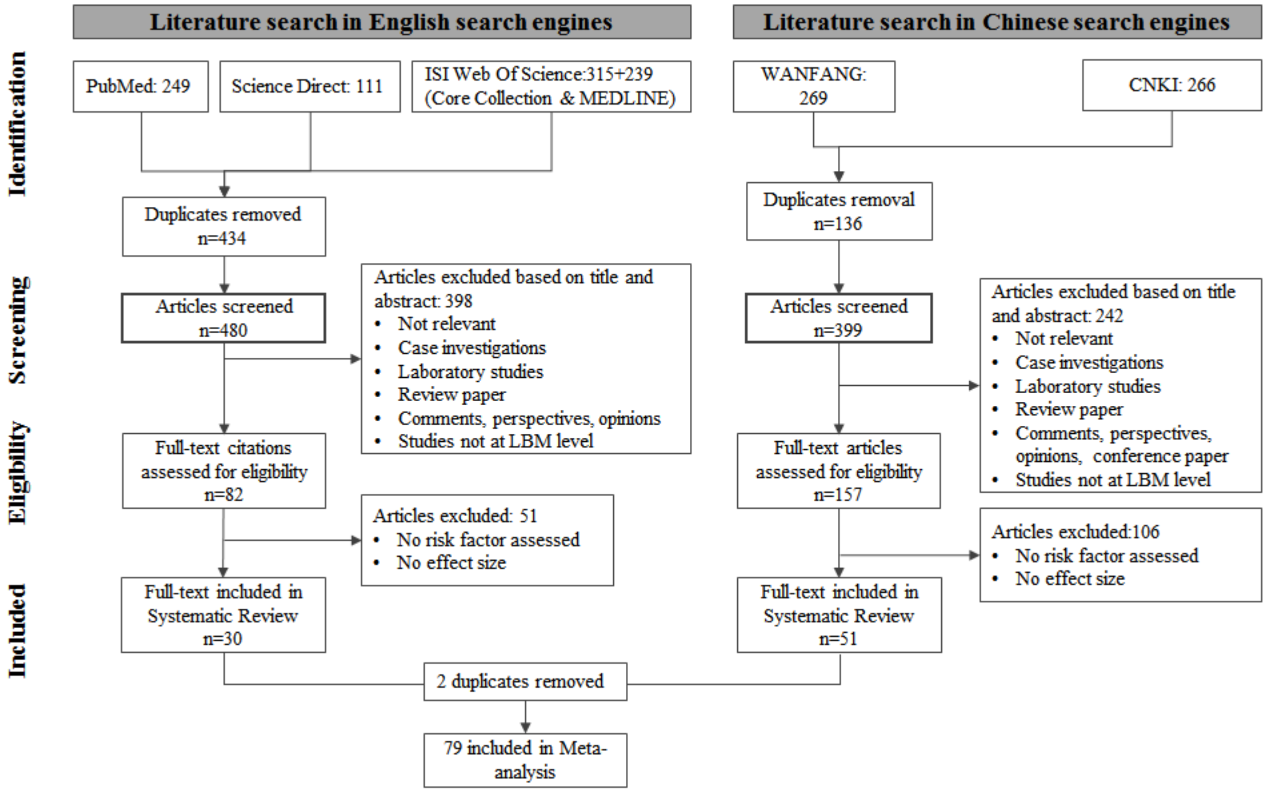


Figure 4-1 Flow diagram of studies selection process. Abbreviation: LBM, live bird market.

4.5.2 Quality and heterogeneity of selected studies

Quality assessments of studies included in the analysis is in Table A-4 and Table A-5. The quality scores of studies related to market infections ranged from 3 to 12 (median is 8, maximum possible is 13), and the scores of studies on market workers' infection ranged from 4 to 12 (median is 8, maximum possible is 13).

Our results indicate that overall studies within biosecurity Groups A, B, C were highly heterogeneous (estimated I^2 values of 90%, 89% and 96% respectively) (see Figure 4-2, Figure 4-3 and Figure 4-4). Moderate heterogeneity was seen within Group D (I2 values of 73%, see Figure 4-5). Very low heterogeneity was seen within Group E (I2 value of 15%) (see Figure 4-6). Of the 79 studies on market infections, there were 60 longitudinal studies, 17 cross-sectional studies, and two case-control studies. A total of 32 studies investigated general AIV and specific subtypes (i.e. H5 or H7 or H9, or their combinations), 23 studies investigated specific AIV subtypes, and 14 studies only studied general AIV. Given the heterogeneity of these studies in relation to the viruses being isolated, the focus of the papers was to report the effect of market-level of biosecurity on AI infection/recovery generally. Of all the 69 studies on market infections, 50 studies investigated only environmental samples in LBMs for AI virus, and 11 studies collected only poultry samples. Eight studies investigated both poultry and environmental samples, and only one study reported the results by sample type. For these reasons, we did not stratify our meta-analysis by type of biological sample (see Table 4-1). Among the 69 studies on risk factors of AI market infection, 64 studies used RT-PCR to detect the AI virus, only five studies conducted virus isolations.

4.5.3 Meta-analysis of the effect of LBM biosecurity indicators on AI market infection

Market characteristics (Group A): The overall effect for optimal market characteristics associated with market infection was protective and statistically significant (OR=0.65, 95%CI: 0.47-0.89) (Figure 4-2). LBMs of smaller size have the significantly lower risk of AI infection compared to those of larger size (OR=0.55, 95% CI: 0.34-0.88), and the presence of single poultry species in the LBM have lower risk of AI infection compared to LBMs with multiple species (OR=0.29, 95% CI: 0.11-0.76). Presence of rabbits and presence of ducks are risk factors of AI infection on LBMs. LBMs that were in central city areas have significantly lower risk than markets located in non-central city areas (OR=0.74, 95% CI: 0.56-0.97).

Market biosecurity management (Group B): The overall effect of market biosecurity management characteristics associated with market infection at LBMs was significantly protective (OR=0.44, 95% CI: 0.32-0.59) (Figure 4-3). The risk of acquiring AI infection was significantly lower in LBMs that practice cleaning and disinfection (OR=0.35, 95% CI: 0.17-0.73) compared to those did not. The risk of AI infection after a rest day is significantly lower than infection before the rest day (OR=0.20, 95%CI: 0.11-0.38). The risk of AI infection is significantly lower in LBMs who ban on overnight storage compared to those did not (OR=0.50, 95% CI: 0.29-0.86). The risk of AI infection in LBMs that source poultry from the local area was significantly lower than LBMs that source poultry from other areas (OR=0.57, 95%CI: 0.35-0.94). Markets that separate different species have lower risks compare to those who did not (OR=0.63, 95%CI: 0.43-0.90). Markets that do not slaughter poultry onsite have lower risk than markets that slaughter onsite although it is not statistically significant (OR=0.54, 95% CI: 0.13-2.25).

Seasonality (Group C): The overall effect of optimal seasonal indicators associated with market infection was protective but not statistically significant (OR=0.98, 95% CI: 0.78-1.23) (Figure 4-4). Summer and autumn months pose a significant lower risk compared to spring and winter seasons (OR=0.65, 95%CI: 0.44-0.96).

4.5.4 Meta-analysis of the effect of LBM biosecurity indicators on poultry workers' AI infection

Socio-demographic characteristics (Group D): The results indicate the human AI infection at LBMs was significantly lower in male workers than for female workers (OR=0.68, 95%CI: 0.54-0.87) and significantly lower in wholesale markets compared to retail markets (OR=0.38, 95%CI: 0.22-0.65) (Figure 4-5). Market workers who did not sell poultry had lower risk of getting AI infection than those who sell poultry (OR=0.34, 95% CI: 0.17-0.70).

Activities involving exposure to poultry (Group E): The overall effect of optimal exposure behaviours was significantly protective (OR=0.37, 95%CI: 0.27-0.51) (Figure 4-6). Our results revealed a significantly lower risk of AI infection in market workers who did not conduct cleaning of feed trays (OR=0.34, 95% CI: 0.13-0.90) and who did not contact ducks (OR=0.28, 95% CI: 0.12-0.64) compare with those that did. Market workers who did not slaughter poultry (OR=0.12, 95% CI: 0.03-0.56), did not defeather poultry (OR=0.19, 95% CI: 0.07-0.51), and who were not involved in poultry evisceration (OR=0.19, 95% CI: 0.07-0.52) had significant lower risk of getting AI infections.

In addition to the risk factors reported above, there were several other different factors, which were assessed only by a single study (Table A-6, Table A-7, Table A-8, Table A-9 and Table A-10). These are not discussed due to the difficulty in interpreting the combined effect of these factors based on the small number of studies.

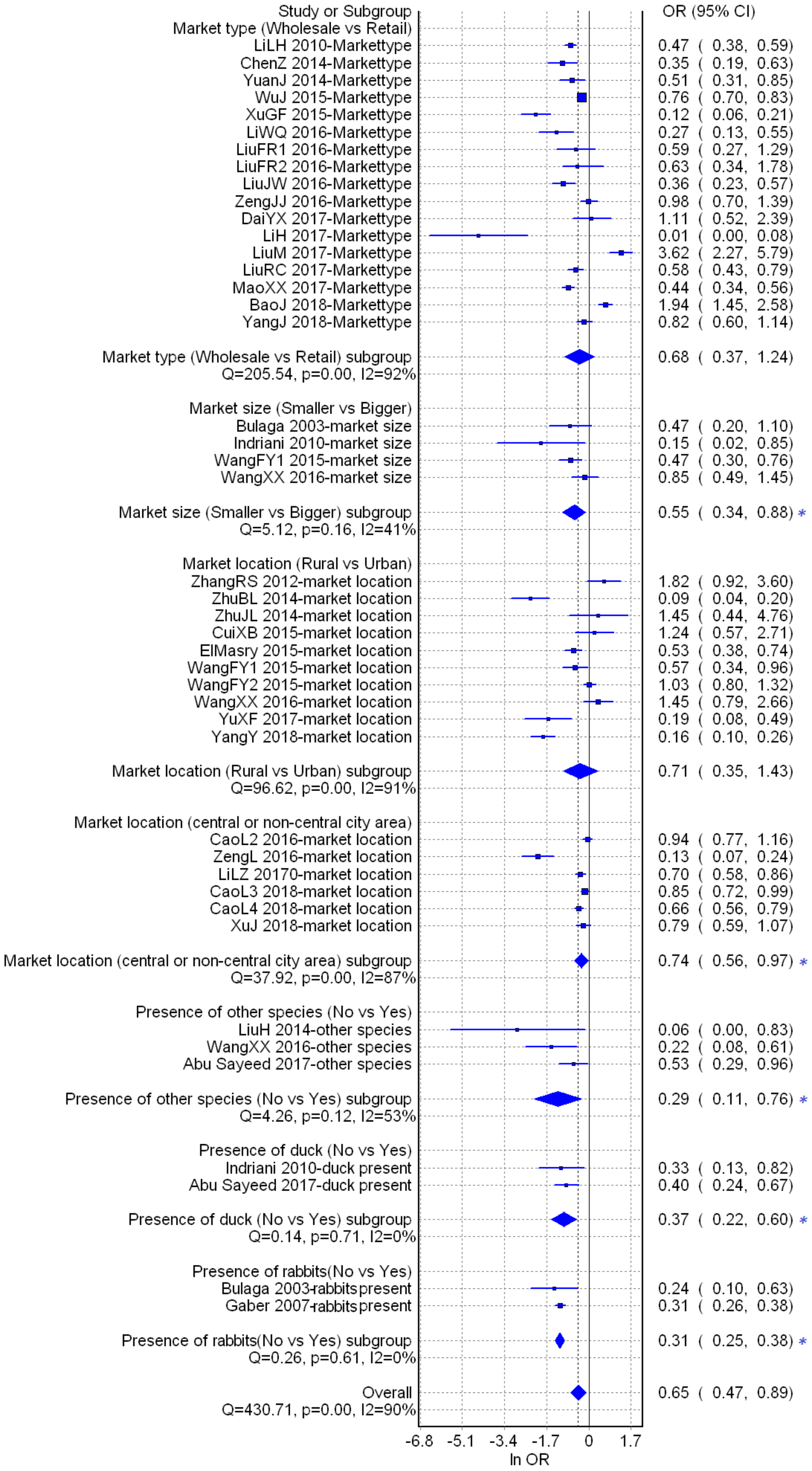

Study or Subgroup
OR (95% CI)
Market type (Wholesale vs Retail)
LiLH 2010-Markettype 0.47 (0.38, 0.59)
ChenZ 2014-Markettype 0.35 (0.19, 0.63)
YuanJ 2014-Markettype 0.51 (0.31, 0.85)
WuJ 2015-Markettype 0.76 (0.70, 0.83)
XuGF 2015-Markettype 0.12 (0.06, 0.21)
LiWQ 2016-Markettype 0.27 (0.13, 0.55)
LiuFR1 2016-Markettype 0.59 (0.27, 1.29)
LiuFR2 2016-Markettype 0.63 (0.34, 1.78)
LiuJW 2016-Markettype 0.36 (0.23, 0.57)
ZengJJ 2016-Markettype 0.98 (0.70, 1.39)
DaiYX 2017-Markettype 1.11 (0.52, 2.39)
LiH 2017-Markettype 0.01 (0.00, 0.08)
LiuM 2017-Markettype 3.62 (2.27, 5.79)
LiuRC 2017-Markettype 0.58 (0.43, 0.79)
MaoXX 2017-Markettype 0.44 (0.34, 0.56)
BaoJ 2018-Markettype 1.94 (1.45, 2.58)
YangJ 2018-Markettype 0.82 (0.60, 1.14)
Market type (Wholesale vs Retail) subgroup 0.68 (0.37, 1.24)
Q=205.54, p=0.00, I2=92%
Market size (Smaller vs Bigger)
Bulaga 2003-market size 0.47 (0.20, 1.10)
Indriani 2010-market size 0.15 (0.02, 0.85)
WangFY1 2015-market size 0.47 (0.30, 0.76)
WangXX 2016-market size 0.85 (0.49, 1.45)
Market size (Smaller vs Bigger) subgroup 0.55 (0.34, 0.88) *
Q=5.12, p=0.16, I2=41%
Market location (Rural vs Urban)
ZhangRS 2012-market location 1.82 (0.92, 3.60)
ZhuBL 2014-market location 0.09 (0.04, 0.20)
ZhuJL 2014-market location 1.45 (0.44, 4.76)
CuiXB 2015-market location 1.24 (0.57, 2.71)
ElMasry 2015-market location 0.53 (0.38, 0.74)
WangFY1 2015-market location 0.57 (0.34, 0.96)
WangFY2 2015-market location 1.03 (0.80, 1.32)
WangXX 2016-market location 1.45 (0.79, 2.66)
YuXF 2017-market location 0.19 (0.08, 0.49)
YangY 2018-market location 0.16 (0.10, 0.26)
Market location (Rural vs Urban) subgroup 0.71 (0.35, 1.43)
Q=96.62, p=0.00, I2=91%
Market location (central or non-central city area)
CaoL2 2016-market location 0.94 (0.77, 1.16)
ZengL 2016-market location 0.13 (0.07, 0.24)
LiLZ 20170-market location 0.70 (0.58, 0.86)
CaoL3 2018-market location 0.85 (0.72, 0.99)
CaoL4 2018-market location 0.66 (0.56, 0.79)
XuJ 2018-market location 0.79 (0.59, 1.07)
Market location (central or non-central city area) subgroup 0.74 (0.56, 0.97) *
Q=37.92, p=0.00, I2=87%
Presence of other species (No vs Yes)
LiuH 2014-other species 0.06 (0.00, 0.83)
WangXX 2016-other species 0.22 (0.08, 0.61)
Abu Sayeed 2017-other species 0.53 (0.29, 0.96)
Presence of other species (No vs Yes) subgroup 0.29 (0.11, 0.76) *
Q=4.26, p=0.12, I2=53%
Presence of duck (No vs Yes)
Indriani 2010-duck present 0.33 (0.13, 0.82)
Abu Sayeed 2017-duck present 0.40 (0.24, 0.67)
Presence of duck (No vs Yes) subgroup 0.37 (0.22, 0.60) *
Q=0.14, p=0.71, I2=0%
Presence of rabbits(No vs Yes)
Bulaga 2003-rabbits present 0.24 (0.10, 0.63)
Gaber 2007-rabbits present 0.31 (0.26, 0.38)
Presence of rabbits(No vs Yes) subgroup 0.31 (0.25, 0.38) *
Q=0.26, p=0.61, I2=0%
Overall 0.65 (0.47, 0.89)
Q=430.71, p=0.00, I2=90%
-6.8
-5.1
-3.4
-1.7
0
1.7
ln OR

Figure 4-2 Forest plots of risk estimates of market characteristics (Group A) on avian influenza market infection.

*Note: * The OR values are statistically significant (P<0.05). Abbreviations: OR, odds ratio; CI, confidence interval.*

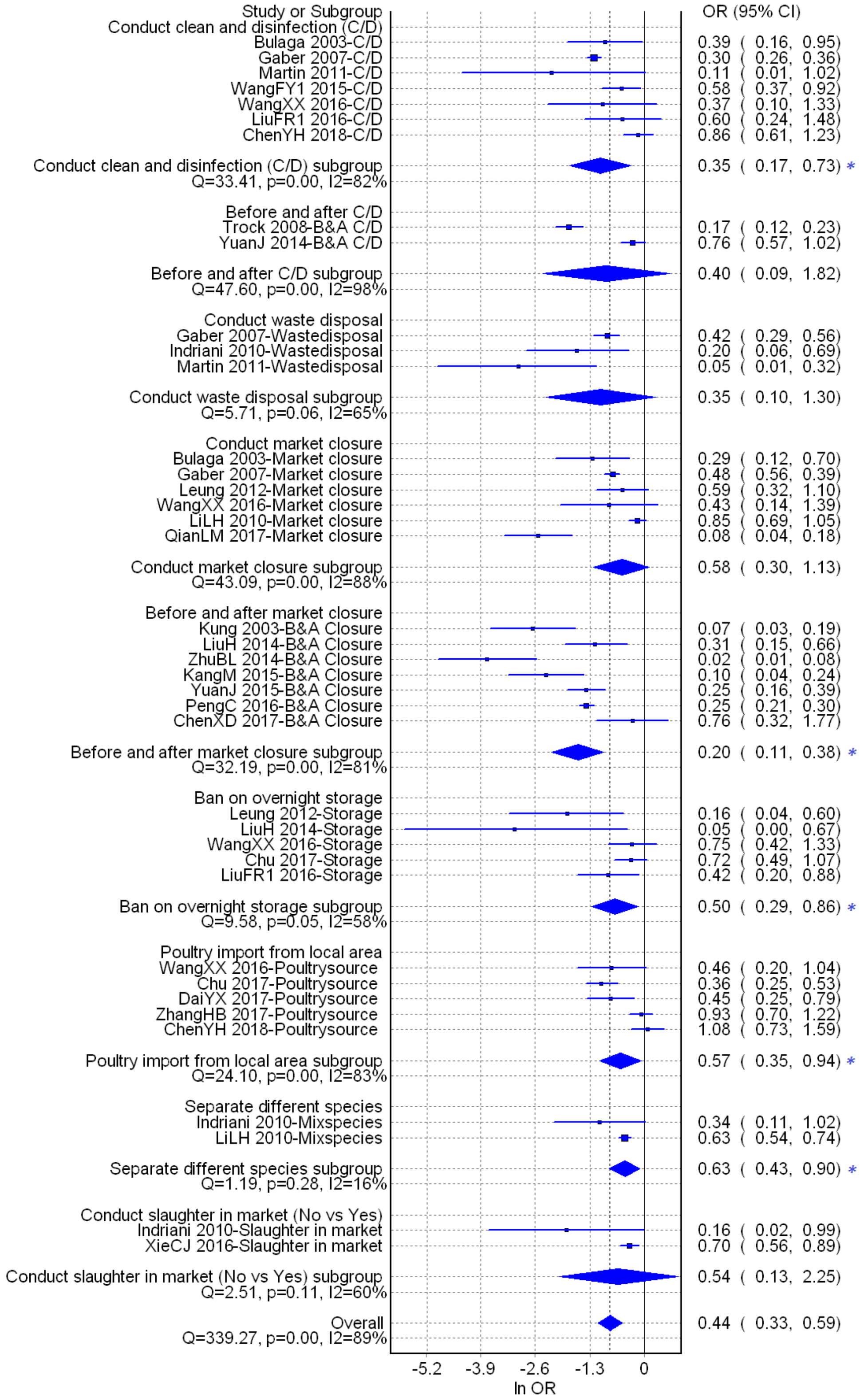


Figure 4-3 Forest plots of risk estimates of market biosecurity management (Group B) on avian influenza market infection.

*Note: * The OR values are statistically significant (P<0.05.) Abbreviations: OR, odds ratio; CI, confidence interval.*

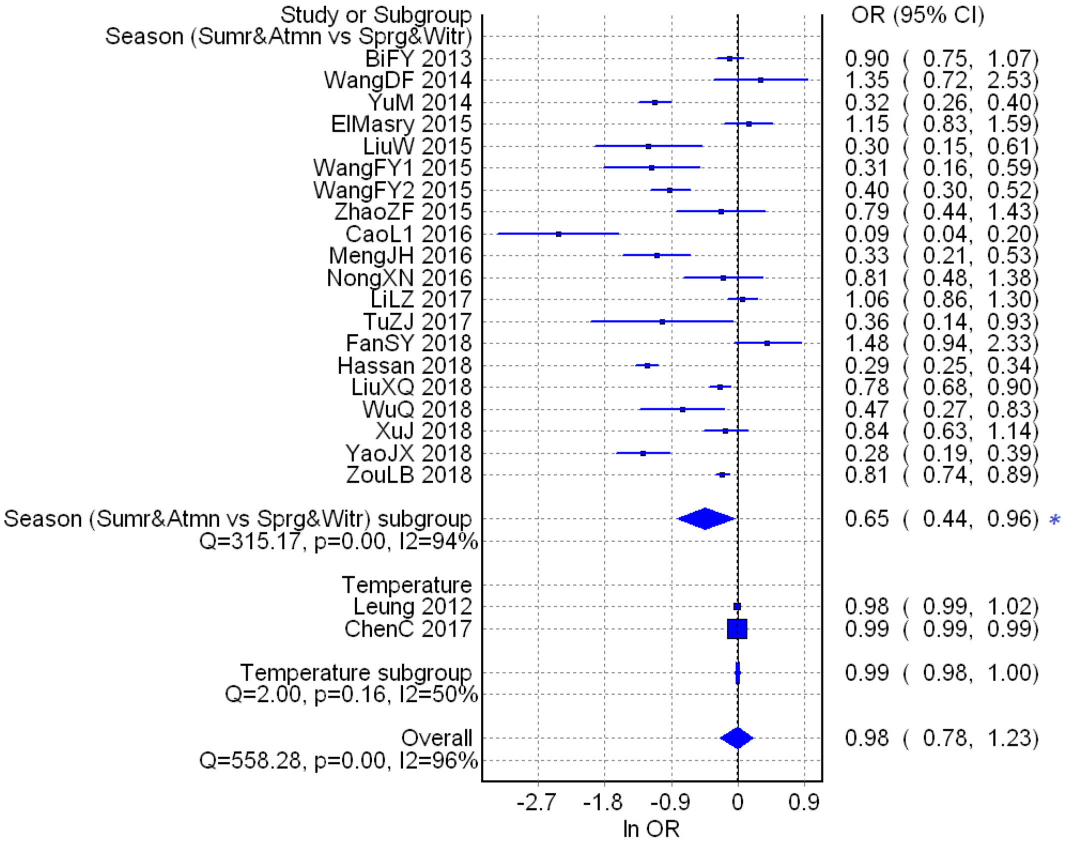


Figure 4-4 Forest plots of risk estimates of seasonality (Group C) on AI market infection.

*Note: * The OR values are statistically significant (P<0.05). Abbreviations: OR, odds ratio; CI, confidence interval.*

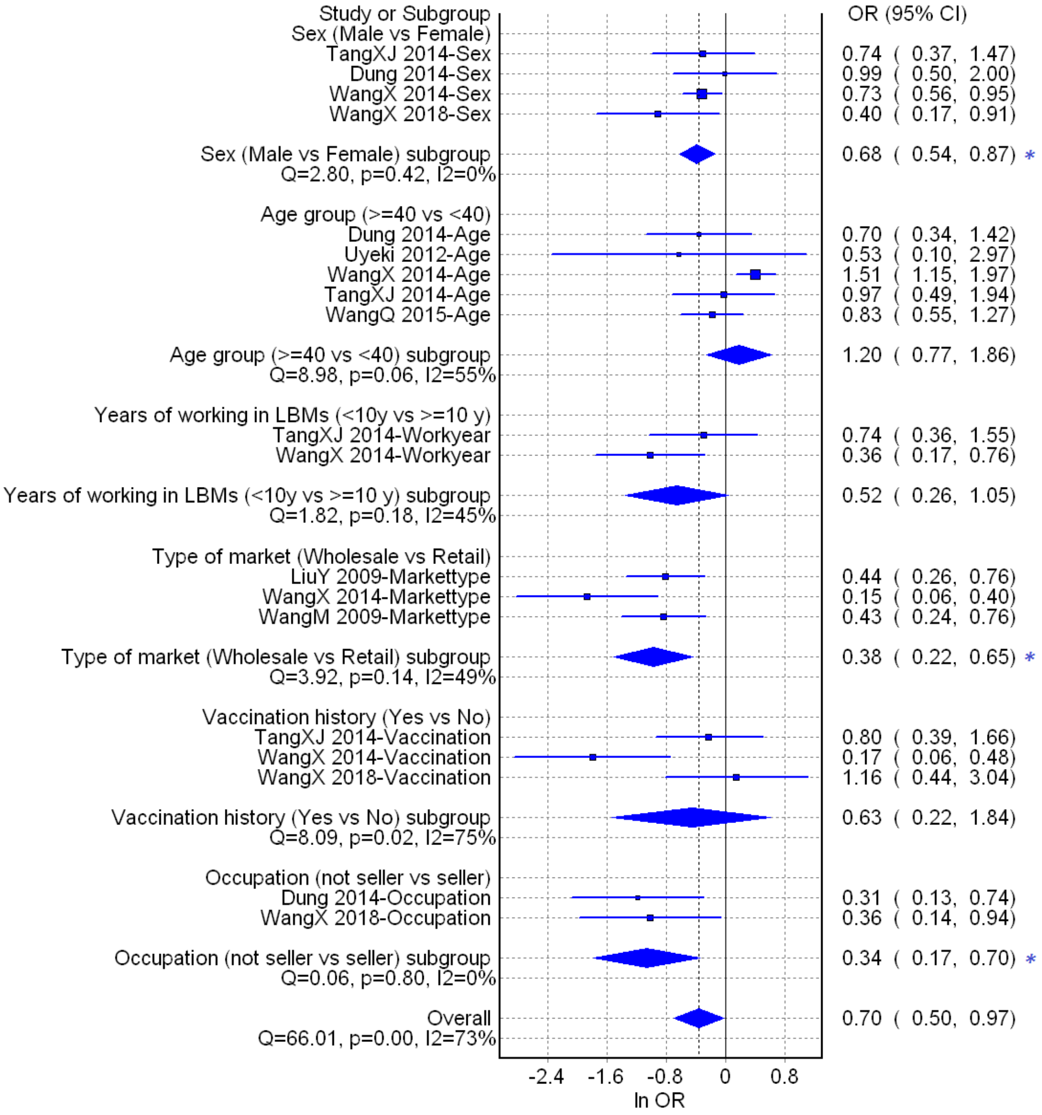


Figure 4-5 Figure 5 Forest plots of risk estimates of socio-demographic characteristics (Group D) on Human avian influenza infection.

*Note: * The OR values are statistically significant (P<0.05). Abbreviations: OR, odds ratio; CI, confidence interval.*

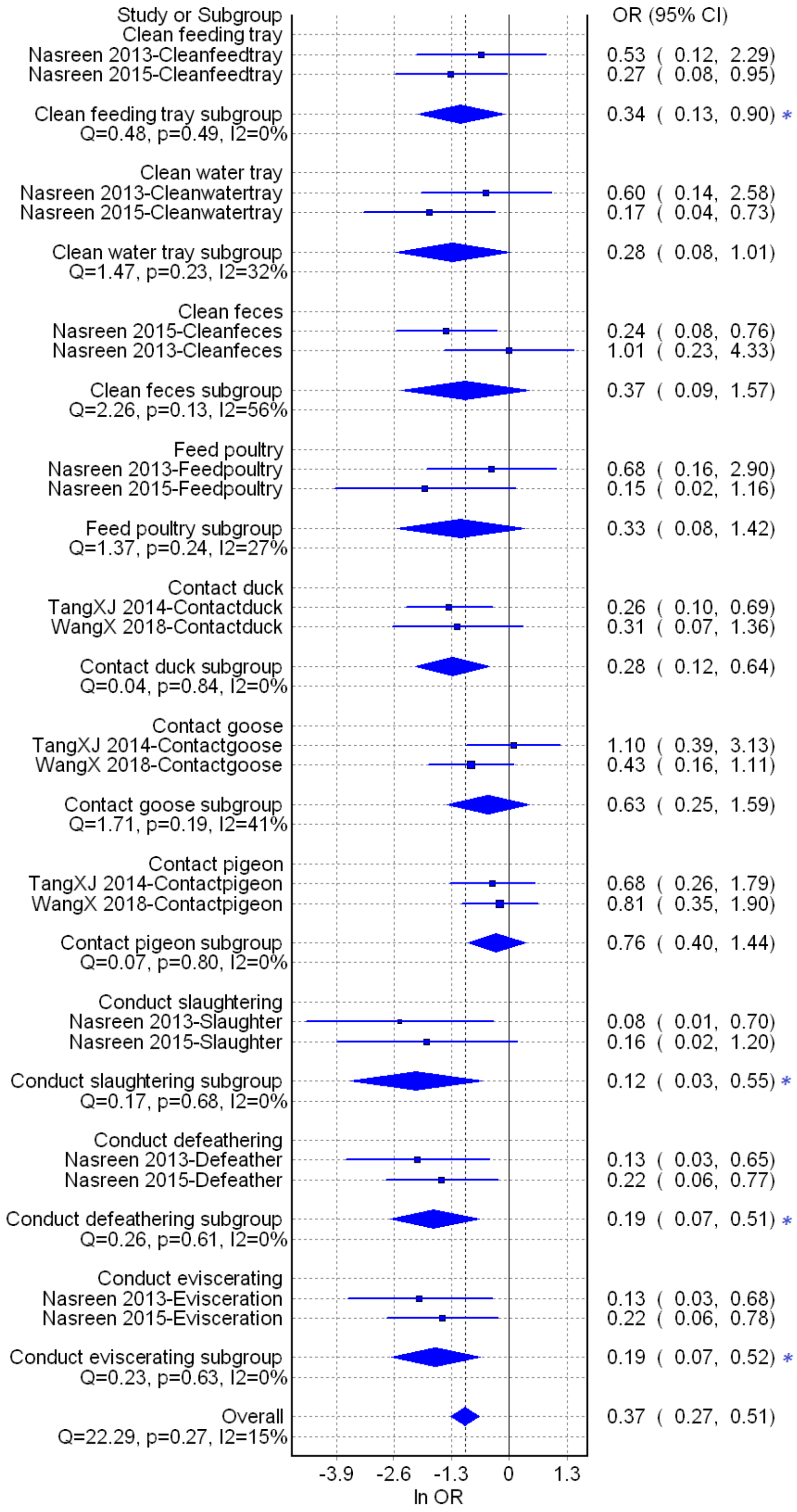


Figure 4-6 Forest plots of risk estimates of activities involving exposure to poultry (Group E) on Human avian influenza infection.

*Note: * The OR values are statistically significant (P<0.05). Abbreviations: OR, odds ratio; CI, confidence interval.*

4.5.5 Publication bias assessment

The Funnel and Doi plots (see Figure A-6 and Figure A-7) demonstrated major negative asymmetry of effects for market characteristics and socio-demographic characteristics only. This is due to the heterogeneity of subgroups that belong to these two categories, although a paucity of negative studies cannot be excluded thus leading to an exaggerated protective effect for factors in these categories.

4.6 Discussion

LBMs are recognized to be reservoirs of AI viruses and a possible source of infection for both domestic poultry and humans working in or visiting them [69, 159-161]. To the best of our knowledge, this is the first meta-analysis of evidence on the effectiveness of market-level biosecurity in both poultry and human AI infections at LBMs. Our analysis of the relevant published English and Chinese research articles provided strong evidence in favour of biosecurity operations at LBMs that are protective for AI infections on both poultry and human infections at LBMs.

Our meta-analysis demonstrates that the odds of detecting AI viruses at LBMs is dependent on a select group of LBM biosecurity characteristics and management measures. Our finding that the presence of multiple poultry species, and presence of rabbits or ducks increased the risk of AI circulation in the LBM, compared to those with single species, can partly be explained by unsafe poultry movements by some traders. A previous study suggested that live poultry traders who sell more than one species are more likely to import birds from multiple sources and may also supply high risk species, e.g. wild animals or birds, without inspection or health checks [21]. LBMs that are located in central city areas have lower risk than those in non-central city areas, this may due to the enhanced LBM management measures in the central city areas (e.g. enhanced clean and disinfection, quarantine etc.) [243] and the massive trade and complex poultry source in the non-central city areas [244]. This suggests the risk of AI virus spread from the non-central areas to the central areas, therefore, enhanced regulations should be emphasized in the non-central city areas.

Cleaning and disinfection procedures are considered to be an important strategy to reduce disease transmission in LBMs, and our meta-analysis of existing evidence supports this view [21, 223]. In addition, our results revealed that the detection of AI viruses was always lower right after market rest days when infectious load is less [163, 224-229]. Closing LBMs can largely eliminate human infection risk [230]. However, studies indicated that recovery of AI viruses can occur shortly after LBMs re-open, presumably following the introduction of AI positive birds [245, 246]. Our study indicates that the combined effect of daily cleaning and disinfection and waste removal are effective ways of reducing AI transmission at LBMs [36, 223, 247]. We also found that LBMs that trade poultry from local areas have lower risk of AI infection compared with those that trade poultry sourced from other areas. Previous research indicates that poultry movements facilitate the transmission and spread of AI viruses between premises as a result of mixing of poultry from different sources and the increased opportunity for virus multiplication during transport [223]. The risk of AI transmission at LBMs posed by cross-regional and long-distance poultry movements could be managed by implementing a market-level traceability system that includes certification of sources of poultry based on their compliance with biosecurity requirements. We found that the risk of AI infection in markets that do not slaughter poultry onsite was lower compared to markets that slaughter poultry onsite [36, 248]. This suggests that poultry slaughter operations at LBMs increases the risk of AI transmission within the LBM environment presumably because of exposure to aerosols arising during the slaughter process where AI virus may be present in large quantities. Improvement of slaughtering and poultry processing operations at LBMs should be an area of investment from market operators through the implementation of standard operating procedures and good manufacturing practices that comply with standard health and safety regulations.

Our previous studies in southern China demonstrated that temporal variation in the intensity of poultry trade and production quantity of live poultry around the Chinese New Year festivities is associated with higher HPAIV H5N1 infection risk in humans and poultry. Our meta-analysis suggested that spring and winter seasons posed significant higher risk of AI infection in the LBM environment compared to summer and autumn [249-255]. This seasonality effect is due, in part, to the fact that lower temperature and humidity can increase virus survival in the environment [237]. These findings demonstrate the need for heightened seasonal targeted surveillance at LBMs to maximize effectiveness.

Our results demonstrate that human infection at LBMs is dependent on important demographic and occupational hazard. The higher risk of human infection in retail markets compared to wholesale markets partly explained by differences in poultry handling operations within the two types of LBMs [256-258]. On one hand, wholesale markets are usually hubs in the poultry market chain where live poultry consignments from different farms congregate before they are sent to other locations, typically retail markets and less frequently slaughterhouses. Poultry handling activities in wholesale markets are limited compared to retail markets in that poultry remain in their cages or assigned area until they are loaded onto trucks on their way to retail markets. One study had noted that live poultry were slaughtered at retail LBMs daily, while many wholesale markets do not slaughter or have separate slaughter areas [259]. On the other hand, retail markets constitute the last step in the LBM chain providing more time for virus to spread and multiply.

Furthermore, our meta-analyses indicate that activities that directly expose LBM workers to AI such as slaughtering, defeathering and cleaning significantly increase the risk of workers AI infection [231-233]. These results are in line with previous observations that the risk of AI infection is greater in LBM workers who clean water containers [226] and those that conduct poultry evisceration with very limited personal protection measures [260]. Several studies have demonstrated that most commonly contaminated sites were located in the poultry slaughtering zone [36, 225]. There is recent evidence on detection of influenza virus in air samples from LBMs, especially with defeathering machines, and risk of airborne transmission [261]. These are all poultry handling activities primarily observed in retail markets which further highlights the need for workers to wear personal protective equipment within retail LBMs.

There are important gender disparities between wholesale and retail markets because wholesale markets tend to be male dominated as opposed to retail markets where women share the poultry value chain with men. Interestingly our results indicate that female workers are at increased risk of AI infection compared to male workers [233, 256, 262]. This contrasts with the situation for H7N9 influenza in humans in the general population where older males are at greater risk than younger females [256]. These results may also reflect gender differentiation in tasks within LBMs, which put female workers at greater risk of AI infection compared to males. Future biosecurity strategies should account for gender differences in risk identified in our study, which could include raising collective awareness through information platforms that target women working at LBMs.

