THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Factors influencing the epidemiology of avian influenza virus circulation on poultry farms in Bangladesh

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A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2019

School of Veterinary Science

Abstract

Highly Pathogenic Avian Influenza (HPAI) virus H5N1 and Low Pathogenic Avian Influenza (LPAI) virus H9N2 are endemic in Bangladesh and pose a threat to both poultry and human health. For effective avian influenza (AI) prevention and control, good knowledge of the factors influencing the epidemiology of avian influenza virus (AIV) circulation is crucial, but no in-depth investigations have thus far been conducted on poultry farms in Bangladesh.

The overall aim of this research was to improve the understanding of the extent of H5 and H9 virus circulation on backyard, and commercial broiler and commercial layer chicken farms in Bangladesh and to identify risk factors associated with the presence of H5 and H9 virus. Furthermore, the research aimed to investigate the perceptions of chicken farmers to implement HPAI prevention and control measures in Bangladesh.

Two cross-sectional studies were conducted in the Chittagong and Cox's Bazaar districts of Bangladesh: 1) between February and April 2016 involving 144 backyard chicken farms in 42 villages, and 2) between February and April 2017 involving 106 commercial broiler and 113 commercial layer chicken farms. Blood samples, oropharyngeal swabs and cloacal swabs were collected from 576 chickens and 204 in-contact ducks on backyard farms, and from 954 broilers and 904 layers on commercial chicken farms. Questionnaires were used to collect data on farm-level and village-level risk factors for H5 and H9 seroprevalence and on farmer's perceptions towards implementation of HPAI prevention and control measures.

Although all sampled birds tested negative for H5 by RT-PCR, H5 seropositive chickens were detected in all three farming systems. The highest H5 seroprevalence was observed in ducks raised with chickens on backyard farms, 14.2% (95% CI: 10.0-19.8), compared to in-contact backyard chickens, 4.2% (95% CI: 2.8-6.1). H5 seroprevalence was lower in unvaccinated broiler chickens, 1.5% (95% CI: 0.9-2.5), than in unvaccinated layer chickens, 7.8% (95% CI: 6.1-9.8). H9 viral infection was detected by RT-PCR in 0.5% (95% CI: 0.2-1.3) and 0.6% (95% CI: 0.3-1.5) of chickens raised in broiler and layer farms, respectively and in 0.2% (95% CI: 0.0-1.2) of chickens on backyard farms suggesting a similar level of exposure to H9 virus is all farming systems. Backyard chickens and ducks showed similar H9 seroprevalence, 16.0% (95% CI: 13.2-19.2) and 15.7% (95% CI: 11.3-21.4) respectively, while it was 5.8% (95% CI: 4.3-7.6) in layers and 1.5% (95% CI: 0.9-2.5) in broilers. Over the course of a production cycle, H5 and H9 seroprevalence increased with the age of backyard and layer chickens. Clustering of H5 seropositivity in ducks was identified, highlighting

that multiple ducks within a flock were H5 seropositive. This was in contrast to backyard and broiler and layer chickens, where only individual birds within flocks developed H5 antibodies.

Using multilevel mixed modelling, farm- and village-level risk factors for AIV exposure for backyard farms were identified. For example, garbage around poultry house or on the farms (a farm-level risk factor) (OR for H5: 9.1, 95% CI: 1.7-48.8; OR for H9: 28.6, 95% CI:3.4-239.8) and crow abundance around garbage dumping places within villages (a village-level risk factor) (OR for H5:3.4, 95% CI: 1.1-10.8; OR for H9:13.1, 95% CI: 2.3-76.8) increased the odds for H5 and H9 seropositivity on backyard farms. Binomial logistic regression was used to identify farm-level risk factors for AIV exposure on commercial farms. For example, visits by workers from other commercial chicken farms during the current production cycle (OR for H5: 15.1, 95% CI: 2.8-80.8; OR for H9: 50.1, 95% CI:4.5-552.7) increased the odds for seropositivity on broiler farms, while access of stray dogs to the sampled farm (OR for H5: 3.1, 95% CI: 1.1-9.1; OR for H9: 4.0, 95% CI:1.1-15.3) increased the odds for seropositivity on layer farms.

Structural Equation Modelling was used to explore direct and indirect effects of farmers' perceptions to implement HPAI prevention and control actions on their farms. Results highlighted that farmers working in different chicken production systems follow different decision-making processes. Perceived barriers to implement prevention and control measures (e.g. wearing protective equipment when handling chickens) refrained both broiler (β=-0.41, $p<0.001$) and backyard farmers (β=-0.52, $p<0.001$) to adopt interventions. Meanwhile perceived benefits (e.g. maintaining high biosecurity to reduce the risk of birds becoming sick) strongly influenced commercial broiler (β=0.44, $p<0.001$) and layer farmers' (β=0.68, $p<0.001$), but not backyard farmers' decisions. Information provided on HPAI control through media, meetings or via information campaigns played an important role in farmers' decision making across all production systems.

Overall, this project provided a holistic picture of the factors influencing the epidemiology of AIV circulation across diverse chicken production systems in Bangladesh. The project described AIV infection patterns, risk factors of infection and farmers perceptions to implement HPAI prevention and control measures. Results from this research project have been used to inform policy makers to develop recommendations and improve current AI prevention and control policies in Bangladesh.

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

No publications included.

Submitted manuscripts included in this thesis

Manuscript 1:

Gupta, S. D., Hoque, M. A., Fournié, G., & Henning, J. (2019). Patterns of avian influenza A (H5) and A (H9) virus infection on backyard, commercial broiler and layer chicken farms in Bangladesh. *Transboundary and Emerging Diseases*. In Press.

The above manuscript was prepared based on the **Chapter 3**. The contribution of the candidate (Gupta, S. D**.**) including other co-authors to this manuscript was described on the page (page number 30) immediately preceding the Chapter 3. This paper has been accepted for publication.

Manuscript 2:

Gupta, S. D., Fournié, G., Hoque, M. A., & Henning, J. (2019). Factors influencing chicken farmers' decisions to implement prevention and control measures to reduce avian influenza virus spread under endemic conditions. *Transboundary and Emerging Diseases*. In Press.

The above manuscript was prepared based on the **Chapter 6**. The contribution of the candidate (Gupta, S. D**.**) including other co-authors to this manuscript was described on the page (page number 95) immediately preceding the Chapter 6. This paper has been accepted for publication.

Other publications during candidature

Conference abstracts

Oral presentations

Gupta, S. D., Fournié, G., Høg, E., Barnett, T., Pfeiffer, D., Hoque, M. A., & Henning, J. (2016). *What drives rural poultry farmers to implement Avian Influenza control in Bangladesh?* Paper presented at the One Health Ecohealth 2016: 4th International One Health Congress and 6th Biennial Congress of the International Association for Ecology and Health, Melbourne, Australia. 3-7 December 2016.

Gupta, S. D., Fournié, G., Hoque, M. A., Høg, E., Barnett, T., Pfeiffer, D., & Henning, J. (2018). *What are drivers that influence backyard and commercial farmers to implement avian influenza control in Bangladesh?* Paper presented at the 15th International Symposium of Veterinary Epidemiology and Economics (ISVEE 15), Epidemiologic principles and methods, session, Chiang Mai, Thailand. 12-16 November 2018.

Gupta, S. D., Hoque, M. A., Fournié, G., Pfeiffer, D., & Henning, J. (2018). *Farm management and environmental village level factors influencing avian influenza circulation on backyard and commercial chicken farms in Bangladesh*. Paper presented at the 15th International Symposium of Veterinary Epidemiology and Economics (ISVEE 15), Special section: Our trend and future, session, Chiang Mai, Thailand. 12-16 November 2018.

Poster presentations

Gupta, S. D., Fournié, G., Hoque, M. A., Høg, E., Barnett, T., Pfeiffer, D., & Henning, J. (2017). *What perceptions, knowledge and attitudes guide backyard poultry farmers to implement Avian Influenza control measures in Bangladesh?* Paper presented at the 9th One Health Bangladesh Conference, Dhaka, Bangladesh. 17-18 September, 2017.

Gupta, S. D., Hoque, M. A., Fournié, G., & Henning, J. (2017). *The extent of H5 and H9 virus circulation on backyard and commercial chicken farms in Bangladesh*. Paper presented at the 9th One Health Bangladesh Conference, Dhaka, Bangladesh. 17-18 September, 2017.

Additional published works by the author relevant to the thesis but not forming part of it

Peer-reviwed paper

Moyen, N., Ahmed, G., **Gupta, S.**, Tenzin, T., Khan, R., Khan, T., . . . Fournie, G. (2018). A large-scale study of a poultry trading network in Bangladesh: implications for control and surveillance of avian influenza viruses. *BMC Veterinary Research, 14*(1), 12. doi:https://doi.org/10.1186/s12917-018-1331-5

Contributions by others to the thesis

Chapter 3: Patterns of avian influenza A (H5) and A (H9) virus infection on backyard, commercial broiler and layer chicken farms in Bangladesh

Suman Das Gupta contributed a total of 80% to the conception and design of the study, field data collection, analysis and interpretation of research data, formatting, drafting and editing of chapter. Dr Joerg Henning contributed 10% to the conception and design of the study, interpretation of results and chapter editing. Dr Guillaume Fournié contributed 6% to the conception and design of the study, interpretation of results and chapter editing. Dr Md. Ahasanul Hoque contributed 4% to the conception and design of the study, field data collection and chapter editing.

Chapter 4: Village and farm-level risk factors associated with avian influenza A (H5) and A (H9) flock-level seroprevalence on backyard chicken farms in Bangladesh

Suman Das Gupta contributed a total of 80% to the conception and design of the study, field data collection, analysis and interpretation of research data, formatting, drafting and editing of chapter. Dr Joerg Henning contributed 12% to the conception and design of the study, interpretation of results and manuscript editing. Dr Guillaume Fournié contributed 6% to the conception and design of the study, interpretation of results and chapter editing. Dr Md. Ahasanul Hoque contributed 2% to the conception and design of the study, field data collection and chapter editing.

Chapter 5: Farm-level risk factors associated with avian influenza A (H5) and A (H9) flock-level seroprevalence on commercial broiler and layer farms in Bangladesh

Suman Das Gupta contributed a total of 80% to the conception and design of the study, field data collection, analysis and interpretation of research data, formatting, drafting and editing of chapter. Dr Joerg Henning contributed 12% to the conception and design of the study, interpretation of results and manuscript editing. Dr Guillaume Fournié contributed 6% to the conception and design of the study, interpretation of results and chapter editing. Dr Md. Ahasanul Hoque contributed 2% to the conception and design of the study, field data collection and chapter editing.

Chapter 6: Factors influencing chicken farmers' decisions to implement prevention and control measures to reduce HPAI virus spread in Bangladesh

Suman Das Gupta contributed a total of 80% to the conception and design of the study, field data collection, analysis and interpretation of research data, formatting, drafting and editing of chapter. Dr Joerg Henning contributed 12% to the conception and design of the study, interpretation of results

and chapter editing. Dr Guillaume Fournié contributed 6% to the conception and design of the study, interpretation of results and chapter editing. Dr Md. Ahasanul Hoque contributed 2% to the conception and design of the study, field data collection and chapter editing.

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 human ethical approval for conducting the interviews with farmers was provided by the University of Queensland (UQ) Human Research Ethics Committee (approval number #2015001703) (**Appendix 7**).

The animal ethical approval for conducting sampling from birds was provided by the University of Queensland Animal Welfare Unit, UQ Research and Innovation (approval number: SVS/465/15/RVC) (**Appendix 8**).

Acknowledgements

First, I would like to express my sincere thanks to the almighty 'GOD' without whom my PhD journey would not have been so smooth and pleasant. 'THANK YOU GOD'

The next genuine gratitude would go to my principal supervisor, Associate Professor Joerg Henning. I wish to write a PhD chapter (!) on this gentleman. I experienced him as a brilliant researcher who taught me how to smartly and efficiently work through complex and complicated research and how to adhere to scientific rigour while addressing the challenges and limitations of field research in a developing country, like Bangladesh. He always provided me with opportunities to develop my professional knowledge by organising participation in training courses (national and international) which enabled me to develop theoretical, methodological and analytical skills in different epidemiological approaches. He also encouraged me to focus on personal self-care, which is essential in the stressful environment of developing an academic career. To sum it up, I appreciated his clarity, his smiley face, his friendship-like mentoring characteristics, which always motivated me and made me feel that Associate Professor Henning is a very approachable colleague (although I was just his student). 'HEADS UP GENTLEMAN'

Then, I would like to thank my co-supervisor Dr Guillaume Fournié for his solid and precise comments, suggestions and feedback provided during meetings, reviewing of study designs, questionnaires, during the development of my research papers which improved unprecedentedly my critical thinking abilities. I am also thankful to my co-supervisor Dr Md. Ahasanul Hoque for his valuable insightful suggestions, guidance and encouragement during the tenure of my PhD. Without his support, my field studies in Bangladesh would have been a lot more difficult. I found Dr Md. Ahasanul Hoque to be a very friendly mentor. 'SALUTES TO BOTH OF MY CO-SUPERVISORS-YOU WERE REALLY GREAT'

I cannot omit acknowledgement of the support I have received from the CVASU BALZAC field research team during field data collection, sample collection and processing. I also thank all farmers for their participation in the research. 'HIGH FIVES TO ALL'

Last but not the least, I would like to thank my family: (1) my parents, in particular my mother who sacrificed herself to always puts her children and family first. My mother is the inspiration for doing my PhD, (2) brothers, sister-in-law, little MOM (daughter of my elder brother), (4) father-in-law, (3) my wife who heartily accepted my busy and sleepless nights before any deadlines, (4) my little cute princess (daughter) 'SHIVDOOTI' who is my new inspiration and breath. 'LOVE YOU ALL'

Financial support

This research was supported by an Australian Government Research Training Program Scholarship.

The field studies of this research was supported by the BALZAC research program "Behavioural adaptations in live poultry trading and farming systems and zoonoses control in Bangladesh" (BB/L018993/1). The BALZAC research program is 1 of 11 programs supported by the Zoonoses and Emerging Livestock Systems, a joint research initiative between the Biotechnology and Biological Sciences Research Council, the Defence Science and Technology Laboratory, the Department for International Development, the Economic and Social Sciences Research Council, the Medical Research Council, and the Natural Environment Research Council.

The living allowance of the author of this thesis was supported by UQ Graduate School Scholarship funded by UQ.

Keywords

avian influenza, backyard, broiler, farm, H5, H9, layer, perception, prevalence, risk factor

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 070704, Veterinary Epidemiology, 70%

ANZSRC code: 070205, Animal Protection (Pests and Pathogens), 20%

ANZSRC code: 111799, Public Health and Health Services not elsewhere classified, 10%

Fields of Research (FoR) Classification

FoR code: 0707, Veterinary Sciences, 70%

FoR code: 0702, Animal Production, 20%

FoR code: 1117, Public Health and Health Services, 10%

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LIST OF ABBREVIATIONS USED IN THE THESIS

Ag:	Antigen
AGID:	Agar Gel Immunodiffusion
AI:	Avian Influenza
AIV:	Avian Influenza Virus
β:	Standardized Regression Coefficient
CCLBM:	Chittagong City Live Bird Market
CI:	Confidence Intervals
CVASU:	Chattogram (previously Chittagong) Veterinary and Animal Sciences
DLS:	Department of Livestock Services
DOC:	Day Old Chick
ELISA	Enzyme-linked Immunosorbent Assay
EU:	European Union
FAO:	The Food and Agriculture Organization of the United Nations
FCD:	Feed and Chick Dealer
FMD:	Foot and Mouth Disease
HBM:	Health Belief Model
HH:	Household
HI:	Haemagglutination Inhibition
HPAI:	Highly Pathogenic Avian Influenza
IBD:	Infectious Bursal Disease

ICC:	Intra-class Correlation or Intra-class Correlation Coefficient
KAP:	Knowledge, Attitudes and Practices
LBM:	Live Bird Market
LMIC:	Low and Middle-Income Country
LPAI:	Low Pathogenic Avian Influenza
ND:	Newcastle Disease
NDV:	Newcastle Disease Virus
NIT:	Neuraminidase Inhibition Test
OIE:	The World Organisation for Animal Health
OR:	Odds Ratio
PA:	Participatory Appraisal
Real-time RT-PCR:	Real-time Reverse Transcription Polymerase Chain Reaction
ROC:	Receiver Operating Characteristics
RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SEM:	Structural Equation Modelling
WHO:	The World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Bangladesh has one of the highest human population (1,240 people/km^2) and poultry population densities (2,400 poultry/km^2) in the world (BBS, 2014b; WB, 2013, 2019b). Unfortunately, Bangladesh is also one of the poorest countries with 24.3% people subsisting under the national poverty line (ADB, 2019). Poultry production plays an important role in the agricultural dominated economy of Bangladesh by providing employment to farmers and workers on poultry farms and by generating an important animal protein source for consumption (Bhuiyan, Bhuiyan, & Deb, 2005; Hamid, Rahman, Ahmed, & Hossain, 2017).

Bangladesh experienced its first Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 outbreak in 2007 and since then a total of 57 outbreaks in backyard and 506 in commercial poultry farms occurred in the country (DLS, 2019). Bangladesh is now considered one of six HPAI H5N1 endemic countries (Bangladesh, China, Egypt, India, Indonesia, and Vietnam) in the world (CDC, 2019b).

A further concern is that the Low Pathogenic Avian Influenza (LPAI) subtype H9N2 has become wide spread in poultry production systems of Bangladesh (Parvin et al., 2019; Parvin et al., 2018). The co-circulation of H5N1 and H9N2 viruses in poultry increases the likelihood for a novel re-assortment of AIV which might spread easily among humans (Marinova-Petkova et al., 2014; Marinova-Petkova et al., 2016). Furthermore, H9 viruses play an important role as a "progenitor" virus for HPAI H5N1 Eurasian lineage viruses and both LPAI and HPAI H7N9 viruses (Peacock, James, Sealy, & Iqbal, 2019; Pu et al., 2015; Su et al., 2018).

To establish effective avian influenza (AI) prevention and control strategies in Bangladesh, a good understanding of the epidemiology of AIV circulation on poultry farms is required. Therefore, the overall aim of this research was to quantify the extent of H5 and also H9 virus which play an important role as a "progenitor" virus for HPAI H5N1 Eurasian lineage viruses and both LPAI and HPAI H7N9 viruses, circulation on backyard, and commercial broiler and layer chicken farms in Bangladesh, to identify risk factors associated with the presence of H5 and H9 and to describe the perceptions of poultry farmers towards HPAI prevention and control measures in Bangladesh.

1.2 Research questions

1) How do the patterns of avian influenza A (H5) and A (H9) virus infection differ between backyard, commercial broiler and layer chicken farms in Bangladesh?

2) What are the village and farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of backyard chicken farms in Bangladesh?

3) What are the farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of commercial broiler and layer farms in Bangladesh?

4) What drives or hinders backyard, commercial broiler and layer chicken farmers to implement HPAI prevention and control measures on their farms?

1.3 General methodology

To answer the outlined research questions, a cross-sectional study design with the following general research methodology was used:

1) To improve the understanding of the pattern of avian influenza A (H5) and A (H9) virus infection on backyard, and commercial broiler and layer chicken farms in Bangladesh
    - Collect blood samples, oropharyngeal and cloacal swabs from backyard chickens, in-contact ducks, commercial broiler and layer chickens
    - Measure antibodies against H5 and H9 in the serum of backyard chickens, in-contact ducks, commercial broiler and layer chickens by Enzyme-linked Immunosorbent Assay (ELISA) & Haemagglutination Inhibition (HI) tests
    - Measure H5 and H9 virus presence in the oropharyngeal and cloacal swabs sample of backyard chickens, in-contact ducks, commercial broiler and layer chickens by Reverse Transcription Polymerase Chain Reaction (RT-PCR) test
    - Estimate bird and flock-level prevalence of current and past H5 and H9 infection
    - Estimate the magnitude or clustering of seroprevalence within flocks
    - Estimate seroprevalence by age groups
    - Estimate the spatial distribution of H5 and H9 infection in backyard flocks

2) To identify village and farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of backyard chicken farms in Bangladesh
    - Collect data on farm-level husbandry, management and marketing practices conducted by backyard chicken farmers

- Collect village-level information on environmental or ecological features, village structure, agricultural production, poultry density, previous disease outbreaks in the villages, where backyard farms were located
- Identify village and farm-level risk factors associated with flock-level H5 and H9 serology status (positive/negative) on backyard farms

3) To identify farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of commercial broiler and layer chicken farms in Bangladesh
    - Collect data on farm-level husbandry, management and marketing practices conducted by commercial chicken farmers
    - Identify farm-level risk factors associated with flock-level H5 and H9 serology status (positive/negative) on commercial farms

4) To describe perceptions of backyard, commercial broiler and layer farmers towards implementation of HPAI prevention and control measures
    - Describe biosecurity measures implemented by poultry farmers operating under different production systems in Bangladesh to prevent HPAI infection in their flocks
    - Collect data on perception of farmers on HPAI risk in chickens and humans, consequences associated with HPAI infection, impact of HPAI prevention and control measures, constraints that refrain farmers to implement HPAI prevention and control measures, engagement of farmers with different sources of information on HPAI prevention and control measures, and the likelihood of farmers to implement HPAI prevention and control measures
    - Identify factors influencing the implementation of the biosecurity measures on backyard, commercial broiler and layer farms

1.4 Structure of the thesis

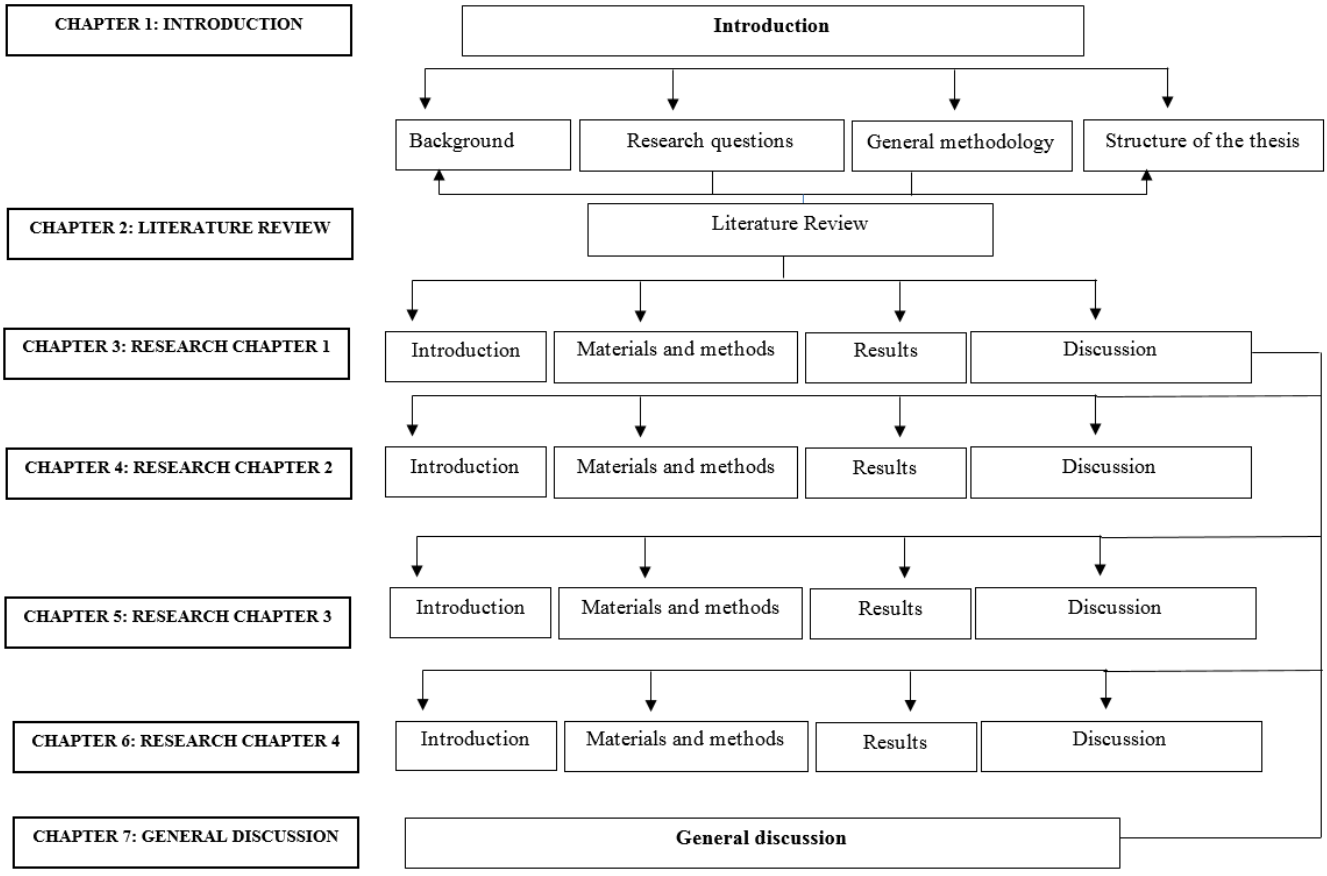

Figure 1.1 Structure of the thesis

This thesis contains 7 Chapters: 'Introduction' (Chapter 1), followed by a 'Literature Review' (Chapter 2), four research Chapters (Chapter 3, Chapter 4, Chapter 5, and Chapter 6), and a 'General discussion' (Chapter 7).