Interpretation of the findings of our study should be done in consideration with its limitations. Firstly, as with all meta-analyses, we were restricted to the data that could be obtained from written reports (all studies included in our meta-analyses were observational studies, given that randomized trials were not available). Secondly, while we meta-analysed studies from different countries, this may overlook the heterogeneity in different study areas, although most of them (almost 95%) were Asian countries. Thirdly, we grouped different types of AIV (H5, H7, H9 etc.); we believe that there will be commonality in spread and transmission in LBMs while there are differences in the epidemiology among these viruses. Fourthly, the estimates presented by the literature reviewed in this study on the effect of sociodemographic analysis of human infection in LBMs were often not adjusted and none reported the interaction between variables. It is difficult to know how much the measured effect of biosecurity indicators could be due to confounding or effect modification; indeed, most effects reported in the studies are unadjusted, and on no occasion did studies explore the presence of effect modification between factors. Finally, we conducted the quality assessment including several study characteristics assuming study quality on a continuous scale and this may not be necessarily the case, and our list of criteria is somewhat arbitrary therefore we also put the results from the random effect model in the Appendix A (Figure A-1 to Figure A-5).

4.7 Conclusion

In conclusion, to minimise market contamination and poultry infection of AI at LBMs, control measures should be targeted to markets that sell and slaughter live poultry and markets with presence of multiple species. Strategies that include daily cleaning and disinfection, regular market closure and ban on overnight storage as well as an emphasis of inspection on cross-regional poultry movements should be put in place. Targeted surveillance programs for AI circulation in LBMs should focus on winter and spring months. Finally, LBM workers directly involved in market cleaning and poultry processing should be provided with occupational health and safety promotion programmes, with emphasis on female workers at retail LBMs.

Table 4-1 General characteristics of the included studies regarding biosecurity indicators of avian influenza infections at live bird markets.

ID	Author, Year and Country	Retrospective time	AIV Subtype	Type of study	Market type	Sampling type	Analysis Methods
Studies of biosecurity indicators related to of poultry infection							
1	Bulaga 2003, USA	2001	H7N2	Cross-sectional	Retail	Poultry and environmental	Multivariate analysis
2	Kung 2003, Hong Kong	2001	H9N2	Longitudinal	Retail	Poultry samples	Univariate analysis
3	Gaber 2007, USA	2004-2005	H5 or H7	Case-control	Not mentioned	Poultry and environmental samples	Multivariate analysis
4	Trock 2008, USA	2002-2003	H5 or H7	Longitudinal	Not mentioned	Poultry and environmental samples	Univariate analysis
5	Indriani 2010, Indonesia	2007-2008	H5N1	Cross-sectional	Both wholesale and retail	Environmental samples	Multivariate analysis
6	LiLH 2010, China	2007-2008	AIV	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
7	Martin 2011, China	2009	H5N1	Cross-sectional	Both wholesale and retail	Poultry samples	Multivariate analysis
8	Leung 2012, Hong Kong	1999-2011	H9N2	Longitudinal	Both wholesale and retail	Poultry samples	Multivariate analysis
9	ZhangRS 2012, China*	2009	H5N1	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
10	BiFY 2013, China	2010-2011	AIV, H5, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
11	Phan 2013, Vietnam	2011	H5N1	Cross-sectional	Not mentioned	Poultry samples	Multivariate analysis
12	ChenZ 2014, China	2013-2014	H7N9	Longitudinal	Both wholesale and retail	Poultry and environmental samples	Univariate analysis
13	LiuH 2014, China	2014	AIV, H5, H7, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
14	WangDF 2014, China	2011-2013	AIV, H5, H9	Longitudinal	Retail	Environmental samples	Univariate analysis
15	YuM 2014, China	2010-2013	AIV, H5, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
16	YuanJ 2014, China	2014	AIV, H5, H9	Longitudinal	Both wholesale and retail	Poultry and environmental samples	Univariate analysis
17	ZhuBL 2014, China	2014	H7N9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
18	ZhuJL 2014, China	2014	H7N9	Cross-sectional	Not mentioned	Environmental samples	Univariate analysis
19	CuiXB 2015, China	2013	AIV, H5, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
20	ElMasry 2015, Egypt	2009-2014	H5N1	Longitudinal	Not mentioned	Poultry samples	Univariate analysis

21	HuangFJ 2015, China	2015	H7N9	Cross-sectional and case-control	Both wholesale and retail	Environmental samples	Univariate analysis
22	KangM 2015, China	2013-2014	H7N9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
23	LiuW 2015, China	2013-2014	AIV, H5, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
24	WangFY1 2015, China	2011-2013	AIV, H5,H9	Longitudinal	Retail	Environmental samples	Univariate analysis
25	WangFY2 2015, China	2014	AIV	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
26	WuJ 2015, China	2013-2015	AIV, H7, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
27	XuGF 2015, China	2013-2014	AIV	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
28	YuanJ 2015, China	2014	AIV, H7N9	Longitudinal	Retail	Environmental samples	Multivariate analysis
29	ZhaoZF 2015, China	2014	AIV, H5,H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
30	CaoL1 2016, China	2015	AIV, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
31	CaoL2 2016, China	2015	AIV, H5, H7, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
32	LiWQ 2016, China	2014-2015	H7N9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
33	LiuFR1 2016, China	2012- 2017	AIV, H5, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
34	LiuFR2 2016, China	2015-2016	AIV,H5,H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
35	LiuJW 2016, China	2013-2015	H7N9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
36	MengJH 2016, China	2013-2015	AIV,H5,H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
37	NongXN 2016, China	2011-2015	AIV	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
38	PengC 2016, China	2015	AIV	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
39	WangXX 2016, China	2014-2015	H7	Longitudinal	Both wholesale and retail	Environmental samples	Univariate and multivariate analysis
40	XieCJ 2016, China	2014-2015	AIV,H5,H7,H9	Longitudinal	Retail	Environmental samples	Univariate analysis
41	ZengJJ 2016, China	2013-2015	AIV,H5,H7,H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
42	ZengL 2016, China	2015-2016	AIV, H5, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
43	Abu Sayeed 2017, Bangladesh	2015	AIV	Cross-sectional	Both wholesale and retail	Environmental samples	Univariate analysis

44	ChenC 2017, China	2016	H7N9	Longitudinal	Both wholesale and retail	Environmental samples	Multivariate analysis
45	ChenXD 2017, China	2017	AIV, H9	Longitudinal	Both wholesale and retail	Poultry and environmental samples	Univariate analysis
46	Chu 2017, Vietnam	2014	AIV	cross-sectional	Both wholesale and retail	Poultry samples	Univariate analysis
47	DaiYX 2017, China	2015	AIV, H5, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
48	LiH 2017, China	2012-2015	AIV	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
49	LiLZ 2017, China	2014-2017	AIV, H5, H7, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
50	LiuM 2017, China	2015-2016	AIV, H5, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
51	LiuRC 2017, China	2009-2014	H5N1	Longitudinal	Both wholesale and retail	Environmental samples	Multivariate analysis
52	MaoXX 2017, China	2016	AIV, H9	Longitudinal	Both wholesale and retail	Poultry samples	Univariate analysis
53	QianLM 2017, China	2013-2015	AIV, H5, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
54	TuZJ 2017, China	2015-2016	AIV	Longitudinal	Retail	Environmental samples	Univariate analysis
55	YuXF 2017, China	Jul-05	H5,H9	cross-sectional	Both wholesale and retail	Environmental samples	Univariate analysis
56	ZhangHB 2017, China	2015-2016	AIV	Longitudinal	Wholesale	Poultry samples	Univariate analysis
57	BaoJ 2018, China	2014-2015	AIV, H5,H9	Longitudinal	Both wholesale and retail	Poultry samples	Univariate analysis
58	CaoL3 2018, China	2016	AIV, H5, H7, H9	Longitudinal	Both wholesale and retail	Poultry and environmental samples	Univariate analysis
59	CaoL4 2018, China	2017	AIV, H5	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
60	ChenYH 2018, China	2017	AIV	Cross-sectional	Wholesale	Poultry samples	Univariate and multivariate analysis
61	FanSY 2018, China**	2014-2016	H7N9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
62	Hassan 2018, Bangladesh	2012-2016	AIV, H5, H9	Longitudinal	Both wholesale and retail	Poultry samples	Univariate analysis
63	LiuXQ 2018, China	2015-2016	AIV, H5, H7, H9	Longitudinal	Both wholesale and retail	Environmental samples	Univariate analysis
64	WuQ 2018, China	2015-2016	AIV, H9	Longitudinal	Not mentioned	Environmental samples	Univariate analysis
65	XuJ 2018, China	2015-2017	H5, H7, H9	Longitudinal	Retail	Environmental samples	Univariate analysis
66	YangJ 2018, China	2014-2015	AIV	Longitudinal	Both wholesale and retail	Environmental samples	Multivariate analysis

67	YangY 2018, China	2017	AIV, H5, H9	Cross-sectional	Not mentioned	Environmental samples	Univariate analysis
68	YaoJX 2018, China	2014-2016	AIV	Longitudinal	Retail	Environmental samples	Univariate analysis
69	ZouLB 2018, China	2012-2017	AIV	Longitudinal	Wholesale	Poultry and environmental samples	Univariate analysis
Studies of biosecurity indicators related to of poultry worker's infection							
1	LiuY 2009, 2009	2007-2009	H5N1, H9N2	cross-sectional	Both wholesale and retail	Market workers	Univariate analysis
2	WangM 2009, 2009	2007-2008	H5, H9	cross-sectional	Both wholesale and retail	Market workers	Univariate analysis
3	ZhangRS 2012, 2012*	2009	H5N1	cross-sectional	Not mentioned	Market workers	Univariate analysis
4	Uyeki 2012, 2012	2001	H9	cross-sectional	Not mentioned	Market workers	Univariate analysis
5	Nasreen 2013, 2013	2009	H5N1	cross-sectional	Both wholesale and retail	Market workers	Univariate analysis
6	Dung 2014, 2014	2011	H5N1	cross-sectional	Not mentioned	Market workers	Univariate analysis
7	TangXJ 2014, 2014	2013	H7N9	cross-sectional	retail	Market workers	Univariate and multivariate analysis
8	WangX 2014, 2014	2013	H7N9	longitudinal	Both wholesale and retail	Market workers	Univariate and multivariate analysis
9	WangQ 2015, 2015	2008-2010	H9N2	cross-sectional	Not mentioned	Market workers	Univariate analysis
10	Nasreen 2015, 2015	2009-2010	H5N1	longitudinal	Both wholesale and retail	Market workers	Univariate analysis
11	FanSY 2018, 2018**	2014-2016	H7N9	Longitudinal	retail	Market workers	Univariate analysis
12	WangX 2018,	2015-2016	H7N9	longitudinal	Both wholesale and retail	Market workers	Univariate and multivariate analysis
* , **Same study Note: The detailed references of all the 79 included studies are cited in the Table A-4 and Table A-5							

Chapter 5 The Role of Live Poultry Movement and Live Bird Market Biosecurity in the Epidemiology of Influenza A (H7N9): A Cross-sectional Observational Study in Four Eastern China Provinces

5.1 Context

In Chapter 4, we quantified the effectiveness of market-level biosecurity at reducing exposure of poultry and humans to avian influenza. The results of Chapter 4 demonstrated that biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs included smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, ban on overnight storage. The results also revealed that sourcing poultry from other areas, i.e. possible long distant poultry movement, is associated with a higher risk of H7N9 infection at LBMs. The results of Chapter 4 indicated that higher risk of exposure to AI infection occurs in workers at retail LBMs, female workers and those who contact ducks, conduct cleaning, slaughtering, defeathering or evisceration.

Despite these key findings from the program of research in Chapter 4, a key limitation was that we were not able to stratify the meta-analysis by different AI strains, so we were unclear whether these effects were true for H7N9 alone. With that gap in knowledge in mind I then designed the study in Chapter 5 which aimed to conduct an empirical investiagtion in the H7N9 affected markets, so to evaluate the biosecurity risk factors within those markets associated with H7N9 infections and quantified the role of live chicken movement in the epidemiology of H7N9 human infections.

Therefore, in the program of research in Chapter 5, we zoomed in to the originally infected area (Shanghai Municipality, Jiangsu, Zhejiang and Anhui provinces). We obtained a unique dataset collected during the emergency epidemiological investigation on the 24 LBMs within one kilometre of H7N9 human infections and those that marketed large quantities of poultry at the time of the outbreak. The dataset included the status of market biosecurity measures adopted on these markets and records of live poultry movements in and out of these markets.

In Chapter 5, we evaluated the biosecurity risk factors associated with H7N9 infections on LBMs during the emergency and identified the role of live poultry movement in the epidemiology of H7N9 human infections. Prior to this research, the sources of infection had yet to be fully clarified and a major challenge for the investigation of sources of exposure is the fact that poultry did not exhibit clinical signs at the time of this study. Epidemiological studies relied on H7N9 infection data from humans and LBMs where infections had been detected. Previous studies in Asia have demonstrated the role of movement of poultry through live bird markets in the circulation and dissemination of HPAI H5N1 virus [41]. This research of the role of LBM and live poultry movement have extended the knowledge of market-level biosecurity risk factors and enabled the stratification of the risk of H7N9 infection geographically.

This Chapter has been published on The Journal of Infection and supporting technical information is presented in Appendix B of this Thesis.

5.2 Abstract

A new reassortant influenza A (H7N9) virus emerged early 2013 in eastern China. Exposure to H7N9 infected poultry at live bird markets (LBM) was implicated as the main risk factor for human infection. We aimed to identify the role of LBM biosecurity indicators and poultry movement in the affected areas. A cross-sectional survey was carried out in 24 LBMs at the beginning of H7N9 outbreak in all affected provinces. We used univariable analysis to identify the biosecurity factors associated with the H7N9 presence in LBMs and social network and spatial analysis to quantify the connectivity and geographic variation in the connectivity of poultry movements. Chickens were the predominant poultry species traded by affected LBMs. The presence of H7N9 in LBMs was significantly associated with the type of LBM and with LBMs that sold chicken to other markets. The chicken movements were significantly spatially clustered and were highest in counties from Jiangsu and Anhui provinces. LBM biosecurity and chicken movement played an important role in the emergence of H7N9. This study identified highly connected areas in eastern China which continue to report human infections highlighting candidate areas for more detailed epidemiological investigations.

Keywords: Influenza A (H7N9), Poultry movement, Live bird markets, Meat Chicken, Social network analysis, Biosecurity

5.3 Introduction

The emergence of a new reassortant influenza A(H7N9) causing human infections without preceding or concomitant outbreaks in poultry was quite unexpected [263]. The sources of infection have yet to be fully clarified and a major challenge for the investigation of sources of exposure is the fact that poultry do not exhibit clinical signs. Epidemiological studies rely on infection data from humans and live bird markets (LBMs) where infections have been detected. The key public health concerns about the novel H7N9 virus are how and where the virus crossed the species barrier and whether it will further adapt to enable sustained human-to-human transmission [264].

Since the report of the first human H7N9 case in Shanghai in March 2013, there have been three major waves of human H7N9 cases in China. The first wave was observed from March to April 2013, starting from Shanghai, Anhui, Jiangsu and Zhejiang provinces in eastern China, then mainly extended to neighbouring provinces: Henan, Shandong, Hunan, Jiangxi and Fujian. The second wave was observed from January to April 2014, affecting initially and primarily the provinces of Zhejiang province in eastern China and Guangdong province in southern China, then extending to Jiangsu, Anhui, Fujian, Hunan and Guangxi provinces. From May to December 2013 (i.e. the period between the two waves), there were sporadic human cases in Shanghai, Jiangsu, Zhejiang, Jiangxi and Guangdong, similar situation during the period from May to October 2014. The third wave began in late 2014, started from Jiangsu and Xinjiang provinces, then massive affected Zhejiang and Jiangsu provinces in eastern China, as well as Fujian and Guangdong provinces in southern China [265, 266].

Exposure to H7N9 infected poultry at LBMs has been implicated as the main risk factor for human infection and chickens are considered to be the species with an important role in the transmission for H7N9 influenza [14-18]. During the first wave, 82% of the 131 reported human H7N9 cases had a history of exposure to live poultry, particularly chickens [102]. Available evidence indicates that LBMs can serve as potential hubs where avian influenza viruses are transmitted and maintained for prolonged periods of time [19-22]. Surveillance and monitoring activities for avian influenza within the poultry market chain (i.e. farms, transport, LBMs and slaughter houses) are an important tool to generate epidemiological evidence on affected species, geographical sources of infection and the role of modifiable risk factors on disease transmission [23].

Previous studies in Asia have demonstrated the role of movement of poultry through live bird markets in the circulation and dissemination of HPAI H5N1 virus [41]. Social network analysis (SNA) techniques can be utilized to build and analyze the network of poultry movements to help identify high-risk premises and offer new insights on disease transmission dynamics, making it possible to develop more effective strategies for disease control. For poultry market chain analysis, SNA of poultry movement is used to quantify the connectivity of sources and markets in the network and quantify the risk associated with HPAIV H5N1 infection along the market chain [40]. These empirical studies have demonstrated the value of coupling data on the social network of the poultry market chain with data on infection along the market chain to develop risk-based, targeted surveillance of farms and markets.

In March 2013, an early emergency investigation was carried out by the MARA of China and this was followed by the establishment of a joint investigation into the source of the outbreak. As a result, a large-scale, cross-sectional survey was carried out from 14 to 19 April 2013 by the Veterinary Bureau of the MARA of China, China Animal Health and Epidemiology Centre (CAHEC) of MARA of China, and the Emergency Centre for Transboundary Animal Diseases of the Food and Agriculture Organization of the United Nations in China (FAO ECTAD China).

The study aimed to identify the role of biosecurity indicators and poultry movement through LBMs in the presence of influenza A H7N9 within LBMs during the first wave of H7N9 infection and to examine the spatial variation in connectivity of counties involved in poultry trade to LBMs in the four affected provinces during the first wave of H7N9 infection.

5.4 Materials and Methods

5.4.1 Ethics Statement

The research proposal leading to the study received ethics approval from the CAHEC of MARA of China. Ethical approval for the questionnaire survey was obtained from the Division of Epidemiology Survey within CAHEC who handles the ethics approval of field studies conducted by their staff in China. Participation in the questionnaire survey was voluntary and oral consent was obtained from market managers at all intervening LBMs. There were no animal samples taken as part of our study, we used secondary information on market positivity to H7N9 infection derived from the official website of MARA of China.

5.4.2 Data sources

During the emergency response to influenza A (H7N9) infection in humans, a cross-sectional survey was conducted in 24 LBMs (15 wholesale markets and 9 retail markets) in the Shanghai provincial-level municipality, Jiangsu, Zhejiang and Anhui provinces from 14 to 19 April 2013. The 24 LBMs included in the study were selected purposively based on two criteria: first, we focused on all LBMs within 1 kilometre in the adjacency of areas with reported H7N9 human infections, and second, we selected all LBMs that marketed more than 20,000 heads of poultry per day at the time of the outbreak. Therefore, the LBMs selected best represented existing production and LBM marketing systems.

This investigation included data collection on live poultry movement and market biosecurity. The managers of the 24 LBMs were interviewed using a standardized, validated questionnaire to capture information relative to trade and general market biosecurity measures. The questionnaires had been used in previous studies by our team [31, 40]. About 90% of live poultry are thought to come through wholesale markets on the way to retail markets. Some wholesale markets that also sell live poultry directly to the consumer have been defined as mixed markets. Information on poultry movements was obtained from poultry movement certificates available at nine of the 24 surveyed markets (four wholesale LBMs and five mixed LBMs), and was recorded for up to three months before the reporting of the first human case in the province, i.e. Shanghai (1 January to 5 April), Jiangsu (1 January to 4 April), Anhui (1 January to 15 April) and Zhejiang (1 January to 11 April). The nine markets were from Shanghai (n=3), Nanjing (n=2), Hangzhou (n=1), Huzhou (n=1), Hefei (n=1) and Chuzhou (n=1). The poultry movement certificates recorded the name of the county of poultry sources, poultry species (e.g. chickens, pigeons, ducks and other types) and the numbers of poultry transported.

Data on the presence of H7N9 in the 24 LBMs was available from the official national emergency surveillance activities conducted during 31 March to 19 April 2013; data on H7N9 infection in poultry and humans used for mapping was up to the end of May 2013, both infection data were extracted from the official website of MARA of China (http://www.moa.gov.cn/zwllm/yjgl/yqfb/index_3.htm) and WHO (http://www.who.int/influenza/human_animal_interface/avian_influenza/archive/en/), and geo-coded based on the reported geographical source of infection.

5.4.3 Social network analysis of live chicken movements

To describe the connectivity pattern within the network dataset consisting of records of paired trading events between a particular LBM and the county of origin of the purchased chickens (termed as “chicken source”), we used social network analysis (SNA), as described previously [31]. We summarised network connectivity using the number of links (indicating the frequency of movement), movement length (representing the catchment area of LBMs), degree centrality, *k*-core and the components of the network. The degree represents the absolute number of unique links of a given node; for example, a degree of two means that the node (poultry market or source) is connected with two other unique markets or sources. The *k*-core represents the maximal group of nodes, it describes the connectivity of different groups in a network; for example, a k-core of two means that the node belongs to a subgroup within the network where all nodes are connected to two other nodes. These two network indicators are important for describing the levels of connectivity and centrality that exist between the different elements of a network and play a key role while identifying the most influential spreaders within a network [267]. The components of the network include a maximal connected sub-graph where all nodes (i.e. chickens’ sources) are connected through paths.

We built one 2-mode binary network (LBM-source network), linking the nine LBMs and chicken sources. The centrality measures at node level (such as the degree and membership of the giant component) have been suggested to be of practical use in the development of effective targeted disease control strategies. The 2-mode LBM-source network was converted into two separate 1-mode binary symmetric networks: one 1-mode binary symmetric network of source nodes linked via a common LBM (source–source network) and a 1-mode binary symmetric network of market nodes linked via a common county (market–market network). All social network analyses were performed using UCINET 6.216 (©Analytic Technologies). All maps were produced using ArcGIS 10.1 (©ESRI).

5.4.4 Analysis of spatial variation in the connectivity of poultry sources

To assess whether there was spatial correlation in the observed pattern of degree centrality and *k*-core among the live chickens sources (source-source network) in the study area we used the Global Moran's Index (Moran’s I), a measure of spatial autocorrelation for spatially aggregated data. Moran's I is positive when nearby areas tend to be similar, negative when they tend to be dissimilar, and approximately zero when attribute values are arranged randomly and independently in space [220]. The estimate of Moran's I value and a Z-score evaluating the significance of the index were estimated using ArcGIS 10.1.

5.4.5 Associations between biosecurity indicators and H7N9 infection status in LBMs

A full range of biosecurity indicators were examined for all the 24 LBMs (Table B-1). The association between LBM biosecurity attributes and infection status (H7N9 presence in the LBM as reported by official reports) was examined for the 24 LBMs with biosecurity data. The biosecurity attributes included market type (wholesale vs. retail markets), waste disposal, manure processing, market disinfection, and market cleaning and trade destination. Firstly, all LBM biosecurity attributes were initially screened for association with market node infection status using univariate statistical analysis based on $P < 0.05$ when applying the Fisher’s exact test (Stata/SE 12.0 ©StataCorp). Secondly, the association between LBM attributes associated with H7N9 infection status of LBMs and other LBM biosecurity practices were examined.

5.5 Results

5.5.1 Social network analysis of live chicken movements

Our results show that live chicken were the main poultry species being traded in the main markets of the affected areas. The results indicate that chicken production in Shanghai and Zhejiang could not meet their local consumption demand, and Jiangsu and Anhui were their main supplementary chicken sources (Table B-2). LBMs in Anhui mainly traded chicken from sources in the same province (55%), Shandong (21%) and Jiangsu (20%) provinces. Similarly, LBMs in Jiangsu traded poultry from sources in the same province (72%) and Anhui (28%) province.

The full extent of the 2-mode network is presented in Figure 5-1. The results of this analysis reveal that there was a giant weak component comprising nine LBMs and 102 chicken sources. Our results indicate that five (55.6%) LBMs had a degree centrality greater or equal to 10. Among LBMs surveyed, we found that eight (89%) had a *k*-core of 3 and one (11%) had a *k*-core of 2. The degree centrality of wholesale LBMs was significantly higher than mixed LBMs, (diff = 15.5, p=0.037, 95% CI =-1.97, 32.97). The catchment area of wholesale LBMs was much larger than mixed LBMs. The frequency of poultry movements (as measured by the number of links) to the wholesale LBMs was higher than mixed LBMs. Among chicken sources captured in our survey, we found that the majority (68.6%; n=70) of chickens' sources had a degree and *k*-core of 1. We found that the mean degree centrality and *k*-core estimates of LBMs were larger than for chicken sources.

5.5.2 Geographical variation in degree centrality and k-core of chicken sources

This analysis indicated that the counties with the highest degree centrality were located in the provinces of Anhui and Jiangsu. The counties with highest degree centrality were Nanling, Changfeng, Quanjiao, Chaohu, Guangde counties in Anhui province and Changzhou, Lishuiqu, Haian, Jiangyan, Dafeng counties in Jiangsu province (Figure 5-1).

The counties with highest *k*-core (=36) were widely distributed across Jiangsu, Anhui, Henan and Shandong provinces and were all connected with each other through the LBMs (counties in red group) The area with the lowest *k*-core (dark green group) was located in Shanghai (Figure 5-2). The spatial pattern of degree centrality and *k*-core of live chicken sources indicated significant spatial clustering of county-specific degree centrality and *k*-core in that the Moran's I value was positive and the Z score greater than 1.96, both statistically significant at the 0.05 level (Table B-3).

5.5.3 Biosecurity of Live Bird Markets and H7N9 positivity

From all the list of biosecurity indicators (Table B-1), only two factors were found significantly associated with the presence of H7N9 in the surveyed markets: type of LBMs (i.e. retail, wholesale or mixed) (P=0.046) and whether the LBMs sold poultry or not to other LBMs (P=0.041) (Table 5-1).

We also found significant variability in biosecurity indicators between different types of LBMs (Table 5-2). The market level biosecurity indicators that statistically associated with different types of LBMs were: type of poultry sold (P=0.013), sell live duck (P=0.039), waste hauled in trash (P=0.016), market disinfection (P=0.043), market closure (P=0.028), sell poultry to other LBMs (P=0.0) and sell poultry directly to consumers (P=0.047).

Our results indicate that the biosecurity indicators that were statistically associated with selling poultry to other LBMs were: type of poultry sold (P=0.001), waste hauled in trash (P=0.019), market disinfection (P=0.031) (Table 5-3).

5.6 Discussion

This study presents evidence in support of the role that LBM poultry movement and biosecurity have played in the seeding of H7N9 infection during the early stages of the first wave of H7N9 infection in LBMs of affected areas in eastern China. The findings of this study also extend previous SNA studies by specifically investigating the geographical variation of the relative connectivity of chicken sources to areas known to have had significant clustering of human infection.

Early emergency investigations and findings from the implementation of the national surveillance and investigation plans have shown that LBMs were likely to play an important role in disease transmission [15]. Our findings demonstrate that the predominant poultry species being traded in the LBMs involved in the first wave of H7N9 infection were live chickens. Prior to mandatory market closure, most live chickens were transported to wholesale LBMs and further to retail LBMs to satisfy cultural and consumer preferences for live chicken, and slaughter usually occurred at LBMs with some being taken for slaughter at home or in restaurants.

Epidemiological evidence from previous researches indicate the indirect transmission via fomites (i.e. equipment, the movement of vehicles during chick delivery) plays an important role in the potential transmission and spread of AI viruses between premises [33, 145, 157, 268]. Our previous studies in South China demonstrated the utility of collecting poultry movement data to understand the epidemiology of avian influenza of the H5N1 subtype in China [31, 40]. Utilizing the materials and data collection protocols from our previous network studies in South China, the joint investigation teams collected poultry movement data from LBMs in the H7N9 infection areas of the first wave. We described the connectivity of poultry trade using the *k*-core (describes the connectivity of different groups in a network) and the degree centrality (describes the connectivity of individual poultry sources).

These measures can theoretically reflect the spread of an infection through a poultry network; using *k*-core as an example, any poultry moved from an infected source can result in the infection of all linked nodes with a maximum *k* distance [40]. Therefore, it is important to identify the location of the subgroup chicken sources with the highest connectivity (*k*-core and/or degree centrality) in order to assist in the selection of areas for a targeted risk-based of surveillance and targeted control. Our results indicate that the counties of Nanling, Changfeng, Quanjiao, Chaohu, Guangde in the province of Anhui and Changzhou, Lishuiqu, Haian, Jiangyan, Dafeng in the province of Jiangsu are the locations with the highest poultry trade connectivity (as measured by the degree centrality and k-core). Our findings also showed significant spatial clustering in terms of degree centrality and *k*-core of live chicken sources, which suggests these areas are epidemiologically significantly correlated.

Furthermore, our study demonstrates the presence of a geographic overlap between the locations with the highest connectivity of live chickens sources and the primary cluster of H7N9 human infections [29]. This geographical overlap occurs in an area straddling the boundaries of the provinces of Anhui and Jiangsu. An analysis of the data from the first wave of H7N9 human infections demonstrated that the primary cluster of influenza (H7N9) overlapped with previous H5N1 human infections [29]. Changes over time in poultry trade and LBM network in terms of extent and volume are likely to occur and could lead to this high-risk area to shift geographically [40]. However, since the third wave of H7N9 infection, there have been 18 human H7N9 outbreaks in Jiangsu province which are well within the highly connected area identified in this study suggesting that our findings are robust to the known changes in poultry trade pattern which occur across the year (Data was derived from http://www.who.int/influenza/human_animal_interface/avian_influenza/archive/en/).

Taken together these findings suggest that the areas identified in our study are of high importance in terms of the epidemiology of influenza A (H7N9). Therefore, continuous surveillance of poultry trade activities should be in place in the areas identified in our study. There is also a need to design and conduct more detailed empirical studies in the provinces of Anhui and Jiangsu to understand live poultry movement data at different points of the poultry marketing chain and integrate that information with data from risk perception and attitudes towards biosecurity of actors in the poultry market chain, particularly poultry farmers, intermediary traders and consumers.

In our previous studies in South China we reported that LBMs with a poor level of biosecurity could play an important role in the dissemination of infected poultry should they be marketed through their network [31]. Other studies have shown that the prevalence of particular avian influenza viruses in retail LBMs is twice as high as the prevalence in wholesale LBMs [269, 270]. However, our results have shown that in the case of H7N9, the market level infection was significantly associated with wholesale and mixed LBMs compared to retail markets suggesting that particular biosecurity practices within wholesale and mixed LBMs may be a good indicator for H7N9 presence. Initial reports had proposed that visits to retail markets during the first wave of H7N9 had posed humans at increased risk of H7N9 infection by facilitating the transmission of avian influenza viruses due to lower biosecurity levels and increased access by consumers [82]. Our results indicate that retail markets were mostly a dead-end for the live chicken trade (i.e. selling poultry directly to the consumer) while all the surveyed wholesale markets reported sending poultry to other LBMs. In addition, all retail markets reported selling both live and slaughtered poultry while over eighty percent of the wholesale markets reported selling live poultry only. Our study also demonstrates the important role that poultry trade profile can have on market positivity to H7N9 in that the presence of H7N9 infection was associated with LBMs that sell live poultry to other LBMs. The results also indicate that LBMs that sell live poultry to other LBMs tended to have insufficient disinfection and cleaning measures and no proper market closure practices.

In our study, eighty-nine percent of the retail markets reported having their waste hauled in the trash while two thirds of wholesale and mixed markets reported not having their waste hauled in the trash. This corroborates the findings in Indonesia where removal of waste contributed to a reduction of HPAI (H5N1) in LBMs [36]. In addition, eighty percent of the wholesale and mixed markets surveyed in our study reported infrequent disinfection practices (none or every three or more days), and only forty-four percent of the retail markets reported not disinfecting stalls. Previous studies have shown that daily cleaning and disinfection are effective at reducing of AI virus in LBMs in southern China and New York [31, 246]. Our results also reveal that all the retail markets and two thirds of wholesale markets reported that they did not perform market closure, while 56% of mixed markets reported that they performed market closures. Studies have shown that the introduction of rest-day in LBMs led to a significant decline in the isolation rate of influenza virus (H9N2) in LBMs [37, 38]. Besides, the increase in selling activities itself could be associated with a lapse in the institution of hygienic measures or even worse the selling of lower quality poultry material left unsold from previous activities [21, 259, 271]. Taken together these results suggest that market biosecurity upgrading, and restructuring could have a significant impact on reducing the level of infection and possibly interrupting the cycle of infection persistence. Nevertheless, the relationship between live bird market biosecurity indicators and the presence of H7N9 is likely to be much more complex and further evidence is necessary to profile the risk of LBMs.

The findings of this study should be interpreted in light of the study limitations. Firstly, this study was conducted during an emergency response and the LBMs were selected to be purposively aligned with the occurrence of H7N9 human infections. Also, while collecting the live poultry movement data, only major LBM's in the large cities in the four provinces were surveyed which may have introduced potential biases. Secondly, data on the presence of H7N9 in LBMs was ascertained by available information from the national emergency surveillance activities. Due to mandatory market closures in the study area, the H7N9 infection status in several markets was not available. Due to the insufficient sample size we were not able to include the biosecurity indicators in a multivariable model nor were we able to detect a significant independent effect of neither of these indicators on H7N9 virus positivity in the LBMs. Thirdly, while the cross-sectional design of our study allows us to identify areas in China involved in poultry trade to LBMs in close proximity to human H7N9 cases, it does not allow us to conclusively explain how the virus emerged and spread over time.

5.7 Conclusion

This study demonstrates that the connectivity of LBMs to particular counties in the provinces of Anhui, Zhejiang and Jiangsu and the level of market biosecurity of LBMs were likely to have played a role in the transmission of H7N9 to humans during the first wave of the epidemic in April 2013. Recent cases are being reported in the areas identified in our study which emphasizes the need to improve our understanding of poultry trading patterns within the counties identified.

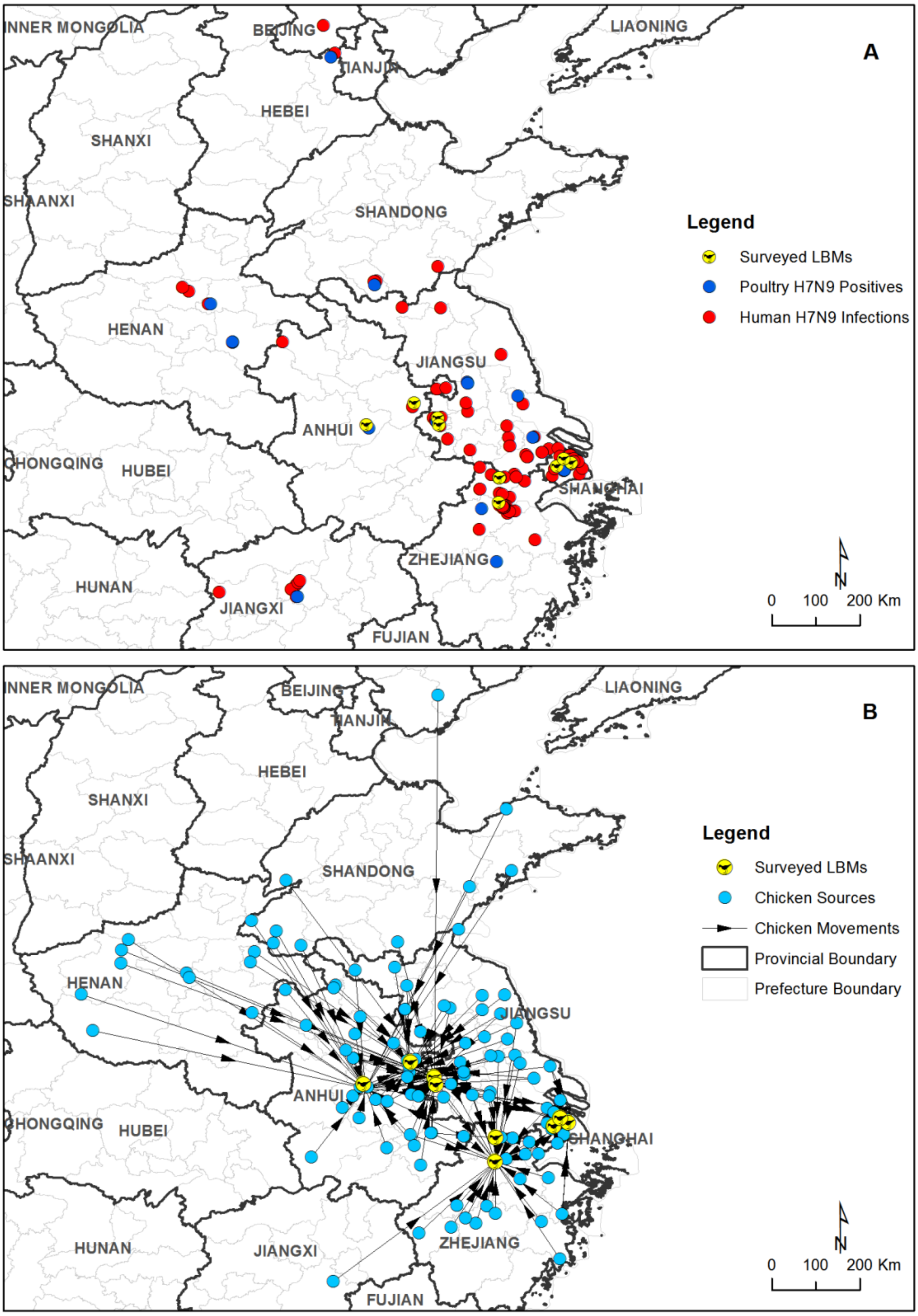


Figure 5-1 Geographical distribution of LBMs surveyed, H7N9 human infections and poultry H7N9 positives (A), and live chicken sources and movement networks included in the study (B). The influenza A (H7N9) human infections (red circle) and poultry positives (blue circle) were updated till end of May 2013. The network represents the 2-mode binary network (LBM-source network), with black arrows linking the nine LBMs (yellow circle with a bird inside) and live chickens' sources (sea-blue circle) from which chicken originate.

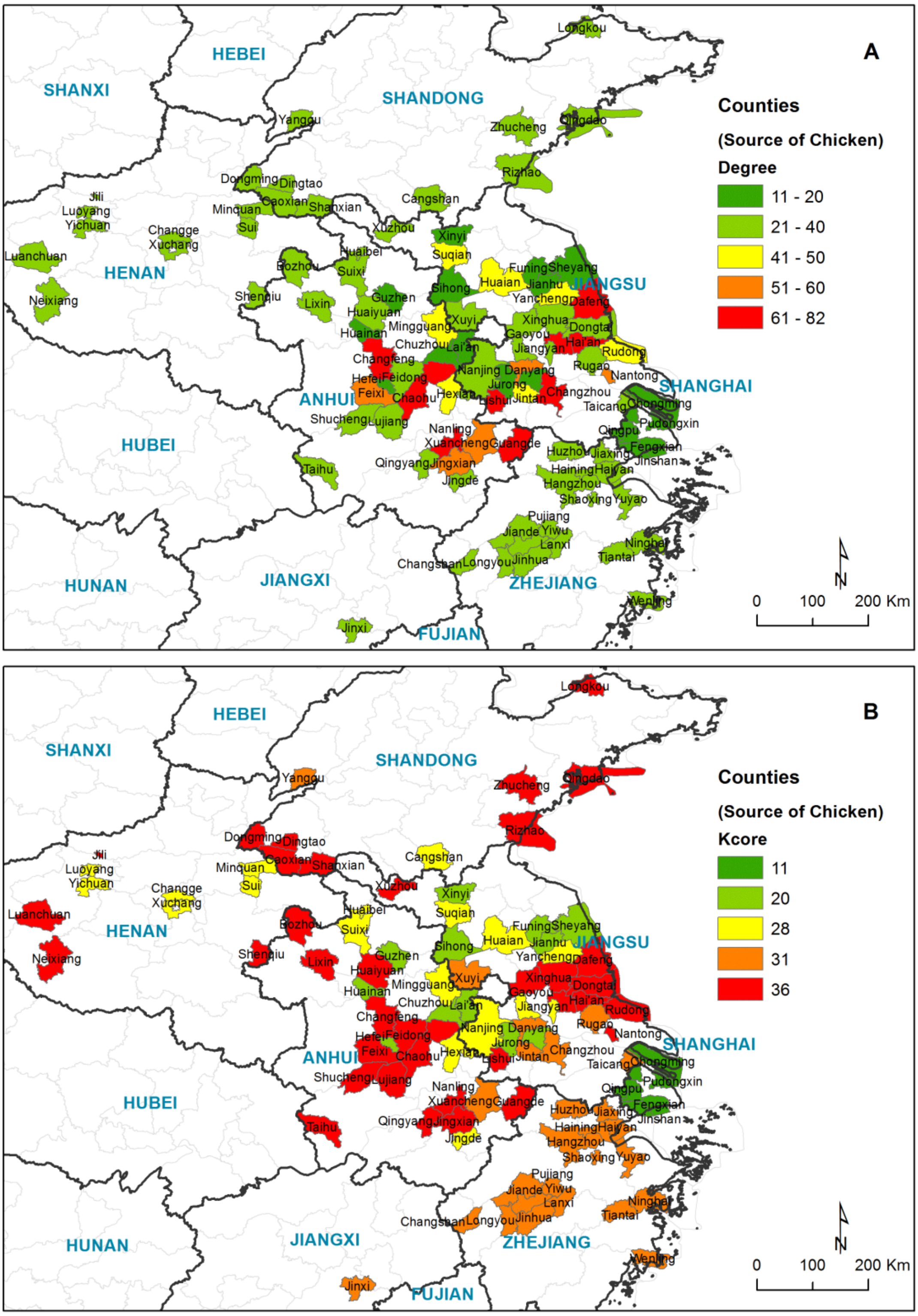


Figure 5-2 Geographical distribution of the degree centrality (A) and k-core (B) of live chicken sources (county level), based on a 1-mode network of chicken sources.

Table 5-1 Market level biosecurity indicators associated with "market H7N9 infections".

Market level biosecurity indicators	**Market H7N9 infection status**			
	No N (row%, col%)	**Yes**	**Total**	**Fisher's exact test**
Type of LBMs				
Retail	9 (100, 47.4)	0 (0, 0)	9 (100, 37.5)	
Wholesale	3 (50, 15.8)	3 (50, 60)	6 (100, 25)	
Mixed	7 (77.8, 36.8)	2 (22.2, 40)	9 (100, 37.5)	
Total	19 (79.2, 100)	5 (20.8, 100)	24 (100, 100)	0.046
Sell poultry to other LBMs				
No	11 (100, 57.9)	0 (0, 0)	11 (100, 45.8)	
Yes	8 (61.5, 42.1)	5 (38.5, 100)	13 (100, 54.2)	
Total	19 (79.2, 100)	5 (20.8, 100)	24 (100, 100)	0.041

Table 5-2 Market level biosecurity indicators associated with "type of LBMs".

Market level biosecurity indicators	**Type of LBMs**				
	Retail N (row%, col%)	**Wholesale**	**Mixed**	**Total**	**Fisher's exact test**
Sell poultry to other LBMs					0.0*
No	9 (81.8, 100)	0 (0, 0)	2 (18.2, 22.2)	11 (100, 45.8)	
Yes	0 (0, 0)	6 (46.2, 100)	7 (53.9, 77.8)	13 (100, 54.2)	
Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)	
Type of poultry sold					0.013*
Live birds only	0 (0, 0)	5 (62.5, 83.3)	3 (37.5, 33.3)	8 (100, 33.3)	
Live and slaughtered poultry	9 (56.3, 100)	1 (6.3, 16.7)	6 (37.5, 66.7)	16 (100, 66.7)	
Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)	
Sell live ducks					0.039*
No	3 (23.1, 33.3)	2 (15.4, 33.3)	8 (61.5, 88.9)	13 (100, 45.8)	
Yes	6 (54.6, 66.7)	4 (36.4, 66.7)	1 (9.1, 11.1)	11 (100, 100)	
Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)	
Waste hauled in trash					0.016*
No	1 (9.1, 11.1)	3 (27.3, 50)	7 (63.6, 77.8)	11 (100, 45.8)	
Yes	8 (61.5, 88.9)	3 (23.1, 50)	2 (15.4, 22.2)	13 (100, 54.2)	
Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)	
Market disinfection					0.043*
No	4 (57.1, 44.4)	1 (14.3, 16.7)	2 (28.6, 22.2)	7 (100, 33.3)	
Every 1-2 days	5 (62.5, 55.6)	1 (12.5, 16.7)	2 (25, 22.2)	8 (100, 37.5)	
Every 3-14 days	0 (0, 0)	4 (44.4, 66.7)	5 (55.6, 55.6)	9 (100, 100)	
Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)	
Market closure					0.028*
No	9 (52.9, 100)	4 (23.5, 66.7)	4 (23.5, 44.4)	17 (100, 70.8)	
Yes	0 (0, 0)	2 (28.6, 33.3)	5 (71.4, 55.6)	7 (100, 29.2)	

Total	9 (37.5, 100)	6 (25, 100)	9 (37.5, 100)	24 (100, 100)

Table 5-3 Market level biosecurity indicators associated with "selling poultry to other LBMs".

Market level biosecurity indicator	**Selling poultry to other LBMs**			
	No **N (row%, col%)**	**Yes**	**Total**	**Fisher's exact test**
Type of poultry sold				0.001*
Live poultry only	0 (0,0)	8 (100, 61.5)	8(100, 33.3)	
Live and slaughtered poultry	11 (68.8, 100)	5 (41.7, 38.5)	16 (100, 16.7)	
Total	11 (45.8, 100)	13 (54.2,100)	24 (100,100)	
Waste hauled in trash				0.019*
No	2 (18.2, 18.2)	9 (81.8,69.2)	11 (100,45.8)	
Yes	9 (69.2, 81.8)	4 (30.8, 30.8)	13 (100, 54.2)	
Total	11 (45.8, 100)	13 (54.17, 100)	24 (100, 100)	
Market disinfection				0.031*
No	5 (71.4, 45.5)	2 (28.6, 15.48)	7 (100, 29.2)	
Every 1-2 days	5 (62.5, 45.5)	3 (37.5, 23.1)	8 (100, 33.3)	
Every 3-14 days	1 (11.1, 9.1)	8 (88.9, 61.5)	9 (100, 37.5)	
Total	11 (45.8, 100)	13 (54.2, 100)	24 (100, 100)	
Market closures				0.078
No	10 (58.8, 90.9)	7 (41.2, 53.9)	17 (100, 70.8)	
Yes	1 (14.3, 9.1)	6 (85.7, 46.2)	7 (100, 29.2)	
Total	11 (45.8, 100)	13 (54.2, 100)	24 (100, 100)	

Table 5-4 Chicken movement network estimates, based on a 2-mode network of LBMs and chicken sources.

Network estimates	**Live bird markets**			**Chicken sources**		
	Mean	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Degree centrality	16.89	3	37	1.49	1	6
K-core	2.89	2	3	1.42	1	3
Distance to human H7N9 cases (in kms)	13.46	0.39	97.31	73.99	0.32	295.78

Chapter 6 Knowledge, Attitudes and Practices Associated with Avian Influenza along The Live Chicken Market Chains in Eastern China: A Cross-Sectional Survey in Shanghai, Anhui and Jiangsu Provinces

6.1 Context

The results of Chapter 5 demonstrated that the level of market biosecurity of LBMs initially affected by H7N9 in 2013 and the connectivity of these LBMs to particular counties in the provinces of Jiangsu, Anhui and Zhejiang provinces were likely to have played a role in the transmission of H7N9 to humans during the first wave of the epidemic in early 2013. We had previously demonstrated in Chapter 4 that people's social demographic and behaviour at LBMs are important indicators of risks in AI human infections. Therefore, I designed the study reported in Chapter 6 to investigate whether these social cognitive indicators are significant in the identified high-risk areas in Southeast China, to identify the level of risk perception towards AI among different actors (farmers, traders and consumers) at LBMs and the risk factors associated with their risk perception in the identified high-risk areas where H7N9 first emerged.

The existing literature indicated that health behaviours and hygiene practices can be influenced by age, gender, education, knowledge and religious beliefs [182]. In recent years there had been a number of studies reporting poultry related workers' KAP for AI in Asia, the US, Europe and Africa [184, 272-279]. To our knowledge, none of the existing studies was conducted on chicken-specific market chains targeting high-risk areas affected by the emergence of the H7N9, and importantly none had compared AI knowledge, attitudes and practices (KAP) simultaneously across chicken farmers, chicken vendors and consumers in the same market chain [10]. So, there was a need to understand the KAP of all actors across the live chicken market chain involved in the H7N9 emergency in China.

In Chapter 6, a primary cross-sectional questionnaire survey was designed and conducted in the hotspot area identified in Chapter 5 during June to July 2014 after the second wave of H7N9 outbreaks in humans in Eastern China. All actors (chicken farmer, vendors and consumers at LBMs) along the live meat chicken market chain were targeted to profile their level of knowledge, attitudes and practices (KAP) towards avian influenza and the risk factors associated with their KAP levels. Multivariable generalized least squares (GLS) random-effects regression models were developed to identify predictors of KAP of AI among different actors along the live chicken market chain.

The study in Chapter 6 analysed determinants of KAP within each actor group. The results of Chapter 6 suggested that interventions to improve KAP towards AI should be promoted among all stakeholders with an emphasis on chicken vendors in LBMs. This further ascertains the findings from Chapter 4 and 5 that LBMs may have played an important role in the dissemination of H7N9 virus in the high-risk area. The results of Chapter 6 also demonstrated that risk-based health promotion interventions should be developed and implemented by both animal health agencies (targeting farmers and vendors) and public health agencies (targeting frequent and male consumers) to prevent transmission of H7N9 along the market chain in China.

This Chapter forms a manuscript published by the Journal of Transboundary and Emerging Diseases, and supporting technical information is presented in Appendix C in this Thesis.

6.2 Abstract

The avian influenza (AI) virus of the H7N9 subtype emerged in China in 2013. Live bird markets (LBMs) selling live meat chickens were indicated to present a high risk for virus dissemination. This study aimed to quantify the level of knowledge, attitudes and practices (KAP) on AI and to measure associated risk factors, among different actors along the live chicken market chain within H7N9-affected Eastern provinces in China. A cross-sectional survey was conducted in these provinces during June - July 2014. Structured questionnaires about KAP for AI were delivered to chicken farmers, chicken vendors and consumers in LBMs. Multivariable generalized least squares (GLS) regression models were developed to identify predictors of KAP scores among different actors.

Our results indicate that KAP scores of chicken farmers were generally higher than those of chicken vendors. Chicken farmers who worked for more than 15 years had significantly lower total KAP scores than those who worked for less than six years. Chicken farmers who worked more than 15 hours in a day had significantly lower attitude scores than those who worked less than six hours. For chicken vendors, females and individuals >35-years-old had significantly lower knowledge scores compared to the reference categories. Practice scores were significantly higher in female vendors and those vendors who also conducted slaughter compared to males and vendors who did not conduct slaughter. Consumers who bought chicken at least once every month had better risk awareness compared to those who bought chicken at least once every week. In addition, female consumers had significantly better practice scores than male consumers.

In conclusion, risk-based health promotion interventions should be developed and implemented by animal health agencies (targeting farmers and vendors) and public health agencies (targeting frequent and male consumers) to prevent transmission of H7N9 along the market chain in China.

Keywords: Avian Influenza, KAP, live chicken market chain, chicken farmers, chicken vendors, consumer, generalized linear regression model

6.3 Introduction

In early 2013, the influenza A (H7N9) virus was first detected in humans in China, and since then six epidemic waves of this avian influenza (AI) subtype in humans have been reported with increasing magnitude in China [280]. During the first wave of human H7N9 infections, chickens were the predominant poultry species traded in affected live bird markets (LBMs) [10]. In a previous study, we demonstrated that H7N9 avian influenza infection in humans was associated with the connectivity of live bird markets (LBMs) located in six counties in the provinces of Jiangsu and Anhui [10].

Continued H7N9 human infections reported in these areas emphasizes the need to conduct further investigations to understand the live chicken trading patterns within the counties identified. From late 2016 to early 2017, there was a sudden increase of human cases and the geographical distribution of cases was more widespread than in the previous four waves. This event was described in the literature as the fifth epidemic wave [11]. As a result, in February 2017, the MARA of China recognising the importance of the live poultry markets in the exposure and dissemination of the virus, established the “1110 policy” on LBMs, i.e. clean once a day, disinfect once a week, close markets once a month, and ensure zero overnight storage in markets. In July 2017, the “National Immunization Program for HPAI” in the poultry sector began with the adoption of a H5 and H7 bivalent inactivated vaccine [138]. While this vaccine has been effective at controlling the number of H7N9 outbreaks in humans, in the long-run vaccination may not help curb the exposure of humans to these viruses in the live meat chicken market chain since these viruses are sporadically detected in poultry [204]. Therefore, a better understanding of the social determinants of exposure is necessary to complement sanitary measures such as vaccination and enhanced LBM biosecurity. In December 2017, a novel influenza subtype A(H7N4) virus emerged and was reported in Changzhou, Jiangsu Province, China [281] demonstrating that these highly connected areas in Jiangsu and Anhui require in-depth investigations, continuous monitoring and surveillance.

As of 5 Sep 2018, there was a total of 1,567 confirmed H7N9 human infections (among whom there were 615 fatalities). Additionally, around 2,500 virological samples from the environment (LBMs, vendors and some commercial or breeding farms), poultry (chickens, pigeons, ducks, turkeys) and wild birds tested positive for H7N9 (since April 2013) [5, 282]. This indicates that vulnerabilities to H7N9 infections are still present in live chicken market chains. An understanding and careful analysis of the views of different actors in the live chicken market chain is necessary to help the design of health promotion interventions and to reduce the risk of exposure. It should be understood that vulnerabilities may be associated with the level of knowledge, attitudes and practices (KAP) of different actors in the live chicken market chain.

Occupational exposure to infected live poultry and contaminated environments is known to be an important risk factor for AI infections in humans [7, 8]. We have recently demonstrated that LBM biosecurity procedures such as cleaning and disinfection, regular market closure, bans on overnight storage and separation of different species are important protective measures for prevention and control of AI [283]. Many interventions are simple and inexpensive, however a lack of knowledge often leads to poor application and unsafe behaviour [144]. The existing literature indicates that health behaviours and hygiene practices can be influenced by age, gender, education, knowledge and religious beliefs [182]. In recent years, there have been a number of studies reporting poultry related workers' KAP for AI in Asia, the US, Europe and Africa [184, 272-279]. To our knowledge, none of these studies was conducted on chicken-specific market chains, none of the studies compared across chicken farmers, chicken vendors and consumers in the same chain, and none of them was targeted on the high-risk areas affected by the emergence of the H7N9 [10]. Therefore, there is a need to understand the knowledge, attitudes and practices (KAP) of all actors across the live chicken market chain involved in the H7N9 emergency in China.

In this study, we aimed to quantify and compare the levels of KAP regarding AI for different actors along the live chicken market chain in areas initially affected by the emergence of H7N9. We aimed to identify risk factors associated with their KAP levels, so as to generate evidence for the design of stakeholder-based AI health promotion interventions in the live chicken market chain in China.

6.4 Materials and Methods

A cross-sectional questionnaire survey was conducted from June to July 2014 targeting meat chicken farmers, live chicken vendors and chicken consumers in LBMs in six counties located in Jiangsu (Lishui, Jintan, Jiangyan) and Anhui (Feixi, Quanjiao and Chaohu) provinces in the east of China. Selection of counties was based on findings from a previous study conducted during the H7N9 emergency response, which demonstrated that chicken movements were highest in those six counties from Jiangsu and Anhui provinces [10]. One county in Shanghai municipality (Fengxian) was also included in this survey because Shanghai was the origin of the first H7N9 human cases reported in 2013 and it is adjacent to Jiangsu province where there was a geographic co-distribution of H7N9 and H5N1 in humans [29]. An initial sample size of 10 commercial meat chicken farms, one wholesale LBM and two to three retail LBMs (subject to actual numbers of LBMs) in the seven high risk counties were included. Within each LBM, 10 chicken vendors and 10 consumers were interviewed.

6.4.1 Survey instruments and participants

Face-to-face interviews were used to elicit knowledge, attitudes and practices of chicken farmers, live chicken vendors and chicken consumers at LBMs in the target counties associated with avian influenza. Structured questionnaires included questions on characteristics of participants (e.g. gender, age, education level, employment status, number of years of work, daily number of hours in contact with chickens), knowledge about AI (e.g. “Is AI an infectious disease?” “what types of animals can be infected with AI” etc.), attitudes towards AI (e.g. “Do you think AI is a severe disease for humans?”, “What do you think is the likelihood for you to get AI?” etc.), and practices regarding AI prevention (e.g. What do you do when you suspect that you have flu symptoms?) (See Table C-1 for the full set of questions).

6.4.2 Statistical analysis

Questions on KAP were scored individually. Scores of all KAP questions were then summed to represent the level of each participant's overall knowledge, overall attitudes and overall practices towards AI (See Table C-1 for details of the scoring method used). For categorical variables, chi-square tests were used to compare frequencies for different groups. To identify predictors of KAP scores among different actors along the live chicken market chain (i.e. chicken farmers, vendors and consumers) we built four multivariable generalized least squares (GLS) random-effects regression models with the KAP scores (overall or individual scores) as the outcome of interest. Due to our study design, we have multiple respondents in the same county, and thereby we need to adjust for the clustering of respondents at this spatial unit of analysis. Therefore, we added a county-specific random effect in our regression model.

Explanatory variables of the KAP scores were carefully selected for different respondents. First, gender and age are important confounders and were included in all the four models. Then, indicated by other studies, education level, employment status and year of work are key factors that may affect the level of KAP of respondents [187, 273, 279, 284-286]. In the case of chicken farmers, we included gender, age, educational level, years of work with chicken and hours of daily chicken contact. In the case of chicken vendors, we included gender, age, educational level, type of stalls, type of vendor, years working with chickens and hours of daily chicken contact. In the case of market consumers, we include gender, age, education level, employment status, the frequency of chicken purchase and type of chickens bought. Model 1 integrated all participants and Models 2-4 were specific to each group of actors (i.e. chicken farmers, chicken vendors and chicken consumers). A two-tailed test with a p-value <0.05 was considered statistically significant. The coefficient of determination (R square) and the distribution of the residuals were estimated to evaluate the performance of the models. Data were analysed using STATA (version 12.0; SPSS Inc., Chicago, IL, USA).

6.4.3 Ethics statement

The study received ethics approval from the Behavioural & Social Sciences Ethical Review Committee of the University of Queensland (Approval number: 2014001167).

6.5 Results

A total of 296 chicken workers (i.e. farmers and vendors) and consumers participated in the study; a total of 274 (92.6%) respondents completed more than 90% of the questionnaire, including 95 chicken farmers (mean age 49, male 77.9%), 104 chicken vendors (mean age 46, male 58.7%) and 75 market consumers (mean age 44, male 42.7%) (Table 6-1) (See Table C-2 for details of the different types of respondents). Most chicken workers (farmers and vendors) have up to secondary (53.5%) or primary (23.6%) levels of education respectively. Chicken vendors had worked for a longer duration in the live chicken industry compared to farmers (13.2 vs 8 years). Daily contact with chickens was longer in chicken farmers than for vendors (11.2 vs. 9.5 hours). The maximum possible score of overall KAP was 42, and the average KAP scores of farmers, vendors and consumers were 16, 13.7 and 15.2 respectively (Table 6-1). The average knowledge, attitudes and practices scores of chicken farmers was generally higher than that of chicken vendors. Our results also show a statistically significant difference between chicken farmers and vendors in terms of the years of working with chickens and hours of contacting chickens per day ($p<0.01$) (Table 6-1).

6.5.1 Factors associated with KAP across different actors (Model 1)

Overall KAP scores of all the three survey groups towards AI were significantly associated with age group and type of respondents (Table 6-2). Females had marginally significantly lower overall KAP scores than males [Coef. =-1.13, 95%CI (-2.28, 0.02), p=0.054]; this is due to their significantly lower knowledge scores compared to males [Coef. =-1.09, 95% CI (-1.82, -0.35), p=0.004]. The oldest age group (>55-years-old) had significantly lower overall KAP scores than the youngest group (≤35-year-old) [Coef. = -2.51, 95% CI (-4.61, -0.4), p=0.02]; this result is due to their attitude scores. Chicken vendors had significantly lower overall KAP scores than chicken farmers [Coef. =-2.26, 95%CI (-3.5, -1.01), p<0.001]; this is due to their knowledge and attitudes scores. We also found that consumers had significantly higher practice scores than chicken farmers [Coef. =-0.66, 95%CI (-1.05, -0.27), p=0.001]. Respondents with secondary school education had lower attitude scores towards AI compared to those with primary school and below education [Coef. = -0.96, 95%CI (-1.67, -0.24), p=0.009] (Table 6-2).

6.5.2 Factors associated with KAP towards AI within chicken farmers (Model 2)

Overall KAP score of chicken farmers towards AI was significantly associated with their years of working with chickens and daily hours of chicken contact (Table 6-3). Chicken farmers who had worked for more than 15 years had significantly lower total KAP scores than those who had worked for less than six years [Coef. =-2.49, 95%CI (-4.93, -0.06), p=0.045]; this result is due to their attitude scores. Farmers with 11 to 15 years working experience have higher knowledge scores than farmers who worked less than 6 years [Coef. =2.32, 95%CI (0~4.65), p=0.050]. Chicken farmers who had worked for more than 15 hours per day had significantly lower total KAP scores than those who had worked for less than 6 hours per day [Coef. =-3.03, 95%CI (-5.48, -0.59), p=0.015]; this result is due to their knowledge scores. Chicken farmers with high school education had lower attitudes scores than those who had primary school or below education [Coef. =-1.15, 95%CI (-2.29, -0.01), p=0.047].

6.5.3 Factors associated with KAP towards AI within chicken vendors (Model 3)

Overall KAP scores of chicken vendors were significantly associated with gender and age (Table 6-4). Female vendors had significantly lower overall KAP scores than male vendors [Coef. =-2.58 (-4.95, -0.22), p=0.032]; this result is due to their knowledge scores. Female vendors had significantly higher practice scores than male vendors [Coef. =0.58,95%CI (0.08, 1.07), p=0.023]. The overall KAP scores towards AI of the 36 to 55 age group [Coef. -3.94, 95%CI (-7.74, -0.14), p=0.042] and over 55 age group [Coef. =-6.00, 95%CI (-11.21, -0.8), p=0.024] were significantly lower than the younger age group (≤35-years-old); this result is due to their knowledge scores. Chicken vendors who also conducted slaughter had higher practice scores than those who did not.

6.5.4 Factors associated with KAP towards AI within market consumers (Model 4)

We found that the overall KAP scores were significantly associated with the frequency of buying chickens (Table 6-5). Consumers who bought chicken at least once every month had better overall KAP scores compared with those who bought chicken at least once every week [Coef. =1.96, 95%CI (0.14, 3.77), p=0.035]; this result is due to their attitudes scores. Female chicken consumers had significantly better practice scores than male consumers [Coef. =0.58, 95%CI (0.12, 1.05), p=0.013].

The coefficient of determination of the four GLS models are summarized in Table C-5. The residuals of all models were all normally distributed except for Model 1. (Figure C-1)

6.5.5 Attitudes towards prevention and control policies on LBMs across chicken farmers, chicken vendors and consumers

Most of the chicken farmers (98%) and vendors (94%) agreed with adopting market rest day and cleaning and disinfection on markets. 55% of chicken farmers agreed on the ban on overnight storage on markets and slaughter all poultry unsold at the end of the day, however, 76% of the chicken vendors did not agree. Despite the fact that 53% of the chicken farmers and 85% of the chicken vendors did not agree with the ban on selling live chicken and central slaughtering of poultry, 62% of consumers were not willing to buy slaughtered chicken at markets. (Table 6-6)

Table 6-1 Socio-demographics of three types of respondents, chicken famers (N=96), chicken vendors (N=108) and consumers (N=75), from the surveyed counties in Jiangsu and Anhui provinces in China.

Demographics		**Chicken farmers (%)**	**Chicken vendor (%)**	**Consumer (%)**	**Pearson chi2**	**P-value**
Gender	Male	74(77.9)	61(58.7)	32(42.7)	22.2	<0.01
	Female	21(22.1)	43(41.3)	43(57.3)		
Age groups	Mean [min~max]	49[33~72]	46[22~70]	44[15~78]		
	≤ 35	7(7.4)	10(10.1)	21(28.8)	28.9	<0.01
	36~55	61(64.9)	80(80.8)	40(54.8)		
	>55	26(27.7)	9(9.1)	12(16.4)		
Education level	Primary School or below	26(27.7)	20(19.8)	8(10.8)	42.6	<0.01
	Secondary school	50(53.2)	55(54.5)	25(33.8)		
	High School	17(18.1)	23(22.8)	25(33.8)		
	University and above	1(1.1)	3(3.0)	16(21.6)		
Years working with chicken	Mean [min~max]	8[0.5~35]	13.2[0.5~33]	-		
	≤ 5 years	54(56.8)	14(14.0)	-	41.7	<0.01
	6~10 years	20(21.1)	31(31.0)	-		
	11~15 years	5(5.3)	19(19.0)	-		
	>15 years	16(16.8)	36(36.0)	-		
Hours of contacting chicken/day	Mean [min~max]	11.2[1~24]	9.5[1~24]	-		
	≤ 5 hours	19(20.4)	11(10.6)	-	15.5	<0.01
	6~15 hours	50(53.8)	83(79.8)	-		
	>15 hours	24(25.8)	10(9.6)	-		
Average KAP Scores (max=42)		16	13.7	15.2		
Average Knowledge Scores (max=18)		8.3	7.6	8.3		
Average Attitudes Scores (max=16)		4.5	3.2	4.3		
Average Practices Scores (max=8)		3.2	2.9	2.6		

Table 6-2 Multivariable GLS model 1 for chicken farmer, vendors and consumers – knowledge, attitudes and practices of avian influenza

Variables	**Total KAP**		**Knowledge**		**Attitudes**		**Practices**	
	Coef. (95% CI)	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**
Gender								
Male	-	-	-	-	-	-	-	-
Female	-1.13 (-2.28, 0.02)	0.054	**-1.09 (-1.82, -0.35)**	**0.004**	-0.24 (-0.82, 0.34)	0.424	0.19 (-0.11, 0.5)	0.213
Age group								
≤ 35								
36~55	-0.57 (-2.21, 1.07)	0.497	0.02 (-1.03, 1.07)	0.966	-0.74 (-1.58, 0.09)	0.079	0.15 (-0.28, 0.59)	0.492
>55	**-2.51 (-4.61, -0.4)**	**0.020**	-1.31 (-2.65, 0.04)	0.058	**-1.34 (-2.41, -0.27)**	**0.014**	0.14 (-0.43, 0.7)	0.632
Education level								
Primary School and below								
Secondary school	-0.79 (-2.19, 0.62)	0.273	0.16 (-0.74, 1.06)	0.730	**-0.96 (-1.67, -0.24)**	**0.009**	0.01 (-0.36, 0.39)	0.955
High School	0.12 (-1.56, 1.8)	0.890	0.71 (-0.37, 1.78)	0.198	-0.50 (-1.35, 0.35)	0.247	-0.09 (-0.53, 0.36)	0.707
University and above	0.63 (-1.97, 3.23)	0.635	0.78 (-0.89, 2.45)	0.360	-0.39 (-1.71, 0.93)	0.560	0.24 (-0.45, 0.94)	0.492
Type of respondents								
Farmer								
Vender	**-2.26 (-3.5, -1.01)**	**<0.001**	**-0.87 (-1.66, -0.07)**	**0.034**	**-1.15 (-1.78, -0.52)**	**<0.001**	-0.24 (-0.58, 0.09)	0.152
Consumer	-0.87 (-2.33, 0.59)	0.243	0.04 (-0.9, 0.98)	0.935	-0.25 (-0.99, 0.49)	0.511	**-0.66 (-1.05, -0.27)**	**0.001**
Intercept	17.69 (15.48, 19.89)	<0.001	8.73 (7.31, 10.14)	<0.001	5.95 (4.83, 7.06)	<0.001	3.01 (2.42, 3.6)	<0.001

Table 6-3 Multivariable GLS model 2 for chicken farmers – knowledge, attitudes and practices of avian influenza

Variables	**Total KAP**		**Knowledge**		**Attitudes**		**Practices**	
	Coef. (95% CI)	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**
Gender								
Male	-	-	-	-	-	-	-	-
Female	-0.79 (-2.87, 1.29)	0.458	-0.75 (-2.11, 0.62)	0.283	0.51 (-0.39, 1.4)	0.270	-0.55 (-1.28, 0.19)	0.143
Age group								
≤ 35								
36~55	2.22 (-0.94, 5.39)	0.169	0.76 (-1.31, 2.83)	0.473	0.99 (-0.38, 2.35)	0.156	0.48 (-0.64, 1.59)	0.403
>55	1.20 (-2.39, 4.8)	0.512	0.42 (-1.93, 2.77)	0.725	0.50 (-1.05, 2.05)	0.525	0.28 (-0.99, 1.55)	0.666
Education level								
Primary School and below								
Secondary school	0.10 (-1.89, 2.08)	0.923	0.64 (-0.66, 1.94)	0.337	-0.50 (-1.36, 0.35)	0.249	-0.03 (-0.73, 0.67)	0.924
High School	0.00 (-2.63, 2.64)	0.998	1.30 (-0.42, 3.03)	0.139	**-1.15 (-2.29, -0.01)**	**0.047**	-0.15 (-1.08, 0.78)	0.754
University and above	-2.24 (-10.33, 5.84)	0.586	0.24 (-5.05, 5.53)	0.929	-2.83 (-6.32, 0.65)	0.111	0.35 (-2.5, 3.2)	0.811
Years working with chicken								
≤ 5 years								
6~10 years	-0.33 (-2.43, 1.78)	0.762	0.56 (-0.82, 1.93)	0.425	-0.62 (-1.53, 0.29)	0.180	-0.27 (-1.01, 0.47)	0.482
11~15 years	2.10 (-1.45, 5.65)	0.246	**2.32 (0, 4.65)**	**0.050**	-0.73 (-2.26, 0.8)	0.353	0.50 (-0.75, 1.75)	0.432
>15 years	**-2.49 (-4.93, -0.06)**	**0.045**	-0.42 (-2.02, 1.17)	0.602	**-2.12 (-3.17, -1.07)**	**<0.001**	0.05 (-0.81, 0.91)	0.909
Hours of contacting chicken								
≤ 5 hours/day								
6~15 hours/day	-1.46 (-3.72, 0.79)	0.202	-1.05 (-2.52, 0.42)	0.162	-0.08 (-1.05, 0.89)	0.869	-0.33 (-1.13, 0.46)	0.412
>15 hours/day	**-3.03 (-5.48, -0.59)**	**0.015**	**-2.28 (-3.87, -0.68)**	**0.005**	-0.93 (-1.98, 0.13)	0.085	0.17 (-0.69, 1.03)	0.702
Intercept	16.52 (12.21, 20.82)	<0.001	8.42 (5.6, 11.24)	<0.001	4.97 (3.11, 6.82)	<0.001	3.13 (1.61, 4.65)	<0.001

Table 6-4 Multivariable GLS model 3 for chicken vendors – knowledge, attitudes and practices of avian influenza

Variables	**Total KAP**		**Knowledge**		**Attitudes**		**Practices**	
	Coef. (95% CI)	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**
Gender								
Male	-	-	-	-	-	-	-	-
Female	**-2.58 (-4.95, -0.22)**	**0.032**	**-2.32 (-3.76, -0.88)**	**0.002**	-0.84 (-2.02, 0.35)	0.167	**0.58 (0.08, 1.07)**	**0.023**
Age group								
≤ 35								
36~55	**-3.94 (-7.74, -0.14)**	**0.042**	**-2.51 (-4.82, -0.2)**	**0.033**	-1.41 (-3.31, 0.5)	0.147	-0.02 (-0.82, 0.78)	0.961
>55	**-6.00 (-11.21, -0.8)**	**0.024**	**-4.65 (-7.82, -1.48)**	**0.004**	-1.35 (-3.96, 1.26)	0.311	-0.01 (-1.1, 1.08)	0.989
Education level								
Primary School and below								
Secondary school	-1.42 (-4.37, 1.53)	0.345	-0.18 (-1.98, 1.61)	0.842	-0.99 (-2.47, 0.49)	0.191	-0.25 (-0.87, 0.37)	0.426
High School	-0.60 (-4.12, 2.91)	0.737	-0.12 (-2.26, 2.03)	0.915	-0.09 (-1.86, 1.67)	0.919	-0.40 (-1.13, 0.34)	0.294
University and above	-1.60 (-8.8, 5.59)	0.662	0.21 (-4.17, 4.6)	0.924	-0.82 (-4.43, 2.79)	0.655	-1.00 (-2.51, 0.52)	0.197
Type of stalls								
Wholesaler								
Retailer	2.37 (-0.32, 5.07)	0.084	1.49 (-0.15, 3.14)	0.074	1.30 (-0.05, 2.66)	0.058	-0.43 (-0.99, 0.14)	0.140
Mixed wholesaler and retailer	-0.91 (-4.65, 2.82)	0.631	-0.52 (-2.8, 1.75)	0.651	-0.33 (-2.21, 1.54)	0.726	-0.05 (-0.84, 0.73)	0.891
Type of vendor								
Vendor only								
Vendor trader	1.14 (-2.24, 4.51)	0.510	0.27 (-1.79, 2.33)	0.800	0.49 (-1.21, 2.18)	0.575	0.38 (-0.33, 1.09)	0.289
Vendor slaughter	-0.02 (-3.25, 3.21)	0.990	0.07 (-1.9, 2.04)	0.944	-0.93 (-2.55, 0.69)	0.261	**0.84 (0.16, 1.52)**	**0.015**
Vendor trader & slaughter	-0.26 (-5.99, 5.48)	0.930	-1.28 (-4.77, 2.22)	0.473	-0.64 (-3.51, 2.24)	0.665	**1.66 (0.45, 2.86)**	**0.007**
Years of working with chicken								
≤ 5 years								
6~10 years	3.27 (-0.64, 7.17)	0.101	1.52 (-0.86, 3.91)	0.209	1.87 (-0.09, 3.83)	0.062	-0.12 (-0.94, 0.7)	0.769
11~15 years	-0.10 (-4.19, 3.99)	0.961	0.11 (-2.38, 2.61)	0.930	0.27 (-1.79, 2.32)	0.800	-0.48 (-1.34, 0.38)	0.274
>15 years	3.49 (-0.42, 7.4)	0.080	1.74 (-0.65, 4.12)	0.154	1.68 (-0.28, 3.64)	0.093	0.08 (-0.74, 0.9)	0.853
Hours of contacting chicken								
≤ 5 hours/day								
6~15 hours/day	-0.79 (-4.16, 2.58)	0.646	-0.08 (-2.13, 1.98)	0.941	-0.57 (-2.26, 1.13)	0.512	-0.15 (-0.85, 0.56)	0.686

>15 hours/day	-1.77 (-6.77, 3.23)	0.489	-0.26 (-3.31, 2.78)	0.865	-2.07 (-4.58, 0.44)	0.105	0.57 (-0.48, 1.62)	0.287
Intercept	16.68 (9.73, 23.63)	<0.001	9.29 (5.06, 13.53)	<0.001	4.21 (0.72, 7.7)	0.018	3.18 (1.72, 4.64)	<0.001

Table 6-5 Multivariable GLS model 4 for chicken consumers – knowledge, attitudes and practices of avian influenza

Variables	**Total KAP**		**Knowledge**		**Attitudes**		**Practices**	
	Coef. (95% CI)	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**	**Coef. (95% CI)**	**P-value**
Gender								
Male	-	-	-	-	-	-	-	-
Female	1.01 (-0.76, 2.79)	0.263	0.40 (-0.88, 1.68)	0.538	0.03 (-1.09, 1.14)	0.963	**0.58 (0.12, 1.05)**	**0.013**
Age group								
≤ 35								
36~55	0.11 (-2.08, 2.29)	0.924	1.03 (-0.54, 2.61)	0.199	-0.96 (-2.34, 0.42)	0.174	0.03 (-0.54, 0.6)	0.921
>55	-2.76 (-8.06, 2.54)	0.308	0.57 (-3.26, 4.39)	0.770	-3.00 (-6.34, 0.34)	0.078	-0.33 (-1.71, 1.05)	0.642
Education level								
Primary School and below								
Secondary school	-0.63 (-4.18, 2.92)	0.728	0.10 (-2.46, 2.66)	0.937	-0.38 (-2.62, 1.86)	0.740	-0.35 (-1.28, 0.57)	0.453
High School	1.23 (-2.27, 4.74)	0.490	0.94 (-1.59, 3.47)	0.466	0.30 (-1.9, 2.51)	0.787	-0.01 (-0.92, 0.9)	0.983
University and above	3.40 (-0.51, 7.31)	0.088	1.85 (-0.97, 4.68)	0.198	1.14 (-1.32, 3.61)	0.363	0.40 (-0.62, 1.42)	0.439
Employment status								
Full-time work								
Part-time work	0.17 (-8.03, 8.37)	0.968	-4.05 (-9.96, 1.87)	0.180	4.70 (-0.47, 9.87)	0.075	-0.48 (-2.62, 1.65)	0.656
Unemployed	1.90 (-1.47, 5.26)	0.270	1.69 (-0.74, 4.12)	0.173	-0.60 (-2.72, 1.53)	0.582	0.80 (-0.07, 1.68)	0.072
Retired	2.05 (-3.18, 7.29)	0.442	-1.24 (-5.02, 2.54)	0.520	2.15 (-1.15, 5.45)	0.201	1.14 (-0.22, 2.5)	0.101
How often do you buy chicken								
≥once per week								
≥once per month and <once per week	**1.96 (0.14, 3.77)**	**0.035**	0.48 (-0.83, 1.79)	0.470	**1.37 (0.23, 2.52)**	**0.019**	0.10 (-0.37, 0.57)	0.671
<once per month	0.93 (-1.76, 3.63)	0.496	-0.31 (-2.25, 1.63)	0.752	1.18 (-0.52, 2.87)	0.174	0.07 (-0.63, 0.77)	0.842
Type of Chicken								
Lived								
Slaughtered	0.57 (-2.32, 3.46)	0.698	-0.41 (-2.5, 1.67)	0.699	1.44 (-0.38, 3.26)	0.121	-0.46 (-1.21, 0.29)	0.231
Intercept	12.62 (8.33, 16.91)	<0.001	6.59 (3.5, 9.69)	<0.001	3.90 (1.2, 6.61)	0.005	2.12 (1.01, 3.24)	<0.001

Table 6-6 Attitudes towards policies from chicken farmers, vendors and consumers

Questions of the acceptance of possible control measures on LBMs	**Options**	**Farmers**			**Vendors**			**Consumers**		
		Number of responses	Positive responses	Positive responses rate	Number of responses	Positive responses	Positive responses rate	Number of responses	Positive responses	Positive responses rate
Regular market rest days and cleaning and disinfection on markets.	Agree	82	80	98%	110	103	94%	-	-	-
	Do not agree		2	2%		7	6%	-	-	-
No overnight poultry in the market, slaughter all poultry unsold at the end of day.	Agree	78	43	55%	109	26	24%	-	-	-
	Do not agree		35	45%		83	76%	-	-	-
Ban on selling live chickens and implement central slaughtering of poultry	Agree	83	39	47%	101	25	25%	-	-	-
	Do not agree		44	53%		86	85%	-	-	-
If selling live bird is forbidden in any market, only fresh slaughtered chicken is allowed, would you still buy?	Yes	-	-	-	-	-	-	85	32	38%
	No	-	-	-	-	-	-	-	53	62%

6.6 Discussion

This study presents a unique insight into the prevailing behavioural conditions of market chain actors of the identified H7N9 high-risk areas in the middle of 2014 after the second wave of H7N9 outbreaks in humans in Eastern China. To our knowledge, this is the first study conducted on KAP of three main stakeholder groups along the live chicken market chain in relation to AI in a high-risk area for both H5N1 and H7N9 infections in Eastern China.