Chapter 1: Introduction

This chapter provides the general background including the aim of this thesis, the research questions and an overview of the research methodology.

Chapter 2: Literature Review

Based on the existing literature, this chapter provides an overview of AIVs, diagnosis of AI and AIV infection pathways. It also provides an overview about poultry production systems in Bangladesh and reviews the AIV infection status in South, South-East Asia and Bangladesh; reviews risk factors for AIV infection on backyard chicken and on commercial chicken farms as well as a review of attitudes and behaviours of livestock farmers and more specifically attitudes, behaviours and practices of backyard and commercial chicken farmers towards AI control measures. The literature review identified the knowledge gaps in the epidemiology of H5 and H9 virus circulation on backyard and commercial broiler and layer chicken farms in Bangladesh. To address the knowledge gaps identified in the literature, four research chapters were develop.

Chapter 3: Patterns of avian influenza A (H5) and A (H9) virus infection on backyard, commercial broiler and layer chicken farms in Bangladesh

This chapter describes bird-level and flock-level H5 and H9 seroprevalence and virus-prevalence on backyard and commercial broiler and layer chicken farms, infection patterns by age groups, the clustering effect for birds being seropositive within a flock, and the spatial distribution of H5 and H9 seropositive backyard flocks. The findings of flock-level serology status (pos/neg) of H5 and H9 on backyard and commercial broiler and layer flocks from this chapter were used as outcome variable in Chapter 4 and Chapter 5 to identify risk factors associated with H5 and H9 seroprevalence.

Chapter 4: Village and farm-level risk factors associated with avian influenza A (H5) and A (H9) flock-level seroprevalence on backyard chicken farms in Bangladesh

This chapter identified farm- and village-level risk factors associated with H5 and H9 seroprevalence on backyard chicken farms.

Chapter 5: Farm-level risk factors associated with avian influenza A (H5) and A (H9) flock-level seroprevalence on commercial broiler and layer farms in Bangladesh

This chapter identified farm-level risk factors associated with H5 and H9 seroprevalence on commercial chicken farms. As many of the risk factors for H5 and H9 infections identified in research Chapters 4 and 5 are related to farmers' perceptions, detailed investigations explored the likelihood of farmers implementing biosecurity and HPAI prevention and control measures in Chapter 6.

Chapter 6: Factors influencing chicken farmers' decisions to implement prevention and control measures to reduce HPAI virus spread in Bangladesh

This chapter identified perceptions of poultry farmers that influenced the implementation of the HPAI biosecurity measures on backyard and commercial farms.

Chapter 7: General discussion

The thesis concludes with a general discussion, where research findings are discussed in a broader aspect, highlighting the significance of the research and providing recommendations for AI control, but also outlining the limitations of the research and providing recommendations for future investigations.

CHAPTER 2

LITERATURE REVIEW

2.1 Avian Influenza (AI) – the pathogen, diagnoses and transmission

2.1.1 Type of avian influenza viruses

AI is a highly contagious viral disease caused by type A influenza virus of the family *Orthomyxoviridae* (Alexander, 2000; OIE, 2019; Paul, Vergne, Mulatti, Tiensin, & Iglesias, 2019). The avian influenza virus (AIV) is single-stranded, negative-sense, pleomorphic in shape (size: 80 to 120 nm) and enveloped RNA virus with eight different gene segments that encode 11 different viral proteins. These include haemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M1), membrane ion channel protein (M2), polymerase proteins (PB1, PB1-F2, PB2, PA), and non-structural proteins (NS1 and NS2) (Nayak et al., 2010; O'Neill, Talon, & Palese, 1998; Palese & Shaw., 2007; Swayne, 2008).

Based on antigenic properties of HA and NA glycoproteins present on the surface of this virus, the AIV is further classified into subtypes. A large number of combinations of 18 HA (H1-18) and 11 NA (N1-11) subtypes have been identified from birds and mammalian hosts (Fouchier et al., 2005; Tong et al., 2012; Tong et al., 2013).

Most importantly, considering the pathogenicity of the AIV in chickens, the virus is generally classified into two categories: HPAI, and LPAI. The World Organization for Animal Health (OIE) described the following methods in their Terrestrial Manual (OIE, 2018) to differentiate between HPAI and LPAI: (1) HPAI virus causes more than 75% mortality within 10 days following intravenous inoculation of a minimum of eight 4 to 8 week old susceptible chickens with infectious virus, or, (2) HPAI virus has an intravenous pathogenicity index greater than 1.2 following inoculation of 10 susceptible 6 week old chickens. In addition, H5 or H7 viruses with amino acid sequences in the HA cleavage site similar to those observed in HPAI viruses are considered as influenza A viruses with high pathogenicity (independent of the outcomes of the pathogenicity experiments conducted to distinguish between HPAI and LPAI) (OIE, 2018).

2.1.2 AI diagnosis

The absence of pathognomonic clinical signs and their variation in different avian species (as well as considerable antigenic variations of AIVs) pose a challenge for precise and rapid diagnosis of AI (Alexander, 2008).

The primary method for virological diagnosis of AIV infections recommended by the European Union (EU) (CEC, 2006a, 2006b) and OIE (OIE, 2018) involve the isolation, identification and

characterization (including estimation of virulence) of the virus. However, as isolation, identification and characterization of AIV is labour intensive and due to an increasing demand for rapid results, molecular techniques, such as the Reverse Transcription Polymerase Chain Reaction (RT-PCR) and real-time RT-PCR are becoming more popular (Alexander, 2008; OIE, 2018). Due to high sensitivity (93.3%) and specificity (98.4%) as well as rapid and cost-effective to test a very large number of samples, the real-time RT-PCR is now the most reliable and widely used virological test for diagnosis of AIV infections (Cattoli et al., 2004).

Recommended serological tests for detecting antibodies against AIV are agar gel immunodiffusion (AGID), HI and ELISA. The preference of using one or more of these recommended serological tests depends on the purpose for the testing (Selleck & Kirkland, 2011). The AGID test is very reliable to detect antibodies against all AIV subtypes in chickens and turkeys, but as not all avian species produce detectable levels of precipitating antibodies, the AGID test is less reliable for avian species in general (Alexander, 2000; Beard, 1970; OIE, 2018; Wright, 2007). In contrast, the HI test is considered as a 'gold standard' for AIV antibody subtyping in all avian species due to its high sensitivity (98·8%) and high degree of haemagglutinin subtype specificity (99·5%). Although the HI test is labour-intensive, it is recommended by EU and OIE for AIV serological diagnosis (Comin, Toft, Stegeman, Klinkenberg, & Marangon, 2013). The ELISA test is considered a potential alternative to the HI test for screening large amounts of avian serum samples (Jensen et al., 2013) and commercial ELISA kits are readily available. ELISA kits are based on indirect and competitive (AIV C-ELISA) or blocking (AIV B-ELISA) strategies (OIE, 2018).

2.1.3 AIV infection pathways

While wild aquatic birds are considered as reservoir for AIV (Olsen et al., 2006; Webster, Bean, Gorman, Chambers, & Kawaoka, 1992), AIV is able to infect a wide range of species of domesticated poultry (e.g. chickens, ducks, gooses, turkeys, quails etc.), pet birds and other wild birds (e.g. crows) (FAO, 2016; OIE, 2019). The virus has also ability to cross the species barrier resulting in sub-clinical to severe infections, including deaths in humans, thus representing a serious public health threat (CDC, 2019c; Webster et al., 2005).

AIV infection in birds naturally occurs via the faecal-oral transmission route (Gilbert, Slingenbergh, & Xiao, 2008). As birds shed AIVs in their saliva, nasal and respiratory secretions, infected feather follicles and in their faeces, direct contact with infected birds can result in the rapid spread of the disease (Nuradji et al., 2016; OIE, 2019). In addition, indirect pathways for example through

contaminated environments (e.g. air, water and dust) and fomites are also considered as an important route for AIV transmission (Webster et al., 1992; Webster, Yakhno, Hinshaw, Bean, & Copal Murti, 1978). Humans are able to carry and spread AIV on fomites (e.g. clothing, equipment, vehicles), while biological vectors (e.g. wild birds, rodents, and insects), have been considered as important pathways for AIV dissemination between poultry farms and live bird markets (Capua and Alexander, 2004; Fusaro et al., 2016; Haase et al., 2010; Hernandez-Jover, Schemann, East, & Toribio, 2015; Poolkhet, Chairatanayuth, Thongratsakul, Kasemsuwan, & Rukkwamsuk, 2013; Ssematimba et al., 2013; Vieira, Hofacre, Smith, & Cole, 2009).

AIV is able to persist in the environment and remain infectious for extended periods of time (Stallknecht, Shane, Kearney, & Zwank, 1990; Webster et al., 1978). It can survive for at least 35 days at 4 °C in faeces and up to 5 weeks within the "environment" of poultry houses (Webster et al., 1978). At 17 °C, HPAI H5N1 virus can survive in water between 14 and 26 days and, at 28 °C between 3 and 5 days (Brown et al., 2006).

2.2 Poultry production systems in Bangladesh

The Food and Agriculture Organization of the United Nations (FAO) has broadly divided the poultry production systems into four different sectors: 1. Industrial and integrated production, 2. Large-scale commercial production, 3. Small-scale commercial production, and 4. Village or backyard production. All of these production systems described exist in Bangladesh, however the small-scale commercial production system (sector 3) and village or backyard production system (sector 4) are preponderating in the country (FAO, 2008) – this is similar to other South and South-East Asian countries (Barua, Biswas, Olsen, & Christensen, 2012; Biswas, Islam, Debnath, & Yamage, 2014). Chickens are the dominant poultry species in Bangladesh: chicken production entails nearly 90% of the total poultry production followed by ducks with about 8% and other poultry species (pigeons, geese and quails) (Das et al., 2008).

2.2.1 Backyard poultry production

Village or backyard chickens comprises almost 90% of the Bangladesh's chicken population (FAO, 2008), and about 80-90% of rural households in Bangladesh rear backyard chickens (FAO, 2008; Fattah, 1999). The predominant backyard chicken breed is a Deshi (meaning 'indigenous' in Bangla) (Barua & Howlider, 1990; Okada et al., 1987). Other backyard chicken "breeds" or strains such as Assel, Naked Neck, Hilly and Red Jungle fowls are rarely seen across the country (Bhuiyan et al., 2005). Village chickens are traditionally reared under scavenging or free ranging conditions, and they

are usually managed by women (SAC, 2017). Many farmers also rear ducks, and sometimes pigeons and geese along with the chickens (Alam, Ali, Das, & Rahman, 2014). The average chicken flock size ranges from 3 to 10 birds (FAO, 2008). During daytime, chickens scavenge around the households, near ponds/wetlands and on agriculture lands (Barua & Yoshimura, 1997). Some farmers may provide backyard chickens with supplementary feed (such as rice polish, rice bran, rice husk, whole rice) (Das et al., 2008). Shelters made of locally available materials (e.g. bamboo, mud, tin, bricks, wood) are used by farmers to protect poultry from predators and extreme weather conditions (Barua & Yoshimura, 1997). Beside the consumption of backyard chickens and their eggs, birds may also be sold, providing households with an important source of income (SAC, 2017). The backyard chicken production system has the lower level of biosecurity compared to commercial chicken production (Conan, Goutard, Sorn, & Vong, 2012; FAO, 2008; Hamilton-West et al., 2012) (**Figure 2.1**).

Figure 2.1 Backyard poultry production system in Bangladesh
(photo taken by author of this thesis)

2.2.2 Commercial poultry production

In Bangladesh, commercial poultry farming started in the 1980s (Begum, Alam, Buysse, Frija, & Van Huylenbroeck, 2012). Since the 1990s, the government of Bangladesh has pursued a policy to expand the national poultry production which resulted in a remarkable upsurge in the number of commercial

poultry farms (ECNEC, 1999). Commercial poultry production plays now a significant role in the socio-economic development of the country (Rana, Rahman, & Sattar, 2013) and chickens are the predominant species raised on commercial poultry farms (FAO, 2008). Bangladesh's commercial chicken production system is broadly divided into two systems: commercial broiler and commercial layer farming (Jabbar, Rahman, Talukder, & Raha, 2007). On commercial broiler farms, chickens are reared for meat, and on layers farms chickens are raised for the production of eggs, although at the end of the production cycle, spent layer hens are sold for meat. The majority of commercial farms in Bangladesh are small-scale (flock-size ≤ 2000 birds) with low to minimal biosecurity (DLS, 2012; Maduka, Igbokwe, & Atsanda, 2016), and only 4% of commercial farms are large-scale units rearing more than 3,000 birds with moderate to high biosecurity (FAO, 2008; Saleque, 2007).

Day old chicks (DOCs) of different exotic broiler chicken strains (e.g. Hybro-PN, Hubbard Classic, Cobb 500, Hybro-PG, Ross etc.) are reared on broiler farms, with the source of DOCs depending on the supplying hatcheries (FAO, 2008; Rana et al., 2013). In addition, Fayoumi and Sonali (a cross between female Fayoumi and male Rhode Island Red) are also popular as meat breed, though they were introduced in the 1980s with different objectives (Das et al., 2008). Broiler chickens are reared on the floor of houses (usually without solid walls), where rice husk, saw dust, wood shavings are used as litter (FAO, 2003) (**Figure 2.2)**.

Figure 2.2 Commercial broiler chicken production system in Bangladesh (photo taken by author of this thesis)

Similar to broilers, layer chickens are also often reared in sheds without solid walls, but their management system is more complex compared to broiler farms (Masud, 2013). Bovan Nera, Shaver 579, Hisex white and brown, Bovinegold line, ISA brown (FAO, 2008) are the common layer chicken strains used in Bangladesh. From DOCs to the grower age (pullets), layer chickens are reared on litter and pullets are then placed into cages where they are reared till the end of the production cycle (Zaman, Sørensen, & Howlider, 2004) (**Figure 2.3**).

Figure 2.3 Commercial layer chicken production system in Bangladesh

(photo taken by author of this thesis)

2.3 AIV infection in poultry production systems

2.3.1 AIV infection in poultry production system - a global overview

HPAI H5N1was first reported in 1959 on a small poultry farm in Scotland, UK (Capua & Alexander, 2007). Since then, several localised outbreaks occurred in different countries across the world. However, since HPAI H5N1 was detected in geese in China in 1996 (Xu, Subbarao, Cox, & Guo, 1999), the virus has been frequently found in domestic poultry and in wild birds resulting in successive epidemics in many countries across Asia, Europe, and Africa (CDC, 2019a; OIE, 2019; WHO, 2016).

Over the past 5 years a total of 7,122 HPAI outbreaks have been reported on domestic poultry farms across 68 different countries, resulting in the loss of approximately 122 million birds, which more than half (58.2%) of the losses being reported from Asia, followed by the Americas (23.0%), Europe (11.6%), Africa (6.8%) and Oceania (0.4%). Of the 12 different AIV subtypes, the greatest diversity was reported from Europe (7 subtypes: H5N1, H5N2, H5N5, H5N6, H5N8, H5N9, H7N7), followed by Asia (6 subtypes: H5N1, H5N2, H5N3, H5N6, H5N8, H7N9) and the Americas (6 subtypes: H5N1, H5N2, H5N8, H7N3, H7N8, H7N9), then Africa (3 subtypes: H5N1, H5N2, H5N8), and Oceania (1 subtype: H7N2). The most widespread subtypes are H5N1, H5N2 and H5N8 (OIE, 2019).

2.3.2 AIV infection in chicken production systems in South, South-East Asia and Bangladesh

In South-East Asia, the first HPAI H5N1 outbreak was officially reported from Vietnam in December 2003 (Martin et al., 2005), but another study indicated that Indonesia experienced an HPAI outbreak in early August 2003 (Morris, Jackson, Stevenson, Benard, & Cogger, 2005). In the South Asian region of India and Pakistan, HPAI outbreaks in domestic poultry were reported shortly in the month of February 2006 (Zhou et al., 2006).

In Bangladesh, the first HPAI H5N1 outbreak in poultry was officially reported in March 2007. Since then, a total of 563 outbreaks (506 on commercial and 57 on backyard farms) were detected in 179 Upazillas or sub-districts (out of 490 Upazillas) of Bangladesh across 52 districts (out of 64 districts) resulting in the culling and destruction of more than 2.87 million birds (and 3.7 million eggs) (DLS, 2019). Since 2013, the reporting of HPAI H5N1 outbreaks has declined, but HPAI H5N1 is still being isolated regularly from live bird markets in Bangladesh (Marinova-Petkova et al., 2016; Rimi et al., 2019; Turner et al., 2017). In addition to the significant economic impact of HPAI on the poultry production in Bangladesh (DLS 2019; FAO, 2014), the country has also experienced eight HPAI H5N1 human cases with one fatality as of September 2019 (WHO, 2019). Bangladesh is now

considered one of the six HPAI H5N1 endemic countries, which also include China, Egypt, India, Indonesia, and Vietnam (CDC, 2019a; FAO 2011, 2013; OIE, 2019). On the other hand, LPAI H9N2 became the second most dominant and geographically widespread Influenza A subtype in commercial and backyard chickens in Bangladesh (Parvin et al., 2018; Parvin et al., 2014; Shanmuganatham et al., 2014). The H5N1 and H9N2 co-circulation in poultry increases the likelihood for a novel re-assortment of AIV, with the potential to infect and spread easily among humans (Marinova-Petkova et al., 2014; Marinova-Petkova et al., 2016).

In 2012, the Government of Bangladesh introduced H5 vaccination on experimental basis in two selected districts, one with high poultry density (Gazipur district) and one with low poultry density (kishoreganj district) (DLS, 2013). Two inactivated vaccines (1) Re-6 from Merial (produced in China), containing the HA gene from a clade 2.3.2.1 H5N1 virus, (2) Nobilis Influenza H5, an inactivated H5N2 vaccine from Intervet (produced in the Netherlands) were promoted. In addition, a live vector vaccine: *Vectormune HVT-AIV* from CEVA-Biomune (produced in the USA), comprising of an innocent vector Marek's disease virus of serotype 3 (Turkey Herpesvirus or HVT) expressing HA gene of a clade 2.2 H5N1 antigen was recommended by the National Technical Expert Committee (the National Technical Expert Committee comprised of renowned scientists, academicians, national experts and international expert from Food and Agriculture Organization of the united Nations FAO), and subsequently approved by Department of Livestock Services (DLS) of Bangladesh. All existing commercial layers and parent stocks are recommended to be vaccinated with two shots of inactivated vaccines at 6-8 weeks interval; whereas, only one shot of live vector vaccine is recommended for the vaccination of day-old layer and broiler chicks (DLS, 2013; Drugs.Com, 2020; Gardin et al., 2015). Since 2014, the Government of Bangladesh has permitted the use of these three vaccines throughout the country (Rimi et al., 2019). However, a study conducted by Ansari et al. (2016) in Bangladesh reported vaccination failures and poor immune responses in layer chickens on H5 vaccinated farms, though the authors did not mention the type of H5 vaccines used to vaccinate the sampled layer chickens. The study reported that out of 221 collected serum samples from vaccinated layer chickens, only a small proportion (8.1%) of vaccinated layer chickens had H5 antibodies. This study recommended to review the currently available vaccines and the overall vaccination program in Bangladesh.

AI is now been recognized as the most important viral poultry disease in the world (OIE, 2019; Swayne, Halvorson, D.A., Saif, Y.M., Swayne, D.E., 2003), with AIV circulation of particular concern in resource limited developing countries (Haque, Giasuddin, Chowdhury, & Islam, 2014; Rahman, Rabbani, Uddin, Chakrabartty, & Her, 2012). A total of 15 studies on the serological and/or

virological status of AIV infections in South and South-East Asia were identified through a review of the available literature (**Table 2.1**). Using a wide range of diagnostic procedures, these studies highlighted varying AIV infection levels across different production systems of farmed poultry. For serological diagnosis, ELISA and/or HI tests were most common approaches to estimate seroprevalence in South and South-East Asia, while rapid AIV antigen (Ag) detection kits and/or RT-PCR were predominately used to estimate AIV prevalence.