Poultry workers at farms and LBMs have been identified as the weakest link in AI prevention and control [183]. Our results indicate that chicken farmers operating in the initial high-risk areas [10, 29] for H7N9 have significantly higher knowledge and attitude scores for AI compared to chicken vendors, and the overall KAP score of consumers is lower than that of chicken farmers but higher than that of chicken vendors. These findings are consistent with a KAP study of H7N9 in Zhejiang province and a qualitative study of H5N1 in Hong Kong indicating that knowledge of AIVs was reasonably high among chicken farmers, but lower among retailers [279, 284]. We also found that chicken farmers have significantly higher practice scores than consumers. An explanation for this is that farmers are more likely to comply with practices that help to protect their flocks and address their financial interests [287]. Our results suggest that interventions to improve KAP towards AI should be promoted among all the stakeholders along the live chicken market chain, with an emphasis on chicken vendors in LBMs.

While these findings provide a good general picture of the level of KAP across all actors in the live chicken market chain, an in-depth analysis into the determinants of KAP within each of the actors is necessary. We therefore analysed determinants of KAP within each actor group and our results for chicken farmers suggests that those who had 11 to 15 years farming experience have higher knowledge scores than those worked less than 6 years. This is consistent with findings from a KAP study of farmers in Italy, which suggested that greater knowledge was found in farmers who worked for a longer time [272]. We also found farmers who had longer farming experience (>15 years) had poorer attitudes towards AI risk compared to those with less experience (<6 years). This finding suggests that knowledge is not a mediator of behaviour in longer practicing farmers. Indeed this is consistent with previous reports indicating that having a better knowledge of AI may lead to a lower perceived risk of infection from poultry among poultry workers [184, 284]. Programmes to increase the awareness of AIVs for chicken famers should not overlook those who have worked for many years in the chicken industry. Our results also indicate that chicken farmers who had longer daily hours of exposure to chickens had significantly lower knowledge scores. It is possible that those farmers who are exposed to poultry for longer periods of time are economically disadvantaged and/or live in more remote areas; in turn, socioeconomic disadvantage and remoteness from urban centres may lead to a lower access to information. Overall, our finding suggests that enhancing their preventive practices are of great importance for chicken farmers.

Our recent meta-analysis [283] of market-level biosecurity risk factors of AI human infections revealed that female workers at LBMs are at significantly higher risk of AI infection compared to male workers. Our analysis for chicken vendors demonstrates that females have significantly lower knowledge scores compared to their counterparts which could explain their higher risk of AI infection reported in our metanalysis [283]. However, the results of the present study also indicate that female chicken vendors had significantly higher practice scores than their counterparts. This finding is consistent with a previous KAP study of H7N9 targeting an unstratified sample of the population that revealed females are more likely to comply with preventive practices than males [288]. A possible explanation for the discrepancy between this study and the results of our metanalysis can be reporting bias of practices highlighting the need for further observational studies on behaviour. In addition, our results reveal that chicken vendors who slaughter poultry at LBMs had significantly higher practice scores than their counterparts. Poultry slaughter operations at LBMs increases the risk of AI transmission within the LBM environment [283]. A possible explanation for our finding is that chicken vendors who conduct slaughter at LBMs are aware of the risk and are thus more likely to wear protective equipment compared to vendors who do not slaughter poultry. This hypothesis requires further empirical observation. Finally, our results suggest that older chicken vendors (>35-years) have lower knowledge of AI compared with younger vendors (≤35-years), which may be attributed to the level of education of older vendors compared to younger vendors. This finding suggests a higher risk of exposure of older vendors as documented by our recent metanalysis [283]. Taken together, our findings highlight the demand for educational interventions about knowledge of AI for female vendors and the less educated and older vendors. There is also a need for male vendors, and those who only sell poultry, but do not conduct slaughter, to wear personal protective equipment within LBMs.

For consumers purchasing chickens in the surveyed markets our results indicate that female consumers had significantly higher practice scores when compared to male consumers, which is in line with our findings for chicken vendors. This is because women are more likely to be responsible for food shopping and cooking for their family, which in turn can lead to better practices. Consumers who bought chicken at least once a month had a higher risk perception of AI than those who buy chicken at least once every week. Interestingly, a study in Taiwan found that consumers with relatively low levels of AI knowledge were likely to prefer not eating chicken at all under a possible threat of AI infection and those with low risk perception levels would be more likely to maintain usual chicken consumption than those with high risk perception levels if outbreaks of AI occurred [188]. This suggests that government administration and industry managers can design effective information communication for educational purposes to prevent a drop-in revenue due to the reduced demand and informing consumers about the safety of chicken products and proper cooking practices.

Despite the high response rate in our study, there are some limitations. Firstly, while this survey occurred after the second wave (2014) in the provinces firstly affected by H7N9 outbreaks in humans, it may not necessarily be generalizable to the conditions in subsequent years. Secondly, we cannot ignore the presence of reporting bias in our sample since responses to the questionnaire were self-reported. In addition, we used close-ended questionnaires which may give limited flexibility in participants' answers. It may miss some specific or relevant local knowledge or preventive practices which may not be included in the questionnaire. Thirdly, some confounding factors (e.g. personal income, place of residence) were not able to be captured in our KAP instrument, and this may have contributed to the differences in KAP towards AI observed among difference groups.

6.7 Conclusion

In conclusion, KAP scores in relation to avian influenza were generally low for all actors in the live chicken market chain at high-risk areas for H7N9 emergence. Risk based interventions should be developed and implemented by both animal health and public health agencies to prevent the spread of AI along the live chicken market chain. Interventions to increase knowledge of AI should be targeted to high-risk chicken vendors in LBMs, with an emphasis on female vendors and older vendors (>35-years-old). Measures for improvement of AI prevention practices should be targeted at male chicken vendors and those who are not engaged in chicken slaughter at LBMs. Programmes to increase the awareness of AI for chicken farmers should not be overlooked and should include those who have worked for many years in the chicken industry. Interventions to improve the knowledge and awareness of AI should be targeting those farmers who work more than 15 hours per day with poultry. There should also be a higher priority on delivering educational programmes about AI to male consumers and those who buy chickens more frequently.

Chapter 7 Geographical variation in the risk of H7N9 human infections in China: implications for risk-based surveillance

7.1 Context

Since 2013, a total of five epidemic waves have affected the country which resulted in the widespread dissemination of H7N9 virus across China. In February 2017, some strains of the 2013 LPAI H7N9 virus mutated to become highly pathogenic in poultry and rapidly spread to other provinces of China [13, 136]. The studies in Chapter 4 and 5 identified the role of LBMs and live poultry movement in the exposure and dissemination of H7N9 viruses during the H7N9 emergency. The study in Chapter 6 demonstrated a better understanding of the social determinants of exposure along the live chicken market chain. However, effective prevention and control of human H7N9 infections relies on identifying spatiotemporal indicators that can anticipate the spatiotemporal variation in infection incidence so to provide essential evidence and recommendations for risk-based AI surveillance programs and appropriate enhancements to current prevention and control policies for H7N9. While several ecological spatial studies aiming at identifying risk factors of H7N9 human cases had been undertaken in China [12, 26, 219, 289-291], none of these studies looked at the effect of more proximal factors such as poultry surveillance results and live chicken movement in affected areas at explaining the geographical variation of human H7N9 infections.

Therefore, in Chapter 7, we assembled the most comprehensive dataset of key risk factors for H7N9 infection in humans, e.g. distribution of LBMs, chicken movement data collected from the study in Chapter 6, and other risk factors based on existing ecological studies (e.g. human population density, chicken density), as well as detailed spatiotemporal data of poultry H7N9 surveillance results. We applied a test of cross-correlation to quantify the temporal relationship between the onset of human H7N9 infections during 2013-2017 and poultry serological and virological surveillance results. We also developed a spatial CAR model that accounted for spatial clustering of incidence to estimate and map the relative risk of H7N9 human incidence in counties in Southeast China by assessing the relationship between human infections as an outcome and poultry surveillance results, live chicken movements and recognized demographic risk factors as predictors.

The findings in Chapter 7 revealed the potential for poultry serological and virologic surveillance to anticipate human H7N9 infections and uncovered important geographical variation in the relative risk of human H7N9 incidence at county level in Southeast China. Specifically, the results indicated that the peak of poultry H7N9 serological positives is followed by human H7N9 infections with a two-month lag, and that poultry H7N9 virological positives is followed by the human H7N9 infections with a one-month lag. The results of the spatial CAR model indicate that human H7N9 incidence at county-level is positively and significantly associated with the presence of wholesale LBMs, higher density of retail LBMs, presence of poultry virological positives, higher poultry movement network connectivity, as well as lower chicken population density and higher human population density.

The map of relative risks of human H7N9 incidence generated in this Chapter indicated that high risk areas of human H7N9 infections were spatially clustered in Southeast China, extending from the Yangtze River delta near Shanghai to the Pearl River delta near Guangzhou and covering most areas of Jiangsu, Zhejiang, Shanghai, Anhui, Fujian, Guangdong and Hunan provinces. Additional “hot spots” for human H7N9 infections were found in the northern region of Guangxi, eastern region of Hubei province, northern and southern region of Jiangxi, northern region of Beijing, and the northern region of Hebei province.

The results of the program of research presented in Chapter 7 is novel in that the geographical model of human H7N9 incorporated key risk factors including poultry virological surveillance results, live chicken movement coming out from the originally H7N9 affected area in Southeast China provinces, and spatial autocorrelation. By doing this, this model provided an approach to identify areas where the likelihood of H7N9 human infections is at its highest, and where spatially targeted control interventions are most needed.

This Chapter is presented as a paper, which has been submitted for publication in Scientific Reports, and supporting technical information is presented in Appendix D in this Thesis.

7.2 Abstract

The influenza A (H7N9) subtype remains a public health problem in China affecting individuals in contact with live poultry, particularly at live bird markets. Despite enhanced surveillance and biosecurity at LBMs H7N9 viruses are now more widespread in China. This study aims to quantify the temporal relationship between poultry surveillance results and the onset of human H7N9 infections from 2013 to 2017 and to estimate risk factors associated with geographical risk of H7N9 human infections in counties in Southeast China. Our results suggest that poultry surveillance data can potentially be used as early warning indicators for human H7N9 notifications. Furthermore, we found that human H7N9 incidence at county-level was significantly associated with the presence of wholesale LBMs, the density of retail LBMs, the presence of poultry virological positives, poultry movements from high-risk areas, as well as chicken population density and human population density. The results of this study can influence the current AI H7N9 control program by supporting the integration of poultry surveillance data with human H7N9 notifications as an early warning of the

timing and areas at risk for human infection. The findings also highlight areas in China where monitoring of poultry movement and poultry infections could be prioritized.

7.3 Introduction

Since the emergence in early 2013 of a low pathogenic avian influenza (LPAI) H7N9 virus [263], there have been six epidemic waves causing about 1,600 human infections in 29 provinces and municipalities in mainland China [5, 292]. During the fifth epidemic wave starting in October 2016, the geographic range of H7N9 human cases expanded and more human cases were reported than any previous wave [12]. In February 2017, strains of the 2013 LPAI H7N9 virus isolated from chickens in Guangdong province mutated to become highly pathogenic avian influenza (HPAI) H7N9 in poultry and rapidly spread to other provinces of China [13, 136]. The rapid evolution, increased pathogenicity and efficient transmissibility of HPAI H7N9 viruses in mammalian models, together with their extended host range, may have increased the threat to public health and the poultry industry [137, 138].

Live bird markets (LBMs) remain the main source of H7N9 virus spreading among poultry, and from poultry to humans [10]. Recognizing the role of LBMs in the exposure and dissemination of H7N9 viruses, in Feb 2017, the MARA of China established the “1110 policy”, which includes mandatory daily market cleaning activities, disinfection, market closure once a month, and no overnight market poultry storage. This policy was followed in July 2017, by the implementation of the National Vaccination Program in the poultry sector through the adoption of a bivalent H5/H7 inactivated vaccine. While this vaccine has largely been effective at controlling H7N9 virus circulation among both chicken and humans [136, 138, 208], the virus is still being occasionally detected by the national animal disease surveillance system [204]. Therefore, a better understanding of the determinants of exposure is necessary to complement sanitary measures such as vaccination and enhanced LBM biosecurity.

The available literature indicates that the primary risk factor for human H7N9 infection in China is exposure to LBMs, and that intervention at this stage of the live poultry market chain is the most effective prevention measure [7, 8, 14-18]. Poultry-to-human transmission is intensified at LBMs, hence as a short term response, LBM closure should be rapidly implemented to substantially reduce the contact of infected poultry with the general population, in areas where the virus is identified in either poultry or humans [293, 294]. However, this may not be favorable to poultry enterprises or individual households due to the associated financial costs. Reactive closure of LBMs may facilitate further dissemination through the opening of unregistered LBMs or illegal poultry movements [295].

Surveillance and monitoring of avian influenza within the poultry market chain (i.e. farms, live bird markets and slaughter houses) generates epidemiological evidence on affected species, geographical sources of infection and the role of modifiable risk factors on disease transmission [23]. Animal health authorities in China have been prompt at identifying the presence of the H7N9 virus within the live poultry market chain and controlling infection transmission at the source since the emergency. The control of H7N9 in chickens through vaccination explains the sudden decrease in the number of human H7N9 infections since October 2017 [138, 208]. Little is known about the relative timing of infections in people and poultry but should peaks in transmission in poultry precede human cases, poultry surveillance results could provide an early warning for the likely timing and location of human H7N9 infections and this requires further evaluation. Furthermore, the role of poultry movements from the originally affected area in Eastern China in disseminating H7N9 infection throughout the country is yet to be quantified.

Several ecological spatial studies aiming at identifying risk factors of H7N9 human cases have been undertaken in China [12, 26, 219, 289-291], and distribution of H7N9 risks were mapped in these studies. Of these, two studies by Fuller et al. and Gilbert et al. attempted to map the suitability for H7N9 human infections in Asian regions. LBM density was demonstrated to be significantly associated with the presence of human H7N9 infections [12, 289, 291]. Human population density and density of both intensively and extensively raised chickens were also found to be predictors of H7N9 presence [291]. A previous study also found there was a major shift of risk factors from anthropological (i.e. LBM and human population density) towards poultry related variables (i.e. poultry density and chicken-to-duck ratio) linked to human H7N9 cases over time [12]. Other studies also evaluated the role of pig density, distance to freeway, distance to national highway, landcover, temperature and relative humidity, etc. [26, 219]. However, none of these studies looked at the effect of more proximal factors such as poultry surveillance results and live chicken movement in explaining the geographical variation of human H7N9 infections.

This study aims to quantify the temporal relationship between the onset of human H7N9 infections during 2013-2017 and poultry serological and virological surveillance results, and to estimate the relative risk of H7N9 human incidence in counties in Southeast China by assessing the relationship between human infections as the outcome and poultry surveillance results, live chicken movements and recognized demographic risk factors as explanatory variables.

7.4 Data sources and methods

7.4.1 Ethics statement

The research proposal leading to the study received ethical approval from the China Animal Health and Epidemiology Centre (CAHEC) of MARA of China. The research proposal leading to the primary data collection of chicken movements received ethics approval from the Behavioral & Social Sciences Ethical Review Committee of the University of Queensland (Approval number: 2014001167). There were no samples from humans or animals taken as part of our study, we used secondary information on human infections and market positivity to H7N9 infection derived from open access websites.

7.4.2 H7N9 human infection data and poultry surveillance data

We obtained all laboratory-confirmed H7N9 human cases reported during 2013-2017, from "Situation Updates - Avian Influenza" of the World Health Organization (WHO) [216] and "Avian Influenza Report" from the Centre for Health Protection of the Department of Health of the Hong Kong Special Administrative Region (SAR) [217]. Case definitions and laboratory testing have been described previously [218, 219]. For each human H7N9 case, information on county of residence and date of onset of symptoms was extracted. Poultry serological and virological surveillance results were obtained from the monthly official Veterinary Bulletin released by the Veterinary Bureau of the MARA of China [204], from which we extracted data on positive identification of H7N9 from the national H7N9 surveillance program between 2013 and 2017. The national H7N9 surveillance program took samples from LBMs, poultry slaughter houses, poultry farms and wild bird habitat, as well as pig farms and slaughter houses. All samples were tested in provincial Centers for Animal Disease Control and Prevention (CADCs) and confirmation of H7N9 virus was based on polymerase chain reaction (PCR). All AI positive samples were sent to the Harbin National Veterinary Research Institute for confirmation, subtyping and virus isolation. All reported H7N9 human cases and poultry virological surveillance positives were then geo-referenced and linked to a county level map of China.

7.4.3 Data on live chicken movement

A cross-sectional survey was conducted from June to July in 2014 targeting the live meat chicken trade in six counties located in Jiangsu (Lishui, Jintan, Jiangyan) and Anhui (Feixi, Quanjiao and Chaohu) provinces. These counties were selected based on the findings from a previous study [10] conducted during the H7N9 emergency response, which demonstrated that connectivity of chicken sources was highest in these six counties. In addition, one county in Shanghai municipality (Fengxian) was added, which was the location of the first H7N9 human case reported in 2013. Shanghai municipality is also adjacent to Jiangsu province where human H7N9 and H5N1 infections were demonstrated to be co-distributed [29]. An initial sample size of one or two wholesale LBMs (subject to actual numbers of LBMs in a county) in the seven high-risk counties were included; typically, there are a total of 1 to 2 wholesale LBMs in each county. Information on live poultry movements was obtained from poultry movement certificates available at wholesale LBMs and live poultry trading platforms (similar to a wholesale LBM) in the selected counties. All available chicken movement certificates in the trading locations surveyed were recorded from January 2014 to July 2014. This dataset records half-year chicken movements and is representative of the extent of live chicken movement within and beyond that region.

7.4.4 Sociodemographic factors

A set of sociodemographic risk factors was considered in the analysis including the presence of wholesale LBMs in each county (a binary variable representing presence/absence), and the density of retail LBMs (markets/100km2), human population density (people/km2) and chicken density (birds/km2). All factors were compiled at the county level. All data sources are summarized in Table 7-1; the source of LBMs is described in the Appendix D.1.

Table 7-1 Risk factor variables used in the analysis.

Variables at county level	***Sources***
Presence of wholesale LBMs	China Animal Health and Epidemiology Centre (see Appendix D.1)
Number of retail LBMs	China Animal Health and Epidemiology Centre (see Appendix D.1)
Poultry virological positives	MARA monthly veterinary bulletin
Network centrality	Primary investigation in Jun-Jul 2014
Human population density	2010 Census
Chicken density	Robinson et al. 2007

7.4.5 Social network analysis

To describe the connectivity pattern within the chicken movement dataset consisting of records of paired trading events between a particular LBM and the county they trade with (termed as “trade county”), we used social network analysis (SNA), as described previously [31]. We summarized network connectivity using degree centrality of the 2-mode binary network (LBM nodes vs trade county nodes). The degree represents the absolute number of unique links of a given node and it is important for describing the levels of connectivity between different actors within a network, thereby allowing identification of the most influential spreaders within a network [267].

7.4.6 Cross correlation analysis

To assess the temporal relationship between the onset of human H7N9 infections and poultry serological and virological surveillance results, we used a time-series cross-correlation analysis to calculate the temporal lags in months between the outcome (human infections) and the surveillance indicator (prevalence of poultry surveillance positives). The dataset was structured by month because the reports of surveillance results were aggregated by month [204]. In order to mitigate potential missing detection and report in early 2013 and potential reporting bias in late 2017 after the adoption of H7 vaccination since July 2017, we only used H7N9 infection data from July 2013 to June 2017. From each time-lagged correlation, only the lag with the highest correlation value was selected for the analysis. Usually, a correlation is significant when the absolute value is greater than 2/sqrt(n-|k|), where n is the number of observations and k is the lag.

7.4.7 Analysis of spatial variation in human H7N9 infections at county level

To assess whether there was spatial autocorrelation in the observed pattern of human H7N9 infections in the study area we used the Global Moran's Index (Moran’s I), a measure of spatial autocorrelation for spatially aggregated data. We used the incidence rate of human H7N9 infections per 1,000 (i.e. estimated by dividing the observed number of human cases by the total human population in the county and multiplied by 1,000) for estimation of Moran’s I. Moran's I is positive when nearby areas tend to be similar, negative when they tend to be dissimilar, and approximately zero when attribute values are arranged randomly in space [220]. The Moran's I value and a Z-score (evaluating the significance of the index) were estimated using ArcGIS 10.1.

7.4.8 Bayesian spatial conditional autoregressive model (CAR)

A Bayesian framework was used to construct a Poisson regression model of the observed incidence of human H7N9 infections in each county using the OpenBUGS software 3.2.3 rev 1012 [221]. The model included all of the explanatory variables described above and a spatially structured random effect. The mathematical notation for the model is provided in the Appendix D.2. It assumed that the observed counts of H7N9 human infections in the county (from 1 to 1181) from 2013 to 2017 followed a Poisson distribution.

The spatially structured random effect was modelled using a conditional autoregressive (CAR) prior structure [222]. This approach uses an adjacency weights matrix to determine spatial relationships between counties. If two counties share a border, it was assumed the weight = 1 and if they do not the weight = 0. The adjacency matrix was constructed using the “Adjacency for WinBUGS tools” in ArcGIS software [221]. A flat prior distribution was specified for the intercept, whereas a normal non-informative prior distribution was used for the coefficients (with a mean = 0 and a precision = 0.001). The priors for the precision of spatially structured random effects were specified using non-informative gamma distributions (0.5, 0.0005). The OpenBugs code is in Appendix D.3.

The first 1,000 iterations were run as a burn-in period and discarded. Subsequent sets of 20,000 iterations were run and examined for convergence. Convergence was determined by visual inspection of posterior density and history plots and by examining autocorrelation plots of model parameters. Convergence occurred at approximately 100,000 iterations for each model. Another 20,000 values from the posterior distributions of the model parameters were stored and summarized for the analysis. Statistical significance was indicated by 95% credible intervals (95% CrI), a variable was considered significant if CrI excluded 0.

Choropleth maps were created using the ArcGIS software to visualize the geographical distribution of crude incidence for the 1181 counties in the study area. The posterior means of the CAR random effects obtained from the models were also mapped.

7.5 Results

The distribution of human H7N9 notifications and poultry surveillance positives from 2013 to 2017 is shown in Figure 7-1. A total of 1,516 human H7N9 infections and 332 poultry virological positives were geocoded at least to county level. The majority of the H7N9 virological positive samples (88.3%, 293 out of 332) were collected from LBMs. A total of 1,181 counties from 14 provinces and municipalities in southeast China were included in this study; about 93.4% of reported human H7N9 infections and 89.5% of reported H7N9 virological positive samples were in these counties.

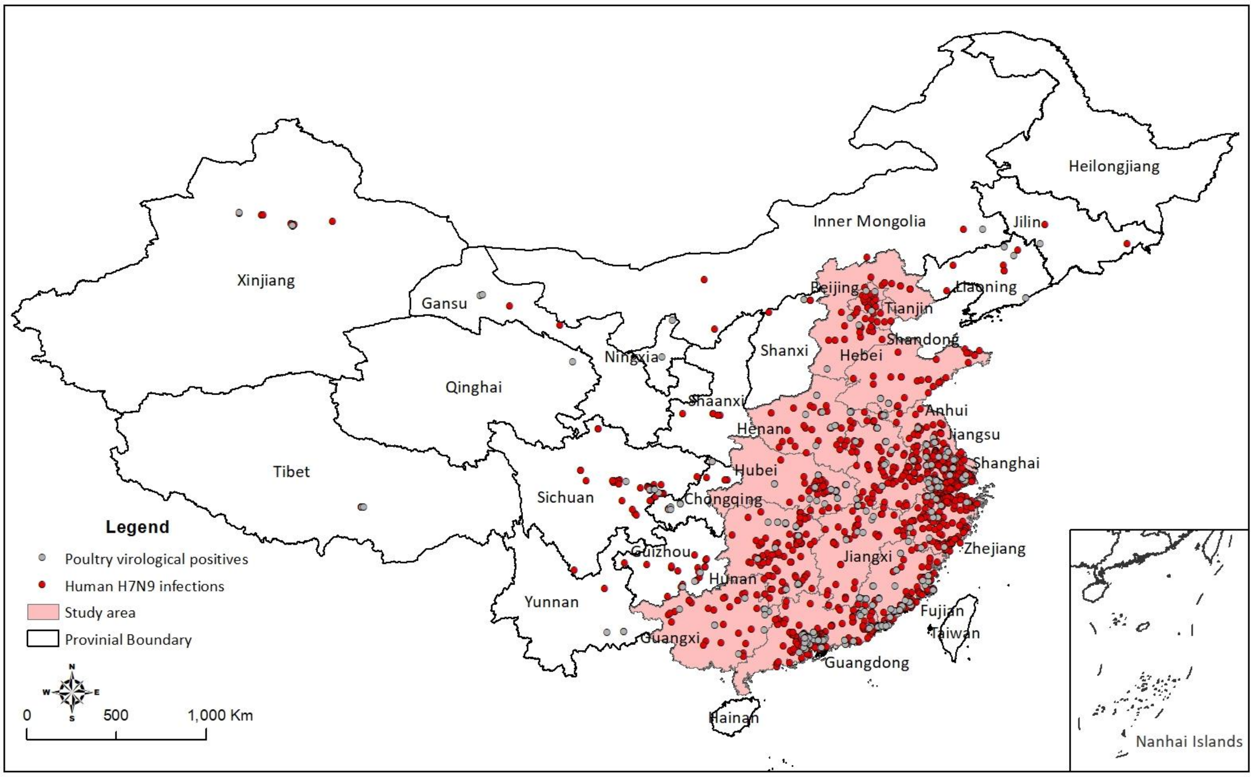


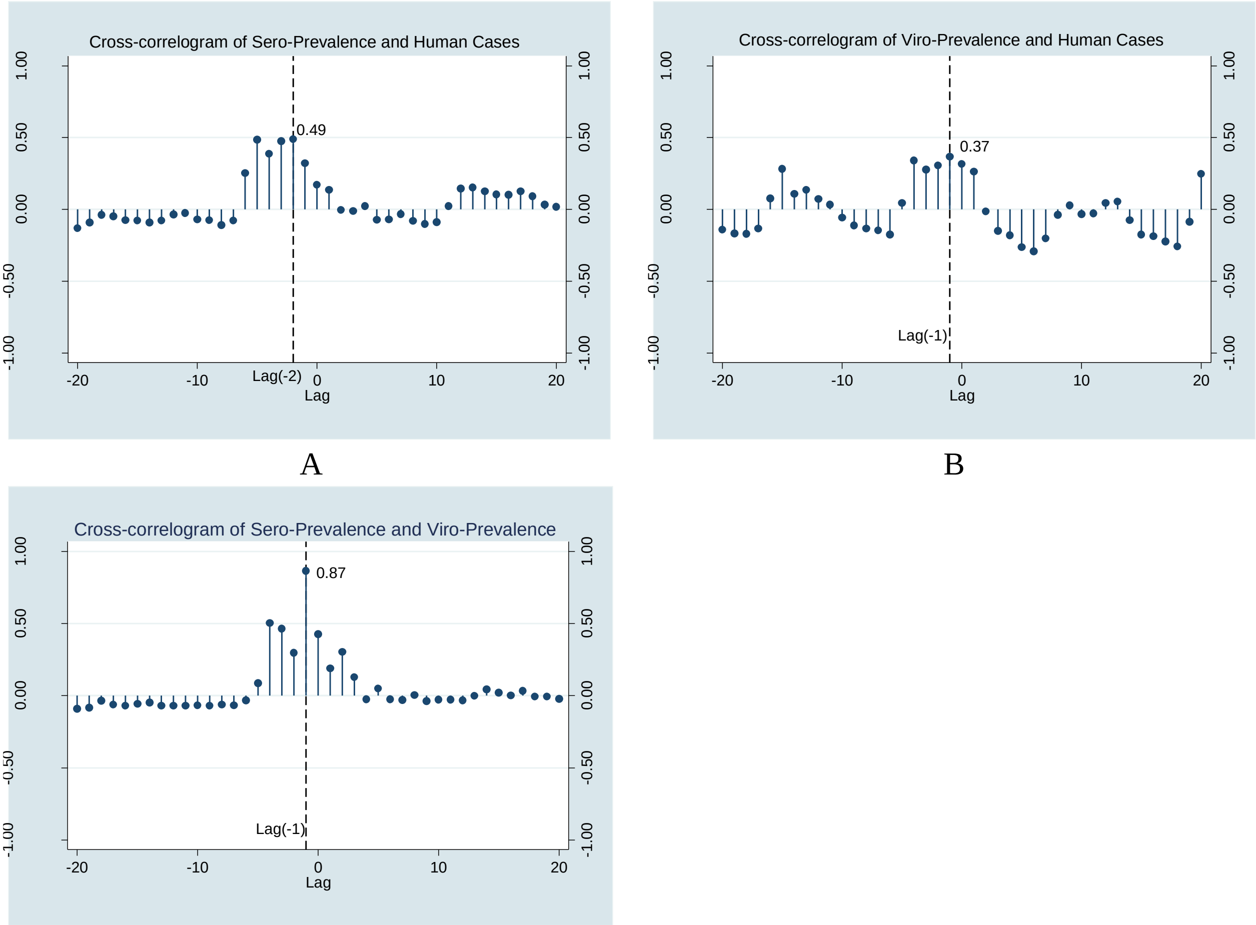
Figure 7-1 Spatial distribution of human H7N9 infections (red dots) and poultry virological surveillance positives (grey dots) from 2013 to 2017. Dots represent either geographic locations of the H7N9 human infections or county centroids when the detailed location is not available.

7.5.1 Social network analysis of chicken movements

In total, we analysed live chicken movement data from four wholesale LBMs and four live poultry trading platforms from Jiangsu, Anhui and Shanghai from January to July 2014 (Table D-1). Chicken movements from live poultry trading platforms tend to involve long-distance and inter-provincial transportation of chickens, while chicken movements from wholesale LBMs are mostly confined to local areas or neighbouring provinces (Figure D-1). The full extent of the 2-mode network (LBMs and chicken source/destination counties) is presented in Figure D-2. The results of this analysis revealed that there was a giant weakly connected component comprising eight wholesale LBMs and 249 chicken source/destination counties. These 249 counties were located mainly in Jiangsu, Anhui and Shanghai, extending to neighbouring provinces Henan, Hubei and Shandong, and further to the south, including Guangdong. The degree centralities of all the county nodes ranged from one to six and the geographic distribution of degree centrality is demonstrated in Figure D-3. The counties with the highest degree centrality were Jintan, Changzhou, Yangzhou in Jiangsu province (degree = 6); and Jiangyan, Lishui, Taixin, Shuyang, Zhenjiang and Nanjing from Jiangsu province, and Huzhou from Zhejiang province and Wuhu from Anhui province (degree = 5).

7.5.2 Temporal associations between human H7N9 notifications and poultry H7N9 surveillance data

Figure 2-16 presents the epidemiological curve of H7N9 human cases, poultry surveillance results by month of onset. We analysed data from July 2013 to June 2017. Results from the time-series analysis indicate that there is a significant temporal relationship between human H7N9 notifications and poultry surveillance results. Our results indicate that the peak of poultry H7N9 serological positives is followed by human H7N9 infections with a two-month lag, poultry H7N9 virological positives are followed by human H7N9 infections with a one-month lag (Figure 7-2). In addition, poultry serological H7N9 positives are followed by poultry H7N9 virological positives with a one-month lag (Figure 7-2).



A

B

C

Figure 7-2 A: Positive lags refer to human H7N9 infections leading by H7N9 sero-prevalence. B: Positive lags refer to human H7N9 infections leading by H7N9 viro-prevalence. C: Positive lags refer to H7N9 viro-prevalence leading by H7N9 sero-prevalence. The correlation of sero-prevalence at Lag -2 is approximately 0.49, and the correlation of viro-prevalence at Lag -1 is approximately 0.37. The correlations are significant because the values are greater than [2/ (Sqrt (n-|lag|))), n=48].

7.5.3 Spatial autocorrelation (Moran's I)

Incidence of human H7N9 infections was significantly spatially clustered, as indicated by a positive Moran's I value (0.152) that was statistically significant at the 0.05 level (Table D-2).