Table 2.1 Studies on prevalence of AI on farms in Bangladesh and South, South-East Asia

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
1	Ansari et al. (2016)	Bangladesh	2013 and 2014	-Commercial layer farms -Live Bird Market (LBM) -Backyard poultry (chickens and ducks)	- Study type: • Part of routine surveillance activities - Samples: • Blood • Cloacal swabs • Oropharyngeal swabs - Diagnostic tests: • ELISA • RT-PCR • Rapid AIV Ag detection kit	**Bird-level H5 seroprevalence:** Layer chicken (vaccinated): 8.1% Layer chicken (unvaccinated): 7.6% Backyard poultry (chicken & duck)*: 34.0% **Bird-level AIV prevalence**:** Duck*: 6.7% Chicken*: 17.2% **Bird-level H5N1 virus prevalence***:** Duck*: 1.7% Chicken*: 6.1%	*Samples collected from LBMs and farms (didn't differentiate) **Tested by rapid AIV Ag detection kit ***Tested by RT-PCR
2	Biswas et al. (2009a)	Bangladesh	- Jan 2002 to May 2003 -Sep.2003 to Aug 2004 - Aug 2005 to March 2006	-Backyard chickens -Small-commercial farms(Sonali chickens)	- Study type: • Cross-sectional study - Sample: • Blood -Diagnostic test: • ELISA	**Bird-level Influenza A seroprevalence:** Chicken*: 20.0% **Flock-level Influenza A seroprevalence:** Chicken*: 23.0%	*Despite separate sample collection from both, backyard and commercial Sonali chickens, the prevalence results were reported together as chickens - AI unvaccinated flocks - Serological status of egg drop syndrome '76 virus infectious bronchitis virus, Newcastle disease virus (NDV), and reovirus was also described

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
3	Haque, Kabir, Ali, Rahman, and Islam (2015)	Bangladesh	Jan to Sep. 2014	-Commercial layer chickens -Commercial broiler chickens -Backyard chickens -Backyard ducks -Backyard geese -Backyard pigeons -Quails	- Study type: • Passive surveillance to collect samples from sick and dead birds - Sample: • Cloacal swabs - Diagnostic tests: • Rapid AIV Ag detection kit • HI test • Neuraminidase Inhibition Test (NIT) • RT-PCR	**Bird-level AIV prevalence*:** Chicken(broiler, layer and backyard chicken): 66.7% Duck: 33.3% Goose: 16.7%	*Tested by rapid AIV Ag detection kit -No pigeons and quails were positive to AIV - Rapid AIV Ag detection +ve samples sub-typed by HI & NIT using mono-specific panel of serum and detected H5N1, H9N2, H7N9. - All collected swabs samples tested by RT-PCR using subtype specific (H5N1, H7N9 and H9N2) specific primers, and detected H5N1, H7N9 and H9N2. -Sub-type specific prevalence for different species didn't quantify
4	Hussain, Islam, Al Mahmud, Islam, and Hasan (2016)	Bangladesh	Jan to June 2015	Commercial chickens*	- Study type: • Passive surveillance to collect sick and dead birds - Samples: • Cloacal swabs from sick birds • Tracheal swabs from dead birds - Diagnostic test: • Rapid AIV Ag detection kit	**Bird-level AIV prevalence:** Chicken***:** 2.0%	*No mentioning of type of chickens sampled (i.e. broilers or layers)

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
5	Khatun et al. (2013)	Bangladesh	Between 2009 & 2012 (Three successive winter seasons, Dec.to Feb.)	Backyard ducks	- Study type: • Active AI surveillance - Samples: • Blood samples • Cloacal swabs - Diagnostic tests: • ELISA • RT-PCR	**Bird-level Influenza A seroprevalence:** Duck: 39.8% **Bird-level H5N1 seroprevalence:** Duck: 0.1% **Bird-level AIV prevalence:** Duck: 22.1%	-AI unvaccinated flocks
6	Nooruddin, Hossain, Mohammaand, Rahman (2006)	Bangladesh	2006 (monsoon ,winter & summer)	Backyard chickens	- Study type: • Cross-sectional study - Samples: • Blood • Cloacal swabs -Diagnostic tests: • ELISA • Rapid AIV Ag detection kit	**Bird-level Influenza A seroprevalence:** Chicken: 9.8% Hen: 10.8% Cock: 8.7%	-AI unvaccinated flocks -All the swab samples were negative to AIV
7	Osbjer et al. (2017)	Cambodia	May 2011, July 2012 and March 2013	-Backyard chickens - Backyard ducks - Backyard pigeons -Backyard pigs	- Study type: • Cross-sectional study - Samples: • Cloacal and tracheal swabs from chicken and duck • Fresh fecal sample from pigeon • Nasal swabs from pig - Diagnostic test: • RT-PCR	**Bird-level AIV prevalence:** Chicken:1.4% Duck: 1.0% Pig: 1.5%	-No pigeons were positive to AIV -Full-length genome sequencing confirmed triple reassortant H3N2 in pigs and various LPAI sub-types in poultry. Chicken:H3N8,H4N6,H6N2, H3N6,H6N2 Duck:H6N8

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
8	Pawar et al. (2012)	India	2009, 2010, 2011	-Backyard poultry(chickens and ducks) -LBM -Wild birds	- Study type: • Cross-sectional study - Samples: • Blood • Tracheal swabs • Cloacal swabs • Environmental samples - Diagnostic tests: • HI • RT-PCR	**Bird-level H5N1 seroprevalence:** Backyard poultry (Chicken and duck):2.2% **Bird-level H7N1 seroprevalence:** Backyard poultry (Chicken and duck):1.9% **Bird-level H9N2 seroprevalence:** Backyard poultry (Chicken and duck):9.0% **Bird-level AIV prevalence:** Backyard poultry (Chicken and duck): 5.4%	-None of the samples collected from wild and migratory birds were positive for AIV
9	Henning et al. (2010)	Indonesia	March 2007 to March 2008	-Smallholder scavenging duck farms • Ducks • In-contact chickens	- Study type: • Longitudinal study - Samples: • Blood • Cloacal swabs • Oropharyngeal swabs - Diagnostic tests: • HI • RT-PCR	**Bird-level H5 seroprevalence:** Duck: 2.6%, Chicken:0.5% **Flock-level H5 seroprevalence**: Duck: 19.5%, Chicken:2% **Flock-level H5 virus prevalence:** Duck: 2.5%, Chicken:1.5%	-AI unvaccinated flocks
10	Gompo et al.(2019)	Nepal	March 2018 to April 2019	-Commercial broiler farms -Commercial layer farms - Backyard poultry farms - Breeder farms	- Study type: • Outbreaks study -Samples: • Cloacal swabs • Tracheal swabs -Diagnostic test: • RT-PCR	**Farm-level H9 virus prevalence:** Commercial broiler: 61.9% Commercial layer: 24.4% Backyard poultry (chicken and duck):11.4% Breeder farm*: 2.44%	-Type of breeder farm was not mentioned

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
11	Karki, Lupiani, Budke, Manandhar, and Ivanek (2014)	Nepal	April to July, 2011	Backyard ducks	- Study type: • Cross-sectional study - Sample: • Blood - Diagnostic test: • ELISA	**Bird-level Influenza A seroprevalence:** Duck: 27.2% **Farm-level Influenza A seroprevalence**: Duck: 42.0%	-AI unvaccinated flocks
12	Zaman, Haleem, Rahman, and Ullah (2019)	Pakistan	2018	Backyard chickens	- Study type: • Cross-sectional study - Sample: • Blood -Diagnostic test: • HI	**Bird-level H5N1 seroprevalence:** Chicken (sick): 76.5% Chicken (healthy): 45.0% Chicken (vaccinated)*: 9.0% Chicken (unvaccinated)*:62.5%	*No differentiation between sick and healthy chickens
13	Jairak (2015)	Thailand	July 2013 to Aug, 2014	-Backyard chickens -Backyard ducks	- Study type: • Surveillance - Samples: • Blood • Oropharyngeal swabs • Cloacal swabs - Diagnostic tests: • ELISA • RT-PCR	**Bird-level Influenza A seroprevalence:** Chicken:1.1% **Bird-level AIV prevalence:** Chicken:99.0% Duck:2.1%	-Bird-level Influenza A seroprevalence for ducks was not specified
14	Serrão et al. (2012)	Timor-Lesté	Dec. 2008 to Feb 2009, March to May 2009, June to August 2009	Backyard chickens	- Study type: • Longitudinal study - Sample: • Blood - Diagnostic tests: • HI • ELISA	**Bird-level Influenza A seroprevalence:** Chicken: 0.4%	- AI unvaccinated flocks - AI +ve serum samples tested by HI using Ag against H5N1, H5N3, H7N3 & H9N2, but results negative

Sl#	Study	Study location	Study time	Population examined	Study type, sample collected & diagnostic test used	Outcome measured	Remarks
15	Henning et al. (2011)	Viet Nam	May 2007 to May 2008	- Backyard & smallholder commercial duck farms • Ducks • In-contact chickens	- Study type: • Longitudinal study - Samples: • Blood • Oropharyngeal swabs • cloacal swabs -Diagnostic tests: • HI • RT-PCR	**Bird-level H5 seroprevalence:** Duck (unvaccinated):17.5% Chicken (unvaccinated):10.7% Duck (vaccinated):54.3% Chicken (vaccinated):55.5% **Flock-level H5 seroprevalence:** Ducks (unvaccinated):42.6% Chickens (unvaccinated):19% Ducks(vaccinated):40%, Chickens(vaccinated): 48% **Flock-level H5 virus prevalence:** Ducks*: 0.7%	* Proportion of flocks with at least one bird positive for H5 virus of the vaccinated and unvaccinated birds tested

2.4 Risk factors for AIV infection in poultry production systems

2.4.1 Risk factors for AIV infection on backyard poultry farms

As backyard chickens are reared under free- roaming scavenging conditions (Huque, Chowdhury, Haque, & Sil, 1999; Spradbrow, 1997), they might be at higher risk of AIV infection compared to commercial poultry (Conan et al., 2012). On contrary, owners of backyard flocks argued that due to the small flock sizes, the risk of AIV introduction into their flocks is substantial lower compared to the commercial flocks (Akey, 2003; Bavinck et al., 2009; Refregier-Petton, Rose, Denis, & Salvat, 2001). Furthermore, backyard farmers usually rear local breeds or strains of birds, which are considered to be less susceptible to diseases than exotic breeds used in commercial production (Barua & Yoshimura, 1997; GRAIN 2006). Nevertheless, there is no experimental and field research evidence that supports the argument that backyard chickens are less susceptible to AIV compared to commercial chicken breeds (FAO, 2019b)

Very few studies have been conducted in resource-limited developing countries of South and South-East Asia to identify possible risks factors associated with AIV infections in backyard chickens. For example, Biswas et al. (2009c) identified that feeding of slaughter remnants of purchased chickens to backyard chickens, contact with pigeons, and presence of water bodies within 0.1km from the backyard farms were associated with H5N1 infection in backyard farms in Bangladesh. In addition, separating chickens and ducks at night reduced the risk of H5N1 infection on backyard farms. This study didn't explore the possible association between the trading of chickens from backyard farms and the risk of AIV infection. A study conducted in Thailand (Paul et al., 2011) illustrated that backyard chicken owners, who bought live chickens from another backyard farm had a higher risk of introducing H5N1 into their own flocks, emphasizing the important relationship between poultry trade and AI. Moreover, this study found that backyard chicken owners, who used disinfectants to clean poultry areas had a reduced risk for H5N1 infection in their flocks. It needs highlighting that since 2004, the Thai government is providing disinfectants composed of aldehydes, chlorine, and quaternary ammonium compounds to villagers (Tiensin et al., 2005), and these disinfectants have been showing a good effectiveness against the AIV (De Benedictis, Beato, & Capua, 2007). Considering the financial constraints faced by backyard farmers, this Thai approach of a centralised distribution of disinfectants might be advisable to other developing countries like Bangladesh to improve their AI prevention and control strategies for village poultry.

The presence of a large number of broiler flocks in the village and presence of at least one poultry trader in the village were identified by Desvaux et al. (2011) as village-level risk factors associated with H5N1 HPAI outbreaks in one province of Northern Vietnam in 2007. Interestingly, this study found that villages with a higher percentage of households keeping poultry had less HPAI outbreaks. The authors argued that villages with a higher percentage of poultry keeping, represented more likely rural backyard farms and a had lower human density and less trading activities compared to others villages where commercial farms were more present. The author's argument on lower human density relating to less HPAI outbreaks in the village is supported by research conducted by Dhingra et al. (2014) in eastern India. Dhingra et al. (2014) found that human population density was associated with HPAI H5N1 outbreaks in backyard poultry. The authors suggested that high demand for poultry products resulted in increased trading and marketing activities at live bird markets, which highlighted the association between human density and HPAI H5N1 outbreaks. In addition to human density, improved connectivity (accessibility) in terms of time taken to access a city with more than 50,000 people, duck density and areas at lower elevation were also identified as factors associated with HPAI H5N1 outbreaks in backyard poultry. Therefore, Dhingra et al. (2014) recommended risk-based surveillance in the areas with high duck density and at all live bird markets in high-throughput areas receiving poultry from diverse locations.

A study conducted in the Netherlands, identified that rearing of different chicken breeds and/or different bird species within the same flocks, and the distance between a backyard farm and an infected commercial farm, also increased the risk of AIV infection in backyard flocks (Bavinck et al., 2009).

Finally, focussing on backyard duck production, a study carried out in Indonesia on home-based stationary ducks (i.e. these are ducks that are allowed to scavenge during the day, but are kept on the farm during the night) identified that duck scavenging around neighbouring houses within the village, and farmer's consumption of birds that died two months prior to the farm visit, were risk factors associated with flocks becoming H5 seropositive. In addition, confinement of flocks overnight in enclosures and reporting of sudden deaths of ducks reduced the likelihood of farms being H5 seropositive (Henning et al., 2013).

2.4.2 Risk factors for AIV infection on commercial chicken farms

Compared to the backyard chicken farms, more studies have been conducted on commercial chicken farms in South, East and South-East Asia to identify and quantify the possible risk factors for AIV infection. However, the majority of these studies focussed on risk factors associated with AI

outbreaks with high mortalities, but did not describe the infection status of any birds (i.e. even healthy birds) within the flocks.

Three case-control studies conducted on commercial poultry farms in Bangladesh identified the following risk factors associated with H5N1 outbreak occurrence on commercial chicken farms: exchange of egg trays with market vendors, farm workers trading chickens, numbers of farm employees, the presence of village chickens scavenging on the commercial farm, mortality observed in backyard chickens reared near commercial farms, access of feral and wild animals to the commercial farm, and dead crows observed near commercial farms (Biswas et al., 2009b; Biswas et al., 2011; Osmani et al., 2014). Surprisingly, one study identified a recommended biosecurity practice (provision of footbaths at the entry to commercial farms or at the entrance of commercial poultry sheds) to be associated with H5N1 outbreaks (Biswas et al., 2009b). However, the author doubted the findings and suggested further investigation to explore whether an effective disinfectant was used in the footbath, the concentration of the disinfectant, frequency of changing the disinfectant in the footbath, and whether the footbath was actually used by visitors and farm employees. More recently, Gompo et al. (2019) used a retrospective case-control approach to explore risk factors associated with H9 outbreaks on poultry farms in Nepal between 2018 and 2019. Birds aged 31-40 days, farms operating for more than 5 years, use of stream water as drinking water supply for birds, the type of poultry production, inadequate fumigation practices, history of H9 outbreaks, visitors not wearing boots on farm, visitors allowed to enter farms and no existence of footbaths at entry of farms were identified as significant risk factors associated with H9 outbreaks on commercial poultry farms.

Trading practices as potential source of infection were highlighted in a study conducted in Pakistan (Chaudhry, Rashid, Thrusfield, Welburn, & Bronsvoort, 2015) with selling of eggs or birds directly to live bird retail stalls being strongly associated with an increased risk of H9N2 infection in commercial poultry farms. One interesting finding observed by this study was that farms having a previous history of Infectious Bursal Disease (IBD) infection were more likely to become infected with AI. Experimental work conducted by Ramirez-Nieto et al. (2010) on the adaptation of a mallard H5N2 LPAI virus in chickens that had a previous history of infection with IBD virus, supported this observation. The authors of the experimental study concluded that previous exposure to IBD virus contributed to the mechanism of adaptation of AIV strains resulting in an altered host range, tissue tropism, and higher virulence of the AIV.

A case-control study conducted by Kung et al. (2007) focussed on risk factors associated with the spread of H5N1 on commercial chicken farms in Hong Kong and identified commercial poultry

owners who did not live on farms, a higher death rate in birds older than 30 days compared to younger birds, sales of chickens directly to retail markets, relatives working in the poultry industry and farms with higher chicken numbers increased the odds of H5N1 infection. Interestingly, wild birds observed in feed troughs was identified as a protective factor for H5N1 infection in farms. The authors concluded that workers on case farm were perhaps more conscious about the role of wild birds in AIV transmission after this issue was discussed with government field officers after the farm was declared as infected. This could have resulted in fewer case farms reporting presence of wild birds compared to control farms.

Overall, a wide range of farm management practices have been identified to be associated with a high risk of AIV infection on commercial chicken farms. For example, farms employing one or more workers, layer flocks older than 400 days and identification of at least one clinical signs (e.g. decreased egg production, respiratory syndromes, and increased mortality) were identified as risk factors associated with higher H9N2 seropositivity in commercial chickens in Korea (Woo & Park, 2008). Presence of neighbouring poultry farms was identified as risk factor associated with H5N1 outbreak occurrence in Nigeria during the 2006–2007 epidemics; however, farm staff washing their hands before handling birds and not allowing traders to enter the farm were protective factors; emphasized the significance of trade and closeness between poultry farms in the transmission of H5N1 as well as the role of biosecurity in AI prevention and control (Metras et al., 2013). In fact, poor biosecurity practices have been often associated with AIV infections on commercial chicken farms, even in developed countries. For instance, incomplete hygienic measures of farm visitors and sharing of farm equipment among farms in Japan (Nishiguchi et al., 2007); disposal of dead birds by rendering and presence of mammalian wildlife on commercial farms in USA (McQuiston et al., 2005); and a high number of contacts between farms through cardboard egg trays in Netherlands (Thomas et al., 2005) were significant risk factors for AIV infections on commercial chicken farms.

Compared to the backyard farms, where risk factor studies focused on the H5 subtype, research on commercial farms paid also attention to risk factors associated with H9 virus spread. This might be due to the great economic importance of LPAI virus in commercial production systems, in addition to the public health concern (Ye & Hu, 2008). LPAI virus infection can result in up to 65 % mortality on commercial broiler farms and in a decrease in up to 70 % in egg production in commercial layer chickens (Azizpour, Goudarzi, Charkhkar, Momayez, & Hablolvarid, 2014; El Houadfi, Fellahi, Nassik, Guérin, & Ducatez, 2016; Seifi, Asasi, & Mohammadi, 2012). Also, as commercial farmers rear larger flocks than backyard farmers, the economic impact of LPAI and HPAI infection on the

commercial poultry industry might influenced the stronger interest in risk factor research on this production type.

2.5 Attitudes, behaviours and practices of farmers

2.5.1 Attitudes and behaviours of livestock farmers – an overview

In livestock production, good biosecurity is considered as the first line of defence to prevent disease occurrence on farms (Burrell, 2002; Palmer, Fozdar, & Sully, 2009) with farmer's behaviours and attitudes towards disease control measures playing a vital role in their decision-making processes (Blackstock, Ingram, Burton, Brown, & Slee, 2010; Fairweather & Keating, 1994; Small, Murphy-McIntosh, Waters, Tarbotton, & Botha, 2005). Thus, the success of any national animal disease control program is strongly influences by attitudes and behaviours of farmers (Delabbio et al., 2005).

Research has highlighted that the information provided to farmers on biosecurity and disease control measures strongly influenced their attitudes, behaviours and practices towards those measures (Heffernan, Nielsen, Thomson, & Gunn, 2008; Olmstead & Rhode, 2007; Palmer et al., 2009), although one study with cattle and sheep farmers in the UK (Heffernan et al., 2008) found that farmers attitudes towards biosecurity were not influenced by any particular source of information provided, although there was a strong negative sentiment towards bio-security information provided in government leaflets. This highlights that a good understanding of farmer's perceptions is required to deliver the most applicable and most useful information to farmers.

Adequate communication strategies are instrumental in delivering good biosecurity outcomes. For example, Oliveira, Anneberg, Voss, Sørensen, and Thomsen (2018) studied attitudes of Danish dairy farmers towards biosecurity and identified that difficult communication between farmers and their employees and visitors, lack of knowledge on disease infection pathways, and economic limitations were constraints for correct biosecurity implementation. Moreover, though farmers received biosecurity information from different sources, veterinarians were considered the key and most trusted source of information. Also, the mass media is an important medium to convey information on avian influenza to backyard and commercial poultry farmers in Bangladesh (Sarker et al., 2016). In addition, a study conducted with Bangladeshi backyard poultry farmers highlighted that information from neighbours and family members strongly influenced their awareness and risk perception on avian influenza (Sultana et al., 2012).

Additional factors impacting on farmers' behaviours relate to the constraints they experience when implement disease control measures. For example a study conducted by Jemberu, Mourits, and

Hogeveen (2015) in Ethiopia on Foot and Mouth Disease (FMD) control identified that constraints such as cost of vaccination, difficulties in isolating herds and movement restriction negatively influenced farmer's intentions to implement FMD control measures on their farms. The study also highlighted that implemented control measures were not uniform, but differed greatly by cattle production systems, such as crop-livestock, pastoral and market-oriented systems.

The impact of benfits of control measues on farmers behaviours were highligted by Valeeva, van Asseldonk, and Backus (2011) who conducted a study with Dutch pig farmers. The authors explored underlying factors that influenced farmers' adoption of two risk management strategies: biosecurity measures and animal health programs. Farmers acknowledged that biosecurity is a more effective strategy than animal health program for preventing and controlling epidemic and endemic diseases. In addition, farmers' perceptions on advantages and the efficacy of these strategies in reducing animal disease risk influenced strongly the implementation of the risk management strategies.

2.5.2 Attitudes, behaviours and practices of backyard and commercial chicken farmers towards AI control measures

The traditional approach to prevent and control of AI includes the implementation of biosecurity measures (for example, separating different poultry species, restricting the movement of visitors and outside vehicles, cleaning and disinfection of farm equipment, wearing of protective clothing while handling poultry etc.) and conducting vaccinations against AI. However, vaccination is only conducted in a few countries with a focus on the commercial poultry industry (Capua & Alexander, 2008; Kandeil et al., 2018; Kapczynski et al., 2015; Marangon, Cecchinato, & Capua, 2008).

Different studies highlighted that the likelihood of implementing AI prevention and control measures is depended on farmer's perception on the susceptibility of birds to AIV infection, the consequences of the disease, the benefits of implementing actions, any constraints or barriers to the implementation of those actions and sources of information that may influence individuals' perceptions (Cui, Liu, Ke, & Tian, 2019a; Cui, Wang, Ke, & Tian, 2019b; Glanz & Bishop, 2010; Glanz, Rimer, & Viswanath, 2008; Høg et al., 2018; Rimi et al., 2017). These factors towards the implementation of AI prevention and control measures will likely differ between backyard, commercial broiler, and commercial layer farmers, as farm management practices, production cycles, flock sizes as well level of education of flock owners differ between these three production systems (Cui, Liao, Lam, Liu, & Fielding, 2017; Cui & Liu, 2016; Jemberu et al., 2015). Unfortunately most of the research conducted, focussed only on one production system. For example, a study conducted with commercial chicken farmers in China observed that farmers' perceived risk of infection of chicken with AIV was significantly higher

compared to the perceived risk of human infection with AIV. The study also highlighted that farmers were less familiar with AIV infection in humans compared to chicken. In addition, biosecurity preventive behaviours and personal protective behaviours have been shown to be positively associated with farm size and farmers' perceived risks of both human and chicken infection (Cui et al., 2019b). Similar findings on risk perception were observed in backyard poultry farmers in Bangladesh (Sultana et al., 2012), where backyard poultry farmers perceived that AIV could transmit from poultry to poultry, but not from poultry to humans resulting in some risky behaviour practiced by the farmers, for example, keeping sick poultry under the bed and slaughter and consumption of sick poultry.

Thus, a number of studies focussed on the level of knowledge of farmers. For example, a study conducted with backyard farmers in Egypt (Ismail & Ahmed, 2010) observed positive attitudes towards AI control measures, but highlighted the need for designing and implementation of educational programs to improve the knowledge and practices of farmers. In general, multiple pathways to communicate AI information are used, and depending on the country and the information content, some of them are preferred by farmers. Umar, Thailagavathi, and Yakubu (2015) found that most of the Nigerian poultry farmers were aware of AI through communication program, and mass media was the primary source of their information. On the other hand, Cui et al. (2019a), observed that multiple sources of information were used by farmers to receive information on AI outbreaks in China but only information received over business networks translated into changes of biosecurity behaviours. A study conducted with Bangladeshi backyard poultry farmers also highlighted that the information from neighbours and family members strongly influenced backyard farmers' awareness and risk perception on AI (Sultana et al., 2012).

2.6 Knowledge gaps identified in the literature

The review of the literature highlighted that the type of assessment (serological vs virological) of an AIV infection status of farms differs between countries, with most of the research conducted focussing on a bird-level analysis of H5 subtype prevalence.

No research had been conducted to compare H5 and H9 infection status across different chicken production systems (backyard, commercial broiler and layer chickens), focussing on both, bird- and farm-level H5 and H9 antibody and virus prevalence.

Furthermore, most of the risk factor research conducted focussed on AI outbreaks and was therefore implemented as case-control studies. In addition, previous risk factor research focussed on

commercial production systems, with little attention been paid to backyard production although this production system is crucial for livelihood generation of rural households in developing countries like Bangladesh. In addition, the impact of both, village-level and farm-level risk factors associated with the risk of both H5 and H9 infection in backyard chickens has not been studied. Finally, although management practices vary greatly between chicken production systems, research comparing factors influencing chicken farmers' decisions to implement HPAI prevention and control measures across these different chicken production systems has not been conducted.

Submitted manuscripts included in this thesis

A manuscript (stated below) was prepared based on the next **Chapter 3**, and submitted to publish on '*Transboundary and Emerging Diseases'* journal. This paper has been accepted for publication.

Gupta, S. D., Hoque, M. A., Fournié, G., & Henning, J. (2019). Patterns of avian influenza A (H5) and A (H9) virus infection on backyard, commercial broiler and layer chicken farms in Bangladesh. *Transboundary and Emerging Diseases*. In Press.