7.5.4 Bayesian spatial conditional autoregressive model of human H7N9 infections

The presence of wholesale LBMs (Coef. = 0.33, 95% CrI: 0.09~0.56) in the county and the density of retail markets (Coef. = 0.88, 95% CrI: 0.52~1.23) were positively and significantly associated with the human H7N9 incidence (Table 7-2). Human H7N9 incidence was positively associated with the presence of poultry virological positives (Coef. = 0.59, 95% CrI: 0.32~0.85) and the connectivity of counties with respect to poultry movements (Coef. = 0.83, 95% CrI: 0.47~1.19; Coef. = 0.89, 95% CrI: 0.28~1.53). While human H7N9 incidence was positively associated with increasing chicken population density (Coef. = 0.34, 95% CrI: 0.02~0.66; Coef. = 0.95, 95% CrI: 0.48~1.4), human H7N9 incidence was inversely proportional to human population density (Coef. = -0.69, 95% CrI: -0.99~-0.38; Coef. = -1.13, 95% CrI: -1.51~-0.72).

A map of adjusted relative risks (RRs) of human H7N9 incidence by county (Figure 7-3) shows that high risk areas of human H7N9 infection were spatially clustered in southeastern China, extending from the Yangtze River delta near Shanghai to the Pearl River delta near Guangzhou and covering most areas of Jiangsu, Zhejiang, Shanghai, Anhui, Fujian, Guangdong and Hunan provinces. Additional hot spots for human H7N9 infections were found in the northern region of Guangxi, eastern region of Hubei province, northern and southern region of Jiangxi, northern region of Beijing, and the northern region of Hebei province (Figure 7-3). The map of the spatially structured random effects demonstrates evidence of clustering around the Yangtze River delta area (Figure D-4).

Table 7-2 Results of spatial conditional autoregressive model of human H7N9 human incidence during 2013-2017. (CrI Credible Interval, a variable was considered significant if CrI excluded 0)

Variables	**Category**	**Coefficient, posterior mean (95%CrI)**
Present of wholesale LBMs	no	Ref.
	yes	0.33 (0.09~0.56)
Retail LBMs density (markets/100km2)	Low density (< 1)	Ref.
	Medium density (1-3)	0.14 (-0.15~0.42)
	High density (>3)	0.88 (0.52~1.23)
Present of poultry virological positive	no	Ref.
	yes	0.59 (0.32~0.85)
Population density (people/km2)	0-200	Ref.
	201-600	-0.69 (-0.99~-0.38)
	>600	-1.13 (-1.51~-0.72)
Chicken density (birds/km2)	<500	Ref.
	500-3000	0.34 (0.02~0.66)
	>3000	0.95 (0.48~1.42)
Network estimate (degree centrality)	0	Ref.
	1~3	0.83 (0.47~1.19)
	4~6	0.89 (0.28~1.53)
Intercept		-1.49 (-1.82~-1.17)
Precision of spatial random effect		0.22 (0.17~0.27)

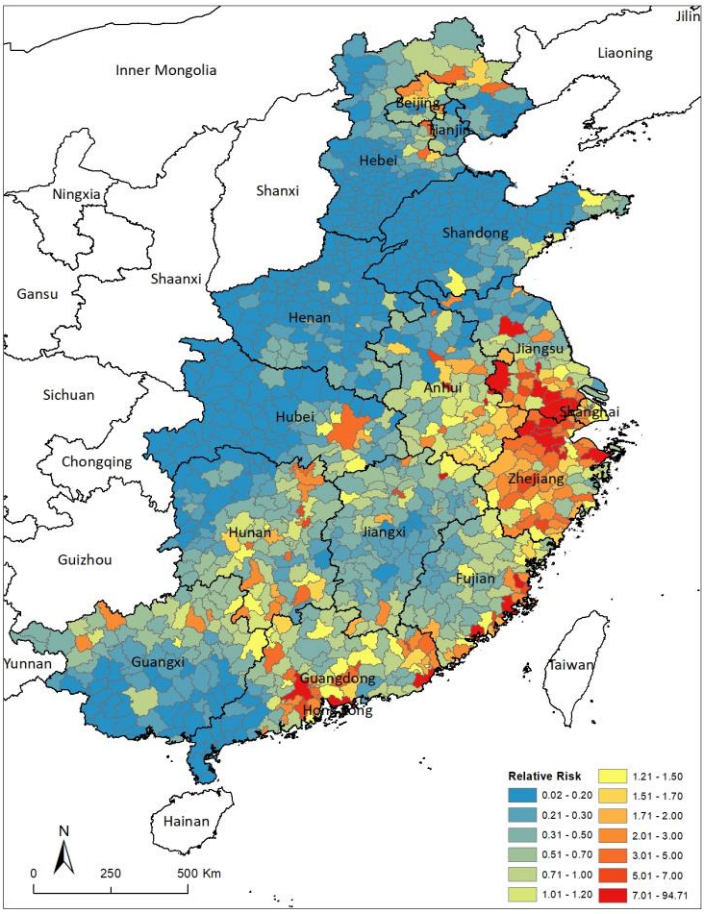


Figure 7-3 Spatial distribution of the relative risks for human H7N9 incidence in counties in southeast provinces. Red and bright colour indicating a higher risk, blue and darker colour indicating a lower risk. The maps were created in ArcGIS 10.1 software (ESRI Inc., Redlands, CA, USA) (http://www.esri.com/).

7.6 Discussion

This study extends current knowledge [12, 26, 219, 289-291] about the spatiotemporal epidemiology of human H7N9 infections in a number of ways. Firstly, using the most complete data on human H7N9 infections and poultry LBM surveillance from 2013-2017, our spatial analyses mapped the spatial distribution of human H7N9 infections and its relationship with poultry serological and virological surveillance results. Second, our human H7N9 relative risk map displayed the distribution of high-risk areas associated with poultry infection status in the county, presence of wholesale LBMs, density of retail LBMs, human population density, chicken density and poultry movement network in the county.

Our analysis identified temporal lags between human H7N9 notifications and poultry surveillance recorded during 2013 to 2017. From examining the temporal relationship between human H7N9 infections and poultry H7N9 surveillance results, we detected a one/two-month temporal lag between the onset of human H7N9 infections and poultry virological/serological surveillance results. These temporal lags may be explained by, firstly, the sensitivity of serological surveillance for H7N9 in poultry is much higher than virological surveillance, and LPAI virus or its genome can be detected in an individual bird for only a few days due to the short period of virus shedding, whereas antibodies elicited by LPAI virus are often present for the entire production life of the infected poultry [296, 297]. Meanwhile, due to low sensitivity, virological positives will be more likely to be detected when the concentration of virus has built up to a more detectable level most likely through the live poultry market chain, i.e. from farms then going through traders, wholesale markets and retail markets. Besides, our results demonstrated that most of the H7N9 virological positive samples were collected in LBMs [204], which is consistent with the consensus that the primary risk factor for human H7N9 infections in China is exposure to LBMs [7, 8, 14-18]. These findings are also consistent with those from our spatial models of human H7N9 incidence, suggesting that the county-level incidence of human H7N9 infections is positively associated with the presence of poultry virological positives in the county. Together these findings have important operational implications for anticipating human H7N9 infections based on current routine LBM H7N9 surveillance in poultry.

Previous studies indicated that LBM density and the number of LBMs were important factors for explaining the risk of H7N9 human infections [12, 16, 26, 219, 289, 291, 298]. In our analysis, both the presence of wholesale LBMs and density of retail LBMs were positively associated with higher relative risk of human H7N9 infections. Wholesale LBMs bring together live birds from large catchment areas and birds are commonly traded to retail LBMs [159, 223]; this results in market networks with numerous trade connections. Higher densities of markets may exacerbate that risk and explain the strong spatial correlation with suitability for H7N9 infection [291]. Closing LBMs appears to be an effective approach for eradicating or reducing H7N9 infections in humans [214]. However, a recent study presented evidence that the closure of LBMs in early waves of H7N9 influenza had resulted in expansion of H7N9 infection to uninfected areas [295]. This implies closing LBMs is a long-term strategy that needs to be further evaluated. Our recent meta-analysis identified biosecurity measures that have been effective for controlling AI viruses at LBMs include smaller market size, selling single poultry species and separating different species, mandatory monthly rest days and bans on keeping live birds overnight, and sourcing poultry from local areas [283]. These identified characteristics of LBMs allow us to better target control efforts.

Furthermore, in our model we included estimates of live chicken movement from areas originally affected by H7N9 in Southeast China, which allowed us to evaluate the effect of live chicken movement from the primary high-risk area on the overall distribution of human H7N9 infections from 2013 to 2017. Our results indicate a positive relationship between human H7N9 incidence and poultry movement estimates (degree centrality) from our CAR model. A previous study of poultry market chains in South China also reported that LBMs where HPAIV H5N1 was isolated were associated with higher degree centrality [31]. Poultry network studies in Vietnam and South China revealed that live poultry traders tend to link poultry sources of similar infection status [31, 39]. These findings suggest that poultry movements from the originally affected area in east China provinces may continue to play a role in disseminating H7N9 virus throughout China. This further demonstrates the importance of evaluating live poultry movement and trading practices to develop appropriate and targeted surveillance recommendations for active H7N9 surveillance program.

After adjusting for poultry marketing system variables (presence of wholesale LBMs and density of retail LBMs) and spatial autocorrelation, our results indicated that human population density was negatively associated with the human H7N9 incidence while chicken density was positively associated with human H7N9 incidence. This can partly be explained by the known epidemiology of H7N9 in humans in that most human cases are a result of animal-to-human transmission, rather than human-to-human transmission. Since most H7N9 cases have been reported in large cities where human population density is very high, it may partially be due to the surveillance effort to detect H7N9 human cases being greater in areas with high population density and better medical facilities [219, 289]. Moreover, higher human population density is usually related to higher biosecurity levels in the LBMs in highly dense urbanized areas. Furthermore, existing evidence indicates that H7N9 is more prevalent in chickens than in other poultry species [10, 12, 102]. Also, while H7N9 can affect other species it is mainly limited to chickens due the characteristics of the industry and the marketing system [10]. Higher chicken density is usually related to high chicken production, chicken trading and transportation which may promote transmission of the pathogen among poultry and increase the chance of humans acquiring H7N9 infection. Our findings suggest that highly connected areas with high chicken density and low human population should be targeted in case the virus continues to evolve or the efficacy of the vaccine is reduced, or even for the emergence of similar viruses in the future.

Moreover, the results of our study demonstrated significant spatial clustering of human H7N9 incidence in the study area, which required the development of a geographical model that incorporated spatial autocorrelation in order to generate a robust risk map of human H7N9 infection across China. Our human H7N9 relative risk map suggests that although H7N9 vaccine for poultry is currently available, continued active surveillance still needs to be strengthened for high-risk areas in China. Our results support strengthening LBM and human surveillance in Southeast area of China (involving Jiangsu, Zhejiang, Anhui provinces and Shanghai Municipality), coastal areas in Fujian and Guangdong provinces, and some inland areas in Hubei, Hunan and Guangxi provinces, as well as Beijing Municipality and the Northern area in Hebei province. According to the National Guidelines on the Prevention and Control of H7N9 influenza in Poultry in China (2018-2020) [299], the current control of H7N9 infections in poultry in China has relied heavily on wide-scale compulsory preventive vaccination combined with biosecurity enhancement in both poultry farms and LBMs, regular surveillance programs, as well as live poultry movement control, quarantine and stamping out. The introduction of live poultry from high-risk areas and sites is strictly restricted [299], however, the delimitation of high risk-areas is unclear. This study attempted a new risk assessment approach and the results provided recommendations to a more targeted risk-based surveillance program, as well as new insights into the role of LBMs and poultry movement in China. However, the map of the spatially structured random effects demonstrates evidence of clustering around the Yangtze River delta area, suggesting that there are other risk factors not included in our spatial models, such as people's behaviour, or indeed other environmental factors that could account for the residual spatial distribution.

The results of this study should be interpreted in light of some limitations. Our analyses were based on laboratory-confirmed cases of human H7N9 infections and reported poultry H7N9 virological surveillance results, and are therefore subject to reporting bias, especially in areas of China with poor surveillance system coverage. In addition, our data for the distribution of LBMs were obtained from local veterinary departments except Shandong and Zhejiang provinces, data for these two provinces were replaced by another dataset clarified in the Appendix D.1, which may bring some reporting bias and uncertainty to the model. Furthermore, our live chicken movement data were collected in selected high-risk areas in Southeast provinces in 2014, representing the live chicken movements coming from and to the originally affected provinces, which may not reflect the current poultry movement situation across the region.

In conclusion, contamination of LBMs with H7N9 is an important determinant of the risk of human H7N9 incidence in China. Moreover, poultry movement from the original areas of H7N9 emergence may be an important driver of the dissemination of H7N9 infections across China, and poultry serological positives and virological positives can serve as a predictor for human H7N9 infections as well as being a guide for the timing of risk management interventions. Highly connected areas with high chicken density and low human population density should be targeted. It is recommended that regular monitoring of poultry movement and poultry infections at the high-risk counties identified in this study will provide essential evidence for the early warning of H7N9 infections across China.

Chapter 8 Discussion and Conclusions

8.1 Introduction

A new reassortant influenza A (H7N9) virus of low pathogenicity (LP) to poultry emerged in eastern provinces of China in early 2013 [263]. In February 2017, some LPAI H7N9 virus mutated to become a highly pathogenic virus (HPAI H7N9) in poultry and rapidly spread to other provinces of China [13]. The rapid evolution, increased pathogenicity and efficient transmissibility of HPAI H7N9 viruses in mammalian models, together with their extended host range, have heightened their pandemic potential, posing an imminent threat to public health and the poultry industry [137]. In addition to its shift in pathogenicity, from 2013 until 2017 there was an increasing number of reported human H7N9 cases throughout China suggesting that vulnerabilities linked to the live meat chicken market chain still remained at that time. In order to better design surveillance programmes and health promotion interventions to prevent poultry and human exposure to HPAI H7N9 virus along the live chicken market chain, the epidemiological determinants for transmission and spread of this virus needed to be better elucidated. The program of research that makes part of this Thesis aimed to address this challenge directly and its findings provide the most comprehensive set of evidence of the epidemiology and control of H7N9 infections along the live chicken market chain in China.

Before the development of the program of research reported in this Thesis there were a number of important knowledge gaps in the epidemiology and control of H7N9 virus infections in live meat chicken market in China. First, the consensus from the literature was that the main risk factors for human infection with H7N9 influenza were exposure to H7N9 infected live meat chickens and exposure to contaminated environments at LBMs [7, 8, 14-18]. Previous studies demonstrated the role of poor biosecurity measures at poultry farms and LBMs and the role of live poultry trade in the dissemination of AI virus [9, 10]. However, the relative efficacy of different biosecurity measures at reducing the transmission of AI to human and poultry at LBMs was uncertain. Furthermore, there was a poor understanding of risk factors associated with human H7N9 infections at LBMs during the H7N9 emergency in early 2013 as well as the role of live meat chicken movement in the epidemiology of H7N9 human infections. Second, a study in China demonstrated that areas of human H7N9 infection overlapped with those that reported H5N1 in an area southeast of Taihu Lake (south of Jiangsu Province), bordering the provinces of Anhui and Zhejiang [29]. This evidence suggested a common high-risk area for novel AI strains which indicated a strong need to design and conduct empirical studies in the identified high-risk areas. Therefore, a better understanding of the social determinants of exposure (including live meat chicken movement data at different points in the poultry marketing chain and data from risk perception and biosecurity practices of actors in the live chicken market chain) was necessary to complement control measures such as vaccination and enhanced LBM biosecurity. Third, while several medical geography studies aiming at identifying H7N9 human risk factors had been undertaken in China [12, 26, 219, 289-291], none had looked at the effect of poultry infection surveillance results and live chicken movement from the initial high-risk areas for H7N9 on the spatiotemporal distribution of human H7N9 incidence across China. In addition, little was known about the potential for poultry surveillance results to provide an early warning for the likely timing and location of human H7N9 infections. The program of research in this Thesis was developed to uncover these identified knowledge gaps.

The overall research aim of this Thesis was therefore to identify and quantify the risks of sustained transmission of H7N9 virus along the live meat chicken market chain in eastern China. To meet the aim, studies were sequentially designed with the following specific objectives: 1) identify the effect of market-level risk factors on AI infections in poultry and humans and generate evidence that will inform AI prevention and control programs at LBMs; 2) to understand the role of market-level live poultry movement and live bird market biosecurity in the epidemiology of H7N9 during the emergency response to the initial outbreaks; 3) to understand the level of knowledge, attitudes and practices (KAP) on avian influenza of different actors along the live meat chicken market chain in the high-risk area for H7N9 emergence and the risk factors associated with their KAP levels; and 4) to quantify the temporal relationship between poultry serological and virological surveillance results and the onset of human H7N9 infections during 2013-2017, and to estimate the relative risk of H7N9 human incidence in counties in Southeast China accounting for poultry surveillance results, live chicken movements and recognized demographic risk factors. The program of research reported in this Thesis is the first of its kind in several ways: it is the first to systematically examine biosecurity risk factors associated with AI infections at LBM level; it is the first to conduct a comprehensive risk-based survey at all the stages of the live meat chicken market chain in the identified highly connected area of live meat chicken movement in eastern China; and it is the first to develop a spatial risk assessment model for the control and early warning of H7N9 in along the live chicken market chain in Eastern China.

The findings of the research studies reported in this Thesis are likely to influence current AI disease control and surveillance interventions within the market chain for live meat chickens in China. This will include evidence for expanding current control policy at LBMs and a spatial-decision support model for targeted planning of resource allocation in counties most at risk of H7N9 infection.

8.2 Key Research findings

The body of this Thesis constitutes the most comprehensive set of evidence on the epidemiology and control of H7N9 infections in the live meat chicken market chain in China. This Thesis has generated important evidence on the effectiveness of market-level biosecurity measures at reducing both poultry and human AI infections. Employing a value chain approach, we identified key geographical areas in China connected via live meat chicken movements originating from the initially affected areas during and after the H7N9 emergency. Within the highly connected areas for live meat chicken movements from the initially affected H7N9 areas we surveyed different stakeholders along the live meat chicken market chain and uncovered important indicators of knowledge, attitudes and practices on AI control that can allow for targeted health promotion programs. We also demonstrated the feasibility of using live chicken movement and poultry surveillance as early warning indicators of H7N9 human infections and identified geographical areas in China more likely for human outbreaks which can be used as candidate areas for enhanced surveillance and control.

To the best of my knowledge, the systematic review and meta-analysis presented in Chapter 4 is the first of its kind to report the combined effectiveness of market-level biosecurity indicators for both poultry and human AI infections at LBMs. The meta-analysis of relevant published English and Chinese research articles provided strong evidence in favour of biosecurity operations at LBMs that are protective for AI infections on both poultry and human infections at LBMs. Before this research, a number of studies attempted to identify biosecurity risk factors associated with AI infection at LBMs [21, 69, 159-161, 163, 223-233]. While some studies had generally demonstrated that biosecurity practices at LBM-level were associated with reduced risk of AI infection, the results from those studies were inconsistent or even contradictory and the relative efficacy of different LBM biosecurity practices at reducing the transmission of AI to both humans and poultry in the LBM setting was still unclear.

The results from Chapter 4 demonstrated that biosecurity measures effective at reducing AI market contamination and poultry infection at LBMs included smaller market size, selling single poultry species and separating different species, performing cleaning and disinfection and market closures, and a ban on overnight storage. These findings are in accordance with the current “1110” system for AI control at LBMs established by the China MARA in Feb 2017, i.e. cleaning once a day, thorough disinfection once a week, one-day market closure once a month and no live poultry remaining at LBMs over night or at closure. Currently there are a number of challenges with the implementation of the “1110” system. For example a recent investigation on the implementation of the “1110” system on LBMs in Guangdong Province [300] revealed that, while all surveyed markets implemented daily cleaning, weekly disinfection, and monthly market closures, only 9.6% of the all stalls surveyed managed to sell all poultry; among the 90.4% that did not sell all poultry during the day, 40% kept unsold live poultry in the LBM overnight and 50.4% carried the unsold poultry out of the market for temporary storage. Several studies in Hong Kong [38, 163, 237] evaluated the effect of rest days and ban on overnight live poultry storage on reducing H9N2 virus isolation in the LBMs, they found that implementation of a ban on overnight storage of live poultry had an even greater effect on reducing viral load in LBM than the intervention of 1 or 2 rest days per month. These findings demonstrated the difficulty as well as the importance of the implementation of overnight ban of storage of live poultry at LBMs.

Previous research indicated that poultry movements facilitate the transmission and spread of AI viruses between premises as a result of mixing of poultry from different sources and the increased opportunity for virus multiplication during transport [223, 299]. The results from Chapter 4 also revealed that markets located at non-central city areas and markets that source poultry from multiple counties, i.e. those engaging possibly in cross-regional and long distant poultry movements, are associated with a higher risk of AI infection in poultry at LBMs. This highlighted the importance and need for automated systematic monitoring of live poultry movement within LBMs for the prevention and control of transmission of AI.

Our meta-analysis also suggested that poultry slaughter operations at LBMs increase the risk of AI transmission within the LBM environment, presumably because of exposure to aerosols arising during the slaughter process where AI virus may be present in large quantities [283]. Several studies have also demonstrated that most commonly contaminated sites were located in the poultry slaughtering zone [36, 225]. This is consistent with the current control measure of promoting the industry upgrade to a new production and consumption model (i.e. scale farming, centralized slaughtering, cold-chain transportation, fresh chilled product in the market) [248, 299]. The results of our meta-analysis also suggested that spring and winter seasons posed significant higher risk of AI infection in the LBM environment compared to summer and autumn [249-255]. This seasonality effect is due, in part, to the fact that lower temperature and humidity can increase virus survival in the environment [237].

In addition, our results demonstrate that human infection at LBMs is dependent on important demographic and occupational hazards. Our results indicate that female workers and retail LBM workers are at increased risk of AI infection compared to male workers and wholesale LBM workers [233, 256-258, 262]. Activities that directly expose LBM workers to AI such as slaughtering, defeathering and cleaning significantly increase the risk of AI infection in market workers [231-233]. These results may reflect gender differentiation in tasks within different LBMs, because wholesale markets tend to be male dominated as opposed to retail markets where women share the poultry value chain with men, plus live poultry were slaughtered at retail LBMs on a daily basis, while many wholesale markets do not slaughter or have separate slaughter areas [259], which might place female workers at greater risk of AI infection compared to males. These high-risk poultry handling activities primarily observed in retail markets which further highlights the need for workers to wear personal protective equipment within retail LBMs, and future biosecurity strategies should account for gender differences in risk identified in our study, which could include raising collective awareness through information sharing and educational training program that target women working at LBMs.

A limitation from the program of research in Chapter 4 was that we were not able to stratify the meta-analysis by different AI strains, so we were unclear whether the identified effects were true for the H7N9 virus alone. With that gap in knowledge in mind we designed the study in Chapter 5 which aimed to conduct an empirical investigation in H7N9-affected LBMs. Therefore, in the program of research in Chapter 5, we focussed our investigation into the originally H7N9-infected area (i.e. Shanghai Municipality, Jiangsu, Zhejiang and Anhui provinces) [102]. We obtained a unique dataset collected during the emergency epidemiological investigation from 24 LBMs within a one-kilometre buffer area from H7N9 human infections and those that marketed large quantities of poultry at the time of the outbreak. We then evaluated biosecurity risk factors within those markets associated with H7N9 infections and quantified the role of live chicken movement in the epidemiology of H7N9 human infections. Prior to this research, the sources of H7N9 infection had yet to be fully clarified and a major challenge for the investigation of source attribution was that poultry did not exhibit clinical signs. Epidemiological studies at the time relied on H7N9 infection data from humans and LBMs where infections have been detected. Previous studies in Asia had demonstrated the role of movement of poultry through live bird markets in the circulation and dissemination of HPAI H5N1 virus [41]. The research in Chapter 5 investigating the role of live poultry movement of affected LBMs extended evidence on the role of market-level biosecurity risk factors and enabled the stratification of the risk of H7N9 infection geographically. The results in Chapter 5 demonstrated that chickens were the predominant poultry species traded by affected LBMs. The presence of H7N9 in LBMs was significantly associated with the type of LBMs and with LBMs that sold chicken to other markets. The results also showed significant spatial clustering in terms of the connectivity of live chicken sources (degree centrality and *k*-core), of affected LBMs. These findings suggested that the connectivity of LBMs to particular counties in the provinces of Anhui, Zhejiang and Jiangsu and the level of market biosecurity of LBMs in these areas were likely to have played a role in the transmission of H7N9 to humans during the first wave of the epidemic in April 2013. Furthermore, an analysis of the data from the first wave of H7N9 human infections demonstrated that the primary cluster of influenza (H7N9) overlapped with previous H5N1 human infections [29]. This geographical overlap occurs in an area straddling the boundaries of the provinces of Anhui and Jiangsu [29]. The identified highly connected areas in eastern China highlighted candidate areas for more detailed epidemiological investigations. Importantly, as seen in Chapter 7, the geographical areas identified in Chapter 5 continued to report human H7N9 infections in subsequent infection waves observed between 2013 and 2017.

We had previously demonstrated in Chapter 4, that people's social demographic and behaviour at LBMs are important indicators of risks in AI human infections. Therefore, we designed the study reported in Chapter 6 to investigate heterogeneity in and risk factors for risk perception towards AI of different actors in the poultry value chain in the high-risk areas where H7N9 first emerged. Existing literature indicated that health behaviours and hygiene practices can be influenced by age, gender, education, knowledge and religious beliefs [182]. There were a number of studies reporting poultry related workers' KAP for AI in Asia, the US, Europe and Africa in recent years [184, 272-279]. To our knowledge, none of these studies were conducted on chicken-specific market chains, none of the studies compared KAP indicators across chicken farmers, chicken vendors and consumers in the same value chain, and none of them targeted the high-risk areas affected by the emergence of the H7N9 [10]. Therefore, in Chapter 6 we report the results of a cross-sectional questionnaire survey conducted in the hotspot area identified in Chapter 5 during June to July 2014 after the second wave of H7N9 outbreaks in humans in Eastern China. All stakeholders along the live meat chicken market chain (i.e. chicken farmers, vendors and consumers at LBMs) were targeted to profile their level of knowledge, attitudes and practices (KAP) towards avian influenza and the risk factors associated with their KAP levels. Multivariable generalized least squares (GLS) random-effects regression models were developed to identify predictors of KAP of AI among different actors along the live chicken market chain. The results of Chapter 6 indicate that chicken vendors at LBMs generally had lower KAP scores than chicken farmers. This finding suggested that interventions to improve KAP towards AI should be promoted among all stakeholders with an emphasis on chicken vendors in LBMs. This further ascertains the findings from Chapter 4 and 5 that LBMs may have played an important role in the dissemination of H7N9 virus in the high-risk area. The study in Chapter 6 analysed determinants of KAP within each actor group; results for chicken farmers demonstrated those who had 11 to 15 years farming experience had significantly higher knowledge scores than those worked for less than six years, and farmers who had longer farming experience (>15 years) had poorer attitudes towards AI risk compared to those with less experience (<6 years). This finding suggests that knowledge is not a mediator of behaviour in longer practicing farmers. Indeed, previous studies also suggested that better knowledge was found in farmers who worked for a longer time [272], but better knowledge of

AI may also lead to a lower perceived risk of infection [184, 284]. The results of Chapter 6 also indicate that farmers who had longer daily hours of exposure to chickens had significantly lower knowledge scores. It is possible that those farmers who are exposed to poultry for longer periods of time are economically disadvantaged and/or live in more remote areas; in turn, socioeconomic disadvantage and remoteness from urban centres may lead to a lower access to information. Overall, our findings suggest that reducing the period of daily contact with chickens and enhancing preventive practices are of great importance for chicken farmers. The results of Chapter 6 also indicated that female chicken vendors had significantly lower knowledge scores compared to male vendors, which could partly explain the findings of our meta-analysis in Chapter 4 of a higher risk of AI infection in female workers in LBMs compared to male workers. However, practice scores were significantly higher in female vendors and those vendors who also conducted slaughter compared to males and vendors who did not conduct slaughter. This conflicts with the findings from Chapter 4 that market workers who slaughter poultry had significant lower risk of getting AI infections. The discrepancy between this study and the results of Chapter 4 can be partly be explained by reporting biases of practices of chicken vendors highlighting the need for further observational studies on vendor behaviour. It is possible that chicken vendors who conduct slaughter at LBMs are more risk aware and are thus more likely to wear personal protective equipment compared to vendors who do not slaughter poultry. This hypothesis requires further empirical observation. The results of Chapter 6 also suggest that older chicken vendors (>35-years-old) had significantly lower knowledge scores compared to younger vendors (≤35-year-old), which may be attributed to the level of education of older vendors compared to younger vendors. This finding suggests a higher risk of exposure of older vendors as documented by Chapter 4. The results of Chapter 6 also indicated that female consumers had significantly better practice scores than male consumers. This may be because women are more likely to be responsible for food shopping and cooking for their family, which in turn can lead to better practices. Consumers who bought chicken less frequent (monthly) had better risk awareness compared to those who bought chicken frequently (weekly). This is consistent with finding of a previous study from Taiwan where consumers with low risk perception levels were reported to be more likely to maintain usual chicken consumption than those with high risk perception levels if outbreaks of AI occurred [188]. This suggests that government administration and industry managers can design effective health promotion packages to prevent a drop-in revenue due to the reduced demand and informing consumers about the safety of chicken products and proper cooking practices.

As mentioned before the effectiveness and practicality of LBM closures and overnight ban on poultry has been disputed [301]. Indeed the results of our study in Chapter 6 also revealed that while most of chicken farmers (98%) and vendors (94%) agreed with adopting regular market closures and cleaning and disinfection on markets, 85% vendors and 53% farmers did not agree with the permanent ban on selling live chicken and central slaughtering of poultry; 62% consumers also reported not being willing to buy slaughtered chicken. The findings in Chapter 4 revealed that the detection of AI viruses was always reduced right after market rest days when infectious load is lower [163, 224-229] and closing LBMs can largely eliminate human infection risk [230]. However, previous studies also indicated that recovery of AI viruses can occur shortly after LBMs re-open, presumably following the introduction of AI positive birds [245, 246], suggesting that the underlying epidemiological and socioeconomic factors of the effect of market closure are complex [171, 301, 302]. Currently, there are four different methods of LBM closures in use by different provinces in China. The first one involves monthly mandatory market closure across all provinces of China. The second one involves emergency market closures during H7N9 outbreaks, which has been adopted by many areas in China, i.e. Zhejiang, Guangdong, Jiangsu, Hunan, Jiangxi provinces. The third one involves seasonal market closure which has been adopted by the Commerce Commission of Shanghai Municipality. This includes suspension of live poultry trading in designated wholesale LBMs and retail LBMs sites from the first day of lunar new year to the end of April. The last method involves permanent LBM closure, which has been adopted in central city areas of Hangzhou city in Zhejiang province. While our findings in Chapter 6 suggest that the current control policy of regular market closure and cleaning and disinfection are acceptable to both farmers and vendors, a policy that aims to permanently shut down LBMs is likely not to find support amongst vendors, farmers and consumers. Key public policy questions that remain unanswered are on what can be done to mitigate the socioeconomic impact of LBM closures and when and where market closure and other market-level interventions should be targeted.

Since 2013 to date, there have been six epidemic waves of H7N9 infection in China which resulted in the widespread dissemination of H7N9 virus across the country. In February 2017, some strains of the 2013 LPAI H7N9 virus mutated to become highly pathogenic in poultry and rapidly spread to other provinces of China [13, 136]. The studies in Chapter 4 and 5 identified the role of LBMs and live poultry movement in the exposure and dissemination of H7N9 viruses during the H7N9 emergency. The study in Chapter 6 demonstrated a better understanding of the social determinants of exposure along the live chicken market chain. However, effective prevention and control of human H7N9 infections relies on identifying indicators that can anticipate the spatiotemporal variation in infection incidence so to provide essential evidence and recommendations for risk-based AI surveillance programs and appropriate enhancements to current prevention and control policies for H7N9. While several ecological spatial studies aiming at identifying risk factors of H7N9 human cases have been undertaken in China [12, 26, 219, 289-291], none of these studies looked at the effect of more proximal factors such as poultry surveillance results and live chicken movement in affected areas at explaining the geographical variation of human H7N9 infections. Therefore, in Chapter 7, we assembled a dataset of important risk factors for H7N9 infection, e.g. distribution of LBMs, chicken movement data collected from the study in Chapter 6, and other risk factors based on existing ecological studies (e.g. human population density, chicken density), as well as poultry surveillance results. In Chapter 6 we aimed to quantify the temporal relationship between the onset of human H7N9 infections during 2013-2017 and poultry serological and virological surveillance results, and to estimate the relative risk of H7N9 human incidence in counties in Southeast China by assessing the relationship between human infections as outcome and poultry surveillance results, live chicken movements and recognized demographic risk factors. The results in Chapter 7 revealed the temporal association between human H7N9 infections and poultry serological and virologic surveillance. Specifically, the results indicated that the peak of poultry H7N9 serological positives is followed by human H7N9 infections with a two-month lag, and that poultry H7N9 virological positives is followed by the human H7N9 infections with a one-month lag.

To map the relative risks of human H7N9 incidence in southeast China, in Chapter 7 we developed a geographical model that accounted for spatial clustering of incidence. The results of our spatial CAR model indicated that human H7N9 incidence at county-level is positively significantly associated with the presence of wholesale LBMs, higher density of retail LBMs, presence of poultry virological positives, higher poultry movement network connectivity, as well as lower chicken population density and higher human population density. This model is novel in that it incorporated poultry virological surveillance results which has never been evaluated as a risk factor in H7N9 risk mapping studies, and the model included estimates of live chicken movement coming out from the originally H7N9 affected area in Southeast China provinces, which evaluated the effect of live chicken movement from the high-risk area on the overall H7N9 infections. By doing so, this model provided an approach to identify areas where the likelihood of H7N9 human infections is at its highest, and where spatially targeted control interventions are most needed. The predictive map of relative risks of human H7N9 incidence generated in Chapter 7 indicated that high risk areas of human H7N9 infections were spatially clustered in Southeast China, extending from the Yangtze River delta near Shanghai to the Pearl River delta near Guangzhou and covering most areas of Jiangsu, Zhejiang, Shanghai, Anhui, Fujian, Guangdong and Hunan provinces. Additional hot spots for human H7N9 infections were found in the northern region of Guangxi, eastern region of Hubei province, northern and southern region of Jiangxi, northern region of Beijing, and the northern region of Hebei province.

8.3 Public health implications of the findings

The major practical contribution of the research program of this Thesis is that it provides much needed empirical evidence on the role of LBMs and live chicken movement in the epidemiology of H7N9 infections and enabled the identification of high-risk areas where health promotion intervention programs and strengthened surveillance for H7N9 are most needed. The results of the research detailed in this Thesis have important operational public health implications in the following ways.

8.3.1 Expansion of market level biosecurity control measures

The findings from the systematic review and meta-analysis in Chapter 4 suggested that AI control measures should be targeted to markets that slaughter live poultry and markets which sell multiple species. Strategies that include daily cleaning and disinfection, separation of different poultry species, regular market closure and ban on overnight storage should be emphasised and strengthened. These findings are important in that they reflect the ongoing "1110" LBM control policy for H7N9 prevention and control established by the Chinese MARA in February 2017 [299]. However, the findings of Chapter 4 also indicated the need for this policy to be expanded to consider the effect of poultry slaughtering in LBMs; our findings demonstrate that workers' involvement in poultry handling activities increases the risk of AI infections at LBMs. Our results indicate that improvement of slaughtering and poultry processing operations at LBMs should be an area of investment from market operators through the implementation of standard operating procedures and good manufacturing practices that comply with standard health and safety regulations. Current regulations indicate that wholesale LBMs and urban agricultural markets require that live poultry slaughter areas should be enclosed and areas where poultry selling, slaughtering and processing occur should be segregated from consumers [210]; this regulation, however, does not apply to markets situated in rural areas. Considering the high risk of slaughtering at LBMs, we suggest that the current "1110" system should be expanded with another "1", i.e. to include the construction of enclosed areas where poultry slaughter can happen in the LBM providing enhanced sewage and waste management as well as PPE (personnel protective equipment) for those who conduct slaughtering or handling of poultry in the separated slaughter area. Overtime, this "1" needs to migrate to a "0" (i.e. no slaughter at markets), this process will allow sufficient time for people's acceptance of consuming slaughtered poultry (chilled poultry) from markets.

The findings of Chapter 4 confirmed the risk of AI transmission at LBMs posed by cross-regional and long-distance poultry movements. In addition, the findings of Chapter 5 and Chapter 7 reinforced the importance of live poultry movement in the emergence of H7N9 and the positive correlation between live chicken movement from the original area of emergence in Eastern China and the current geographical distribution of H7N9 human notifications. The “Regulations of prevention and control of HPAI in LBMs” jointly released in 2006 by MARA, NHC and SAIC stipulated that wholesale LBM should check the quarantine certificates of poultry entering the LBM. However, this regulation does not apply to retail LBMs. Besides, it is market vendor’s responsibility to demonstrate the quarantine certificate at the stall for publicity and the quarantine certificate should be kept for more than six months [210]. Furthermore, the animal health supervision agencies are responsible for the quarantine supervision over live poultry that are transported out of the LBM. Together our findings and current policy challenges indicate that the current “1110” system can also be expanded with another “0”, i.e. no entry of poultry at LBMs without an animal quarantine confirmative certificate. The proposition of an enhanced LBM control policy from the current “1110” to a “4-2” policy (i.e. 1111 and 00) is indeed necessary to help mitigate the residual risk of poultry and human H7N9 exposure and dissemination at LBMs.