Gupta, S. D. (Suman Das Gupta, the candidate) contributed a total of 80% to the conception and design of the study and field data collection, 90% to the analysis and interpretation of the questionnaire and laboratory test results data, 85% to the formatting, drafting and editing of the manuscript. Hoque, M. A. (Dr Md. Ahasanul Hoque, the co-supervisor of the candidate) contributed 4% to the conception and design of the study and field data collection, 1% to the analysis and interpretation of the data and 1% to the manuscript editing. Fournié, G. (Dr Guillaume Fournié, the co-supervisor of the candidate) contributed 6% to the conception and design of the study, 3% to the analysis and interpretation of the data and 4% to the manuscript editing. Henning, J. (Dr Joerg Henning, the principal supervisor of the candidate) contributed 10% to the conception and design of the study, 6% to the analysis of the data and interpretation of results and 10% to the manuscript editing.

CHAPTER 3

PATTERNS OF AVIAN INFLUENZA A (H5) AND A (H9) VIRUS INFECTION ON BACKYARD, COMMERCIAL BROILER AND LAYER CHICKEN FARMS IN BANGLADESH

3.1 Introduction

HPAI H5N1 virus is now considered to be endemic in Bangladesh, China, Egypt, India, Indonesia and Vietnam (CDC, 2019a; FAO, 2011), causing sporadic cases in humans, generally associated with exposure to infected poultry or contaminated environments (Fournié, Høg, Barnett, Pfeiffer, & Mangtani, 2017). However, it is feared that the ongoing co-circulation of LPAI virus subtype H9N2 in H5N1-endemic areas might promote the emergence of reassortants able to spread effectively among humans (Marinova-Petkova et al., 2016; Parvin et al., 2019; Parvin et al., 2018; Thuy et al., 2016). H5N1 infection had also an severe impact on poultry populations in endemically infected countries, resulting for example in the death and culling of more than 2.7 million poultry in Bangladesh between 2007 and 2019 (DLS, 2019).

The number of notified outbreaks is now low in H5N1-endemic countries, such as Bangladesh, where the annual average number of reported outbreaks dropped from 92 in 2007-12, to 2 in 2013-19 (DLS, 2019). Underreporting might be one reason for this decline, as compensation policies were interrupted (Chattopadhyay et al., 2018) or because farmers might have accepted the ubiquity of HPAI outbreak occurrence similar to the endemicity of Newcastle Disease (ND) in many developing countries (Spradbrow, 1996).

Investigations of HPAI outbreaks have generated insights in possible risk factors associated with sudden deaths of birds (Biswas et al., 2009b; Loth, Gilbert, Osmani, Kalam, & Xiao, 2010; Osmani et al., 2014), but they don't provide information about the circulation of AIV in farmed poultry populations in endemically infected countries. Furthermore, in such countries, studies aiming to assess the level of viral circulation in poultry are generally conducted in LBMs (ElMasry et al., 2017; Kim et al., 2018; Negovetich et al., 2011; Thuy et al., 2016), and rarely in poultry farms. This can partly be explained by the ease of sampling, as birds raised under different production systems are brought together in a single location. However, prevalence of infection estimated in marketed poultry populations cannot be extrapolated to farmed populations. In addition, the very few studies conducted in farms focused on a unique production system (Haider et al., 2015; Henning et al., 2011; Henning et al., 2010), and comparison of the level of infection across poultry production systems is lacking.

In Bangladesh, about 80-90% of rural households (HHs) rear small flocks of poultry in their backyard. Backyard chickens, referred to as Deshi, 'indigenous' in Bangla (Barua & Yoshimura, 1997; FAO, 2008) are usually reared under scavenging or free ranging conditions. Many backyard chicken farmers also rear ducks, and sometimes pigeons and geese (Alam et al., 2014; FAO, 2008). In contrast, in commercial broiler and layer farms, exotic strains or cross-breeds of chickens (e.g. Cobb 500 strain,

Hisex brown strain, Sonali cross-breed) are usually reared intensively, under confinement, with provision of commercially available feed (FAO, 2008; Huque, Saleque, & Khatun, 2011).

In order to control and prevent the spread of H5N1 and H9N2 viruses in chickens, a detailed understanding of infection patterns at bird- and flock-level is required. It is hypothesized that different poultry species as well as different poultry husbandry systems might play different roles in the transmission and maintenance of those viruses (Alexander, 2000; Zhang et al., 2014). Furthermore, H9 viruses play an important role as a "progenitor" virus for HPAI H5N1 Eurasian lineage viruses and both LPAI and HPAI H7N9 viruses (Peacock, James, Sealy, & Iqbal, 2019; Pu et al., 2015; Su et al., 2018). Thus, this study aims to quantify the extent of H5 and H9 virus circulation in backyard chicken farms, and in commercial broiler and layer chicken farms in Bangladesh. Two cross-sectional studies were conducted. We estimated 1) bird and flock-level prevalence of current and past H5 and H9 infection, 2) the magnitude of spread of the infection within flocks, and 3) variations in prevalence with age. Finally, we assessed 4) the spatial distribution of H5 and H9 infection in backyard flocks.

3.2 Materials and methods

3.2.1 Study design

Two cross-sectional studies were conducted in Chittagong and Cox's Bazaar districts, which were identified as the main districts supplying chickens to Chittagong City Live Bird Markets (CCLBMs) (Moyen, 2019; Moyen et al., 2018). Backyard chicken farms were surveyed between February and April 2016, and commercial broiler and layer chicken farms between February and April 2017.

3.2.1.1 Sample size

H5 and H9 bird- and flock-level seroprevalence were assumed to differ according to poultry species and production systems. For each poultry species (i.e. ducks and chickens) and each production system (i.e. backyard, commercial broiler and layer), a two stage sampling approach was used to estimate 1) the number of farms, and 2) the number of birds per farm to be sampled (Humphry, Cameron, & Gunn, 2004). Input parameters for sample size calculations and estimated sample sizes are listed in **Table 3.1**. The assumed design prevalence, i.e. the expected bird and flock-level H5 seroprevalence for backyard and commercial birds, were based on Henning et al. (2011) and Hassan (2017); respectively.

Table 3.1 Input parameters for sample size calculations and estimated sample sizes. Flock sensitivity: the probability that at least one sampled bird in an infected flock is found positive, assuming that the flock is infected at a prevalence equal to or greater than the specified design prevalence (Sergeant & Perkins, 2015)

Parameters	Backyard chickens	Backyard in-contact ducks	Commercial broiler chickens	Commercial layer chickens
Test sensitivity (%)	98.0	98.0	98.0	98.0
Confidence level (%)	95.0	95.0	95.0	95.0
Design bird-level H5 seroprevalence (%)	15.0	35.0	15.0	35.0
Design flock-level H5 seroprevalence (%)	25.0	50.0	25.0	45.0
Flock size	10	3	1500	1500
Tolerance (%)	10.0	10.0	10.0	10
Minimum desired flock sensitivity (%)	65.0	95.0	75.0	95.0
Calculated flock sensitivity (%)	65.6	98.7	76.2	96.6
Farms to be sampled	123	99	103	102
Birds to be sampled	4	2	9	8

3.2.1.2 Selection of administrative areas

For backyard farms, the selection of sub-districts (upazillas) in the Chittagong district was based on features identified to influence AIV transmission (Ahmed et al., 2012): 1) their density of backyard poultry farms, 2) their density of backyard chickens, 3) their location in the district, 4) their environmental characteristics, and 5) their distance to Chittagong City, where most live bird markets are located (as distance to LBM is a crude proxy for value chain interactions). The density of backyard poultry farms and backyard chickens per square kilometre was calculated based on census data: the number of rural households (BBS, 2014a, 2015), with the assumption that 80% of households operated as backyard poultry farms (FAO, 2008), and the number of backyard chickens (BBS, 2011a, 2011b). Quartiles of the density of backyard poultry farms and backyard chickens across the Chittagong district were computed and each upazilla was assigned to one of those quartiles. To cover most of a district's geographical area, the Chittagong district was divided into regions (south, north, east, west, middle), and upazillas were identified from each of these regions. We also aimed to recruit upazillas differing according to the presence of water reservoirs (sea/river/canal/lake/wetland), woodlands (forest/hill/jungle), and their distance to Chittagong city. A ranking matrix was then developed for all upazillas in the Chittagong district, and eight upazillas were selected representing combinations of all five selection criteria.

Two upazillas in the Cox's Bazaar district, which were the main suppliers of poultry for CCLBMs, were also selected (Moyen, 2019).

In order for the studied backyard and commercial farms to be from the same geographical areas, the upazillas selected for the backyard farm research were also selected for the subsequent commercial farm research.

3.2.1.3 Selection of villages and backyard chicken farms

We then calculated quartiles for the number of households (or farms) per village across each district.

In each selected upazilla, each village was assigned to a quartile according to their number of households, and one village was randomly selected from each quartile (using syntax RANDBETWEEN in Microsoft Excel 2013, Microsoft Corporation, USA).

Four villages were thus selected from each of the 8 selected upazillas in the Chittagong district, and 5 villages were selected from each of the 2 selected upazillas in Cox's Bazaar district.

We aimed to sample at least 123 backyard chicken farms, of which 99 also raised ducks. To sample 3 farms per village, 2 farms had to raise both chicken and ducks, and 1 farm only chicken. Following this strategy, we would need to sample 84 farms (42*2= 84) that raised both chicken. Thus, to reach our estimated sample size (N=99), for 24 villages, we sampled 3 farms per village (2 farms raised both chicken and ducks, and 1 farm only chicken), and for 18 villages, we sampled 4 farms per village (3 farms raised both chicken and ducks, and 1 farm only chicken). Following this procedure, we selected 144 backyard farms of which 102 also raised ducks. Starting from one the village entrance, we counted farms as we walked through the village, and recruited farms matching numbers which were randomly generated before the field visit. If a selected farm owner was not available or had an insufficient number of birds to be sampled, the neighbouring farm was used as a replacement.

3.2.1.4 Selection of commercial chicken farms

For each selected upazilla, a list of commercial broiler and layer farmers was generated through consultations with upazilla livestock officers, feed and chick dealers, veterinary pharmaceutical representatives, private veterinarians, feed company representatives and hatchery representatives. Information about the flock sizes of those farms was not available.

Then, simple random sampling (using syntax RANDBETWEEN in Microsoft Excel 2013, Microsoft Corporation, USA) was used to select broiler or layer farms within each upazilla. In order to sample at least 102 layer farms, 10-11 farms were required per upazilla. As only six and eight layer farms were identified in two upazillas, all of those farms were selected in these two upazillas, and 10-15 farms were selected from the other eight upazillas. To sample at least 103 broiler farms, 10-13 farms were randomly selected in each selected upazilla.

3.2.1.5 Selection of birds

As backyard chickens and in-contact ducks were free-ranging, birds were conveniently recruited in each selected farm, with the backyard flock owner capturing available birds until the sample size was reached. A total of 4 chickens and 2 in-contact ducks were selected from farms that had both, chickens and ducks, and 4 chickens were selected from farms that had chickens only.

For commercial farms, chickens were selected from different parts of the poultry shed until eight layer and nine broiler chickens were obtained. Bird characteristics that could have made them appear as different from other birds in the same flock were not accounted for (e.g. clinical signs, plumage colour, body weight etc.). If several sheds were present on a commercial farm, the shed with oldest

birds was selected assuming that these birds had a higher chance of being exposed to AIV throughout their production cycle.

3.2.2 Sample collection and processing

Informed written consent (signature or thumb impression) was provided by each farmer before sampling the birds and conduct of the interview. A blood sample, a cloacal and an oropharyngeal swabs were then collected from each bird, and bird's age, sex and apparent clinical signs (if any) were recorded.

Depending on the body weight, 1-3 ml blood were collected from wing or jugular vein of each bird and transferred to individual sterile plastic tube immediately after collection. Oropharyngeal swabs were taken by gently rolling the swab tip around the inside of the bird's mouth and behind the tongue. Cloacal swab were collected by inserting the swab into the cloaca and rotating it several times. Swabs were placed into separate cryovials containing viral transport media. Tubes and cryovials collected in Chittagong district were kept in a cool box filled with ice packs and transported to the Chattogram (previously Chittagong) Veterinary and Animal Sciences (CVASU) laboratory within the same day. And cryovials were stored at -80^{0}C. Blood samples were refrigerated overnight, then the serum was separated by centrifugation at 10,000 rpm for 30 minutes at 4^{0}c and transferred to Eppendorf tubes. The serum was stored at -20^{0}C until further processing. In Cox's Bazaar, samples were transported immediately to the local office of the Department of Livestock Services (DLS). Blood samples were processed as indicated above, while cryovials were stored in liquid nitrogen for up to 8 days before their transfer and storage in a -80^{0}C freezer at CVASU.

3.2.3 Diagnostic tests

3.2.3.1. Serological tests

The serum samples were first screened for the presence of antibodies against Influenza A virus using a commercially available ELISA. For backyard chicken and duck samples the IDEXX® AI MultiS-Screen ELISA (Product Code: 5004.20, IDEXX Laboratories, Inc.,USA) and for commercial chicken samples the ID Screen® Influenza A Antibody Competition Multi-Species ELISA (Product Code: FLUACA ver 1216 GB, ID.vet, FRANCE) or the IDEXX® AI ELISA (Product Code: 5004.00, IDEXX Laboratories, Inc.,USA) were used. Positive samples were then tested for the presence of H5 and H9 specific antibodies using the HI test. Due to the unavailability of local AIV H5N1 and H9N2 antigens from field viruses collected in Bangladesh, inactivated antigens prepared by the Animal and Plant Health Agency in Surrey, United Kingdom were used in the HI test (H5N1-A/Ck/Scot/59,

H5N3-A/Teal/Eng/7394-2805/06, H9N2-A/Tky/Wisc/1/66, H9N9-A/knot/Eng/SV497/02). A serum sample was positive if there was an inhibition at a dilution of 1/16 (2^4) or more against 4 haemagglutinating units of antigen (OIE, 2015).

3.2.3.2. Virological tests

Swab samples were pooled at the CVASU laboratory with respect to their type (cloacal and oropharyngeal), bird species and farm of origin, with a maximum of five samples per pool. RNA was extracted from the pooled samples using the MagMax^{TM}-96 extraction kit (Ambion Life Technologies Corporation®, 2013). Real-time RT-PCR tested for the presence of AIV Matrix gene (M-gene). For all M-gene positive pools, RNA was extracted from the corresponding individual samples and tested by real-time RT-PCR for H5 and H9 genes (AAHL, 2014). A bird was positive if its cloacal and/or oropharyngeal swabs were positive.

3.2.4 Data analyses

Laboratory test results were entered into Microsoft Excel 2013 spreadsheets, coded and checked for integrity, with the final dataset exported into STATA 14.1(Stata Corporation, College Station, Texas, USA).

3.2.4.1 Bird and flock-level prevalence

Bird and flock-level apparent virus prevalence were calculated separately for Influenza A (M-gene positive), H5 and H9. A flock was positive for a specific serological or virological test if at least one of its birds was positive. The 95% logit confidence intervals (CI) for prevalence (Dean & Pagano, 2015) were calculated using the *-prop-* command in STATA 14.1. If the prevalence was zero, the 97.5% binomial exact or Clopper-Pearson confidence interval (Clopper & Pearson, 1934; Dean & Pagano, 2015) was calculated using the *–cii prop-* command in STATA 14.1. To describe infection patterns over the duration of a production cycle, the bird-level seroprevalence was stratified by age groups and presented with 95% confidence intervals.

3.2.4.2 Relationship between bird-level and flock-level seroprevalence

We assessed the correlation between the serological statuses of individual birds within a flock (Shrout & Fleiss, 1979) by computing the individual intra-class correlation (ICC):

$$\rho = ICC = Corr(y_{ij}, y_{ij'}) = \frac{\sigma_r^2}{\sigma_r^2 + \sigma_\epsilon^2}$$

Where, σ_r^2= variance between flocks and σ_ϵ^2= error variance or variance within flocks

In our study, chickens (backyard, commercial broiler, layer) and in-contact ducks were considered as "raters" for the serological status of flocks (represented as "targets") in a one-way random effects model:

$$y_{ij} = \mu + r_i + \epsilon_{ij}$$

We observed y_{ij}, where *i*= 1,…, *n; j*=1,…, *k*; *where* y_{ij} is the j^{th} rating on the i^{th} target; μ is the mean rating; r_i is the target random effect and ϵ_{ij} is the random error (StataCorp., 2019).

3.2.4.3 Spatial clusters for H5/H9 seropositivity of backyard farms

To explore spatial patterns in viral transmission, we assessed whether H5/H9 seropositive birds were randomly distributed across the two study districts. The total number of seropositive and seronegative birds on each farm was used as the outcome of a discrete Bernoulli probability model implemented in the SaTScan software version 9.4.4 (SaTScan™, 2016, Boston, USA). Spatial clusters of infection were identified based on 999 Standard Monte Carlo replications. The coordinates of the visited backyard farms were used as spatial information in the analysis. The maximum size of a spatial cluster was 25% of the population at risk (Kulldorff, 1997). The analysis was conducted separately for backyard chickens and in-contact ducks, and for both H5 and H9 subtypes.

3.3 Results

A total of 576 backyard chickens and 204 in-contact ducks were sampled across 144 backyard flocks, and a total of 954 broiler and 904 layer chickens were sampled from 106 broiler and 113 layer chicken flocks. None of the sampled backyard (N=144) and commercial broiler flocks (N=106) was vaccinated against H5 (using inactivated or live H5 virus strains). Of the total sampled commercial layer flocks (N=113), 13 layer flocks were vaccinated against H5 (using inactivated or live H5 strain), and the remaining 100 layer flocks were not vaccinated against H5 (using inactivated or live H5 strain).HPAI outbreaks or mass mortality events were not reported in any of the backyard and commercial farms in the 12 months preceding the sampling.

The average (minimum, maximum) flock size of sampled backyard poultry, commercial broiler, unvaccinated and vaccinated commercial layer flocks were 21 (5, 73), 1,657 (200, 6,000), 2,118 (60, 7,500) and 2,831 (975, 10,500), respectively.

3.3.1 Bird-level virus prevalence

Influenza A virus prevalence was 0.2% (95% CI: 0.0-1.2) for backyard chickens, 1% (95% CI: 0.2-3.9) for backyard in-contact ducks, 1.8% (95% CI: 1.1-2.8) for broiler chickens, 1.6% (95% CI: 0.9-2.8) for unvaccinated layer and 1.9% (95% CI: 0.5-7.4) for H5 vaccinated layer chickens (**Figure 3.1**).

None of the sampled birds on backyard and commercial farms was H5 virus positive.

On backyard farms, 0.2 % (95% CI: 0.0-1.2) of chickens, but none of the in-contact ducks were H9 virus positive (**Figure 3.1**). Similarly, low bird-level H9 virus prevalence was observed for broiler and unvaccinated commercial layer chickens, with 0.5% (95% CI: 0.2-1.3) and 0.6% (95% CI: 0.3-1.5), respectively. None of the H5 vaccinated commercial layer chickens was H9 virus positive (**Figure 3.1)**.

3.3.2 Flock-level virus prevalence

The flock-level Influenza A virus prevalence was 0.7% (95% CI: 0.1-4.9) for backyard flocks, 7.5% (95% CI: 3.8-14.5) for broiler flocks, 5.0% (95% CI: 2.1-11.6) for unvaccinated layer and 7.7% (95% CI: 1.0-41.6) for H5 vaccinated layer flocks (**Figure 3.1**).

Relatively more unvaccinated commercial flocks were H9 virus positive compared to backyard flocks, with 1.9% (95% CI: 0.5-7.4) and 2.0% (95% CI: 0.5-7.8) of broiler and unvaccinated layer flocks being positive compared to 0.7% (95% CI: 0.1-4.9) backyard flocks. None of the H5 vaccinated commercial layer flocks was H9 virus positive (**Figure 3.1**).

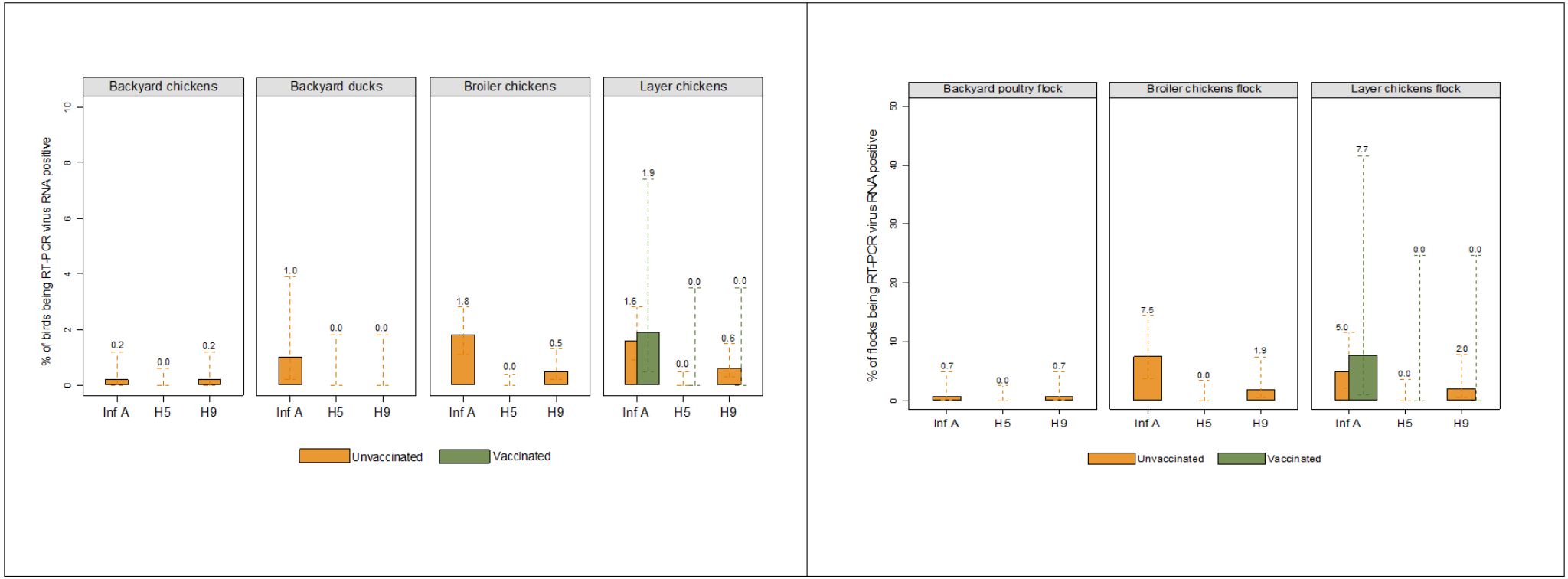

Figure 3.1 Bird-level (left panel) and flock-level (right panel) Influenza A (M-gene), H5 and H9 virus RNA prevalence detected by Reverse Transcription Polymerase Chain Reaction (RT-PCR) in backyard and commercial chicken production systems in Bangladesh (2016-2017). Data labels represent the prevalence values. The confidence intervals are shown as dashed lines. Confidence intervals represent 95% limits if prevalence was >0% and 97.5% limits if prevalence was 0%.

3.3.3 Bird-level seroprevalence

Bird-level Influenza A seroprevalence was 71.7% (95% CI: 67.9-75.2) for backyard chickens, 75.5% (95% CI: 69.1-80.9) for backyard in-contact ducks, 9.3% (95% CI: 7.6-11.3) for broiler chickens, 33.1% (95% CI: 29.9-36.5) for unvaccinated layer chickens, and 69.2% (95% CI: 59.7-77.4) for H5 vaccinated (using live or inactivated H5 virus strains) layer chickens (**Figure 3.2**).

In backyard chickens, bird-level H5 seroprevalence was lower compared to H9 seroprevalence - it was 4.2% (95% CI: 2.8-6.1) and 16.0% (95% CI: 13.2-19.2) respectively; while bird-level H5 and H9 seroprevalence were similar in in-contact ducks, with 14.2% (95% CI: 10.0-19.8) and 15.7% (95% CI: 11.3-21.4), respectively (**Figure 3.2**).