8.3.2 One health interventions for health promotion and surveillance

The analysis outlined in Chapter 6 suggested that health interventions should be promoted among all stakeholders along the live chicken market chain, with an emphasis on chicken vendors operating at LBMs. Efforts for interventions in chicken farmers should be focused on reducing the period of daily contact with chickens and enhancing their preventive practices, as well as improving the awareness of AI for chicken farmers who have worked for many years in the chicken industry. The findings of Chapter 6 also highlighted the demand for health educational interventions about knowledge of AI for the less educated and older vendors. The findings of Chapter 6 revealed that consumers who bought chicken more frequently had lower risk awareness scores compared to those who bought chicken less frequently, suggesting that public health administration and industry managers can design effective health promotion programmes to inform consumers about the safety of chicken products and proper cooking practices. The findings on gender disparities in the epidemiology of AI in Chapter 4 and 6 suggested that future biosecurity strategies should account for gender differences in risk identified in our study, which could include raising collective awareness through information platforms that target women working at LBMs. Together these findings indicate the need for a One health approach to health promotion at LBMs. It is the responsibility of veterinary administration departments for the supervision and administration of animal health in LBMs, and the public health administration departments are responsible for the population health in LBMs. Therefore, a one-health approach to health promotion is needed so efforts can be articulated along the same market chain that has actors that are under different jurisdictions.

In Chapter 7, the identified temporal relationship between human H7N9 infections and poultry H7N9 surveillance also demonstrates the need for a One Health approach to the data flow between MARA and NHC. To allow the development of an operational model for the anticipation of human H7N9 infections based on current routine LBM H7N9 surveillance in poultry we suggest that poultry H7N9 surveillance and notification system to be linked to human H7N9 notification system in order to allow the early warning of human infections moving forward. The predictiveness of the poultry surveillance to the incidence of H7N9 human infection will rely on the sensitivity and specificity of the surveillance system, as well as the traceability of the poultry market chain for precautions to the prevention of H7N9 transmitted to humans. Collaboration on risk communication and establishment of disease notification data sharing mechanisms between animal and human health administration departments are paramount to understand the risk of transmission between poultry and humans and to develop effective programs to control and prevent the spread of H7N9 within poultry populations and onwards to humans.

8.3.3 Targeted risk-based surveillance

In Chapters 4, 5 and 7 we demonstrated the importance of seasonality of infection, that high risk areas for poultry movement were found to be associated with the emergence of H7N9 infections and that live poultry movement is indeed an important driver of the spatiotemporal dissemination of H7N9 human infections. To target temporally and geographically the current H7N9 surveillance resources we suggest that live poultry movement data be embedded into a day-to-day centralized information system established and managed by the animal health departments. The system will allow disease control managers to estimate the connectivity of different poultry locations (can be at “county” level, or individual poultry farms and LBMs) and so to identify high-risk areas for H7N9 infections in terms of poultry movement. Meanwhile, this system can also be a poultry traceability system that includes certification of sources of each batch of poultry. Once a H7N9 positive has been identified or an outbreak notified, this system will allow tracing back to the poultry source location and generation of a list of areas with high-risk for immediate actions and targeted risk-based surveillance. In addition, the spatial model of H7N9 presented in Chapter 7 extends existing approaches to risk mapping and has important public health implications for the planning, design and implementation of geographically targeted interventions. The present model or a more advanced version thereof could be used to guide decision making processes for mass vaccination and surveillance strategies in combination with local knowledge and programme needs. Based on the key findings and methodology developed in Chapter 7, we suggest that animal health departments build up a H7N9 Spatial Risk Assessment System that integrates data on poultry surveillance results, live poultry movements and local socio-demographic data (distribution of LBMs, poultry density, human population) into a comprehensive spatial decision support system. This system will allow disease control program managers to produce operational maps that can assist decisions on how to best allocate their limited resources to implement spatially targeted H7N9 programs within the regions of high relative risk of human infections.

8.4 Limitations

The findings reported in each of the research Chapters in this Thesis must be considered within the context of potential limitations of the data and the methodological assumptions of the data analysis techniques used in each Chapter. Each key limitation is listed and discussed below.

8.4.1 Reporting bias

In the meta-analysis presented in Chapter 4, all included studies were restricted to those that could be obtained from published literature in English and Chinese. A common limitation is that only studies with significant results are published which may introduced publication biases to the results presented in Chapter 4.

In Chapter 5, our epidemiological investigation was conducted during the H7N9 emergency response, therefore, LBMs were selected purposively and aligned with the occurrence of H7N9 human infections. Due to mandatory market closure in the study area, the infection status of several LBMs was not available. I would have been able to quantify more accurately risk factors associated with the presence of H7N9 in LBMs, if more LBMs in the affected area had been investigated during the emergency response. In spite of these limitations, I am confident that the dataset I obtained was the most up-to-date, extensive and representative dataset to allow the research question to be addressed. Likewise, live poultry movements were only collected from large LBMs, which may have introduced potential biases to the findings in Chapter 5, it may also influence KAP study in Chapter 6, and may propagate bias to the study in Chapter 7.

In the KAP study in Chapter 6, I used a close-ended questionnaire which may have given limited flexibility to participants' answers. It may have missed some contextual/specific or relevant local knowledge on additional preventive practices.

In Chapter 7, data on the distribution of LBMs were obtained from local veterinary departments except for two provinces (Shandong and Zhejiang), data for these two provinces were replaced by another database clarified in Appendix D.1, which may bring some reporting bias and uncertainty to the model. The study in Chapter 7 used notifications of H7N9 human cases that required clinical care collected through a passive surveillance system. Therefore, it has the potential to underestimate the actual incidence rate of H7N9 exposure as the system only captures individuals who seek medical treatment. The study in Chapter 7 used poultry serological and virological surveillance results from the regular national poultry surveillance system, however, there was missing information in terms of geographic location of some of the positives. This could lead to misrepresentation of the extent of the disease.

8.4.2 Diagnostic uncertainty

In Chapter 4, among eligible studies included in the meta-analysis, most of the studies used RT-PCR for detecting H7N9 virus, and some studies adopted PCR or virus isolation. Due to the adoption of different diagnostic methods, diagnostic uncertainty may have been introduced to the results. As mentioned above, in Chapter 7, the presence of H7N9 infections in human was obtained through a passive surveillance system, therefore, the results reported in the study may have underestimated the incidence of H7N9 infections in humans, in an area where the capacity for diagnosis among physicians and hospitals is relatively low. Meanwhile, the study in Chapter 7 used poultry serological and virological surveillance results from the regular national poultry surveillance system. Due to the initial low pathogenicity of H7N9 in poultry, and variance in the awareness and diagnostic capacity among veterinary departments over time and geographically, this could lead to misrepresentation of the extent of the disease.

8.4.3 Confounding factors

The effects reported in these studies were adjusted for important confounding factors, but they do not represent the complete multifactorial nature of infections. This limitation exists in Chapter 6 and Chapter 7. In the research in Chapter 6, I accounted for the most important characteristics of study participants, however, some other confounding factors (e.g. personal income [303, 304], place of residence [182, 305]) were not captured which may also have contributed to differences in KAP towards AI observed among difference groups. In the research in Chapter 7, I controlled for poultry surveillance data, live chicken movements and sociodemographic factors that are related to H7N9 human infections. Other possible contributors not included in the model, included people’s behaviour [288], some environmental factors and ecoclimatic factors [306]. In the researches in Chapter 5 and Chapter 7, I used degree centrality and k-core as indicators of network connectivity for chicken movement, however, the trading volume of chicken movement was not factored, which may overlook the effect of trading volume in the risk of H7N9. This can be improved in the future researches.

8.4.4 Modelling limitation

Another limitation of the studies in this Thesis is the use of ecological designs that do not measure individual exposure. The study in Chapter 7 used an ecological approach, using secondary data on predictors of outcome variables such as human population density and chicken density. Some of these proxies are imprecise measurements of exposure, resulting in regression dilution bias leading to underestimation of the observed effects [307, 308]. Future interactions of spatial models should observe the impact of individual-level modelling approaches at reducing the effect of regression dilution bias compared to that of the ecological approach.

8.5 Recommendations and future pathways

There are considerable opportunities for future research in the epidemiology of H7N9 infections. Considering the findings of this Thesis and above limitations, we propose the following directions to be incorporated in future research.

First, there remains considerable scope for underreporting of human and poultry H7N9 infections and therefore data is still lacking on the risk factors for human H7N9 infection. It would be constructive to conduct a large-scale seroprevalence study of poultry related workers who are in regular contact with poultry. Based on the findings from Chapter 4 this seroprevalence study should target larger LBMs that are located at non-central city areas, and include poultry workers, especially individuals that are responsible for selling and processing poultry at the LBMs (i.e., market sellers that slaughter, defeather, handle internal organs etc.,) in China. This study could evaluate the risk factors identified or discussed by studies in Chapter 4, Chapter 5 and Chapter 6 in this Thesis. Careful attention to sample size should be considered when designing such a study to adequately evaluate risk factors for infection.

The relationship between LBM biosecurity indicators and the presence of H7N9 is likely to be much more complex and further evidence is necessary to profile the risk for LBMs. The effects of control measures at LBMs (e.g., improving bio-security measures, stamping out, restricting movement, educational promotions), which have been implemented in some areas of China to control the spread of H7N9, were not well understood as there are very few peer-reviewed and published reports that have evaluated such control programs. The meta-analysis of the effectiveness of market-level biosecurity in Chapter 4 also demonstrated that most of the included studies were observational studies given that data from randomized trials were not available. A future study could be designed as a randomised controlled intervention trial (RCTs) whereby a group of markets would be randomised to the intervention group while another group of markets which would not be given the intervention would serve as a control group. This approach allows disentangling the effect of potential confounders while estimating the causal relationship between infection and the risk factor being manipulated. Several biosecurity measures that can be selected for intervention include the ban on overnight storage of live poultry at LBMs, upgrading and segregation of the slaughter area at LBMs, or no entry of live poultry without animal quarantine confirmative certificate. RCTs are very popular in human epidemiology and often underutilised in veterinary epidemiology. Another major advantage of RCT's over traditional longitudinal follow-up studies is that these studies can be coupled with economic models to quantify the cost-effectiveness of the interventions under study. Therefore, designing RCTs would constitute a way forward for conducting translational research with direct relevance to policy formulation for avian influenza disease control.

In Chapter 7, the predictive map of the relative risks of H7N9 human infections in Southeast China could be utilised as a spatial decision support tool to guide the local integrated H7N9 prevention and control programs and to help conduct individual-level studies within the predicted high-risk areas in Southeast China. Besides, the map of spatially structured random effect demonstrates evidence of clustering around the Yangtze River Delta area, suggesting that there are other risk factors not included in this spatial CAR model. In addition to the identified strongly correlated variables (live chicken movements, poultry surveillance data and sociodemographic factors), other factors such as environmental factors (the role of temperature, water, transportation) could be also evaluated in further researches.

Future research is recommended to integrate the H7N9 poultry surveillance results from national human surveillance systems and national poultry surveillance programs in China to evaluate the predictiveness of surveillance in poultry for the early warning of AI infections in humans. Advanced analytical approaches applied in this Thesis such as SNA and spatial CAR modelling could also be applied to controlling and reducing other AI virus subtypes, such as H5, H9 and other H7 subtypes.

8.6 Conclusions

The results of this Thesis demonstrate that deficiency in LBM biosecurity management and live meat chicken movement played an important role in the emergence and spread of H7N9. This Thesis presents comprehensive evidence of biosecurity measures on reducing both poultry and human AI infections on LBMs. This Thesis also identified highly connected areas in eastern China in terms of live poultry movements during the H7N9 emergency highlighting candidate areas for more detailed epidemiological investigations.

It also provided recommendations on risk targeted intervention programs for improving the knowledge, attitudes and practices of AI of different stakeholders along the live meat chicken market chain in Southeast China. The findings suggested that risk-based health promotion interventions should be developed and implemented by both animal health (i.e. targeting farmers and vendors) and public health agencies (i.e. targeting consumers) to prevent further human cases of H7N9 along the live chicken market chain in China.

Furthermore, the study in Chapter 7 attempted a new risk assessment approach and generated a predictive map of the relative risks of H7N9 human infections in Southeast China. The results from Chapter 7 provided recommendations to a more targeted risk-based surveillance program, as well as new insights into the role of LBM biosecurity and poultry movement in China. It revealed the feasibility of employing poultry serological and virological surveillance results in the early warning and timing of prevention and control interventions of H7N9 human infections in China. It also highlighted that those highly connected areas with high chicken density and low human population should be targeted. The risk map indicated that high risk areas of human H7N9 infections were spatially clustered in Southeast China, which highlighted regular monitoring of poultry movement and poultry infections at these identified high-risk areas will provide essential evidence for the early warning of H7N9 infections across China.

Appendix A Chapter 4 Supplementary Information

A.1 Tables

Table A-1 PICO/PEO method to define key search terms

PICO	Contents
Population	Poultry/environment, poultry workers at LBM
Exposure	Biosecurity indicators
Comparator	Not Applicable
Outcomes	Evidence of Avian influenza exposure in poultry and in workers at LBM. For market infection: virologically positive poultry or environmental samples. For market worker's infection: seropositive or seroconversion.

Notes: We used the following terms together and in various combinations: "avian influenza", "risk factors" and "market". For "avian influenza", we selected "flu or influenza" associated with any of the following susceptible population: "avian", "bird", "poultry", "workers", "consumers" etc. For "risk factors", we searched with the following terms: "biosecurity", "risk factors", "Knowledge", "Attitude" or "Practice", we expected that "Knowledge, Attitude and Practice" (KAP) studies describe biosecurity practices of poultry workers or poultry consumers at LBMs. Secondly, we also translated the same set of keywords into Chinese language and applied the search in CNKI (the China Academic Journals full-text database) and WANFANG database (includes most comprehensive online full-text Chinese medical journals).

Here is our search strategy:
Market* AND (flu OR influenza) AND (avian OR bird* OR poultry OR duck* OR chicken OR quail OR pigeon OR geese OR goose OR consumer* OR worker* OR trader OR vendor OR intermediary OR seller) AND (risk OR risks OR factor* OR reduce* OR effect OR role OR influence OR associat* OR impact OR evaluat*) AND (biosecurit* OR bio-securit* OR clean* OR disinfect* OR "market closure" OR "rest day" OR rest-day OR dispos* OR slaughter* OR intervention* OR protective OR age OR gender OR sex OR education OR occupation* OR knowledge OR attitude* OR practice OR behavio* OR perception OR surplus OR overnight OR transport)
CNKI: SU=('家禽'+'活禽'+'禽类'+'涉禽'+'禽交易') * ('市场'+'集市') * ('禽流感'+'甲型流感'+'A 型流感') * ('风险'+'因子'+'影响'+'因素'+'生物安全'+'监测')
Wanfang: 主题:((家禽 + 活禽 + 禽类 +涉禽 + 禽交易) * (市场 + 集市) * (禽流感 + 甲型流感 + A 型流感) * (风险 + 因子 + 影响 + 因素 + 生物安全 + 监测))

Table A-2 Summary of the biosecurity indicators categories that were considered.

Group of biosecurity indicators	Subgroup
Group A: Market Characteristics	Market type
	Market size
	Market location (Rural or urban)
	Market location (Central city or non-central city area)
	Presence of other species
	Presence of ducks
	Presence of rabbits
Group B: Market Biosecurity Management	Conduct cleaning and disinfection (C/D)
	Before and after C/D
	Conduct waste disposal
	Conduct market closure
	Before and after market closure
	Ban on overnight storage
	Poultry import from local area
	Separate different species
	Conduct slaughter in market
Group C: Seasonality	Seasonality
	Temperature
Group D: Socio-demographic Characteristics	Sex
	Age group
	Years of working in LBMs
	Type of market
	Vaccination history
	Occupation
Group E: Exposure to Poultry	Clean feeding tray
	Clean water tray
	Clean feces
	Feed poultry
	Contact duck
	Contact goose
	Contact pigeon
	Conduct slaughtering
	Conduct defeathering
	Conduct eviscerating

Table A-3 Criteria used in quality assessment and allocated scoring system

No	Criteria	Options	Score
C1	Type of study	Case-control	0
		Cross-sectional	1
		Longitudinal	2
C2	Type of analysis	Univariable analysis	0
	(Univariable or Multivariable models)	Multivariable analysis	1
C3	Timing of study*	not cover peak season of AI	0
	(Whether it covers high risk season for AI)	close to peak season of AI	1
	(Peak season is defined from Nov to Feb.)	cover peak season of AI	2
C4	Type of markets studied	Retail only	0
	(Wholesale, Retail or Both)	Wholesale only	1
		Both	2
C5	Number of counties surveyed	1	0
	(Geographical extent of study)	2~5	1
		>5	2
C6	Number of LBMs sampled	<=5	0
		6~10	1
		>10	2
C7	Biological samples analyzed	poultry only	0
	(Environmental samples or poultry samples within LBMs)	environment only	1
		both poultry and environment	2
C8	Number of workers sampled	<=100	0
		100~300	1
		>300	2

Note: The criteria were derived based on three aspects, 1) Study design: a longitudinal study is more powerful than a cross-sectional study or a case-control study. 2) Study size: the more counties/markets/poultry or environmental samples/market workers were sampled, the more power the study will be. 3) Study representativeness: studies that involved both wholesale and retail LBMs, collected both poultry and environmental samples, covered the peak season of AI infections are more representative than those who didn’t. Studies that recorded a higher score were considered higher quality.

*Peak season was defined as November, December, January and February. If a study covers all these months, it covers peak seasons of AI, if a study covers one or two of these months, it is close to peak season of AI, if a study doesn’t cover any of these months, it doesn’t cover peak seasons of AI.

Table A-4 Quality assessment for studies reporting effect of biosecurity indicators on poultry/environmental infection at LBMs

ID	Study Name	C1: Study type	C1: Score	C2: Type of analysis	C2: Score	C3: Timing of study	C3: Score	C4: Market type	C4: Score	C5: Number of counties	C5: Score	C6: Number of LBMs	C6: Score	C7: Sample type	C7: Score	Total Score
1	Bulaga 2003[21]	Cross-sectional	1	Multivariate	1	no	0	Retail	0	>5	2	109	2	Both	2	8
2	Kung 2003[163]	Longitudinal	2	Univariate	0	no	0	Retail	0	N/A, HK	1	8	1	Poultry	0	4
3	Gaber 2007[247]	Case-control	0	Multivariate	1	yes	2	N/A, both	1	7 area	2	78	2	Both	2	10
4	Trock 2008[246]	Longitudinal	2	Univariate	0	close to	1	N/A, both	1	N/A, NY city area, 4	1	70	2	Both	2	9
5	Indriani 2010[36]	Cross-sectional	1	Multivariate	1	yes	2	Both	2	16	2	83	2	Environmental	1	11
6	LiLH 2010[309]	Longitudinal	2	Univariate	0	yes	2	Both	2	7	2	42	2	Environmental	1	11
7	Martin 2011[223]	Cross-sectional	1	Multivariate	1	no	0	Both	2	> 5	2	30	2	Poultry	0	8
8	Leung 2012[237]	Longitudinal	2	Multivariate	1	yes	2	Both	2	3	1	8	1	Poultry	0	9
9	ZhangRS 2012[310]	Longitudinal	2	Univariate	0	close to	1	Both	2	2	1	4	0	Environmental	1	7
10	BiFY 2013[311]	Longitudinal	2	Univariate	0	yes	2	Both	2	14+	2	14+	2	Environmental	1	11
11	Phan 2013[312]	Cross-sectional	1	Multivariate	1	close to	1	N/A, both	1	39	2	78	2	Poultry	0	8
12	ChenZ 2014[259]	Longitudinal	2	Univariate	0	yes	2	Both	2	12	2	24	2	Both	2	12
13	LiuH 2014[225]	Longitudinal	2	Univariate	0	close to	1	N/A, both	1	3	1	5	0	Environmental	1	6
14	WangDF 2014[313]	Longitudinal	2	Univariate	0	yes	2	Retail	0	1	0	4	0	Environmental	1	5
15	YuM 2014[252]	Longitudinal	2	Univariate	0	yes	2	N/A, both	1	10	2	30	2	Environmental	1	10
16	YuanJ 2014[245]	Longitudinal	2	Univariate	0	no	0	Both	2	>5	2	144	2	Both	2	10
17	ZhuBL 2014[227]	Longitudinal	2	Univariate	0	no	0	N/A, retail	0	1	0	10	1	Environmental	1	4

18	ZhuJL 2014[314]	cross-sectional	1	Univariate	0	close to	1	N/A, retail	0	9	2	49	2	Environmental	1	7
19	CuiXB 2015[260]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	1	0	N/A, <5	0	Environmental	1	5
20	ElMasry 2015[249]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	24	2	257	2	Poultry	0	8
21	HuangFJ 2015[315]	case-control	1	Univariate	0	no	0	Both	2	1	0	1	0	Environmental	1	4
22	KangM 2015[224]	Longitudinal	2	Univariate	0	yes	2	Both	2	4 prefectures	1	31	2	Environmental	1	10
23	LiuW 2015[316]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	6	2	6	1	Environmental	1	8
24	WangFY1 2015[317]	Longitudinal	2	Univariate	0	yes	2	Retail	0	9	2	21	2	Environmental	1	9
25	WangFY2 2015[318]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	10	2	109	2	Environmental	1	9
26	WuJ 2015[319]	Longitudinal	2	Univariate	0	yes	2	Both	2	21	2	369	2	Environmental	1	11
27	XuGF 2015[320]	Longitudinal	2	Univariate	0	yes	2	Both	2	1	0	2	0	Environmental	1	7
28	YuanJ 2015[226]	Longitudinal	2	Multivariate	1	close to	1	Retail	0	1	0	4	0	Environmental	1	5
29	ZhaoZF 2015[321]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	1	0	N/A, <5	0	Environmental	1	5
30	CaoL1 2016[254]	Longitudinal	2	Univariate	0	yes	2	Both	2	12	2	>22	2	Environmental	1	11
31	CaoL2 2016[322]	Longitudinal	2	Univariate	0	yes	2	Both	2	12	2	20+	2	Environmental	1	11
32	LiWQ 2016[323]	Longitudinal	2	Univariate	0	yes	2	Both	2	1	0	22	2	Environmental	1	9
33	LiuFR1 2016[324]	Longitudinal	2	Univariate	0	yes	2	Both	2	1	0	16	2	Environmental	1	9
34	LiuFR2 2016[325]	Longitudinal	2	Univariate	0	yes	2	Both	2	1	0	37	2	Environmental	1	9
35	LiuJW 2016[326]	Longitudinal	2	Univariate	0	yes	2	Both	2	12	2	>=319	2	Environmental	1	11
36	MengJH 2016[327]	Longitudinal	2	Univariate	0	yes	2	Both	2	4	1	10	1	Environmental	1	9
37	NongXN 2016[328]	Longitudinal	2	Univariate	0	yes	2	N/A	0	N/A	0	N/A	0	Environmental	1	5

38	PengC 2016[329]	Longitudinal	2	Univariate	0	No	0	Both	2	1	0	3	0	Environmental	1	5
39	WangXX 2016[330]	Longitudinal	2	Multivariate	1	close to	1	Both	2	11 prefectures	2	237	2	Environmental	1	11
40	XieCJ 2016[248]	Longitudinal	2	Univariate	0	yes	2	Retail	0	4 districts	1	40	2	Environmental	1	8
41	ZengJJ 2016[331]	Longitudinal	2	Univariate	0	yes	2	Both	2	7	2	N/A	0	Environmental	1	9
42	ZengL 2016[243]	Longitudinal	2	Univariate	0	yes	2	Both	2	10	2	31	2	Environmental	1	11
43	Abu Sayeed 2017[332]	Cross-sectional	1	Univariate	0	no	0	Both	2	1	0	40	2	Environmental	1	6
44	ChenC 2017[333]	Longitudinal	2	Multivariate	1	yes	2	Both	2	12	2	5~6	1	Environmental	1	11
45	ChenXD 2017[229]	Longitudinal	2	Univariate	0	close to	1	Both	2	1	0	3	0	Both	2	7
46	Chu 2017[334]	cross-sectional	1	Univariate	0	close to	1	Both	2	4	1	7	1	Poultry	0	6
47	DaiYX 2017[335]	Longitudinal	2	Univariate	0	close to	1	Both	2	5	1	30	2	Environmental	1	9
48	LiH 2017[336]	Longitudinal	2	Univariate	0	yes	2	Both	2	N/A, 2+	1	5	0	Environmental	1	8
49	LiLZ 2017[337]	Longitudinal	2	Univariate	0	yes	2	Both	2	9	2	10+	2	Environmental	1	11
50	LiuM 2017[338]	Longitudinal	2	Univariate	0	yes	2	Both	2	15	2	15+	2	Environmental	1	11
51	LiuRC 2017[339]	Longitudinal	2	Multivariate	1	yes	2	Both	2	9	2	64	2	Environmental	1	12
52	MaoXX 2017[340]	Longitudinal	2	Univariate	0	close to	1	Both	2	4	1	4	0	Poultry	0	6
53	QianLM 2017[341]	Longitudinal	2	Univariate	0	yes	2	N/A	0	1	0	N/A		Environmental	1	5
54	TuZJ 2017[342]	Longitudinal	2	Univariate	0	close to		Retail	0	1	0	6	1	Environmental	1	4
55	YuXF 2017[343]	cross-sectional	1	Univariate	0	close to	1	Both	2	13	2	>13	2	Environmental	1	9
56	ZhangHB 2017[344]	Longitudinal	2	Univariate	0	yes	2	wholesale	1	1	0	2	0	Poultry	0	5

57	BaoJ 2018[345]	Longitudinal	2	Univariate	0	yes	2	Both	2	N/A 2+	1	N/A	0	Poultry	0	7
58	CaoL3 2018[346]	Longitudinal	2	Univariate	0	yes	2	Both	2	11	2	11+	2	Both	2	12
59	CaoL4 2018[244]	Longitudinal	2	Univariate	0	close to	1	Both	2	6	2	18	2	Environmental	1	10
60	ChenYH 2018[347]	Cross-sectional	1	Multivariate	1	no	0	wholesale	1	1	0	2	0	Poultry	0	3
61	FanSY 2018[348]	Longitudinal	2	Univariate	0	yes	2	N/A, retail	0	1	0	16	2	Environmental	1	7
62	Hassan 2018[349]	Longitudinal	2	Univariate	0	yes	2	Both	2	N/A 2+	1	10	1	Poultry	0	8
63	LiuXQ 2018[350]	Longitudinal	2	Univariate	0	yes	2	Both	2	15	2	15	2	Environmental	1	11
64	WuQ 2018[351]	Longitudinal	2	Univariate	0	yes	2	N/A	0	N/A 1	0	11	2	Environmental	1	7
65	XuJ 2018[352]	Longitudinal	2	Univariate	0	yes	2	Retail	0	1	0	31	2	Environmental	1	7
66	YangJ 2018[353]	Longitudinal	2	Multivariate	1	yes	2	Both	2	7	2	N/A		Environmental	1	10
67	YangY 2018[354]	Cross-sectional	1	Univariate	0	no	0	N/A, wholesale	0	5	1	105	2	Environmental	1	5
68	YaoJX 2018[355]	Longitudinal	2	Univariate	0	yes	2	Retail	0	1	0	1	0	Environmental	1	5
69	ZouLB 2018[356]	Longitudinal	2	Univariate	0	yes	2	wholesale	1	9	2	10	1	Both	2	10

Table A-5 Quality assessment for studies reporting effect of biosecurity indicators on human infection

ID	Study Name	C1: Study type	C1: Score	C2: Type of analysis	C2: Score	C3: Timing of study	C3: Score	C4: Market type	C4: Score	C5: Number of counties	C5: Score	C6: Number of LBMs	C6: Score	C8: Number of workers	C8: Score	Total Score
1	LiuY 2009[257]	cross-sectional	1	Univariate analysis	0	yes	2	both	2	12	2	59	2	702	2	11
2	WangM 2009[258]	cross-sectional	1	Univariate analysis	0	yes	2	both	2	11	2	61	2	496	2	11
3	ZhangRS 2012[310]	cross-sectional	1	Univariate analysis	0	close to	1	NA, both	1	2	1	4	0	102	1	5
4	Uyeki 2012[357]	cross-sectional	1	Univariate analysis	0	no	0	NA, both	1	1 province	0	11	2	200	1	5
5	Nasreen 2013[232]	cross-sectional	1	Univariate analysis	0	yes	2	both	2	1	0	3	0	210	1	6
6	Dung 2014[262]	cross-sectional	1	Univariate analysis	0	no	0	NA	0	3 provinces	1	5	0	607	2	4
7	TangXJ 2014[358]	cross-sectional	1	Univariate and multivariate	1	close to	1	retail	0	10	2	10+	2	250	1	8
8	WangX 2014[256]	longitudinal	2	Univariate analysis	0	close to	1	both	2	10	2	24	2	96	0	9
9	WangQ 2015[359]	cross-sectional	1	Univariate analysis	0	yes	2	NA	0	2	1	137	2	826	2	8
10	Nasreen 2015[231]	longitudinal	2	Univariate analysis	0	yes	2	both	2	4	1	12	2	290	1	10
11	FanSY 2018[348]	Longitudinal	2	Univariate analysis	0	yes	2	retail	0	1	0	16	2	290	1	7
12	WangX 2018[360]	longitudinal	2	Univariate and multivariate	1	close to	1	both	2	10	2	13+	2	366	2	12

Table A-6 Effect size of biosecurity factors in Group A: Market Characteristics on Market Infection

Study ID	Category	Group	Factor ID	Factor Name	ES	Lo 95% CI	Hi 95% CI	Qi
6	A	Market type (Wholesale vs Retail)	Market type 1	LiLH 2010-Markettype	0.47	0.38	0.59	0.92
12	A		Market type 2	ChenZ 2014-Markettype	0.35	0.19	0.63	1.00
16	A		Market type 3	YuanJ 2014-Markettype	0.51	0.31	0.85	0.83
26	A		Market type 4	WuJ 2015-Markettype	0.76	0.70	0.83	0.92
27	A		Market type 5	XuGF 2015-Markettype	0.12	0.06	0.21	0.58
32	A		Market type 6	LiWQ 2016-Markettype	0.27	0.13	0.55	0.75
33	A		Market type 7	LiuFR1 2016-Markettype	0.59	0.27	1.29	0.75
34	A		Market type 8	LiuFR2 2016-Markettype	0.63	0.34	1.78	0.75
35	A		Market type 9	LiuJW 2016-Markettype	0.36	0.23	0.57	0.92
41	A		Market type 10	ZengJJ 2016-Markettype	0.98	0.70	1.39	0.75
47	A		Market type 11	DaiYX 2017-Markettype	1.11	0.52	2.39	0.75
48	A		Market type 12	LiH 2017-Markettype	0.01	0.00	0.08	0.67
50	A		Market type 13	LiuM 2017-Markettype	3.62	2.27	5.79	0.92
51	A		Market type 14	LiuRC 2017-Markettype	0.58	0.43	0.79	1.00
52	A		Market type 15	MaoXX 2017-Markettype	0.44	0.34	0.56	0.50
57	A		Market type 16	BaoJ 2018-Markettype	1.94	1.45	2.58	0.58
66	A		Market type 17	YangJ 2018-Markettype	0.83	0.60	1.14	0.83
1	A	Market size (Smaller vs Bigger)	Market size 1	Bulaga 2003-market size	0.47	0.20	1.10	0.67
5	A		Market size 2	Indriani 2010-market size	0.15	0.02	0.85	0.92
24	A		Market size 3	WangFY1 2015-market size	0.47	0.30	0.76	0.75
39	A		Market size 4	WangXX 2016-market size	0.85	0.49	1.45	0.92
9	A	Market location (Rural vs Urban)	Location rural vs urban 1	ZhangRS 2012-market location	1.82	0.92	3.60	0.58
17	A		Location rural vs urban 2	ZhuBL 2014-market location	0.09	0.04	0.20	0.33
18	A		Location rural vs urban 3	ZhuJL 2014-market location	1.45	0.44	4.76	0.58
19	A		Location rural vs urban 4	CuiXB 2015-market location	1.24	0.57	2.71	0.42

20	A		Location rural vs urban 5	ElMasry 2015-market location	0.53	0.38	0.74	0.67
24	A		Location rural vs urban 6	WangFY1 2015-market location	0.57	0.34	0.96	0.75
25	A		Location rural vs urban 7	WangFY2 2015-market location	1.03	0.80	1.32	0.75
39	A		Location rural vs urban 8	WangXX 2016-market location	1.45	0.79	2.66	0.92
55	A		Location rural vs urban 9	YuXF 2017-market location	0.19	0.08	0.49	0.75
67	A		Location rural vs urban 11	YangY 2018-market location	0.16	0.10	0.26	0.42
31	A	Market location (central or non-centra cityl area)	Market in central city 1	CaoL2 2016-market location	0.94	0.77	1.16	0.92
42	A		Market in central city 2	ZengL 2016-market location	0.13	0.07	0.24	0.92
49	A		Market in central city 3	LiLZ 20170-market location	0.70	0.58	0.86	0.92
58	A		Market in central city 4	CaoL3 2018-market location	0.85	0.72	1.00	1.00
59	A		Market in central city 5	CaoL4 2018-market location	0.66	0.56	0.79	0.83
65	A		Market in central city 6	XuJ 2018-market location	0.79	0.59	1.07	0.58
13	A	Presence of other species (No vs Yes)	Other species 1	LiuH 2014-other species	0.06	0.00	0.83	0.50
39	A		Other species 2	WangXX 2016-other species	0.22	0.08	0.61	0.92
43	A		Other species 3	Abu Sayeed 2017-other species	0.53	0.30	0.96	0.50
5	A	Presence of duck (No vs Yes)	Duck present 1	Indriani 2010-duck present	0.33	0.13	0.82	0.92
43	A		Duck present 2	Abu Sayeed 2017-duck present	0.40	0.24	0.67	0.50
1	A	Presence of rabits (No vs Yes)	Presence of rabbits 1	Bulaga 2003-rabits present	0.24	0.10	0.63	0.67
3	A		Presence of rabbits 2	Gaber 2007-rabits present	0.31	0.26	0.38	0.83
39	A	Other	Structure 1	WangXX 2016-market structure	0.64	0.3674	1.1	0.92
39	A		Tradestructure 1	WangXX 2016-poultry trading area-closed	0.71	0.4027	1.26	0.92
39	A		Tradestructure 2	WangXX 2016-poultry trading area close to other products	1.28	0.7123	2.3	0.92

Table A-7 Effect size of biosecurity factors in Group B: Market Biosecurity Management on Market Infection

Study ID	Category	Group	Factor ID	Factor Name	ES	Lo 95% CI	Hi 95% CI	Qi
1	B	Conduct clean and disinfection (C/D)	C/D 1	Bulaga 2003-C/D	0.39	0.16	0.95	0.73
3	B		C/D 2	Gaber 2007-C/D	0.30	0.26	0.36	0.91
7	B		C/D 3	Martin 2011-C/D	0.11	0.01	1.02	0.73
24	B		C/D 4	WangFY1 2015-C/D	0.58	0.37	0.92	0.82
39	B		C/D 5	WangXX 2016-C/D	0.37	0.10	1.33	1.00
33	B		C/D 6	LiuFR1 2016-C/D	0.60	0.24	1.48	0.82
60	B		C/D 7	ChenYH 2018-C/D	0.86	0.61	1.23	0.27
4	B	Before and after C/D	B&A C/D 1	Trock 2008-B&A C/D	0.17	0.12	0.23	0.82
16	B		B&A C/D 2	YuanJ 2014-B&A C/D	0.76	0.57	1.02	0.91
3	B	Waste disposal	Waste disposal 1	Gaber 2007-Wastedisposal	0.42	0.29	0.56	0.91
5	B		Waste disposal 2	Indriani 2010-Wastedisposal	0.20	0.06	0.69	1.00
7	B		Waste disposal 3	Martin 2011-Wastedisposal	0.05	0.01	0.32	0.73
1	B	Conduct market closure	Market closure 1	Bulaga 2003-Market closure	0.29	0.12	0.70	0.73
3	B		Market closure 2	Gaber 2007-Market closure	0.48	0.56	0.39	0.91
8	B		Market closure 3	Leung 2012-Market closure	0.59	0.32	1.10	0.82
39	B		Market closure 4	WangXX 2016-Market closure	0.43	0.14	1.39	1.00
6	B		Market closure 5	LiLH 2010-Market closure	0.85	0.69	1.05	1.00
53	B		Market closure 6	QianLM 2017-Market closure	0.08	0.04	0.18	0.45
2	B	Before and after market closure	B&A Closure 1	Kung 2003-B&A Closure	0.07	0.03	0.19	0.36
13	B		B&A Closure 2	LiuH 2014-B&A Closure	0.31	0.15	0.66	0.55
17	B		B&A Closure 3	ZhuBL 2014-B&A Closure	0.02	0.01	0.08	0.36
22	B		B&A Closure 4	KangM 2015-B&A Closure	0.10	0.04	0.24	0.91
28	B		B&A Closure 5	YuanJ 2015-B&A Closure	0.25	0.16	0.39	0.45
38	B		B&A Closure 6	PengC 2016-B&A Closure	0.25	0.21	0.30	0.45
45	B		B&A Closure 7	ChenXD 2017-B&A Closure	0.76	0.32	1.77	0.64