In broiler chickens, bird-level seroprevalence was 1.5% (95% CI: 0.9-2.5) and 1.5% (95% CI: 0.9-2.5) for H5 and H9, respectively. In unvaccinated layer chickens, bird-level seroprevalence was 7.8% (95% CI: 6.1-9.8) for H5 and 5.8% (95% CI: 4.3-7.6) for H9, while in H5 vaccinated (using live or inactivated H5 virus strains) layer chickens bird-level seroprevalence was 10.6% (95% CI: 5.9-18.2) for H5 and 4.8% (95% CI: 2.0-11.1) for H9 (**Figure 3.2**).

3.3.4 Flock-level seroprevalence

The flock-level Influenza A seroprevalence was 97.2% (95% CI: 92.8-99.0) for backyard flocks, 17.9% (95% CI: 11.7-26.6) for broiler flocks, 52.0% (95% CI: 42.1-61.7) for unvaccinated layer flocks, and 84.6% (95% CI: 53.0-96.4) for H5 vaccinated (using live or inactivated H5 virus strains) layer flocks (**Figure 3.2**).

In backyard poultry, flock-level seroprevalence was 27.8% (95% CI: 21.0-35.7) for H5 and 60.4% (95% CI: 52.1-68.2) for H9 (**Figure 3.2**). In contrast to backyard poultry, the flock-level H5 seroprevalence in broiler and unvaccinated layers flocks was relatively higher than H9: it was 9.4% (95% CI: 5.1-16.8) and 5.7% (95% CI: 2.5-12.2) in broilers and 31.0% (95% CI: 22.6-40.9) and 22.0% (95% CI: 14.9-31.3) in unvaccinated layer flocks, respectively. The flock-level seroprevalence was 38.5% (95% CI: 16.2-66.9) for H5 and 23.1% (95% CI: 7.2-53.8) for H9 in H5 vaccinated (using live or inactivated H5 virus strains) layer flocks (**Figure 3.2)**.

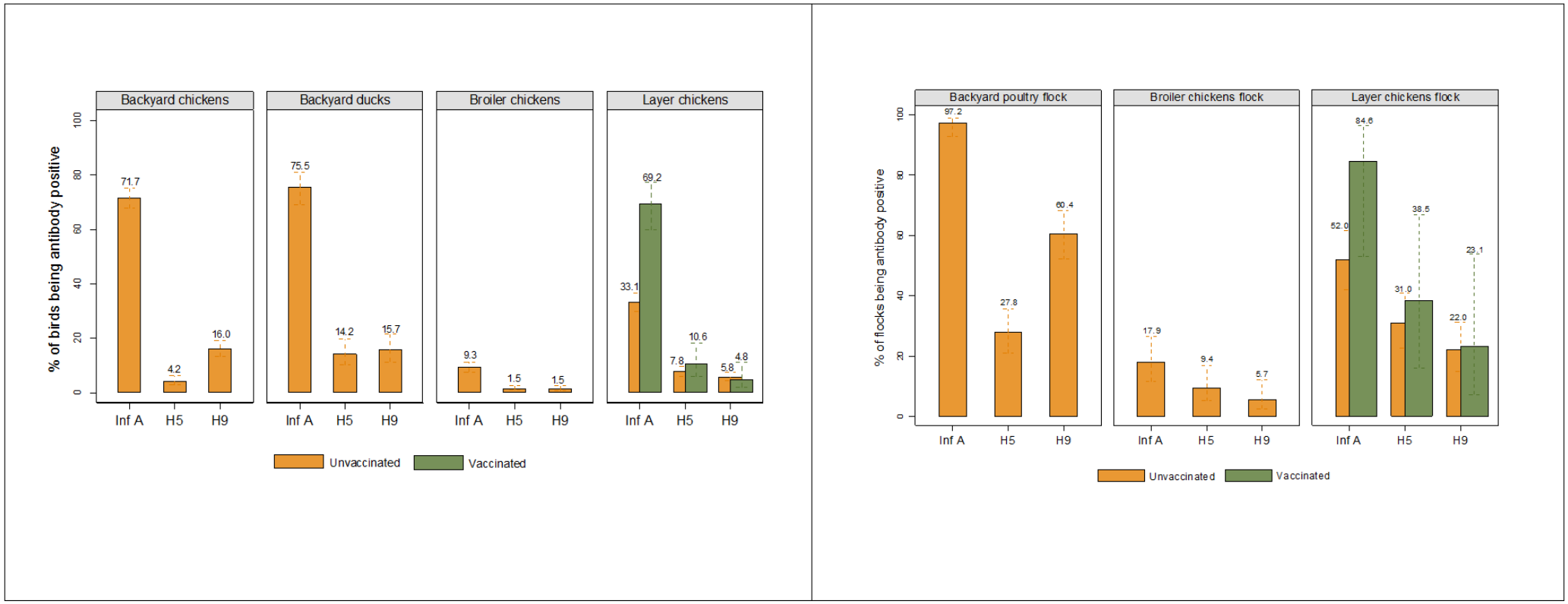

Figure 3.2 Bird-level (left panel) and flock-level (right panel) Influenza A, H5 and H9 seroprevalence in backyard and commercial chicken production systems in Bangladesh (2016-2017). Influenza A antibodies were detected by Enzyme Linked Immunosorbent Assay (ELISA), and H5 and H9 antibodies were detected by Haemagglutination Inhibition (HI) test (≥1/16 dilution). Data labels represent the prevalence values. The 95% confidence intervals are shown as dashed lines.

3.3.5 Relationship between bird-level and flock-level seroprevalence

The majority of H5 and H9 seropositive backyard flocks (70.0% of H5 and 66.7% of H9 seropositive flocks) had only a single bird (either chicken or duck) that tested positive within the flock. Interestingly, in only 5.0% of backyard flocks, both chickens and ducks, were found to be H5 seropositive, while in 13.8% of backyard flocks, both chickens and ducks, were H9 seropositive.

The clustering effect of birds being seropositive within a flock is represented by the ICC displayed in **Figure 3.3**. As often only single chickens were H5 or H9 seropositive within a backyard flock, the ICC was low for backyard chickens (Backyard chicken H5: ICC=0.07, 95% CI: 0.0-0.2; Backyard chicken H9:0.04, 95% CI: 0.0-0.1). In contrast, often several ducks with a backyard flock were seropositive (Backyard duck H5: ICC=0.48, 95% CI: 0.3-0.6; Backyard duck H9: ICC=0.19, 95% CI: 0.0-0.4), which highlights that H5 and H9 seropositivity of backyard flocks are identified with multiple in-contact ducks that are H5 and H9 positive within the same flock.

For all broiler flocks (100%) only 1-2 chickens tested H5 seropositive across the 9 birds sampled per flock (Broiler H5: ICC=0.06, 95% CI: 0.0-0.1), whereas for H9 serpositivity, 50% of broiler flocks had 1-2 birds, and 50% had 3-4 birds being positive (Broiler H9: ICC=0.22, 95% CI: 0.2-0.3).

As similar low clustering effect for H5 and H9 seropositivity was observed for unvaccinated layer flocks with 1-2 birds of the 8 birds sampled tested H5 seropositive in 71% of flocks and H9 seropositive in 73% of flocks (Unvaccinated layer H5: ICC=0.15, 95% CI: 0.1-0.2; Unvaccinated layer H9: ICC=0.17, 95% CI: 0.1-0.2). In 60% of vaccinated layer flocks only 1-2 birds tested H5 seropositive (Vaccinated layer H5: ICC=0.19, 95% CI: 0.1-0.5).

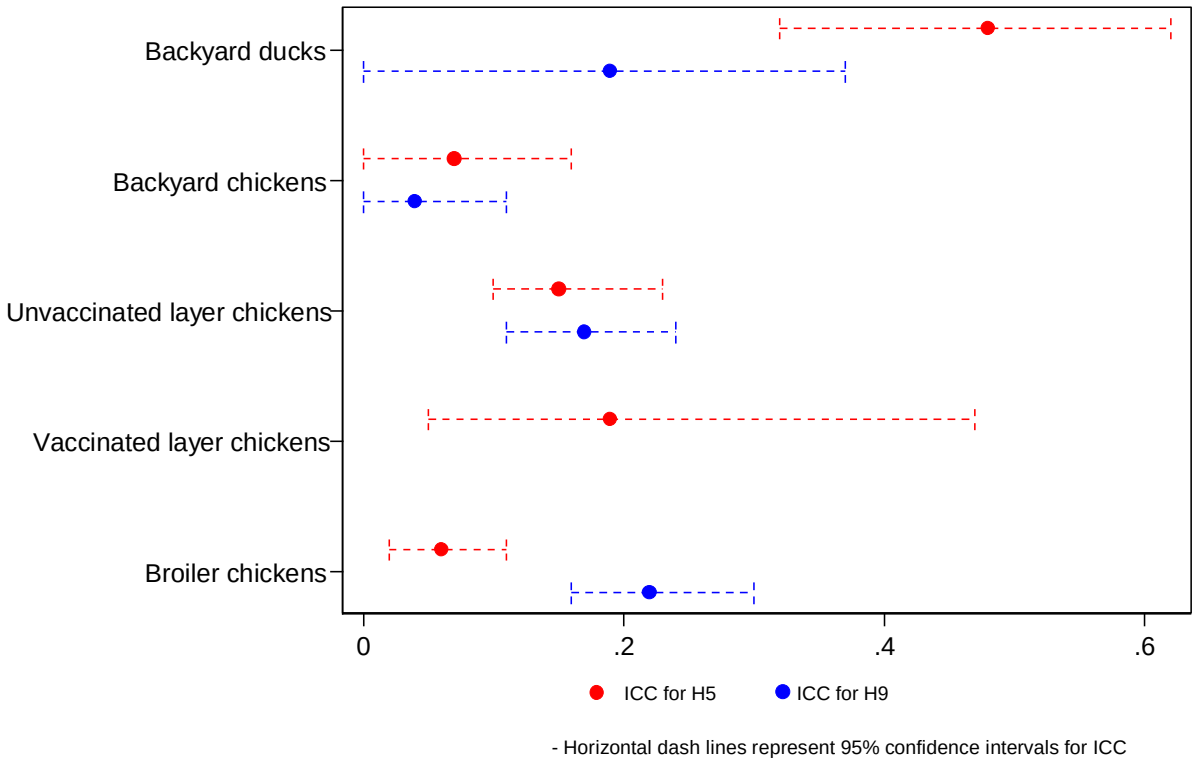

Figure 3.3 Intra-class Correlation Coefficients (ICCs) for H5 and H9 seropositivity in backyard and commercial chicken production systems in Bangladesh (2016-2017). The 95% confidence intervals are shown as dashed lines.

3.3.6 Infection patterns by age groups

Over the course of a production cycle, higher bird-level H5 and H9 seroprevalence was in general observed in older backyard chickens and ducks as well as in older unvaccinated layers (**Figure 3.4**). Interestingly, H5 seroprevalence peaked around 1.5 years in backyard chickens and unvaccinated layers, but then declined afterwards. A similar decline in older birds was not observed for H9 seropositivity in backyard chickens and unvaccinated layers.

An increase in H5 and H9 titres with age was not as prominent in broilers.

Surprisingly, H5 titres in vaccinated layers were low in the first year of age (when vaccination of layers was conducted) and only peaked at 1.5 years of age and drastically declined afterwards.

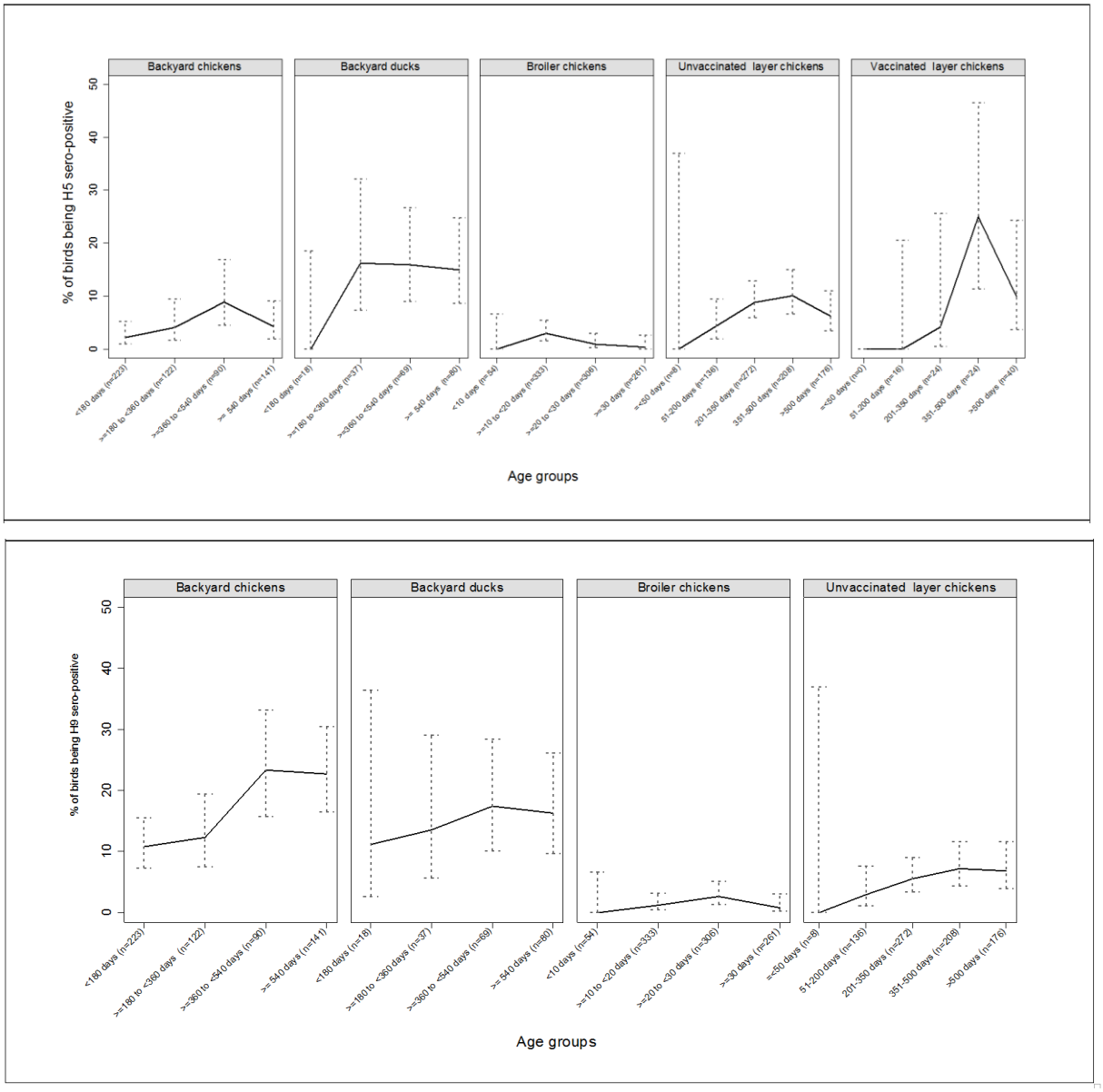

Figure 3.4 Bird-level H5 and H9 seroprevalence by age group in backyard and commercial birds and flocks in Bangladesh (2016-2017). The confidence intervals are shown as dashed lines. Confidence intervals represent 95% limits if prevalence was >0% and as 97.5% limits if prevalence was 0%.

3.3.7 Spatial clusters for H5/H9 seropositivity of backyard farms

The relatively high flock-level seropositivity of backyard flocks and a strong clustering of ducks being seropositive within backyard flocks, intrigued us to further explore the spatial distribution of H5 and H9 seropositivity of backyard poultry within our study area.

When analysing the locations of chickens being positive within backyard farms, a high risk cluster for H5 seropositivity (Relative Risk=5.4, p=0.004, radius=16.8 km) and a spatially overlapping high risk cluster for H9 seropositivity (Relative Risk=15.2, p=0.036, radius=15.2 km) were identified in the central part of the Chittagong district (**Figure 3.5**). This area is represented by high densities of backyard poultry farms, proximity to the Chittagong city, where most live bird markets are located, and most importantly, the largest river in Chittagong district, the Karnaphuli river, passes through the clusters.

Interestingly, when locations of ducks being positive within a backyard farm were analysed, only a small high risk cluster for H9 (Relative Risk=7.7, p=0.048, radius=0.6 km) was identified, highlighting that the risk of ducks being H5 and H9 seropositive was uniform throughout the study area (**Figure 3.6**).

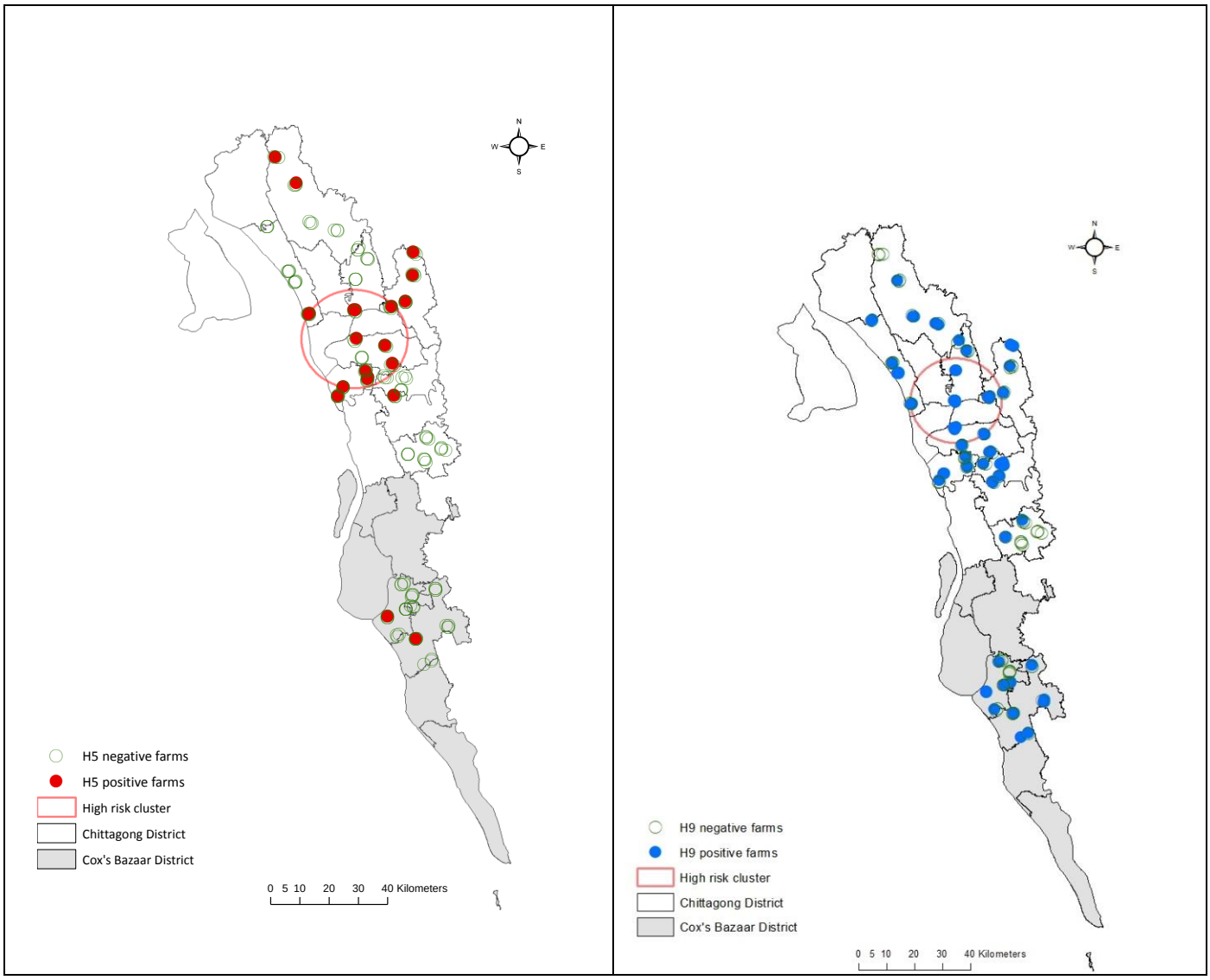

Figure 3.5 Spatial distribution and high-risk clusters of H5 and H9 seropositivity for chickens on backyard farms in the Chittagong and Cox's Bazaar districts of Bangladesh (2016-2017).

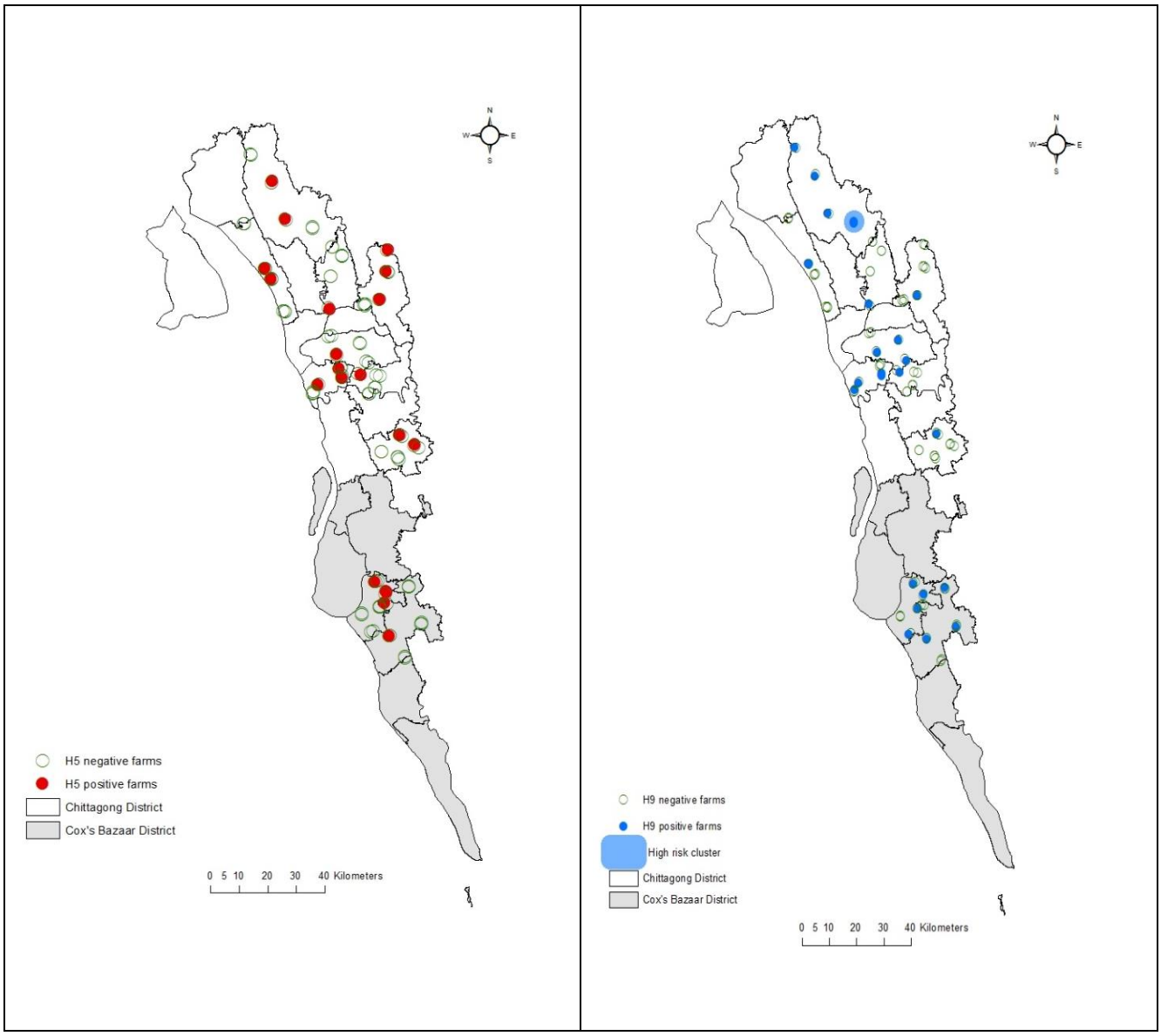

Figure 3.6 Spatial distribution of H5 and H9 seropositivity for ducks on backyard farms in the Chittagong and Cox's Bazaar districts of Bangladesh (2016-2017).