8	B	Ban on overnight storage	Overnight storage 1	Leung 2012-Storage	0.16	0.04	0.60	0.82
13	B		Overnight storage 2	LiuH 2014-Storage	0.05	0.00	0.67	0.55
39	B		Overnight storage 3	WangXX 2016-Storage	0.75	0.42	1.33	1.00
46	B		Overnight storage 4	Chu 2017-Storage	0.72	0.49	1.07	0.55
33	B		Overnight storage 5	LiuFR1 2016-Storage	0.42	0.20	0.88	0.82
39	B	Import poultry from local or other area	Poultrysource 1	WangXX 2016-Poultrysource	0.46	0.20	1.04	1.00
46	B		Poultrysource 2	Chu 2017-Poultrysource	0.36	0.25	0.53	0.55
47	B		Poultrysource 3	DaiYX 2017-Poultrysource	0.45	0.25	0.79	0.82
56	B		Poultrysource 4	ZhangHB 2017-Poultrysource	0.93	0.70	1.22	0.45
60	B		Poultrysource 5	ChenYH 2018-Poultrysource	1.08	0.73	1.59	0.27
5	B	Separate different species	Mixspecies 1	Indriani 2010-Mixspecies	0.34	0.11	1.02	1.00
6	B		Mixspecies 2	LiLH 2010-Mixspecies	0.63	0.54	0.74	1.00
5	B	Conduct slaughter in market (No vs Yes)	SlaughterInMarket 1	Indriani 2010-Slaughter in market	0.16	0.02	0.99	1.00
40	B		SlaughterInMarket 2	XieCJ 2016-Slaughter in market	0.70	0.56	0.89	0.73
46	B	Others	Washhands	Chu 2017	1.73	1.18	2.54	0.50
21	B		Wearmask 1	HuangFJ 2015	0.69	0.10	4.72	0.33
46	B		Weargloves 1	Chu 2017	0.70	0.45	1.06	0.50
24	B		Quarantine 1	WangFY1 2015	0.91	0.59	1.39	0.75
11	B		Multisource 1	Phan 2013	0.41	0.22	0.75	0.67
20	B		Poultryorigin 1	ElMasry 2015	0.58	0.36	0.94	0.67
60	B		Poultryorigin 2	ChenYH 2018	0.59	0.41	0.85	0.25
39	B		Tradehour 1	WangXX 2016	0.72	0.38	1.37	0.92
5	B		Clearzone 1	Indriani 2010	0.16	0.03	0.86	0.92
47	B		Disposal	DaiYX 2017	0.94	0.54	1.65	0.75
5	B		Table 1	Indriani 2010	0.26	0.10	0.65	0.92
43	B		Bird hodling area	Abu Sayeed 2017	0.53	0.29	0.91	0.50
5	B		Stackcages 1	Indriani 2010	0.38	0.13	1.10	0.92

1	B		Mixnewbird 1	Bulaga 2003	0.43	0.16	1.19	0.67
43	B		Seperation sick	Abu Sayeed 2017	0.59	0.25	1.39	0.50
5	B		Pigeon present	Indriani 2010	0.33	0.10	1.04	0.92
43	B		Access of wild birds	Abu Sayeed 2017	0.18	0.09	0.37	0.50
11	B		Duckage 1	Phan 2013	0.20	0.08	0.51	0.67
11	B		Sellpublic 1	Phan 2013	0.53	0.29	0.97	0.67
43	B		Hygien of stalls	Abu Sayeed 2017	0.32	0.18	0.59	0.50
46	B		Sex	Chu 2017	0.57	0.27	1.22	0.50
46	B		Workyear	Chu 2017	1.09	0.70	1.70	0.50
46	B		Education	Chu 2017	2.62	1.33	5.13	0.50
8	B		Ventilation 1	Leung 2012	0.71	0.42	1.22	0.75
33	B		Ventilation 2	LiuFR1 2016	0.51	0.24	1.08	0.75

Table A-8 Effect size of biosecurity factors in Group C: Seasonality

Study ID	Category	Group	Factor ID	Factor	ES	Lo 95% CI	Hi 95% CI	Qi
10	C	Season (Sumr&Atmn vs Sprg&Witr)	Season 1	BiFY 2013	0.90	0.75	1.07	1.00
14	C		Season 2	WangDF 2014	1.35	0.72	2.53	0.45
15	C		Season 3	YuM 2014	0.32	0.26	0.40	0.91
20	C		Season 4	ElMasry 2015	1.15	0.83	1.59	0.73
23	C		Season 5	LiuW 2015	0.30	0.15	0.61	0.73
24	C		Season 6	WangFY1 2015	0.31	0.16	0.59	0.82
25	C		Season 7	WangFY2 2015	0.40	0.30	0.52	0.82
29	C		Season 8	ZhaoZF 2015	0.79	0.44	1.43	0.45
30	C		Season 9	CaoL1 2016	0.09	0.04	0.20	1.00
36	C		Season 10	MengJH 2016	0.33	0.21	0.53	0.82
37	C		Season 11	NongXN 2016	0.81	0.48	1.38	0.45
49	C		Season 12	LiLZ 2017	1.06	0.86	1.30	1.00
54	C		Season 13	TuZJ 2017	0.36	0.14	0.93	0.36
61	C		Season 14	FanSY 2018	1.48	0.94	2.33	0.64
62	C		Season 15	Hassan 2018	0.29	0.25	0.34	0.73
63	C		Season 16	LiuXQ 2018	0.78	0.68	0.90	1.00
64	C		Season 17	WuQ 2018	0.47	0.27	0.83	0.64
65	C		Season 18	XuJ 2018	0.84	0.63	1.14	0.64
68	C		Season 19	YaoJX 2018	0.28	0.19	0.39	0.45
69	C		Season 20	ZouLB 2018	0.81	0.74	0.89	0.91
8	C	Temperature	Temperature 1	Leung 2012	0.98	0.99	1.02	0.82
44	C		Temperature 2	ChenC 2017	0.99	0.99	1.00	1.00

Table A-9 Effect size of biosecurity factors in Group D: Socio-demographic Characteristics on Human Infection

Study ID	Category	Group	Factor ID	Factor	ES	Lo 95% CI	Hi 95% CI	Qi
7	D	Sex (Male vs Female)	Sex 1	TangXJ 2014-Sex	0.74	0.37	1.47	0.8
6	D		Sex 2	Dung 2014-Sex	0.99	0.50	2.00	0.4
8	D		Sex 3	WangX 2014-Sex	0.73	0.56	0.95	0.8
12	D		Sex 4	WangX 2018-Sex	0.40	0.17	0.91	1
6	D	Age group (>=40 vs <40)	Age 1	Dung 2014-Age	0.70	0.34	1.42	0.4
4	D		Age 2	Uyeki 2012-Age	0.53	0.10	2.97	0.3
8	D		Age 3	WangX 2014-Age	1.51	1.15	1.97	0.8
7	D		Age 4	TangXJ 2014-Age	0.97	0.49	1.94	0.8
9	D		Age 5	WangQ 2015-Age	0.83	0.55	1.27	0.6
7	D	Years of working in LBMs (<10y vs >=10 y)	Workyear 1	TangXJ 2014-Workyear	0.74	0.36	1.55	0.8
8	D		Workyear 2	WangX 2014-Workyear	0.36	0.17	0.76	0.8
1	D	Type of market (Wholesale vs Retail)	Markettype 1	LiuY 2009-Markettype	0.44	0.26	0.76	0.8
8	D		Markettype 2	WangX 2014-Markettype	0.15	0.06	0.40	0.8
2	D		Markettype 3	WangM 2009-Markettype	0.43	0.24	0.76	0.8
7	D	Flu vaccination history (Yes vs No)	Vaccination 1	TangXJ 2014-Vaccination	0.80	0.39	1.66	0.8
8	D		Vaccination 2	WangX 2014-Vaccination	0.17	0.06	0.48	0.8
12	D		Vaccination 3	WangX 2018-Vaccination	1.16	0.44	3.04	1
6	D	Occupation (not seller vs seller)	Occupation 1	Dung 2014-Occupation	0.31	0.13	0.74	0.4
12	D		Occupation 2	WangX 2018-Occupation	0.36	0.14	0.94	1
7	D	Others	Other occupation	TangXJ 2014-Occupation	0.42	0.17	1.06	0.67
6	D		Education 1	Dung 2014-Education	1.68	0.39	7.20	0.33
3	D		Marketlocation 1	ZhangRS 2012-Marketlocation	0.25	0.09	0.74	0.42
6	D		Medicalhistory 1	Dung 2014-Medicalhistory	0.76	0.31	1.88	0.33
11	D		Infection status of markets	FanSY 2018-Marketinfection	0.47	0.29	0.76	0.58
10	D		Smoke 1	Nasreen 2015-Smoke	2.21	0.83	5.88	0.83

Table A-10 Effect size of biosecurity factors in Group E: Exposure to Poultry on Human Infection

Study ID	Category	Group	Factor ID	Factor	ES	Lo 95% CI	Hi 95% CI	Qi
5	E	Clean feeding tray	Clean feeding tray 1	Nasreen 2013-Cleanfeedtray	0.53	0.12	2.29	0.60
10	E		Clean feeding tray 2	Nasreen 2015-Cleanfeedtray	0.27	0.08	0.95	0.90
5	E	Clean water tray	Clean water tray 1	Nasreen 2013-Cleanwatertray	0.60	0.14	2.58	0.60
10	E		Clean water tray 2	Nasreen 2015-Cleanwatertray	0.17	0.04	0.73	0.90
10	E	Clean feces	Clean feces 1	Nasreen 2015-Cleanfeces	0.24	0.08	0.76	0.90
5	E		Clean feces 2	Nasreen 2013-Cleanfeces	1.01	0.23	4.33	0.60
5	E	Feed poultry	Feed poultry 1	Nasreen 2013-Feedpoultry	0.68	0.16	2.90	0.60
10	E		Feed poultry 2	Nasreen 2015-Feedpoultry	0.15	0.02	1.16	0.90
7	E	Contact duck	Contact duck 1	TangXJ 2014-Contactduck	0.26	0.10	0.69	0.80
12	E		Contact duck 2	WangX 2018-Contactduck	0.31	0.07	1.36	1.00
7	E	Contact goose	Contact goose 1	TangXJ 2014-Contactgoose	1.10	0.39	3.13	0.80
12	E		Contact goose 2	WangX 2018-Contactgoose	0.43	0.16	1.11	1.00
7	E	Contact pigeon	Contact pigeon 1	TangXJ 2014-Contactpigeon	0.68	0.26	1.79	0.80
12	E		Contact pigeon 2	WangX 2018-Contactpigeon	0.81	0.35	1.90	1.00
5	E	Conduct slaughtering	Slaughter 1	Nasreen 2013-Slaughter	0.08	0.01	0.70	0.60
10	E		Slaughter 2	Nasreen 2015-Slaughter	0.16	0.02	1.20	0.90
5	E	Conduct defeathering	Defeathering 1	Nasreen 2013-Defeather	0.13	0.03	0.65	0.60
10	E		Defeathering 2	Nasreen 2015-Defeather	0.22	0.06	0.77	0.90
5	E	Conduct eviscerating	Evisceration 1	Nasreen 2013-Evisceration	0.13	0.03	0.68	0.60
10	E		Evisceration 2	Nasreen 2015-Evisceration	0.22	0.06	0.78	0.90
10	E	Others	Contactfeces	Nasreen 2015-Contactfeces	4.11	0.54	31.61	0.83
5	E		Contactdead	Nasreen 2013-Contactdead	3.58	0.81	15.79	0.50
10	E		Stuff poultry into bag	Nasreen 2015-Stuffpoultry	0.20	0.07	0.63	0.83
7	E		Contact chicken	TangXJ 2014-Contactchicken	0.41	0.05	3.26	0.67
7	E		Contact wildbird	TangXJ 2014-Contactwildbird	1.70	0.49	5.92	0.67

10	E		Medicate poultry	Nasreen 2015-Medicatepoultry	0.47	0.10	2.22	0.83
10	E		Isolate sick	Nasreen 2015-Isolatesick	0.73	0.28	1.91	0.83
10	E		Wash hand 1	Nasreen 2015-Washhand	0.77	0.29	2.00	0.83
12	E		Wash hand 2	WangX 2018-Washhand	1.03	0.44	2.42	1.00
10	E		Eat raw poultry or egg	Nasreen 2015-Eatrawpoultry	1.22	0.44	3.35	0.83
12	E		Disinfection frequency	WangX 2018-Disinfection	0.25	0.10	0.61	1.00

A.2 Figures

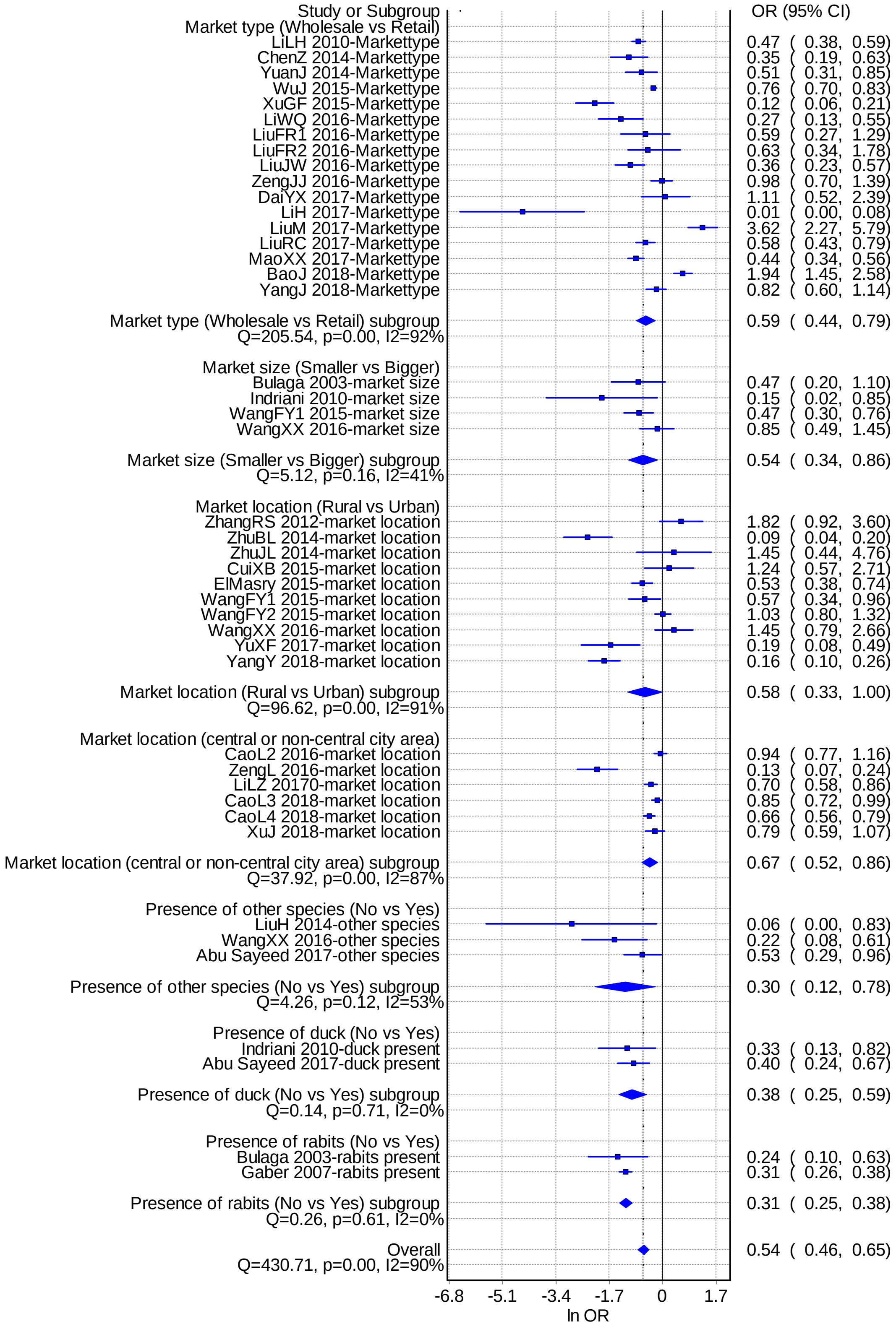


Figure A-1 Forest plots of risk estimates of market characteristics (Group A) on AI market infection using Random Effect Model.

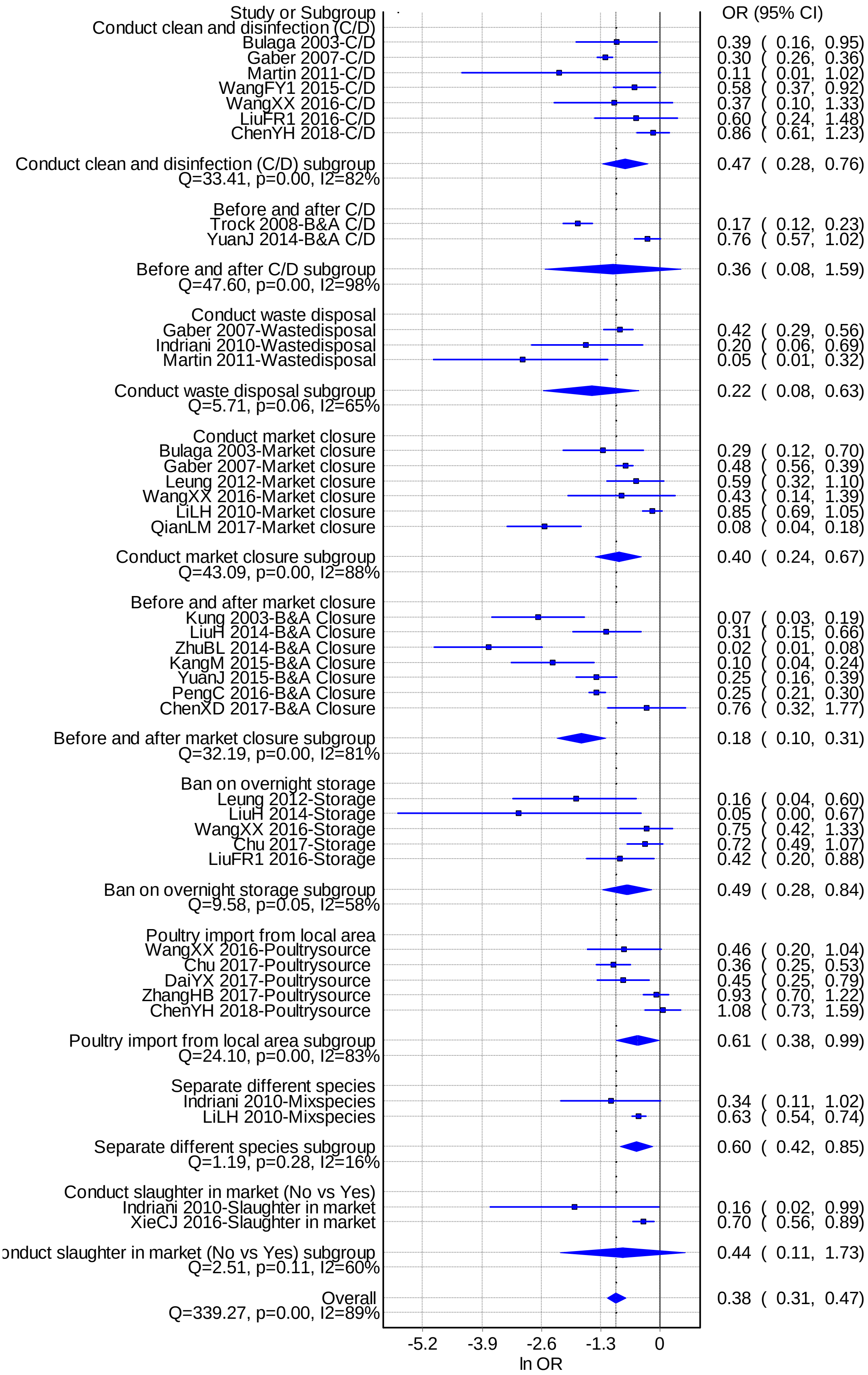


Figure A-2 Forest plots of risk estimates of market biosecurity management (Group B) on AI Market Infection using Random Effect Model.

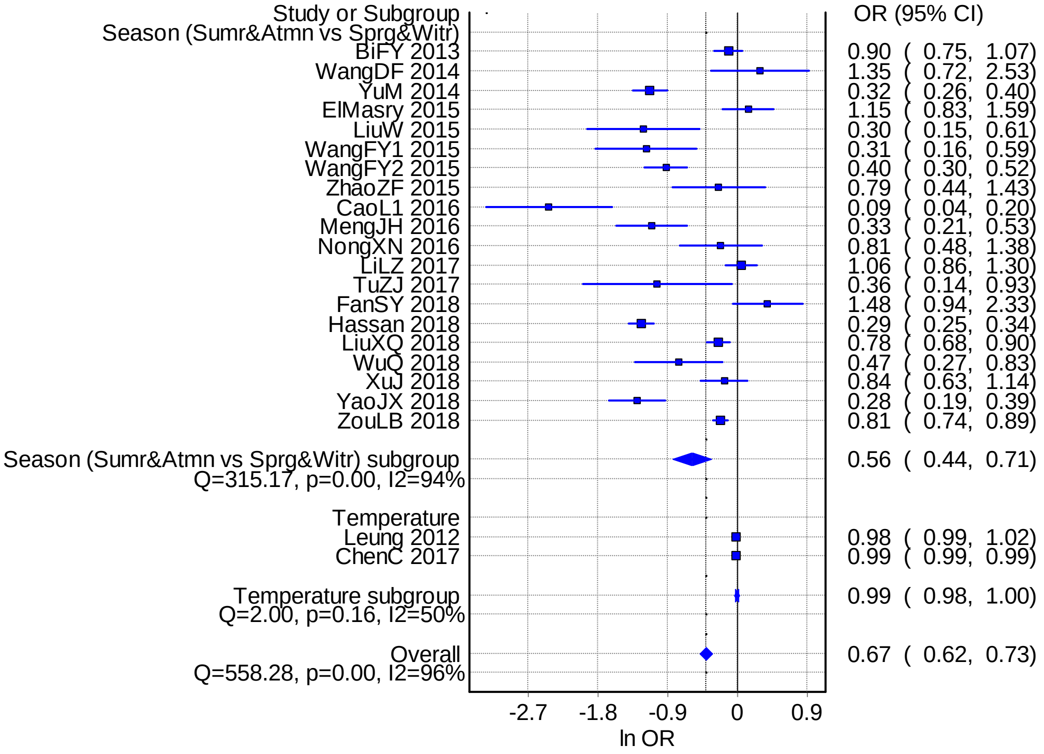


Figure A-3 Forest plots of risk estimates of seasonality (Group C) on AI market infection using Random Effect Model.

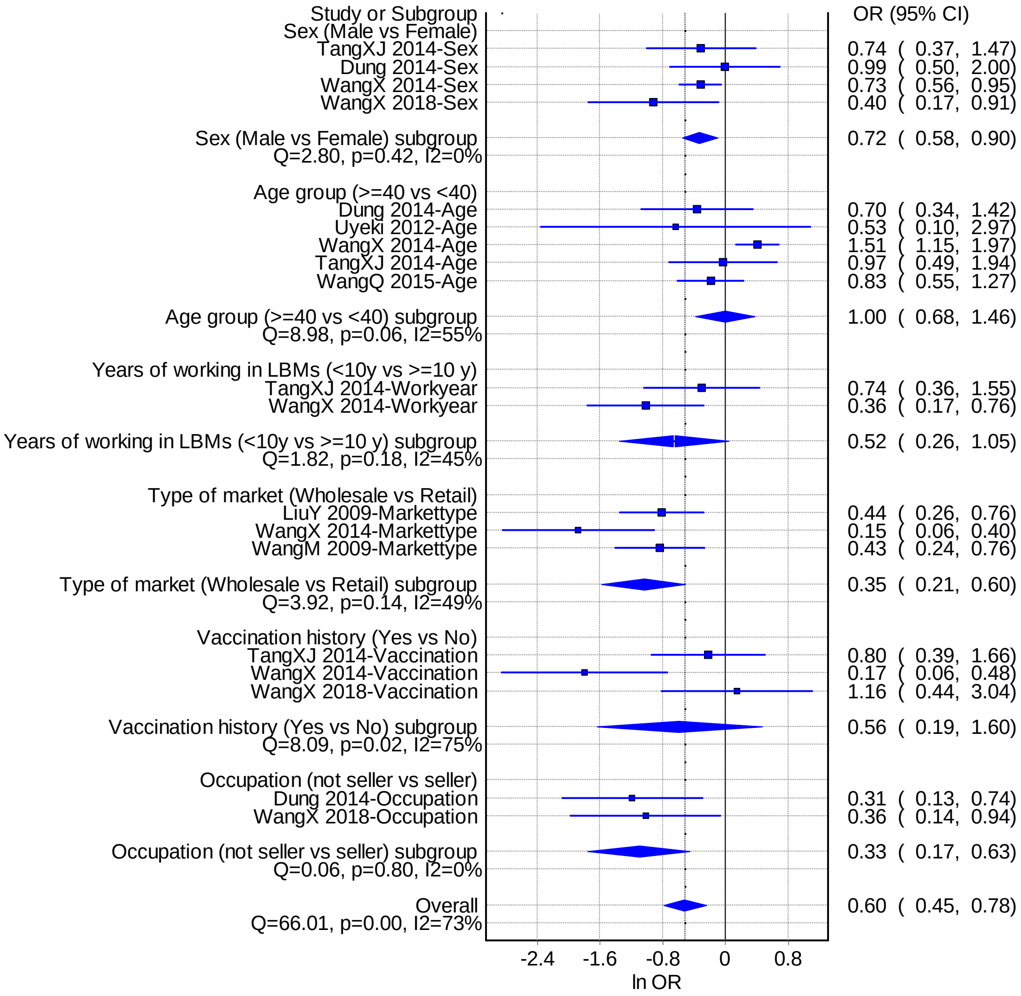


Figure A-4 Forest plots of risk estimates of socio-demographic characteristics (Group D) on Human AI infection using Random Effect Model.

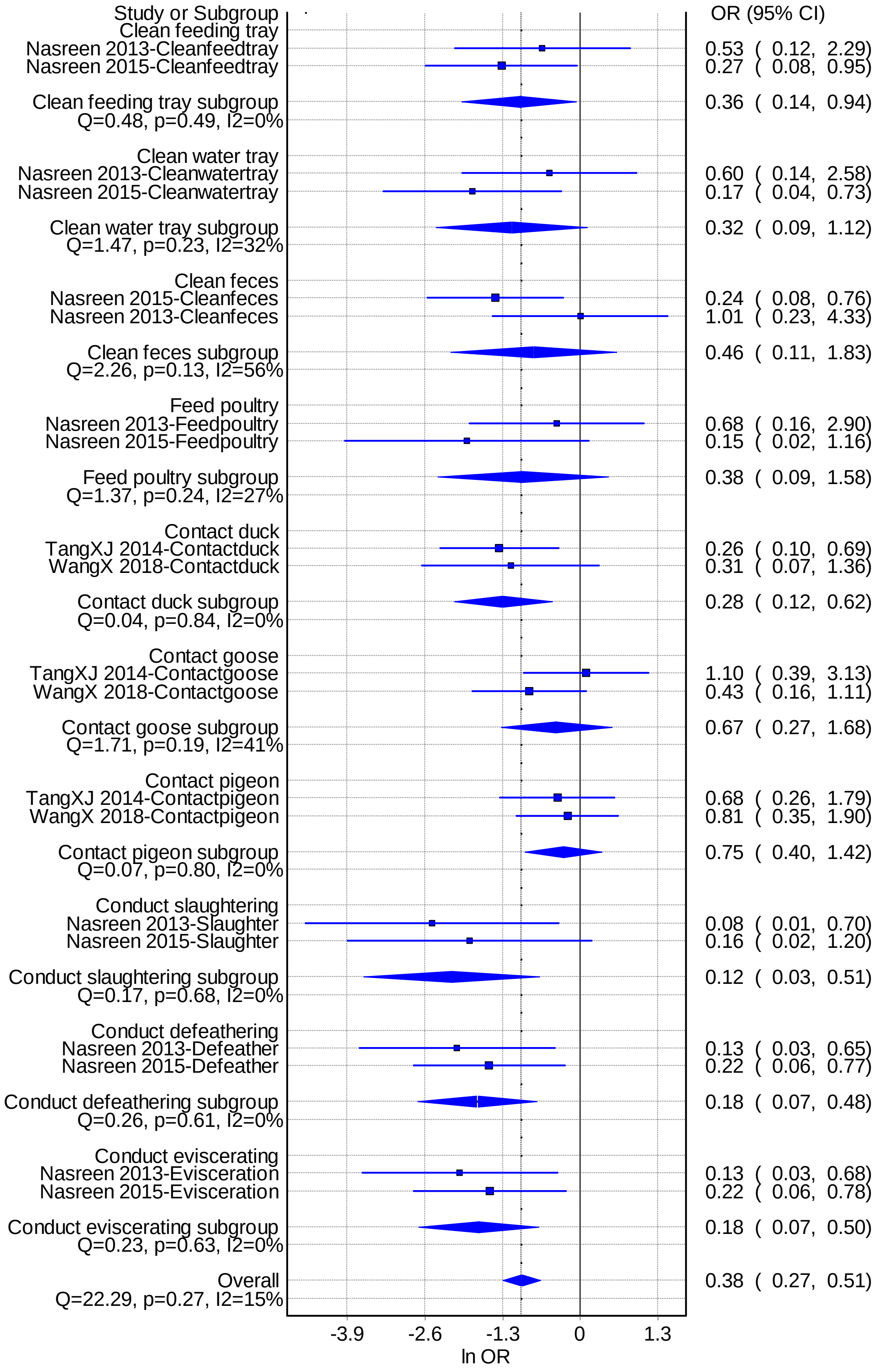


Figure A-5 Forest plots of risk estimates of activities involving exposure to poultry (Group E) on Human AI infection using Random Effect Model.

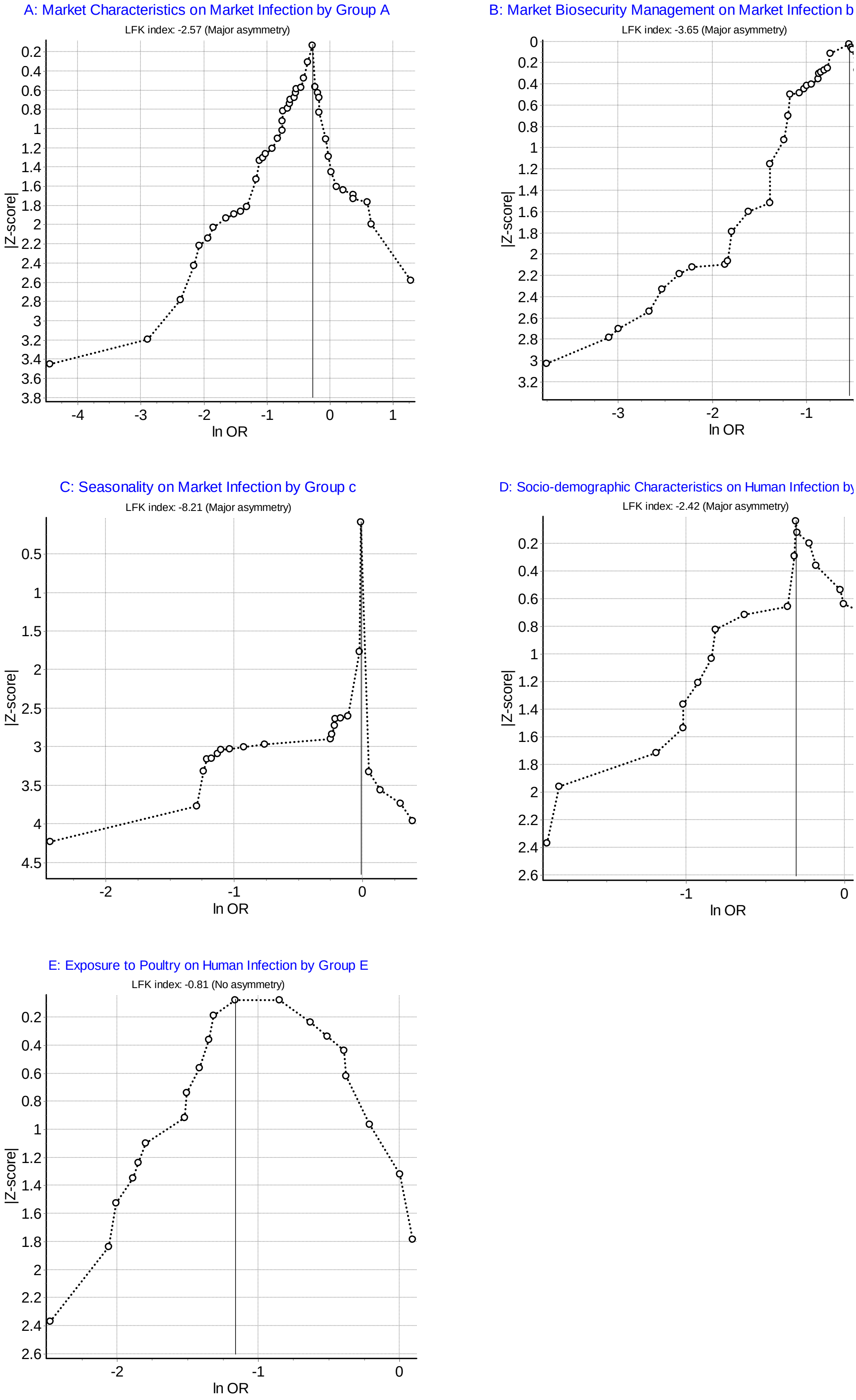


Figure A-6 Doi plots of the five biosecurity groups

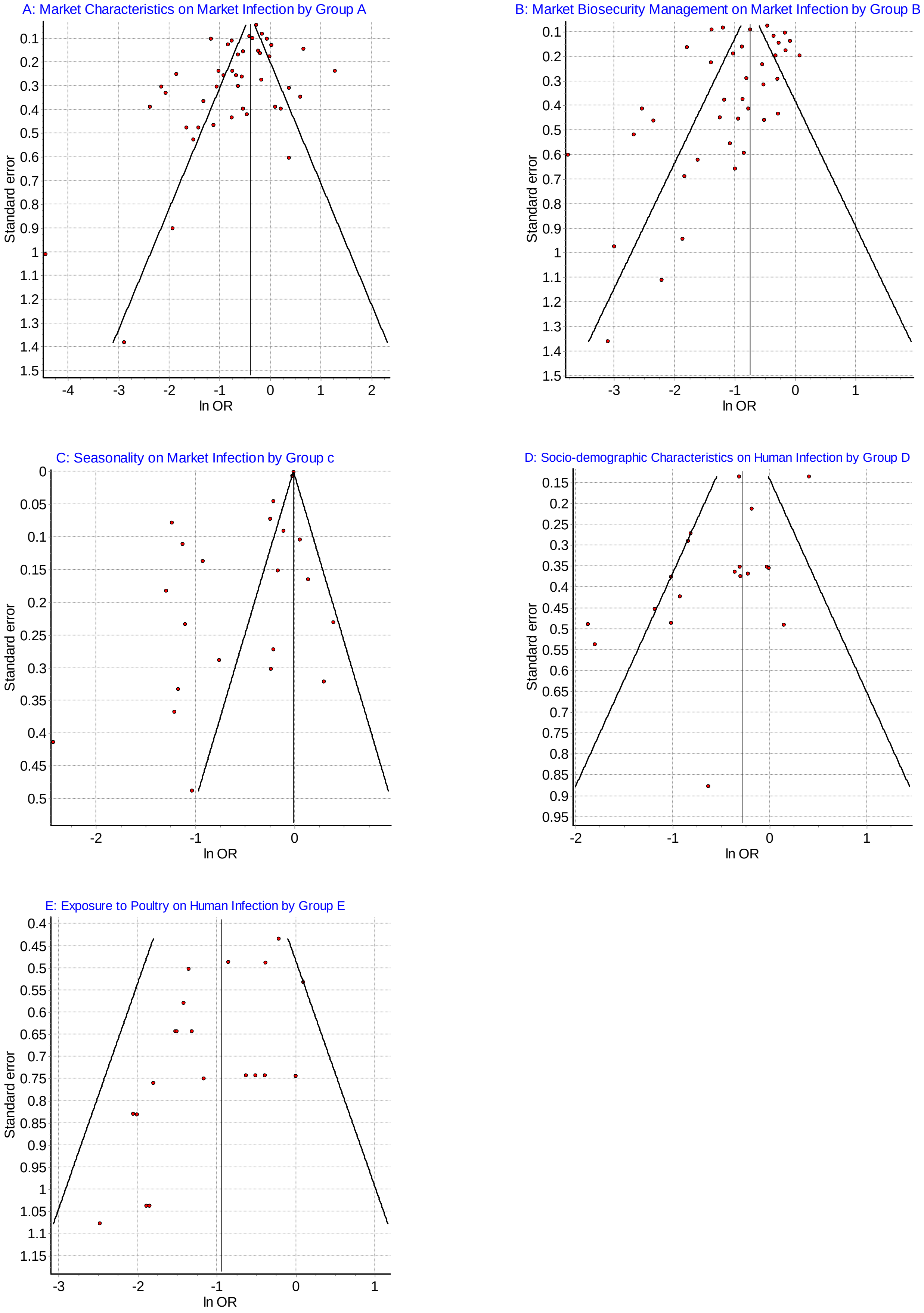


Figure A-7 Funnel plots of the five biosecurity groups

Appendix B Chapter 5 Supplementary Information

B.1 Tables

Table B-1 A full list of the biosecurity indicators included in the univariate analysis.

Market level biosecurity indicators	**Category**
Type of LBMs	Retail
	Wholesale
	Mixed
Type of poultry sold	Sell live birds directly
	Sell live birds and slaughter
Sell multiple species	Yes/No
Volume of yellow fat chicken	Numerical
Sell spent hens	Yes/No
Sell ducks	Yes/No
Sell geese	Yes/No
Sell pigeons	Yes/No
Sell quail	Yes/No
Market structure	Outdoor market
	Indoor market
Wastes collected by specially-assigned person, and then process	Yes/No
Wastes handled by themselves	Yes/No
Wastes hauled in trash	Yes/No
Wastes burned on site	Yes/No
Waste water of market dumped in sewer	Yes/No
Waste water of market dumped everywhere	Yes/No
Waste water from processing birds dumped in sewer	Yes/No
Waste water from processing birds dumped everywhere	Yes/No
Drainage system is covered	Yes/No
Drainage system is flooded with water	Yes/No
Cleaning times per week	Frequency
Disinfection times per week	Frequency
Market closure	Yes/No
Quarantine again before into the market	Yes/No
Sampling regularly	Yes/No
Live birds move to retail markets	Yes/No
Live birds move to other wholesale markets	Yes/No
Sell to consumers directly	Yes/No

Table B-2 Number of chicken source counties and volume of chicken that transported to the surveyed LBMs by provinces.

LBMs in the province of	**Market Name**	**Import chicken from the province**	**Number of source counties**	**Volume of chicken moved**
Shanghai	Sanguantang	Jiangsu	4	1,022,583
		Shanghai	7	130,950
		Zhejiang	1	72,068
	Nongchanpin	Jiangsu	4	513,037
		Shanghai	3	184,600
		Zhejiang	2	133,510
	Huhuai	Jiangsu	1	66,537
		Zhejiang	1	50,816
		Shanghai	1	25,800
Zhejiang	Chengbei	Zhejiang	17	1,199,820
		Jiangsu	10	583,850
		Anhui	3	30,500
		Shandong	1	12,000
		Jiangxi	1	3,000
	Zhebei	Zhejiang	2	122,910
		Jiangsu	1	33,300
		Anhui	1	20,500
Anhui	Huishangcheng	Anhui	15	409,570
		Shandong	8	272,700
		Jiangsu	10	157,500
		Henan	4	49,400
	Fenghuang	Anhui	10	281,170
		Jiangsu	11	93,000
Jiangsu	Zijinshan	Anhui	13	314,700
		Jiangsu	8	214,817
		Shandong	1	20,780
		Hebei	1	16,340
		Henan	6	12,400
	Tianyinshan	Jiangsu	3	86,640
		Anhui	2	33,900

Table B-3 Summary of Moran's I index of degree centrality and k-core of chicken sources.

	Degree of source Counties	k-core of source Counties
Moran's Index:	0.118	0.189
Expected Index:	-0.009	-0.009
Variance:	0.0005	0.0005
z-score:	5.711	8.841
p-value:	0.000	0.000

Appendix C Chapter 6 Supplementary Information

C.1 Tables

Table C-1 Scoring method of questions about knowledge, attitudes and practices in relation to avian influenza among all participants.

Question ID	Type of question	Question contents	Scoring method	Score range
Knowledge Q1	Binary	K1. Have you heard about AI infection in animals?	'yes' 1 point, 'no' 0 point	0-1
Knowledge Q2	Categorical	K2. Is AI an infectious disease?	'yes' 1 point, 'no' 0 point	0-1
Knowledge Q3	Categorical	K3. What season do you think AI is most likely to occur?	winter 2 points, spring +1, autumn +1, summer 0	0-4
Knowledge Q4	Open answer	K4. What types of animals can be infected with AI?	mention any poultry 2 points, mention birds +1, mention any other mammals only +1	0-4
Knowledge Q5	Categorical	K5. Do you think that people can be infected with AI?	'yes' 1 point, 'no' or 'don't know' 0 point	0-1
Knowledge Q6	Open answer	K6. How can people get AI?	contact with poultry or market +2 points, answer eating +1, answer virus +1, answer air transmitted +1, answer low immunity and others 0	0-4
Knowledge Q7	Categorical	K7. Can AI be transmitted from one person to the other?	'yes' 0 point, 'not sure' or 'don't know' 1 point, 'no' 2 points	0-2
Knowledge Q8	Open answer	K8. What do you think are the symptoms of people infected with AI?	mention any of the symptoms' 1 point, 'no' or 'don't know' 0 point	0-1
Attitudes Q1	Categorical	A1. Do you think AI is a severe disease for humans?	'yes' 1 point, 'no' 0 point	0-1
Attitudes Q2	Categorical	A2. Do you think people who are infected with AI can be cured?	'yes' 0 point, 'not sure' or 'don't know' 1 point, 'no' 2 points	0-2
Attitudes Q3	Categorical	A3. What do you think is the likelihood for you to get AI?	'No risk' 0, 'Low risk' 1, 'Moderate risk' 2, ‘High risk' 3	0-3
Attitudes Q4	Categorical	A4. In your opinion, which of the following is the riskiest place for poultry to get AI	add 1 point for each checked option	0-5
Attitudes Q5	Categorical	A5. In your opinion, which of the following is the riskiest place for humans to get AI	add 1 point for each checked option	0-5
Practices Q1	Categorical	What do you do when you suspect that you have flu symptoms?	Do nothing' 0 point, 'take medicine at home' +2, 'rural health clinic' +1, 'county level hospital' +1	0-3
Practices Q2	Categorical	Before contacting live birds, do you usually take any protection measures?	'Do nothing' 0, 'wear gloves'+1, 'wear masks' +1, 'wear protective clothes'+1, 'wash hands' +1, 'wash hands with soap' +1	0-5

Table C-2 Summary of the 15 KAP questions for three types of respondents.

Variable	Score	Chicken farmers (N=95)		Chicken vendors (N=104)		Consumers (N=75)		All respondents (N=274)	
		n	%	n	%	n	%	n	%
K1. Have you heard about AI infection in animals?	0	0	0.0	1	1.0	7	9.3	8	2.9
	1	95	100.0	103	99.0	68	90.7	266	97.1
K2. Is AI an infectious disease?	0	10	10.5	47	45.2	15	20.0	72	26.3
	1	85	89.5	57	54.8	60	80.0	202	73.7
K3. What season do you think AI is most likely to occur?	0	8	8.4	11	10.6	11	14.7	30	10.9
	1	24	25.3	40	38.5	46	61.3	110	40.1
	2	35	36.8	32	30.8	13	17.3	80	29.2
	3	25	26.3	17	16.3	4	5.3	46	16.8
	4	3	3.2	4	3.8	1	1.3	8	2.9
K4. What types of animals can be infected with AI?	0	14	14.7	37	35.6	5	6.7	56	20.4
	1	8	8.4	9	8.7	3	4.0	20	7.3
	2	36	37.9	23	22.1	26	34.7	85	31.0
	3	31	32.6	33	31.7	34	45.3	98	35.8
	4	2	2.1	0	0.0	3	4.0	5	1.8
K5. Do you think that people can be infected with AI?	0	30	31.6	51	49.0	13	17.3	94	34.3
	1	65	68.4	53	51.0	57	76.0	175	63.9
K6. How can people get AI?	0	64	67.4	62	59.6	25	33.3	151	55.1
	1	6	6.3	4	3.8	11	14.7	21	7.7
	2	19	20.0	17	16.3	25	33.3	61	22.3
	3	3	3.2	8	7.7	4	5.3	15	5.5
	4	0	0.0	1	1.0	0	0.0	1	0.4
K7. Can AI be transmitted from one person to the other?	0	28	29.5	15	14.4	36	48.0	79	28.8
	1	45	47.4	32	30.8	22	29.3	99	36.1
	2	19	20.0	47	45.2	16	21.3	82	29.9
K8. What do you think are the symptoms of people infected with AI?	0	40	42.1	34	32.7	17	22.7	91	33.2
	1	48	50.5	57	54.8	54	72.0	159	58.0
A1. Do you think AI is a severe disease for humans?	0	54	56.8	81	77.9	33	44.0	168	61.3
	1	40	42.1	20	19.2	42	56.0	102	37.2
A2. Do you think people who are infected with AI can be cured?	0	24	25.3	53	51.0	49	65.3	126	46.0
	1	61	64.2	34	32.7	18	24.0	113	41.2
	2	4	4.2	5	4.8	5	6.7	14	5.1
A3. What do you think is the likelihood for you to get AI?	0	27	28.4	41	39.4	15	20.0	83	30.3
	1	28	29.5	38	36.5	28	37.3	94	34.3
	2	29	30.5	11	10.6	27	36.0	67	24.5
	3	8	8.4	11	10.6	5	6.7	24	8.8
	0	12	12.6	29	27.9	55	73.3	96	35.0

A4. In your opinion, which of the following is the riskiest place for poultry to get AI?	1	69	72.6	66	63.5	15	20.0	150	54.7
	2	12	12.6	7	6.7	2	2.7	21	7.7
	3	2	2.1	0	0.0	2	2.7	4	1.5
	4	0	0.0	2	1.9	0	0.0	2	0.7
	5	0	0.0	0	0.0	1	1.3	1	0.4
A5. In your opinion, which of the following is the riskiest place for humans to get AI?	0	10	10.5	41	39.4	4	5.3	55	20.1
	1	68	71.6	51	49.0	37	49.3	156	56.9
	2	14	14.7	4	3.8	20	26.7	38	13.9
	3	3	3.2	2	1.9	6	8.0	11	4.0
	4	0	0.0	3	2.9	7	9.3	10	3.6
	5	0	0.0	3	2.9	1	1.3	4	1.5
P1. What do you do when you suspect that you have flu symptoms?	0	3	3.2	11	10.6	8	10.7	22	8.0
	1	74	77.9	66	63.5	42	56.0	182	66.4
	2	18	18.9	27	26.0	22	29.3	67	24.5
	3	0	0.0	0	0.0	3	4.0	3	1.1
P2. Before contacting live birds, do you usually take any protection measures?	0	0	0.0	0	0.0	2	2.7	2	0.7
	1	52	54.7	59	56.7	51	68.0	162	59.1
	2	12	12.6	19	18.3	18	24.0	49	17.9
	3	12	12.6	21	20.2	4	5.3	37	13.5
	4	15	15.8	5	4.8	0	0.0	20	7.3
	5	4	4.2	0	0.0	0	0.0	4	1.5

Table C-3 Demography characteristics of different type of chicken vendors from the surveyed counties in Jiangsu and Anhui provinces in China.

Characteristics		Vender only (n=64)	Vender trader (n=22)	Vender slaughter (n=14)	Vendor trader and slaughter (n=4)	Chi-square	P-value
Male (%)		28 (43.8)	20 (90.9)	10 (71.4)	3 (75)	16.68	0.001
Female (%)		36 (56.2)	2 (9.1)	4 (28.6)	1 (25)		
Age	<=35	7	3	0	0	10.28	0.113
	36~55	51	15	10	4		
	>55	3	2	4	0		
Education level	Primary School or below	13	4	3	0	16.29	0.061
	Secondary school	29	15	8	3		
	High School	19	1	3	0		
	University and above	2	0	0	1		
Average KAP Scores		13.3	14.5	14.4	14.0		
Average Knowledge Scores		7.3	8.2	7.9	7.0		
Average Attitudes Scores		3.2	3.4	3.2	2.8		
Average Practices Scores		2.7	3.0	3.3	4.3		

Table C-4 Correlation matrix of all KAP variables

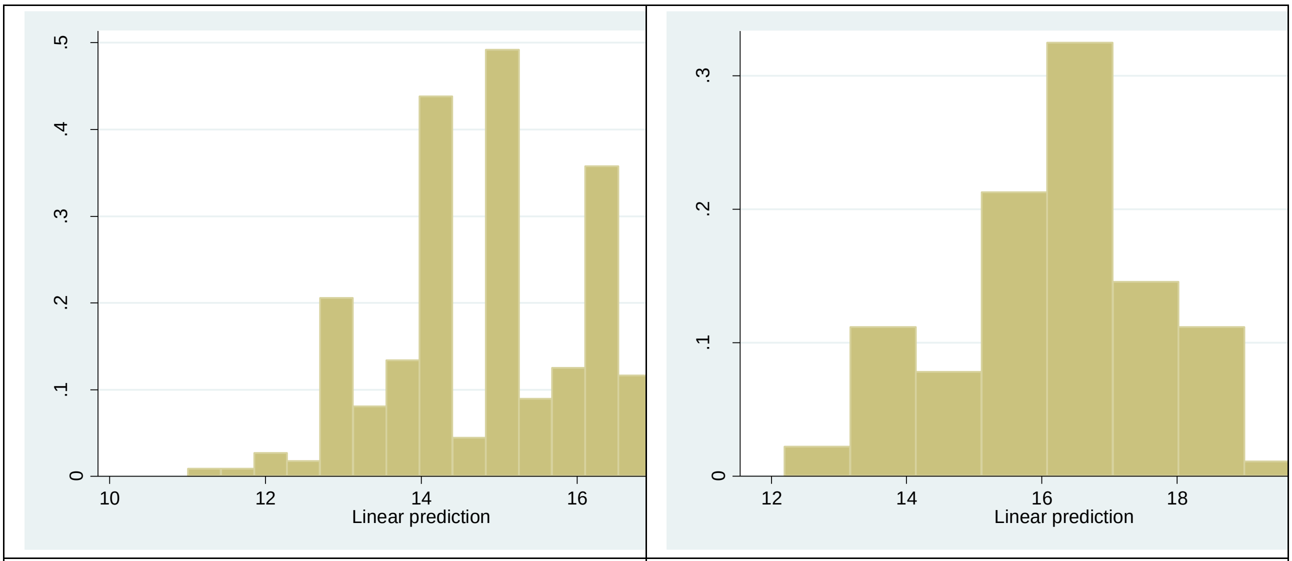
Corr.	scorek1	scorek2	scorek3	scorek4	scorek5	scorek6	scorek7	scorek8	scorea1	scorea2	scorea3	scorea4	scorea5	scorep1	scorep2
scorek1	1.00														
scorek2	0.03	1.00													
scorek3	0.15	0.15	1.00												
scorek4	0.05	0.35	0.07	1.00											
scorek5	0.00	0.41	0.01	0.22	1.00										
scorek6	0.01	0.22	0.03	0.27	0.31	1.00									
scorek7	0.06	-0.28	0.04	-0.07	-0.41	-0.29	1.00								
scorek8	0.03	0.23	0.05	0.34	0.33	0.35	-0.04	1.00							
scorea1	-0.06	0.12	-0.19	0.10	0.25	0.06	-0.34	0.00	1.00						
scorea2	0.09	0.06	0.05	-0.13	0.14	-0.12	-0.13	-0.12	0.16	1.00					
scorea3	-0.05	0.12	0.02	0.12	0.29	0.10	-0.24	0.02	0.36	0.11	1.00				
scorea4	0.13	0.19	0.27	0.14	0.09	0.17	-0.03	0.03	-0.04	0.03	0.16	1.00			
scorea5	-0.10	0.15	-0.02	0.26	0.25	0.32	-0.10	0.22	0.10	0.07	0.15	0.22	1.00		
scorep1	0.01	0.07	0.00	0.02	0.03	-0.11	-0.07	-0.01	0.06	-0.06	-0.04	-0.11	-0.05	1.00	
scorep2	0.02	0.01	0.16	0.09	0.00	0.26	-0.05	0.10	-0.07	-0.10	0.06	0.25	0.06	-0.11	1.00

Table C-5 The coefficient of determination (R-squared) of the four GLS regression models.

R-squared	Mode 1: All respondents	Model 2: Farmers	Model 3: Vendors	Model 4: Consumers
within	0.08	0.14	0.15	0.21
between	0.31	0.24	0.66	0.65
overall	0.10	0.17	0.24	0.31

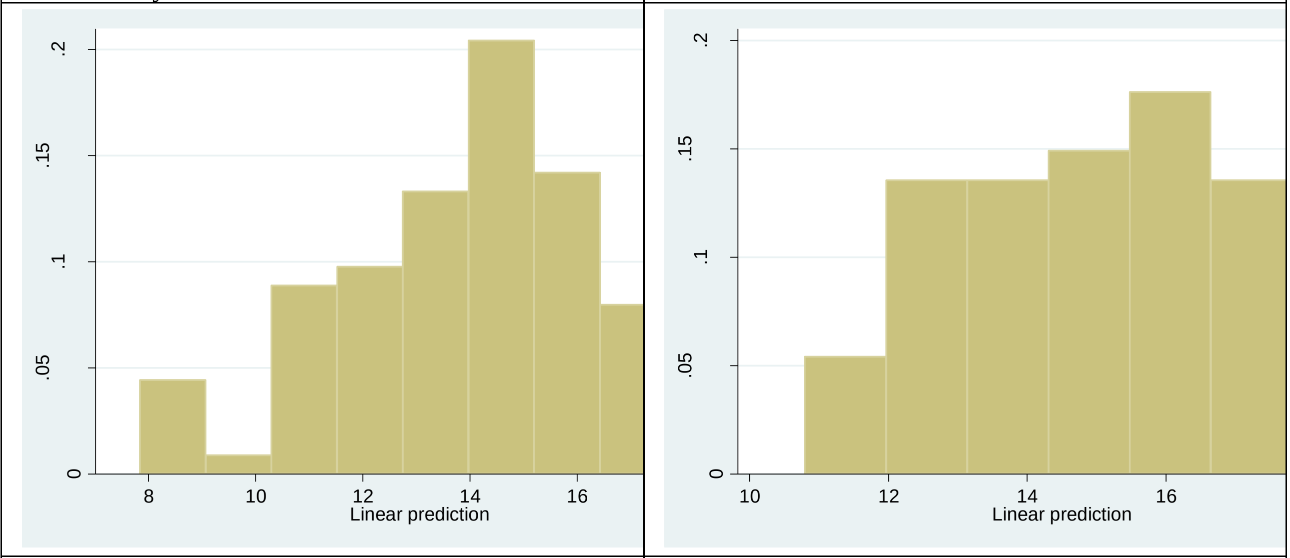
Note: The between R2 is "How much of the variance between separate counties does my model account for", the within R2 is "How much of the variance within the county does my model account for", and the overall R2 is a weighted average of these two.

C.2 Figures



A: Histogram of residuals from Model 1 - All respondents
Not normally distributed

B: Histogram of residuals from Model 1 – Farmers
Normally distributed



C: Histogram of residuals from Model 1 – Vendors
Normally distributed

D: Histogram of residuals from Model 1 – Consumers
Normally distributed

Figure C-1 Distribution of the residuals from four GLS regression models

Appendix D Chapter 7 Supplementary Information

D.1 Data source of wholesale LBMs and retail LBMs

The presence of wholesale LBMs and number of retail LBMs were obtained from China Animal Health and Epidemiology Centre (CAHEC). The provincial Center of Animal Diseases Control and Prevention (CADC) were approached by CAHEC to provide with number of wholesale and retail LBMs in each county in the province. However, LBMs data in Zhejiang and Shandong provinces is missing. Therefore, we used the Points of Interest (POI) data from year 2012 for these two provinces as a database, and we searched with different combinations of terms, i.e., live bird markets, famers' markets, agricultural products markets and wet markets. We then carefully screened each market name and make sure all included markets are agriculture product related markets. We use this dataset as a substitute to live bird markets in the two provinces.

D.2 Mathematical notation for the Bayesian spatial CAR model

It assumed that the observed counts of the H7N9 human infection, for the ith county ($i = 1$ to 1181) followed a Poisson distribution with mean (μi), that is,

$Yi \sim \text{Poisson}(\mu i)$

$\log(\mu i) = \log(\text{Exp}i) + \theta i$

$\theta i = \alpha + x * \gamma + \sum \beta z * \lambda zi + si$

where Exp i is the expected number of human H7N9 cases in county i (acting as an offset to control for population size) and θ i is the mean log relative risk (RR); α is the intercept, γ is the coefficient for temporal trend, β is a vector of z coefficients, λ is a matrix of z environmental covariates, and si is the spatially structured random effect with mean zero and variance σ_s^2. Standardization of environmental variables was used to allow comparability of the effects and provide a more meaningful interpretation on the results.

D.3 OpenBUGS code

The OpenBUGS code used to develop the Bayesian spatial model for H7N9 human infections from 2013 to 2017.

```
model {

#CAR prior distribution for spatial random effects:
s[1:1181] ~ car.normal(adj[], weights[], num[], tau.s)
for(k in 1:sumNumNeigh) {
weights[k] <- 1
}

for (i in 1:1181) {
O[i] ~ dpois(mu[i])
log(mu[i]) <- log(E[i]) + log.RR[i]
log.RR[i] <- alpha + U[i] + s[i]
U[i] <- beta1 * WsM[i] + beta2 * ReMDen1[i] + beta3 * ReMDen2[i] + beta4 * VPos[i] + beta5 * Pop1[i] + beta6* Pop2[i] + beta7 * Ck1[i] + beta8 * Ck2[i] + beta9 * Deg1[i] +
beta10 * Deg2[i]
RR[i] <- exp(log.RR[i])
}

#Other priors
tau.s ~ dgamma(0.5, 0.0005)
alpha ~ dflat()
beta1 ~ dnorm(0,0.00001)
beta2 ~ dnorm(0,0.00001)
beta3 ~ dnorm(0,0.00001)
beta4 ~ dnorm(0,0.00001)
beta5 ~ dnorm(0,0.00001)
beta6 ~ dnorm(0,0.00001)
beta7 ~ dnorm(0,0.00001)
beta8 ~ dnorm(0,0.00001)
beta9 ~ dnorm(0,0.00001)
beta10 ~ dnorm(0,0.00001)
}

#Initial values
list(alpha = 0, beta1 = 0, beta2 = 0, beta3 = 0, beta4 = 0, beta5 = 0, beta6 = 0, beta7 = 0, beta8 = 0, beta9 = 0, beta10 = 0, tau.s=0.5)
```

D.4 Tables

Table D-1 List of surveyed sites of chicken movements.

No.	Province	Records From	NameEN	Start Date	End Date	Incoming records	Outgoing records	Degree
1	Shanghai	Wholesale LBM	Shanghai Nongpi	1/01/2014	16/07/2014	1247	6061	23
2	Jiangsu	Wholesale LBM	Changzhou Lingjiatang	1/01/2014	24/07/2014	2540	*	43
3	Jiangsu	Wholesale LBM	Zhenjiang Nongfuchanpin	9/01/2014	24/07/2014	1674	*	42
4	Jiangsu	Wholesale LBM	Lishui Wenshi	1/01/2014	22/07/2014	715	*	24
5	Jiangsu	Trading platform	Changzhou Tianmu Lihua	1/1/2014	30/06/2014	*	8693	124
6	Jiangsu	Trading platform	Jiangyan Heyin	2/02/2014	25/07/2014	*	703	46
7	Anhui	Trading platform	Chaohu Zhengkang	1/01/2014	16/06/2014	*	323	33
8	Anhui	Trading platform	Feixi Wenshi	1/01/2014	20/06/2014	*	1383	105

Table D-2 Summary of Moran's I index of incidence rate of human H7N9 infections.

	Number of observed human H7N9 infections
Moran's Index:	0.1525
Expected Index:	-0.00085
Variance:	0.00018
z-score:	11.3533
p-value:	0.0000

Table D-3 Results of multivariable logistic model applied to H7N9 Human infections in each epidemic wave during 2013-2017.

Variables in each county	**Category**	**Coef.**	**[95% Conf.**	**P>z**
Epidemic wave of human H7N9 infections	Wave 1	Ref		
	Wave 2	**1.03**	**(0.72~1.34)**	**0**
	Wave 3	**0.72**	**(0.41~1.04)**	**0**
	Wave 4	0.31	(-0.02~0.63)	0.062
	Wave 5	**2.09**	**(1.8~2.39)**	**0**
Present of wholesale LBMs	no	Ref		
	yes	**0.65**	**(0.39~0.9)**	**0**
Retail LBMs density (100km2)	Low density (< 1)	Ref		
	Medium density (1-3)	**0.38**	**(0.07~0.68)**	**0.015**
	High density (>3)	**0.93**	**(0.57~1.29)**	**0**
Present of poultry virological positive	no	Ref		
	yes	**1.94**	**(1.49~2.38)**	**0**
Network estimate (Degree centrality)	0	Ref		
	1~3	**0.76**	**(0.47~1.04)**	**0**
	4~6	**1.51**	**(0.88~2.15)**	**0**
Human population density (km2)	0~200	Ref		
	201~600	0.19	(-0.16~0.54)	0.295
	>601	0.41	(-0.01~0.82)	0.054
Chicken density (km2)	0~500	Ref		
	500~3000	0.14	(-0.18~0.46)	0.399
	>3000	**-0.57**	**(-0.99~-0.15)**	**0.008**
Constant		-3.94	(-4.3~-3.59)	0

Notes: The presence of wholesale LBMs, higher retail LBMs density, presence of poultry virological positives, higher network centrality, as well as higher human population density were significantly associated with the presence of human H7N9 infections. Specifically, our result indicated the presence of wholesale LBM in the county increased 65% of the probability of human H7N9 infections in the county (Coef. = 0.65, 95% CI: 0.39-0.9). Counties with higher density of retail LBMs had more chance to observe human infections compared to counties with no retail LBMs. The presence of poultry virological positives almost tripled the probability of human H7N9 infections in the county (Coef. = 1.94, 95% CI: 1.49-2.38). Counties with a degree centrality of 1 to 3 had 76% more probability of having human infections than counties with 0-degree centrality, and counties with a degree centrality of 4 to 6 had 151% more probability of having human infections than counties with 0-degree centrality. Counties with more than 3,000 chicken/km2 had 60% less probabilities of having human infections in the county compared to counties with less than 1,000 chicken/km2 (Coef. = 0.57, 95% CI: -0.99--0.15).

D.5 Figures

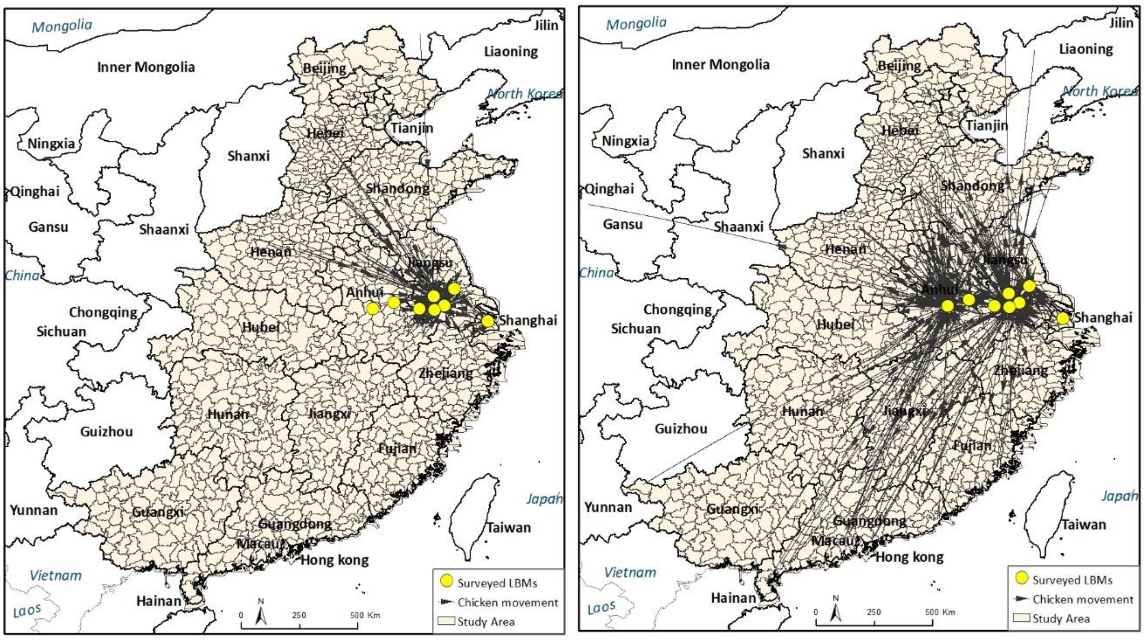


Figure D-1 Geographic distribution of live chicken movements from wholesale LBMs (left) and live poultry trading platforms (right).

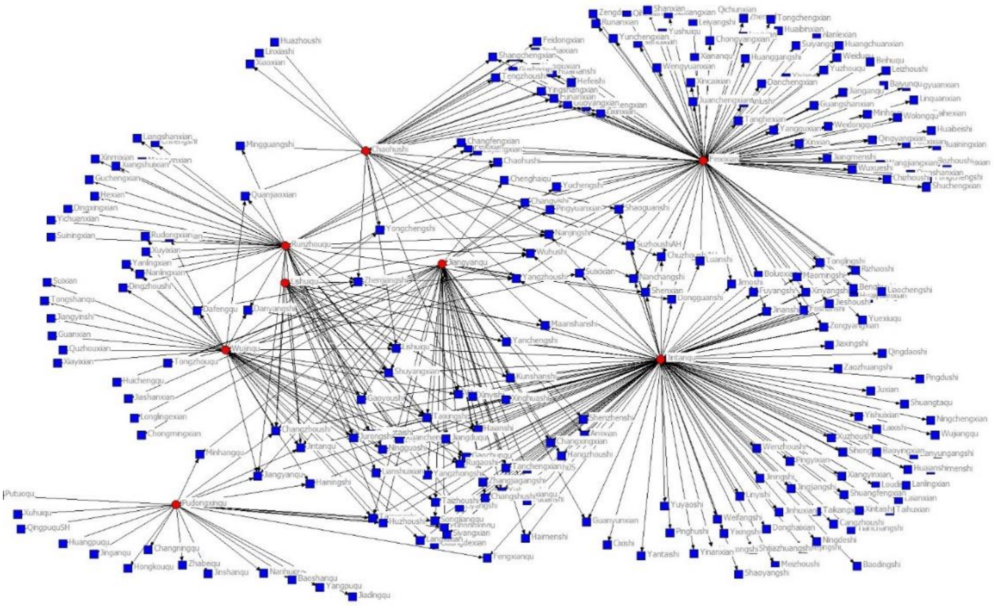


Figure D-2 2-mode Network between surveyed LBMs/poultry trading platforms and counties of live chicken sources/destinations.

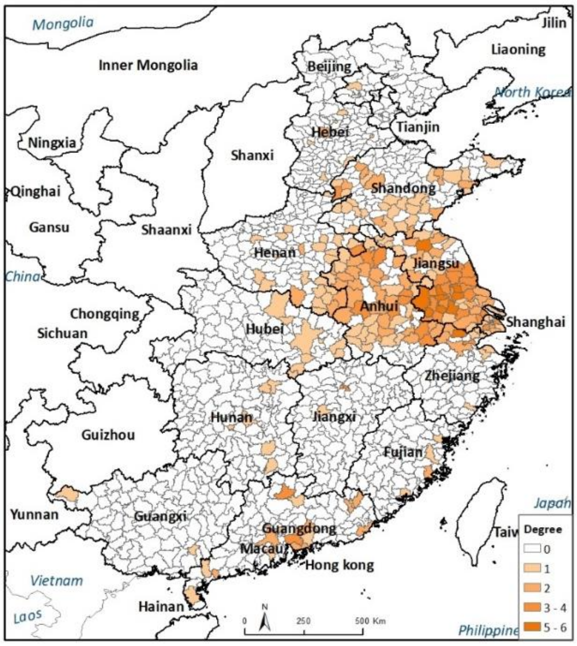


Figure D-3 Geographical distribution of the degree centrality of live chicken sources/destinations (county level), based on a 2-mode network of live chicken movements.

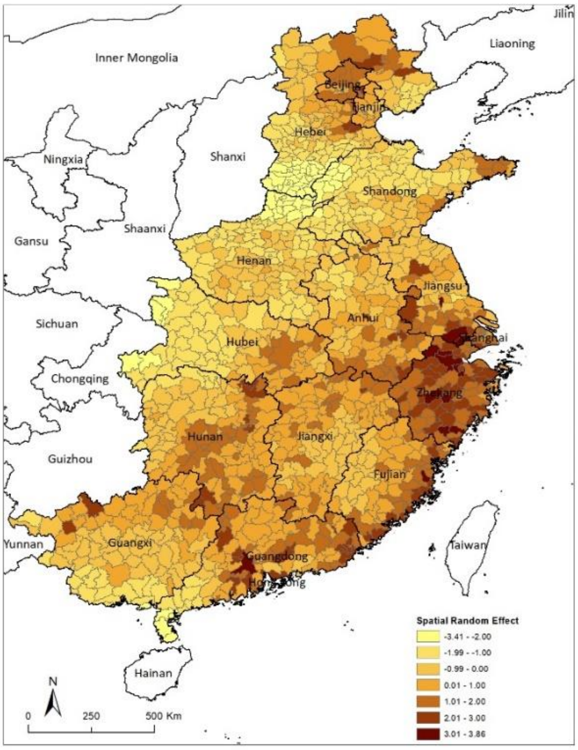
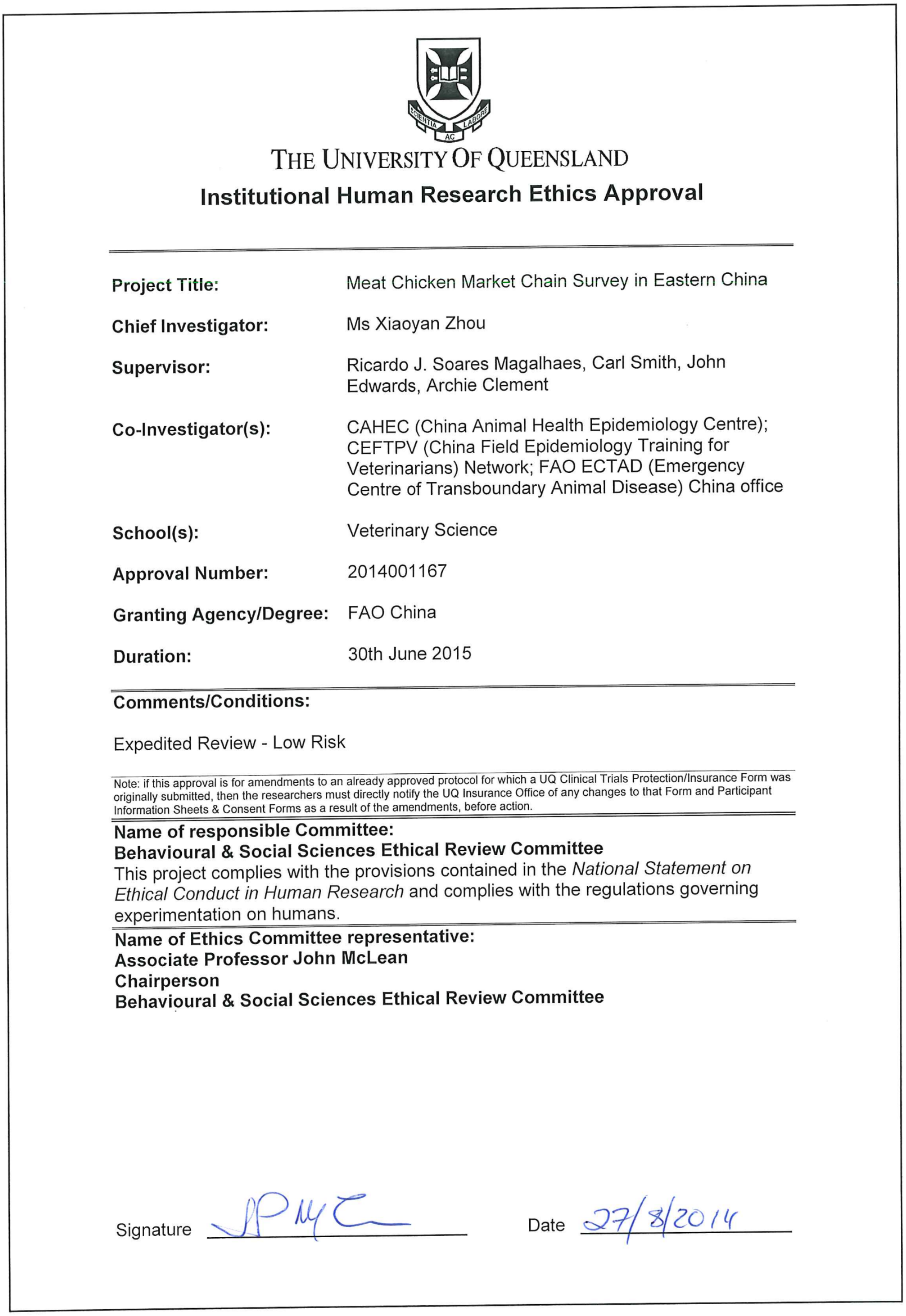


Figure D-4 Spatial distribution of spatially structured random effects of the CAR model for human H7N9 infections. The maps were created in ArcGIS 10.1 software (ESRI Inc., Redlands, CA, USA) (http://www.esri.com/).

Appendix E Ethics Approval Letter



THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	Meat Chicken Market Chain Survey in Eastern China
Chief Investigator:	Ms Xiaoyan Zhou
Supervisor:	Ricardo J. Soares Magalhaes, Carl Smith, John Edwards, Archie Clement
Co-Investigator(s):	CAHEC (China Animal Health Epidemiology Centre); CEFTPV (China Field Epidemiology Training for Veterinarians) Network; FAO ECTAD (Emergency Centre of Transboundary Animal Disease) China office
School(s):	Veterinary Science
Approval Number:	2014001167
Granting Agency/Degree:	FAO China
Duration:	30th June 2015

Comments/Conditions:

Expedited Review - Low Risk

Note: if this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
Behavioural & Social Sciences Ethical Review Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Associate Professor John McLean
Chairperson
Behavioural & Social Sciences Ethical Review Committee

Signature ____________________ Date 27/8/2014

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