3.4 Discussion

This is the first study comprehensively investigating the extent of H5 and H9 virus circulation among populations of backyard, commercial broiler and layer chickens in a H5N1-endemic country.

In a recent study, the proportion of birds positive for the AIV was estimated in Bangladeshi LBMs, including in CCLBMs which are supplied by our farm study population. The proportion of birds positive for H5 and H9 virus reached 1.3% and 8.3 % in backyard chickens, 7.6% and 3.4% in waterfowl (including ducks and geese), 0.9% and 13.1% in broiler chickens, respectively (Kim et al., 2018). In contrast, H9 prevalence was here estimated to be much lower in farmed poultry, and all sampled birds tested negative for H5. This may be due to the amplification of the AIV in LBMs (Kung et al., 2007), or along the trading networks through which poultry are moved from farms to LBMs.

Interestingly, the proportion of flocks positive for the Influenza A and H9 virus was similar for broiler and layer farms. This suggests that the level of exposure of broiler flocks to AIVs may be similar to layer flocks. Whereas AI vaccination programmes often focus on layer farms, these results suggest that a vaccination aiming to reduce the transmission of AIVs in a poultry population should consider the vaccination of broilers. A detailed cost-benefit analysis would be required before such a prevention strategy can be implemented. In addition, as vaccinating commercial broilers is a high-cost approach, vaccinating valuable broiler breeder parent and grandparent stock with HVT-AIV vaccine might be a more reasonable and acceptable option for poultry producers in Bangladesh. However, the potential risk of antigenic variants of HPAIV H5N1 viruses evolving due to extensive use of H5 vaccine need to be considered as this may result in the failure of a vaccination programme (Setiawaty, Pratiwi, Pawestri, Ibrahim, & Soebandrio, 2013).

As infected birds may shed AIVs for 3-7 days, serological testing can be useful to assess past infection patterns (Achenbach & Bowen, 2011; Leigh Perkins & Swayne, 2002; Saito et al., 2009; Spackman, Pantin-Jackwood, Swayne, & Suarez, 2009; Sturm-Ramirez et al., 2004). For example, it has been shown that H5 antibodies persist up to 40 weeks post-vaccination in H5N3 vaccinated chickens and ducks (Boltz et al., 2009), while H9N2 antibodies had been detected for up to 15 weeks in unvaccinated chickens (Imai et al., 2007).

In our study, H5 bird-level seroprevalence was higher in in-contact ducks than chickens in backyard farms. Such pattern was also described in Vietnam and Indonesia (Henning et al., 2011; Henning et al., 2010). One plausible explanation is that chickens infected by some clades of HPAI H5 (for

instance, A/chicken/Yamaguchi/7/04 (H5N1, clade 2.5)) will most likely die, whereas ducks may survive (Kishida et al., 2005; WHO/OIE/FAO, 2008), although farmers did not report any significant increase in chicken mortality over the year preceding the sampling. Other possible explanations for the higher seroprevalence in ducks are that antibodies might persist for longer in ducks, or ducks might be exposed more frequently to H5 viruses as they are more likely to mingle with (potentially infected) wild waterfowls in water bodies (Hill et al., 2015; Khatun et al., 2013). Ducks are considered to be a natural reservoir for most AI subtypes due to their immunological characteristics (Hinshaw, Webster, & Turner, 1980; Vanderven et al., 2012; Webster, Bean, Gorman, Chambers, & Kawaoka, 1992). As suggested by former studies, ducks may be a major source of H5 virus for backyard chickens and other poultry (Henning et al., 2011; Hulse-Post et al., 2005; Kishida et al., 2005; Sarkar et al., 2017).

Whereas previous studies (Ansari et al., 2016; Karki et al., 2014; Khatun et al., 2013) generally only reported bird-level prevalence, we estimated the clustering of seropositive birds within a flock. The significant clustering effect of H5 seropositivity in ducks suggests that if one duck was H5 seropositive in a flock, other ducks were also likely to be H5 seropositive. This was in contrast to backyard and commercial broiler and layer chickens, highlighting only individual birds within these flocks developed H5 antibodies.

Similarly to a study on ducks in Vietnam (Henning et al., 2011), H5 and H9 seroprevalence increased with the age of backyard and layer birds. Indeed, it is expected that the likelihood of having been exposed to endemic viruses increases over time. Although seroprevalence marginally increased with age in broilers, we did not observe the same magnitude of increased H5 seroprevalence in broilers compared to layers and backyard chickens. The short lifespan of broiler reflects a shorter duration of exposure to AIV compared to layers or backyard chickens, and thereby highlights a lower risk of AIV infection in broilers (Tombari et al., 2013). Field research highlighted that high H9 antibodies titres were only observed after 2 weeks past infection in broilers (Nili & Asasi, 2002)**.** Hence, considering the short production cycle of broilers there are limited opportunities to observe a significant rise of antibody titres in broilers under field conditions. We also did not find any H5 antibodies in very young broiler and layer chicks indicating may be there were no maternal antibodies persisting in this age group for commercial birds. For birds of the same age, H9 seroprevalence was higher in backyard than layer chickens, which indicates a higher level of exposure to H9 virus as well as lower mortality due to LPAI. This would need to be further explored through longitudinal studies.

Interestingly no major mortalities or clinical HPAI symptoms were observed in backyard and commercial chicken flocks although birds developed antibodies. The survival of some chickens to

infection by H5 viruses, and their subsequent seropositivity, might result from infection by LPAI H5 strains and other LPAI H5 subtypes (such as, H5N2, H5N3, H5N8). A number of studies reported LPAI H5 viruses (H5N2, H5N3, H5N8) occurring in Asia (Duan et al., 2007; Nguyen et al., 2005), including LPAI H5N2 virus in Bangladesh (Gerloff et al., 2016). Another explanation might be a reduction in H5N1 pathogenicity due to viral evolution (Li et al., 2017; Londt, Banks, & Alexander, 2007) and the development of cell-mediated immunity that contributes to host resistance (Kapczynski, 2008; Wang, Loh, Kedzierski, & Kedzierska, 2016).

In Bangladesh, two inactivated vaccines are used for commercial layers and parent stocks: (1) *Re-6* from Merial (produced in China), containing the HA gene from a clade 2.3.2.1 H5N1 virus, (2) *Nobilis Influenza H5,* an inactivated H5N2 vaccine from Intervet (produced in the Netherlands). The Department of Livestock Services (DLS) of Bangladesh approved these two vaccines for the initial vaccination of commercial layers and parent stocks, irrespective of their ages. Then, 6-8 weeks after the initial vaccination, a booster vaccination is recommended.

In addition, the live vector vaccine *Vectormune HVT-AIV* from CEVA-Biomune (produced in the USA), comprising of an innocent vector Marek's disease virus of serotype 3 (Turkey Herpesvirus or HVT) expressing HA gene of a clade 2.2 H5N1 antigen is used for vaccination of day-old layer and broiler chicks. Due to the development of life-long immunity of *Vectormune HVT-AIV* (as claimed by the manufacturer of vaccine), booster vaccination is not required (DLS, 2013; Drugs.Com, 2020; Gardin et al., 2015).

However, in our study, none of the sampled broiler flocks were vaccinated against H5, and out of the 113 sampled layer flocks only 13 flocks (11.5%) were vaccinated against H5 (using live or inactivated H5 vaccine strains). This low uptake of vaccination might be due to the high cost of the vaccine, which is Bangladeshi Taka 5 (US$0.06) for a single dose of the vaccine. Education of farmers about the benefits of vaccination and the payment of incentives (through the Government of Bangladesh) could potentially increase the uptake of AI vaccinations (DhakaHerald, 2013; DLS, 2013; Rimi et al., 2019).

Surprisingly, we found that a substantial proportion of vaccinated layer chickens developed no immune response. This findings was consistent with an earlier study in Bangladesh, which reported that a small proportion (8.1%) of vaccinated layer chickens had H5 antibodies, although the type of H5 vaccine used was not mentioned in this study (Ansari et al.; 2016). This poor immune response might be due to improper vaccination, poor vaccine quality, immunosuppressive diseases (for

example, Marek's disease, infectious bursal disease) or the administration of other vaccines at the time of the AI vaccination (for instance, Marek's vaccine) (van den Berg et al., 2008). Nevertheless, higher bird-level H5 seroprevalence was observed in older vaccinated birds, which might be due to repeated vaccinations, or exposure to LPAI H5 field viruses.

Our spatial cluster analysis revealed consistent H5 (past) infection of ducks across the whole study areas. In contrast, for backyard chickens, the spatial distribution of H5 and H9 seropositive cases were clustered in the same area, with the Karnaphuli River, the largest river in Chittagong district, passing through this cluster. Indeed, river systems in Bangladesh have been hypothesized as being as potential risky areas for HPAI H5N1 infection, although no biological sampling of birds had been reported in those areas (Ahmed et al., 2012; Muzaffar et al., 2008).

There are some limitations in our study. Firstly, the HI test antigens were prepared from field virus isolated from a range of countries, but not from a field virus collected in Bangladesh. This might have reduced the sensitivity and the specificity of HI test used in this study. Due to the unavailability of local AIV H5N1 and H9N2 antigens prepared from field viruses collected in Bangladesh, we were unable to explore the impact of the source of antigen on test characteristics of the HI test. However, a study conducted by Yamamoto et al. (2007) estimated sensitivity and specificity of the HI test to be 99% and 90%, respectively, when different antigens were used. Considering this good specificity of the HI test using different antigens, we are confident that our estimated antibody prevalence was not overestimated due to many false-positive results. Secondly, recall bias may have led to a miss-estimation of the age of chickens. This would only relate to backyard farmers, as commercial flock owners usually record the dates when they start their production cycle with day-old chicks. Unfortunately, the exact dates of vaccinations were not recorded by layer farmers, and, therefore we could not assess the patterns of seropositivity according to farm-specific vaccination programmes.

In conclusion, this research provided unique insights into current and past H5 and H9 infection pattern across all chicken production systems in Bangladesh. Our findings can support the development of targeted preventions and control measures for chicken production systems and provide import parameters for mathematical models exploring the infection dynamics of AIVs in endemic settings.

CHAPTER 4

VILLAGE AND FARM-LEVEL RISK FACTORS ASSOCIATED WITH AVIAN INFLUENZA A (H5) AND A (H9) FLOCK-LEVEL SEROPREVALENCE ON BACKYARD CHICKEN FARMS IN BANGLADESH

4.1 Introduction

Backyard chickens reared in a traditional scavenging system are an important source of high quality nutrition (Axe, 2016; Islam, Seeland, Bulbul, & Howlider, 2002) and self-employment for rural households in low and middle-income countries (LMICs), such as Bangladesh (Huque, 1999; Islam, Begum, Kausar, Hossain, & Kamruzzaman, 2015; SAC, 2017). However, the low level of biosecurity in this farming system may put backyard poultry at a high risk of infection by AIVs (Conan et al., 2012) and backyard poultry are often considered to promote the spread and persistence of AIVs (Bavinck et al., 2009; Tiensin et al., 2005). It has been hypothesised that, due to their small flock size, the risk of viral introduction into backyard flocks may be substantially lower than in commercial flocks (Akey, 2003; Refregier-Petton et al., 2001), and that local breeds raised on backyard farms may be less susceptible to infection than exotic breeds reared in commercial chicken farming systems (Barua & Yoshimura, 1997; GRAIN 2006). However, there is no experimental and observational evidence supporting these hypotheses (FAO, 2019b).

HPAI H5N1 was first reported in Bangladesh in 2007. It is now endemic in Bangladesh with multiple AIV subtypes, including LPAI H9N2, circulating in the country's poultry population raising concerns for the emergence of a new AIV variant of significant public health concern (Marinova-Petkova et al., 2016; Parvin et al., 2018).

A recent study conducted in Bangladeshi LBMs estimated a prevalence of H5 and H9 AIVs of 1.3% and 8.3 % in backyard chickens, 7.6% and 3.4% in waterfowl (ducks and geese), respectively (Kim et al., 2018). In contrast, none of the chickens and ducks that we sampled on backyard flocks tested positive for H5 AIV, and only 0.2% of chickens tested positive for H9 AIV (Chapter 3). The H5 and H9 seroprevalence was 4.2% and 16.0% in backyard chickens, and 14.2% and 15.7% in ducks, respectively, indicating a past exposure to circulating H5 and H9 virus (Chapter 3).

The implementation of comprehensive biosecurity practices is notoriously challenging, if at all feasible, in a backyard farming system (Rimi et al., 2019). The lack of adherence to recommended biosecurity practices is likely influenced by backyard farmers' belief that their poultry does not play a significant role in AIV transmission (Bavinck et al., 2009). It is therefore essential to identify risk factors contributing to AIV infection in backyard flocks, in order to develop extension messages on AI prevention and control that are tailored to backyard farmers.

To our knowledge, only one case-control study was conducted in Bangladesh, more than 10 years ago, to identify farm-level factors associated with H5N1 outbreak occurrence on backyard farms (Biswas et al., 2009c). Risk factors for current H5 and H9 circulation on largely outbreak free backyard farms have not been described. Therefore, this study aimed to identify farm- and village-level factors associated with current H5 and H9 seroprevalence on backyard farms in Bangladesh.

4.2 Materials and methods

4.2.1 Overview of the study design

A cross-sectional study was conducted in the Chittagong and Cox's Bazaar districts of Bangladesh between February and April 2016. The study was conducted on 144 backyard chicken farms across 42 villages. The sample size calculations and the selection of study units are described in Chapter 3.

4.2.2 Data collection

We used two types of questionnaires to collect information on farm-level and village-level risk factors potentially associated with AIV circulation. The questionnaires were developed based on causal diagrams constructed using the software MindMaple Lite version 1.3 (MindMaple Inc., Tustin, USA) visualising the hypothesized relationships between the flock-level serological status and potential farm- (**Figure 4.1**) and village-level risk factors (**Figure 4.2**).

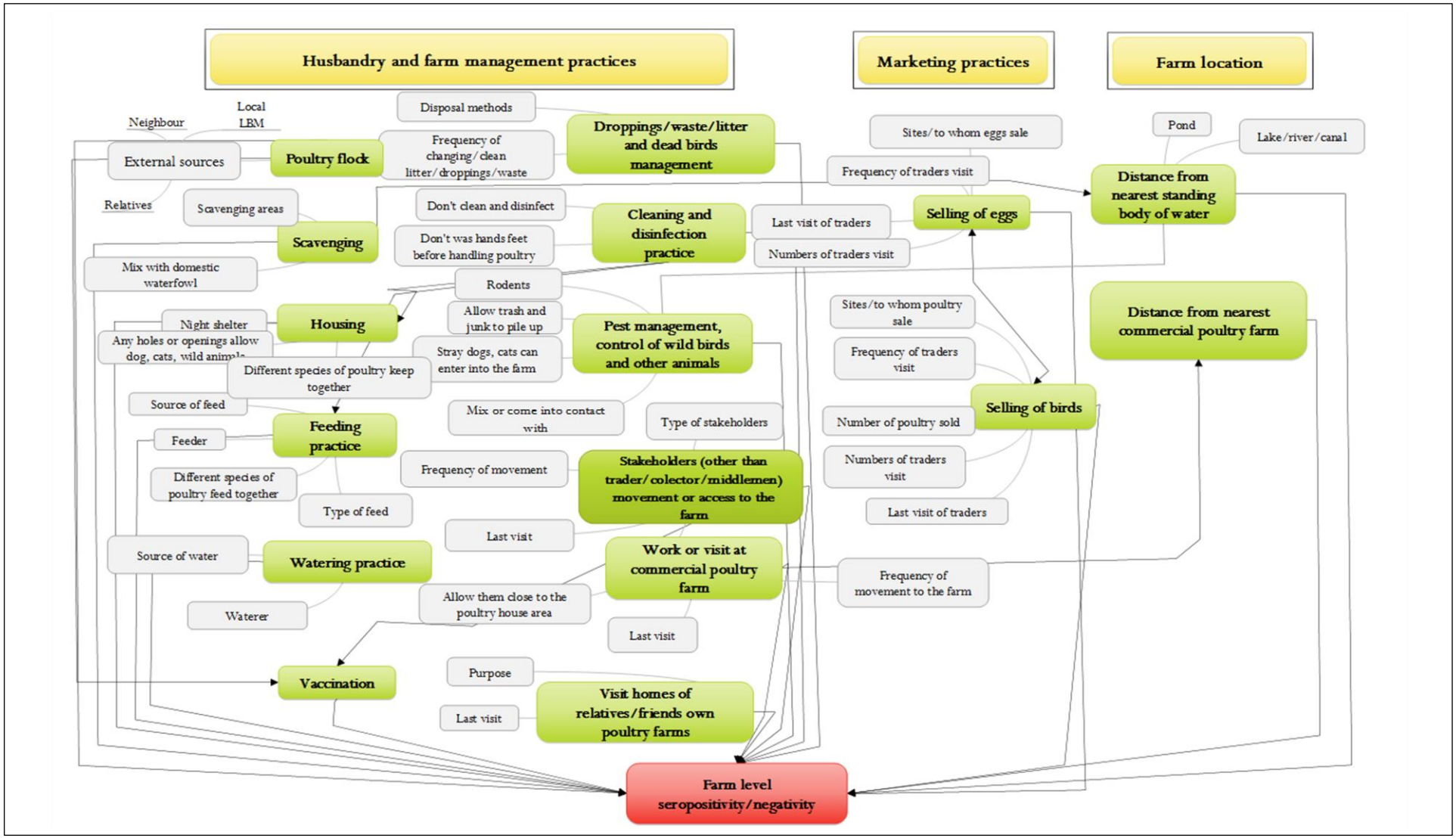

Figure 4.1 Hypothesized causal pathways for farm-level risk factors associated with AI infection on backyard farms in Bangladesh. The red box represent the outcome (farm-level seropositivity) in the risk factor analysis, green boxes represent individual risk factors with grey boxes indicating additional categories/levels within the risk factor. Yellow-brown headings represent themes or categories under which risk factors can be combined. The causal pathways were used to inform the development of questions used in the interviews with backyard farmers and to guide the inclusion of potential confounders and interactions in the final multivariable model.

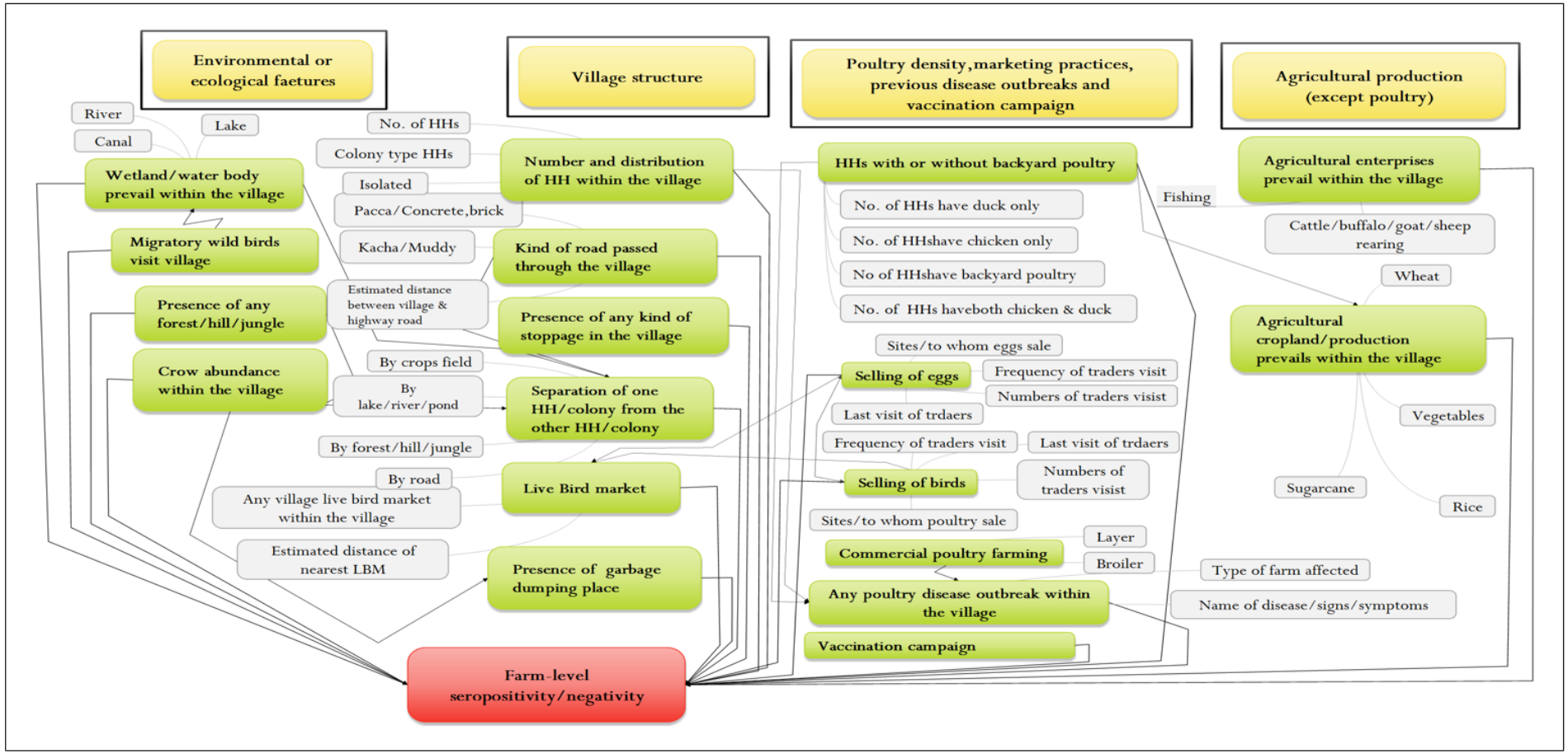

Figure 4.2 Hypothesized causal pathways for village-level risk factors associated with AI infection on backyard farms in Bangladesh. The red box represent the outcome (The red box represent the outcome (farm-level seropositivity) in the risk factor analysis, green boxes represent individual risk factors with grey boxes indicating additional categories/levels within the risk factor. Yellow-brown headings represent themes or categories under which risk factors can be combined. The causal pathways were used to inform the development of questions used in the interviews with village-key informants including backyard farmers and to guide the inclusion of potential confounders and interactions in the final multivariable model.

The farm-level questionnaire was designed to collect detailed information on husbandry and farm management practices, marketing practices, and the farm location. The questionnaire was pilot-tested on five backyard farms that were not part of the farms recruited for the study. Twelve out of 58 questions were subsequently modified as their initial formulation was unclear for interviewed farmers. For example, farmers in the pilot study had difficulties understanding the question '*Do you maintain any quarantine measures for newly introduced poultry into the flock*?' and we modified the question to '*Do you keep newly introduced poultry separate from other poultry in a safe and separate place/house before introducing into the existing poultry flock*?'.

A second village-level questionnaire was designed to collect information on environmental or ecological features, the village structure, type of agricultural production in the village, poultry density, previous disease outbreaks and vaccination campaign in the village. The village-level questionnaire contained 26 questions and was divided into two parts. The first part comprising of 15 sections, summarized information that were made during observations while walking through the village and examining environmental and agricultural village characteristics. The second part included 11 questions on village demographics, poultry production and marketing within the village. The observational information was collected by the author of this thesis, while key informants (see below) provided answers to questions in the second part of the questionnaire during a Participatory Appraisal (PA). The village-level questionnaire was also pilot-tested with key informants from 2 villages which were not included in the final study. The pilot testings resulted in the modification of five questions in the village-level questionnaire. For example, the original question, '*Is there a live bird market in this village?*' was modified to '*Is there any market within the village where trading of poultry is conducted?*'. The reason for the misunderstanding of the original question was that interviewees considered live birds markets as markets that operate daily (while village markets usually operate several days per week).

Both questionnaires were developed in English and then translated into Bengali language.

A total of 144 backyard chicken farmers were interviewed using the farm-level questionnaire. An interview lasted about 35 minutes. The interviews were conducted by one female and one male veterinarians trained in data collection.

The PA were conducted as group discussions involving 5-7 key informants in each village. The key informants included at least one village headman, two backyard chicken farmers, one Veterinary Field Assistant from the local livestock office, one school/college teacher or/and religious leader and/or a

commercial poultry farmer. The key informants were contacted one week before of the visit to the village. The PA lasted 20 minutes in each village.

Informed consent (signature/thumb impression) was obtained from each farmer and village key informant before the commencement of the interview/PA and sample collection of birds.

Of the interviewed 144 backyard chicken farmers, 102 raised both chickens & ducks, and 42 only raised chickens. Blood samples were collected from 4 chickens and 2 in-contact ducks from farms that had both chickens and ducks, and from 4 chickens from farms that had chickens only. Depending on the body weight, 1-3 ml of blood were collected from the wing or jugular vein of each chicken/duck and transferred to an individual sterile plastic tube immediately after collection. The tube was kept in cool box filled with ice packs and transported to the CVASU laboratory (for samples collected in Chittagong) and transported to the local office of the DLS (for samples collected in Cox's Bazaar). Samples were refrigerated overnight, then the serum was separated by centrifugation at 10,000 rpm for 30 minutes at 4^0c and transferred to Eppendorf tubes.

All the serum samples were further processed at the CVASU laboratory, where the samples were first screened for the presence of antibodies against Influenza A virus using commercially available ELISA kits. Influenza A positive samples were then tested for the presence of H5 and H9 specific antibodies using the HI test. A serum sample was considered positive if there was an inhibition at a dilution of 1/16 (2^4) or more against 4 haemagglutinating units of antigen (OIE, 2015).

4.2.3 Data analyses

Farm- and village-level data were entered in Microsoft Access 2013 databases (Microsoft Corporation, USA). Data analysis was conducted in STATA 14.1 (Stata Corporation, College Station, Texas, USA). We used the farm-level H5 and H9 serological status as binary outcome variable: a farm was considered positive for a given AIV subtype if at least one chicken or duck on that farm had a HI titre of $\geq 2^4$. Data analysis was conducted separately for H5 and H9.

A total of 281 farm-level and 96 village-level dichotomous/binary and ordinal categorical variables were derived from questionnaire data. For each AIV subtype, the proportion of positive and negative farms for each risk factor was calculated.

To reduce the number of predictors we used correlation analysis and screening of variables based on bivariate unconditional associations in the univariate analysis (Dohoo, Martin, & Stryhn, 2009). In the univariate analysis associations between the H5/H9 flock-level serological status and each

potential risk factor were explored using a mixed-effect logistic regression approach with the village as random effect. For predictors with at least 3 modalities p-values were computed using Wald tests ('*testparm*' command).

All farm- and village-level predictors associated with a p-value ≤0.15 in the univariate analysis were screened for pairwise correlations. Considering the dichotomous/binary and ordinal nature of the predictors, pairwise correlations were examined by estimating the polychoric correlations coefficients (UCLA, 2019; Uebersax, 2006) using the –***polychoric***- command in STATA. If high correlation was identified (≥0.9 for H5/H9) one of the two variables which was less biologically justified and/or was highly correlated with another variable was excluded.

Multivariable mixed-effects logistic regression models were built for each AIV subtype with village as a random effect, using a backward stepwise elimination procedure. Farm- and village-level predictors were considered together in the same models. At each step, the predictors with the highest p-value was removed, until all predictors remaining in the model had p-values <0.05. Wald test were applied using –*testparm*- command to test the overall significance of predictors with at least 3 modalities.

We also evaluated potential confounding by subsequently adding, eliminated risk factors that were considered biological plausible and important based on the hypothesized casual diagrams. A change in the Odds Ratio (OR) >30% (Dohoo et al., 2009) for any of the added predictors in the model was considered as an indication for confounding. Biologically plausible 2-way interactions of risk factors significant at p<0.05 in the final main effect model were also explored (Dohoo et al., 2009).

Furthermore the residual Intra-class Correlation was estimated. Finally, to identify any specific observations that impact or do not fit the models, normality and heteroscedasticity plots of the residuals were developed.

4.3 Results

4.3.1 H5 and H9 flock-level serology status

None of the sampled flocks were vaccinated against AI. The farmers reported no HPAI outbreaks or abnormal mortalities in chickens or ducks on their farms within the last 12 months before the sampling. The flock-level prevalence 27.8% (N=40) for H5 and 60.4% (N=87) for H9.

4.3.2 Farm and village-level risk factors associated with H5 and H9 flock-level seroprevalence on backyard chicken farms

Table 4.1 Results of the univariate and multivariable analysis for village-level and farm-level risk factors associated with H5 and H9 flock-level seroprevalence on backyard chicken farms

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Village-level factors (N=144 farms, N=42 villages)													
Environmental or ecological features													
Crow abundance around a garbage dumping place in the village	No or absence of garbage dumping place	20 (19.6)	82 (80.4)	Reference	0.001	53 (52.0)	49 (48.0)	Reference	0.004	Reference	0.039	Reference	0.004
	Yes	20 (47.6)	22 (52.4)	3.7 (1.7-8.1)		34 (81.0)	8 (19.1)	4.3 (1.6-11.5)		3.4 (1.1-10.8)		13.1 (2.3-76.8)	
Migratory wild birds visiting the village	No	14 (18.4)	62 (81.6)	Reference	0.009	37 (48.7)	39 (51.3)	Reference	0.006	-	-	Reference	0.007
	Yes	26 (38.2)	42 (61.8)	2.7 (1.3-5.9)		50 (73.5)	18 (26.5)	3.1 (1.4-7.1)		-		5.8 (1.6-21.1)	
Forest or jungle is present in the village	No	23 (33.8)	45 (66.2)	Reference	0.128	-	-	-	-	-	-	-	-
	Yes	17 (22.4)	59 (77.6)	0.6 (0.3-1.2)		-	-	-		-		-	
Village structure													
A pond/river/lake/canal was present between HHs in the village	No	10 (19.6)	41 (80.4)	Reference	0.108	-	-	-	-	-	-	-	-
	Yes	30 (32.3)	63 (67.7)	2.0 (0.9-4.4)		-	-	-		-		-	
Estimated distance between the village and the closest main road	>3.5 km	5 (14.3)	30 (85.7)	Reference	0.047	13 (37.1)	22 (62.9)	Reference	0.004	-	-	-	-
	≤3.5 km	35 (32.1)	74 (67.9)	2.8 (1.0-7.9)		74 (67.9)	35 (32.1)	3.8 (1.5-9.7)		-		-	

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Presence of isolated HHs within the village	No	-	-	-	-	16 (80.0)	4 (20.0)	Reference	0.090	-	-	-	-
	Yes	-	-	-		71 (57.3)	53 (42.7)	0.3 (0.1-1.2)		-		-	
Road passing through the village was mainly muddy	No	-	-	-	-	60 (68.2)	28 (31.8)	Reference	0.036	-	-	-	-
	Yes	-	-	-		27 (48.2)	29 (51.8)	0.4 (0.2-0.9)		-		-	
Presence of any kind of public vehicle stop (e.g. bus train) in the village	No	-	-	-	-	36 (52.2)	33 (47.8)	Reference	0.090	-	-	-	-
	Yes	-	-	-		51 (68.0)	24 (32.0)	2.1 (0.9-4.9)		-		-	
Poultry density													
At least one commercial poultry farm present in the village	No	7 (18.4)	31 (81.6)	Reference	0.138	-	-	-	-	-	-	-	-
	Yes	33 (31.1)	73 (68.9)	2.0 (0.8-5.0)		-	-	-		-		-	
Number of HHs rearing backyard poultry in the village	≤300	12 (19.4)	50 (80.7)	Reference	0.052	-	-	-	-	-	-	-	-
	>300	28 (34.2)	54 (65.9)	2.2 (1.0-4.7)		-	-	-		-		-	
Number of HHs rearing both chickens and ducks	<50	-	-	-	-	15 (45.5)	18 (54.6)	Reference	0.080	-	-	-	-
	≥50	-	-	-		72 (64.9)	39 (35.1)	2.5 (0.9-6.7)		-		-	
Farm-level factors (N=144 farms)													
Trading practices													
Number of chickens bought from LBMs in the last 12 months	0	25 (20.5)	97 (79.5)	Reference	0.000	-	-	-	-	Reference	0.016	-	-
	1 to 3	6 (54.6)	5 (45.5)	4.7 (1.3-16.5)		-	-	-		9.5 (1.3-69.9)		-	
	>3	9 (81.8)	2 (18.2)	17.5 (3.5- 86.0)		-	-	-		8.8 (1.2-65.9)		-	

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Farmed poultry was obtained from LBM in the last 12 months	No	-	-	-	-	60 (54.6)	50 (45.5)	Reference	0.015	-	-	-	-
	Yes	-	-	-		27 (79.4)	7 (20.6)	3.6 (1.3-10.0)		-		-	
Farmed poultry was obtained from neighbours in the last 12 months	No	-	-	-	-	69 (56.1)	54 (43.9)	Reference	0.025	-	-	Reference	0.020
	Yes	-	-	-		18 (85.7)	3 (14.3)	4.7 (1.2-17.9)		-		8.1 (1.4-46.9)	
Number of LBM visits by farmers or HH members in the last month for any purpose rather than selling poultry and eggs	0 times	-	-	-	-	12 (46.2)	14 (53.9)	Reference	0.045	-	-	Reference	0.038
	1 to 5 times	-	-	-		64 (61.0)	41 (39.1)	2.3 (0.8-6.4)		-		3.8 (0.9-16.1)	
	>5times	-	-	-		11 (84.6)	2 (15.4)	12.7 (1.6-97.2)		-		47.2 (2.4-933.3)	
Purchase of poultry for consumption from LBM and processing on backyard farm	No purchase of poultry for consumption from LBM; or if purchase processing at LBM	-	-	-	-	8 (38.1)	13 (61.9)	Reference	0.015	-	-	Reference	0.021
	Purchase of poultry for consumption from LBM and processing on backyard farm	-	-	-		79 (64.2)	44 (35.8)	5.1 (1.4-18.7)		-		9.3 (1.4-62.1)	
Frequency of sales of eggs, chicken or ducks within the last 12 months	0 times	8 (15.1)	45 (84.9)	Reference	0.014	-	-	-	-	-	-	-	-
	1 to 5 times	10 (25.6)	29 (74.4)	2.0 (0.7-5.7)		-	-	-		-		-	
	>5 times	22 (42.3)	30 (57.7)	4.3 (1.6-11.8)		-	-	-		-		-	

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Number of chicken eggs sold in the last 12 months	0 to 10	-	-	-	-	62 (55.4)	50 (44.6)	Reference	0.048	-	-	-	-
	>10	-	-	-		25 (78.1)	7 (21.9)	2.7 (1.0-7.3)		-		-	
Number of poultry sold in the last 12 months	0	7 (18.0)	32 (82.1)	Reference	0.064	-	-	-	-	-	-	-	-
	1 to 30	26 (28.3)	66 (71.7)	1.9 (0.7-5.0)		-	-	-		-		-	
	>30	7 (53.9)	6 (46.2)	5.8 (1.3-25.2)		-	-	-		-			
Number of visits to LBMs to sell poultry in the last 12 months	0 times	-	-	-	-	42 (50.6)	41 (49.4)	Reference	0.034	-	-	-	-
	1 times	-	-	-		8 (80.0)	2 (20.0)	4.0 (0.7-22.1)		-		-	
	>1 times	-	-	-		37 (72.6)	14 (27.5)	2.6 (1.1-6.0)		-		-	
Cleaning practices													
Frequency of cleaning (dry or wet cleaning) of the poultry house or places where were poultry were kept	Daily	2 (10.0)	18 (90.0)	Reference	0.073	-	-	-	-	-	-	-	-
	≥A Week	38 (30.7)	86 (69.4)	4.0 (0.9-18.0)		-	-	-		-		-	
Disposal of garbage, droppings/litter and dead birds													
Garbage piled up around the poultry house or on the farm	No	23 (19.0)	98 (81.0)	Reference	0.000	66 (54.6)	55 (45.5)	Reference	0.003	Reference	0.010	Reference	0.002
	Yes	17 (73.9)	6 (26.1)	30.8 (5.6-168.7)		21 (91.3)	2 (8.7)	12.3 (2.3-65.8)		9.1 (1.7-48.8)		28.6 (3.4-239.8)	
Disposal of litter/droppings by throwing them into nearby rivers, lakes or canals	No	32 (25.0)	96 (75.0)	Reference	0.046	-	-	-	-	-	-	-	-
	Yes	8 (50.0)	8 (50.0)	3.1 (1.0-9.2)		-	-			-		-	

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Disposal of dead birds by throwing them into nearby bushes/jungle	No	-	-	-	-	57 (55.9)	45 (44.1)	Reference	0.112	-	-	-	-
	Yes	-	-	-		30 (71.4)	12 (28.6)	2.0 (0.9-4.8)		-		-	
Indirect contact with other animals													
Feeding of different poultry species in the same feeder or in the same location	No	10 (13.0)	67 (87.0)	Reference	0.000	41 (53.3)	36 (46.8)	Reference	0.033	Reference	0.003	-	-
	Yes	30 (44.8)	37 (55.2)	5.8 (2.4-14.3)		46 (68.7)	21 (31.3)	2.5 (1.1-5.6)		5.2 (1.7-15.7)		-	
Pond water used as for source of drinking water for poultry	No	13 (16.3)	67 (83.8)	Reference	0.002	42 (52.5)	38 (47.5)	Reference	0.029	Reference	0.010	-	-
	Yes	27 (42.2)	37 (57.8)	4.1 (1.7-10.2)		45 (70.3)	19 (29.7)	2.7 (1.1-6.4)		4.6 (1.4-14.9)		-	
Holes in the poultry house or places where poultry were kept, allowing feral/wild animals to enter	No	-	-	-	-	23 (45.1)	28 (54.9)	Reference	0.010	-	-	Reference	0.001
	Yes	-	-	-		64 (68.8)	29 (31.2)	2.7 (1.3-5.9)		-		10.8 (2.8-41.9)	
Outbreak responses													
Selling of sick birds at the local LBM	No	-	-	-	-	62 (65.3)	33 (34.7)	Reference	0.123	-	-	-	-
	Yes	-	-	-		25 (51.0)	24 (49.0)	0.5 (0.2-1.2)		-		-	
No separation of healthy chickens during disease outbreaks	No	-	-	-	-	75 (58.1)	54 (41.9)	Reference	0.152	-	-	-	-
	Yes	-	-	-		12 (80.0)	3 (20.0)	2.8 (0.7-11.6)		-		-	
If a disease outbreak occurs on a neighbouring farm, restricting of the scavenging area of own birds	No	-	-	-	-	56 (65.1)	30 (34.9)	Reference	0.090	-	-	-	-
	Yes	-	-	-		31 (53.5)	27 (46.6)	0.5 (0.2-1.1)		-		-	

Risk factors (listed in risk groups)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Visit of commercial poultry farms													
Frequency of visits of commercial poultry farms in the last 12 months by farmer or family members	0 times	29 (26.4)	81 (73.6)	Reference	0.051	-	-	-	-	-	-	-	-
	1 to <50 times	4 (18.2)	18 (81.8)	0.6 (0.2-2.0)		-	-	-		-		-	
	≥50 times	7 (58.3)	5 (41.7)	3.9 (1.2-13.3)		-	-	-		-		-	
Consumption of own reared poultry													
Number of home-reared poultry consumed in the last 12 months	0 to 15	39 (30.5)	89 (69.5)	Reference	0.073	-	-	-	-	-	-	-	-
	>15	1 (6.2)	15 (93.8)	0.1 (0.0-1.2)		-	-	-		-		-	

A total of 281 farm-level and 96 village-level predictors were screened by univariate analyses and by calculating pairwise correlations. For H5, 271 farm-level and 89 village-level predictors were excluded resulting in the inclusion of 10 farm-level, and 7 village-level predictors in the multivariable analysis. For H9, 14 farm-level and 7 village-level predictors were included in the multivariable analysis after excluding 267 farm-level and 89 village-level predictors by univariate analyses and by evaluating pairwise correlations (**Table 4.1**).

The final multivariable model for H5 contained 1 village-level and 4 farm-level predictors as risk factors for H5 seroprevalence on backyard farms. For H9 seroprevalence on backyard farms, 2 village-level and 5 farm-level predictors remained in the final model (**Table 4.1**). Of the final village-level factors, two related to the environmental or ecological features within the village, which one common village-level risk factor for both H5 and H9 seropositivity. The final farm-level risk factors related to trading practices by backyard farmers and contact with other animals. A common farm-level risk factor for both H5 and H9 seropositivity was the existence of garbage piled up around the poultry house or on the backyard farm. We did not observe any confounding effect of initially eliminated variables that were added to final multivariable models. We also did not identify any significant 2-way interactions.

The estimated residual ICCs were 0.11 (95% CI: 0.00-0.87) for the H5 and 0.18 (95% CI: 0.02-0.75) for the H9 model, indicative that after including village as random effect, little clustering was observed between villages. In general, there were comparatively higher heterogenecity in H5 serology status among farms within a village compared to H9 serology. Finally, the normality and heteroscedasticity plots of the residuals identified no undue influence of any observations on the final models.

4.4 Discussion

This is the first study that explored farm and village-level risk factors associated with H5 and H9 flock-level serological statuses of backyard chicken farms in Bangladesh.

4.4.1 Village-level risk factors for H5 and H9 infection on backyard chicken farms

4.4.1.1 Factors relating to environmental or ecological village features

Our study identified crow abundance around village garbage dumping places as a risk factor for both H5 and H9 seropositivity. The spatial distribution of crows is influenced by the availability of food. In Bangladesh, the main sources of food for crows are household scraps and garbage from LBM (Biswas et al., 2011). The presence of crows may be indicative of locations, where poultry farming-related waste, including dead birds, offal, poultry droppings have been disposed. Backyard chickens are likely to scavenge around these dumping places, and might be exposed to AIV-contaminated material. Indeed, rather than being vectors of infection, crows may well act as sentinels of the AIV-contaminated environment, and might become infected themselves. Infection of crows with H5N1 virus resulting from their exposure to garbage dumping places have been previously reported (Khan et al., 2014; Tanimura et al., 2006) and H9N2 (Iqbal Yaqub, Mukhtar, Shabbir, & McCauley, 2013; Umar et al., 2016).

The presence of migratory wild birds in villages was associated with increased odds of farms being positive for H9. Bangladesh is located in the river delta of two major rivers, the Jamuna (Brahmaputra) and Padma (Ganges). These waterways attract many migratory wild birds that travel along two major flyways, the 'Southeastern end of the Central Asian Flyway' and the 'Southwestern end of the East Asian—Australasian flyway', to overwinter in Bangladesh. Thus, more than 30 species of migratory wild birds visit Bangladesh during the winter months, including the Lesser Whistling Teals, Greater Whistling Teals, Cotton Pigmy Goose, Pochards, Darters (Snake bird), Pintail Ducks, Herons, Comb Duck Gurganis, Kingfishers, Egrets, Bitterns, Storks, and Flycatchers (Lepage, 2014; Olsen et al., 2006). Migratory wild birds mingle frequently with domestic water birds and thereby provide a potentially source for AIV spread. In fact, active surveillance conducted in wild birds and backyard flocks in Northern Italy between 2004 and 2006 confirmed that contacts between migratory birds and free-range backyard poultry was a likely route of AIV transmission (Terregino et al., 2007). Mixing between chickens and migratory birds may be direct, or more likely mediated by domestic ducks, which often share the same water bodies as migratory birds, and eventually introduce H9 into backyard farms.

4.4.2 Farm-level risk factors for H5 and H9 infection on backyard chicken farms

4.4.2.1 Factors relating to the disposal of garbage on the backyard farm

Similarly to the village-level risk factor related garbage disposal, garbage piled up on farms was also a risk factor for both H5 and H9 seropositivity. A survey in the United States identified garbage as an important source of HPAI virus infection for commercial poultry farms. In this study, potential HPAI contaminated or infectious material (poultry carcasses, egg shells, dead wildlife) were disposed near poultry farms. The study also suggested that a garbage collection service shared by commercial and backyard poultry farmers might have been one of the potential pathways for HPAI virus spread (Walz et al., 2018). Considering that AIVs shed in the environment remain infectious at ambient temperatures for weeks or months, untreated garbage can play a significant role in the AIV epidemiology (Guan et al., 2009; Kurmi et al., 2013; Sakaguchi et al., 2010; Swayne et al., 2008; Tiwari, Patnayak, Chander, Parsad, & Goyal, 2006; Wood, Choi, Chappie, Rogers, & Kaye, 2010; Yamamoto, Nakamura, Yamada, & Mase, 2010). However, the sometimes extreme seasonal variations in rainfall, humidity and temperatures in Bangladesh might impact on the survival of AIV in this country (Khatun, Rashid, & Hygen, 2016).

4.4.2.2 Factors relating to trading practices by backyard farmers

Visiting LBMs to purchase poultry to be raised as part of farmers' backyard flocks, and purchase of poultry at LBM to be processed and consumed on the backyard farm was associated with H5 and/or H9 seropositivity. Indeed, AIVs have been found to circulate at high prevalence in LBMs in countries where live bird trading is a common practice, including Bangladesh (Turner et al., 2017), making LBMs a likely source of AIV infection for poultry farms. Purchasing poultry from neighbouring farms was also found to increase the odds of a farm being positive. Similar observations were made in Thailand, further emphasizing the importance of poultry trade in the spread of AIVs (Paul et al. (2011).

4.4.2.3 Factors relating to indirect contact of backyard chickens with other animals

Farms associated with husbandry practices promoting inter-species contacts were more likely to be serologically positive for H5. Using the same equipment to feed multiple species of poultry promotes contacts between these species. Furthermore, chickens and ducks are often reared together on backyard farms (Alam et al., 2014), and left to scavenge for food during the day (Barua & Yoshimura, 1997), promoting contacts between domestic poultry and wild birds in the village environment, but also along waterways (Terregino et al., 2007).

In addition, the type of drinking water supplied to backyard chickens, can play a role in the AIV epidemiology, with pond water increasing the odds of both H5 and H9 seropositivity. In Bangladesh, ponds are a habitat for ducks and migratory water birds, increasing the likelihood of pond water contaminated with AIV. Indeed, an experimental laboratory-based study conducted by Mihai et al. (2011) reported that H5N1 virus can remain infective in water for 12 days at 22-35 0C and up to 20 days at 4 0C. Ponds might also become contaminated by the disposal of dead poultry, a common practice conducted by fish farmers in Bangladesh providing a feed source for their fish.

Access of feral and wild animals to poultry houses identified in this study, has been described previously as a plausible route for H9 virus transmission (Kuiken et al., 2004; Reperant et al., 2008; Songserm et al., 2006).

4.4.3 Limitations of the study

There are some limitations in our study. Firstly, as in any research involving interviews, recall bias may have affected farmers' responses. However, as we only interviewed the people actually caring for the chickens, thus we are confident to have minimised this bias. Secondly, due to the cross-sectional nature of the study, we could not assess the impact of detailed seasonal variations of some predictors. Thirdly, interviewees may have given socially acceptable answers to some sensitive questions, for example on those related to cleaning and disinfection practices, which may explain why those variables were not selected in the final multivariable model. Finally, chickens infected by HPAI H5 are expected to die, although backyard farmers did not report any abnormal mortalities or HPAI outbreaks on their farms over the year preceding the sampling. Although, we also collected swabs from birds to monitor virus shedding, virus prevalence was very low (flock-level H9 virus prevalence was 0.7% and no flocks were H5 virus positive) we were not able to use it as an outcome variable in the risk factor analysis. We therefore considered H5 and H9 serological flock status as a surrogate for past virus exposure to identify risk factors associated with HPAI infection.

4.4.4 Recommendations

The following recommendations based on the findings of this study can reduce the risks of infection of AIVs of backyard poultry:

- Backyard farmers should be encouraged to not pile up garbage. Garbage should be disposed as far as possible from farms by burning or burying it deep in the ground, so scavengers are not able to access it.
- Backyard farmers who rear multiple poultry species within the farm should be discouraged to feed different poultry species with the same feeder or trough.

- As open water sources (e.g. ponds) might be contaminated with AIV excreted by ducks or wild birds or through the disposal of poultry carcasses, alternative water sources such as tube-well water should be provided to backyard poultry.
- Backyard farmers should be encouraged to purchase poultry to supplement their own flocks from reliable sources. In particular, sources of live poultry with likely contact to infected birds (e.g. poultry at LBM) should be avoided. Backyard farmers should be encouraged to hatch their own birds in a bio-secure environment.
- Live poultry bought from LBM for family consumption should not be slaughtered and/or processed at home – it should be slaughtered and processed at LBM.
- As much as practical and feasible, the movement of backyard farmers or their family members to LBM should be minimized. Changing of clothes, disinfecting of shoes and washing of hands and feet after returning from LBM is recommended.
- Backyard farmers need to be encouraged to avoid dumping of poultry droppings or even dead birds in waste areas within villages and on their farms as this might attract wild birds (e.g. crows). Backyard farmers should also be encouraged to restrict the scavenging of their poultry in waste areas.
- An analytical value chain study is recommended to explore the risks of infection of AIVs in backyard poultry along the poultry value chain."

We believe, the recommendations based on risk factors identified in this study could help policy makers to develop more specific and practical biosecurity measures aiming to mitigate the risk of AIV infection in backyard chickens.

CHAPTER 5

FARM-LEVEL RISK FACTORS ASSOCIATED WITH AVIAN INFLUENZA A (H5) AND A (H9) FLOCK-LEVEL SEROPREVALENCE ON COMMERCIAL BROILER AND LAYER FARMS IN BANGLADESH

5.1 Introduction

Commercial poultry production is the main supplier for the consumption of animal protein in Bangladesh, which 6.3 kg of broiler meat (WPSA, 2019) and 103 eggs (Abdullah, 2019) consumed per capita annually. Due to the increasing demand for poultry meat and eggs, the commercial broiler and layer chicken production has undergone a rapid growth in Bangladesh, resulting in a 2.5 fold increase in commercial poultry farm density between 1995 and 2017 (Daily Star, 2017; Rahman, Jang, & Yu, 2017).

However, since 2007 the circulation of HPAI H5N1 and LPAI H9N2 subtypes became a major threat to commercial chicken production in Bangladesh (Parvin et al., 2018). In response to the first HPAI outbreak waves in the Bangladesh, the Government of Bangladesh, with technical assistance from the WHO and FAO, developed the first National Avian Influenza and Human Pandemic Influenza Preparedness and Response Plan for the period 2006-2008 (DGHS, 2006). The second National Avian and Human Pandemic Influenza Preparedness and Response Plan was drafted in 2008 covering the period 2009-2011(DGHS, 2009), but unfortunately this draft had not been approved (Chattopadhyay et al., 2018), leaving Bangladesh without any national policy framework to tackle the threat of AI. The decline of reported H5N1 outbreaks in poultry since 2013 and only one human fatality since the emergence of HPAI are the main reasons why the development and implemenation of HPAI policies are not considered as a priority in Bangladesh (Rimi et al., 2019). On the other hand, farm and LBM investigations in Bangladesh confirmed that H5N1 and H9N2 virus subtypes are widely circulatig in commercial broiler and layer flocks (Ansari et al., 2016; Kim et al., 2018), although fewer outbreaks and less severe clincal signs in birds are reported by farmers. This might also be indicative for an underreporting of cases by farmers, which could favour the persistence and transmisison of H5 and H9 viruses in the commerical poulty value chain (Parvin et al., 2018; Rimi et al., 2019).

Improved biosecurity is considered to be an important tool for controlling and preventing H5N1 and H9N2 dissimenation in poultry production systems (FAO, 2011, 2013; Kelly, Hawkins, Sandrock, & Boyce, 2008). Thus, biosecurity guidelines to prevent and control infectious poultry diseases, including AI, were developed in 2010 for commercial poultry flocks (DLS, 2010). However many of these recommendations are considered not to be practical for small-scale commercial farmers in Bangladesh (Rimi et al., 2017).

Case-control studies conducted before 2011 highlighted biosecurity-related risk factors that were associated with H5N1 outbreaks in commercial chicken flocks in Bangladesh (Biswas et al., 2009b; Biswas et al., 2011; Osmani et al., 2014), but risk factors associated with the current circulation of

AIVs in outbreak-free commercial flocks have not been described. Furthermore, commercial broiler and layer chicken farmers following different production cycles and have different management systems that might provide different pathways for H5N1 and H9N2 introduction into their flocks (Ali et al., 2013; Artois et al., 2018). A meta-analysis conducted by Wang et al. (2014) also highlighted that risk factors for AI infections differ between types of poultry production.

Thus, an in-depth understanding of factors associated with the ongoing risk of H5 and H9 circulation in commercial broiler and in commercial layer chickens is essential to develop an effective avian influenza prevention and control strategy for Bangladesh. Therefore the aim of this study was to identify and quantify potential farm-level risk factors associated with ongoing H5 and H9 infections in apparently healthy layer and broiler chickens in Bangladesh.

5.2 Materials and methods

5.2.1 Overview of the study design

Between February and April 2017, a cross-sectional study was conducted in the Chittagong and Cox's Bazaar districts of Bangladesh involving 106 commercial broiler and 113 commercial layer chicken farms. Of the 113 layer chicken farms, 13 farms had their chickens vaccinated against H5 – these 13 farms were excluded from the analysis. Details on sample size calculation and the selection of study units are described in Chapter 3.

5.2.2 Questionnaire design

Using the software MindMaple Lite version 1.3 (MindMaple Inc., Tustin, USA) hypothesized causal pathways, that could potentially increase the risk of H5 and H9 infections for broiler (**Figure 5.1**) and layer farms (**Figure 5.2**), were developed. Based on these hypothesized causal pathways, questions were developed focussing on husbandry, management and marketing practices conducted by commercial farmers. The questions were then incorporated in a digital questionnaire application using the CommCare software (Dimagi, Inc., Cambridge, USA). Although causal pathways were not used to inform the structured construction of multivariable statistical models in a dynamic acrylic causal framework (Dohoo et al., 2009), they were used to guide the inclusion of confounders and potential interactions between risks factors in the data analysis.

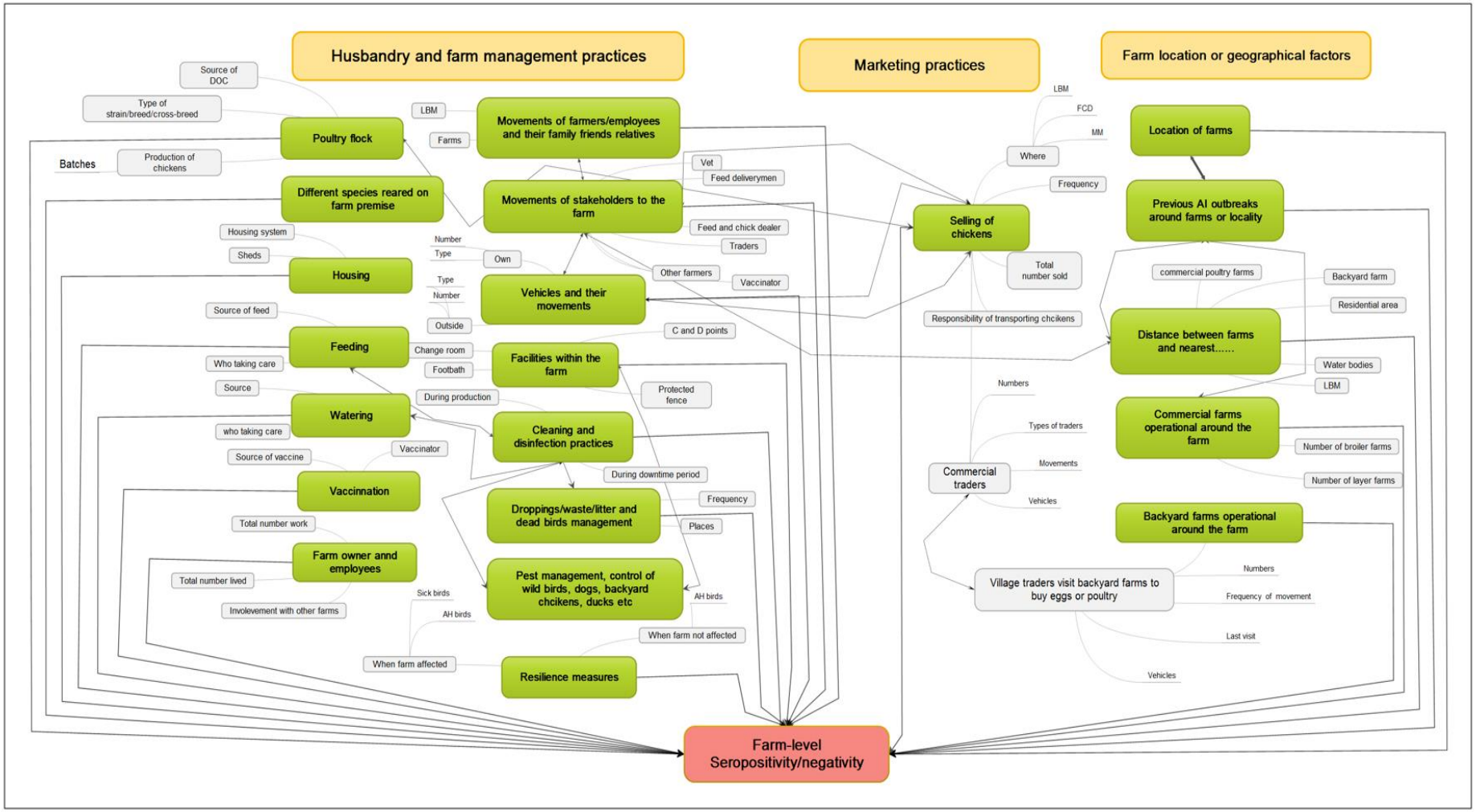

Figure 5.1 Hypothesized causal pathways for farm-level risk factors associated with AI infection on commercial broiler farms in Bangladesh. The red box represent the outcome (farm-level seropositivity) in the risk factor analysis, green boxes represent individual risk factors with grey boxes indicating additional categories/levels within the risk factor. Yellow-brown headings represent themes or categories under which risk factors can be combined. The causal pathways were used to inform the development of questions used in the interviews with broiler farmers and to guide the inclusion of potential confounders and interactions in the final multivariable model.

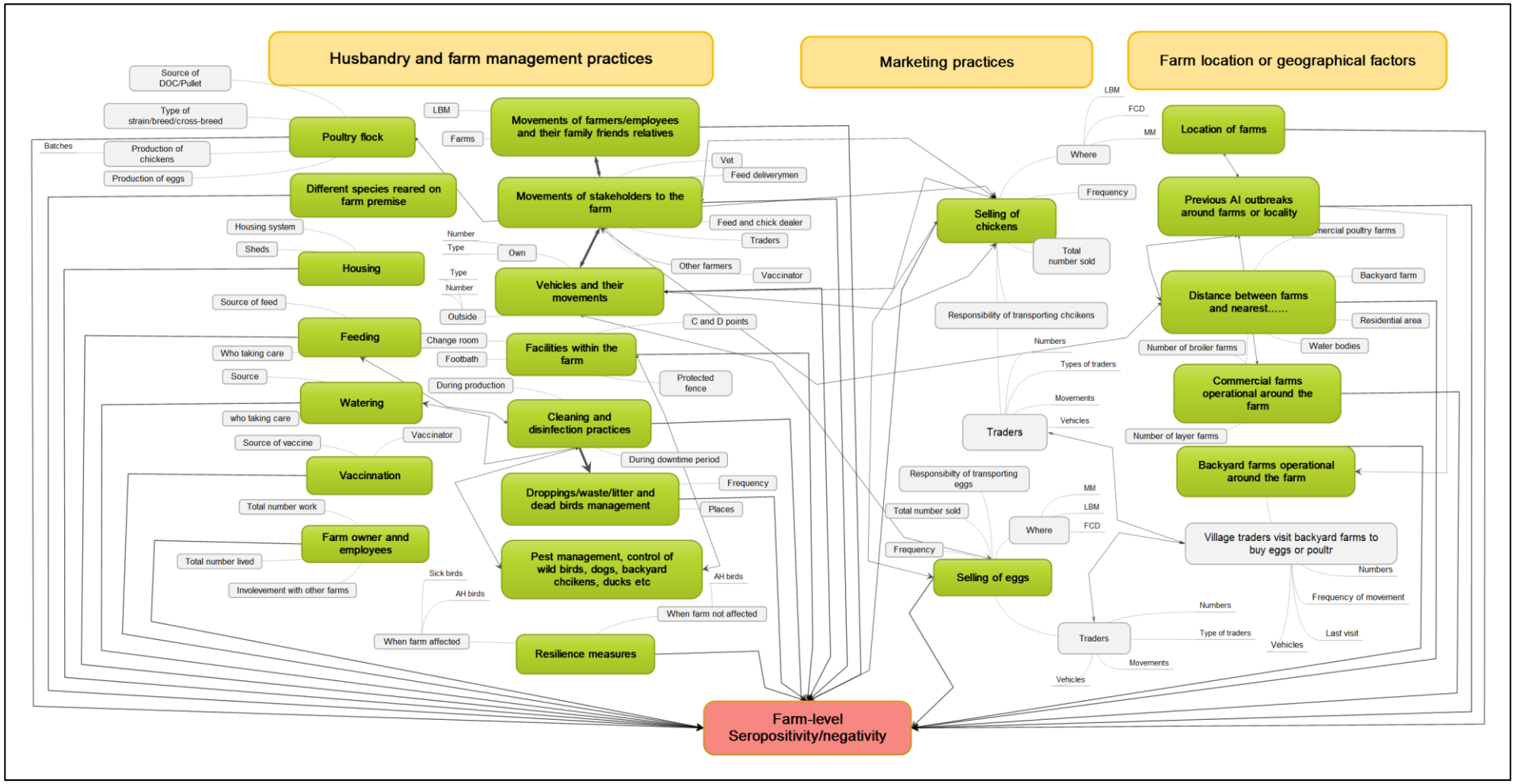

Figure 5.2 Hypothesized causal pathways for farm-level risk factors associated with AI infection on commercial layer farms in Bangladesh. The red box represent the outcome (farm-level seropositivity) in the risk factor analysis, green boxes represent individual risk factors with grey boxes indicating additional categories/levels within the risk factor. Yellow-brown headings represent themes or categories under which risk factors can be combined. The causal pathways were used to inform the development of questions used in the interviews with layer farmers and to guide the inclusion of potential confounders and interactions in the final multivariable model.

The questionnaire contained 84 questions that were identical for both, broiler and layer farmers. Six additional questions in the layer farm questionnaire focussed on the sale of eggs on layer farms. The questionnaire was pilot-tested on five broiler farms and on five layer farms that were not part of the finally selected farms. After pitot testing, minor modifications were made to nine questions.

5.2.3. Data collection

A total of 106 broiler and 100 unvaccinated layer commercial chicken farmers were interviewed. Interviews were conducted with the owner of the farm and each interview lasted about 30 minutes. Before each interview and sampling of chickens, consent via signature or by thumb impression was obtained in a consent form. All interviews were conducted by one female and one male field veterinarians who were trained in data collection using questionnaires.

Blood samples were collected from 9 and 8 chickens from each layer and broiler farm, respectively (Chapter 3). Depending on the body weight, 1-3 ml of blood were collected from the wing or jugular vein and transferred into individual sterile plastic tube immediately after collection. The tube was then kept in a cool box filled with ice packs and transported to the CVASU laboratory (for samples collected in Chittagong) and to the local office of the DLS (for samples collected in Cox's Bazaar). Samples were refrigerated overnight, then the serum was separated by centrifugation at 10,000 rpm for 30 minutes at 4^0c and transferred to Eppendorf tubes.

All the serum samples were further processed at the CVASU laboratory, where the samples were first screened for the presence of antibodies against Influenza A virus using commercial ELISA kits. Influenza A positive samples were then tested for the presence of H5 and H9 specific antibodies using the HI test. A serum sample was considered positive if there was an inhibition at a dilution of 1/16 (2^4) or more against 4 haemagglutinating units of antigen (OIE, 2015).

5.2.4 Data analyses

The questionnaire data were downloaded as a csv file from the CommCare web platform and imported into STATA 14.1 (Stata Corporation, College Station, Texas, USA) for data analysis.

A flock/farm was considered seropositive for H5/H9 if at least one chicken within a flock/farm had an H5/H9 HI titre of $\geq 2^4$. The analysis was conducted separately for H5 and H9 with a positive farm coded as 1 and negative farm coded as 0.

In the data analysis, we explored risk factors associated with H5 and H9 flock-level serpositivity separately for broiler farms and layer farms. A total of 344 and 421 dichotomous and ordinal risk factors were derived from the questionnaire data for broiler and layer chicken farms, respectively.

To reduce the number of predictors to be considered in the regression models, we used correlation analysis and screening of variables based on bivariate unconditional (Dohoo et al., 2009). As all the risk factor variables were dichotomous and ordinal, pairwise correlations were examined by estimating polychoric correlations (UCLA, 2019; Uebersax, 2006) using the –***polychoric***- command in STATA. If the correlation was ≥0.9 for H5/H9 in layer flocks, and ≥0.9 for H5 and ≥0.7 for H9 in broilers flocks, the biologically more plausible variable was maintained, while the other variable was removed.

Binomial logistic regression was used to evaluate the unconditional association between H5/H9 flock-level serology status (positive/negative) and each risk factors in the univariate analysis. For both, broilers and unvaccinated layer farms, risk factors associated with H5 and H9 seropositivity at a p-value ≤0.15 in the univariate analysis were included in the multivariable analysis.

The multivariable binominal logistic regression models were built using a backward stepwise elimination procedure. At each step, the risk factor with the highest p-value was removed until all factors retained in the final model had p-values <0.05. To test the overall significance of the risk factors with more than two levels, Wald test were conducted using the -*testparm*- command in STATA. We also evaluated potential confounding by subsequently adding eliminated risk factors that were considered biological plausible based on the hypothesised casual pathways. Biologically plausible 2-way interactions of risk factors in the final main effect model were also explored.

The Hosmer-Lemeshow goodness-of-fit statistic was used to access the fit of the final model (Hosmer & Lemeshow, 2000). Pearson and Deviance residuals and Pregibon leverage were examined to explore if any specific observations influenced the fit of the models. Finally, to evaluate power of the model in predicting the outcome, the area under the curve for the receiver operating characteristics (ROC) was calculated (Hosmer & Lemeshow, 2000).

5.3 Results

During the 12 months prior to the sampling, no HPAI outbreaks or abnormal mortalities were reported on any of the sampled broiler (N=106) and layer (N=100) farms.

5.3.1 H5 and H9 flock-level serology status

Among the sampled broiler flocks, 9.4% (N=10) were H5 and 5.7% (N=6) were H9 seropositive. Similar to the broiler flocks, H5 seroprevalence was higher than H9 seroprevalence in unvaccinated layer flocks: it was 31.0% (N=31) and 22.0% (N=22) respectively.

5.3.2 Farm-level risk factors associated with H5 and H9 flock-level seroprevalence on commercial, unvaccinated broiler farms

Table 5.1 Results of the univariate and multivariable analysis of farm-level risk factors associated with H5 and H9 flock-level seroprevalence on unvaccinated broiler farms

Risk factors (listed in risk groups) (N=106)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR (95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR (95% CI)	H9 P value	H5 OR (95% CI)	H5 P value	H9 OR (95% CI)	H9 P value
Access to backyard ducks													
Access of ducks from neighbouring backyard farms to the sampled farm	No	1(1.5)	67(98.5)	Reference	0.005	1(1.5)	67(98.5)	Reference	0.038	Reference	0.007	-	-
	Yes	9(23.7)	29(76.3)	20.8(2.5-171.7)		5(13.2)	33(86.8)	10.2(1.1-90.4)		21.5(2.3-201.1)		-	
Farm management													
Owner involved in taking care (feeding, watering, cleaning etc.) of chickens on sampled farm	No	4(18.2)	18(81.8)	Reference	0.128	-	-	-	-	-	-	-	-
	Yes	6(7.1)	78(92.9)	0.3(0.1-1.4)		-	-	-		-		-	
Disposal of litter/waste/droppings													
Litter/droppings/waste are disposed on sampled farm	No	7(6.9)	94(93.1)	Reference	0.003	4(4.0)	97(96.0)	Reference	0.008	-	-	-	-
	Yes	3(60.0)	2(40.0)	20.1(2.9-141.2)		2(40.0)	3(60.0)	16.2(2.1-125.5)		-		-	
In- and out farm movements													
Farm owner works or manages another commercial poultry farm	No	-	-	-	-	2(2.6)	76(97.4)	Reference	0.040	-	-	-	-
	Yes	-	-	-		4(14.3)	24(85.7)	6.3(1.1-36.8)		-		-	

Risk factors (listed in risk groups) (N=106)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR (95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR (95% CI)	H9 P value	H5 OR (95% CI)	H5 P value	H9 OR (95% CI)	H9 P value
Workers from another commercial chicken farm visited the sampled farm during the current production cycle	No	4(4.4)	87(95.6)	Reference	0.000	1(1.1)	90(98.9)	Reference	0.001	Reference	0.002	Reference	0.001
	Yes	6(40.0)	9(60.0)	14.5(3.4-61.2)		5(33.3)	10(66.7)	45.0(4.8-424.5)		15.1(2.8-80.8)		50.1(4.5- 552.7)	
Private veterinarians visited the sampled farm in the current production cycle	No	8(13.6)	51(86.4)	Reference	0.122	-	-	-	-	-	-	-	-
	Yes	2(4.3)	45(95.7)	0.3(0.1-1.4)		-	-	-		-		-	
Total number of vehicles (rickshaw van, pick-up, motorized vehicle etc.) used by traders to collect the last batch of chickens on the sampled farm	0 to 5	4(6.1)	62(93.9)	Reference	0.139	2(3.0)	64(97.0)	Reference	0.150	-	-	-	-
	> 5	6(15.0)	34(85.0)	2.7(0.7-10.4)		4(10.0)	36(90.0)	3.6(0.6-20.4)		-		-	
Total number of workers on the sampled farm	0 to 1	5(6.1)	77(93.9)	Reference	0.040	2(2.4)	80(97.6)	Reference	0.021	-	-	Reference	0.041
	≥2	5(20.8)	19(79.2)	4.1(1.1-15.4)		4(16.7)	20(83.3)	8.0 (1.4-46.8)		-		9.4(1.1-80.6)	
Marketing practices													
Sale of the last batch of broiler chickens to a Feed and Chick Dealer (FCD)	No	2(4.1)	47(95.9)	Reference	0.100	-	-	-	-	-	-	-	-
	Yes	8(14.0)	49(86.0)	3.8(0.8-19.0)		-	-	-		-		-	
Farm characteristics													
Total number of sheds on the sampled farm	1 to 2	-	-	-	-	3(3.5)	84(96.6)	Reference	0.054	-	-	-	-
	3 to 4	-	-	-		3(15.8)	16(84.2)	5.3(1.0-28.4)		-		-	
History of AI outbreaks near farm													
AI outbreaks near the sampled farm or within the village within the last 12 months	No	7(7.1)	91(92.9)	Reference	0.013	4(4.1)	94(95.9)	Reference	0.033	-	-	-	-
	Yes	3(37.5)	5(62.5)	7.8(1.5-39.6)		2(25.0)	6(75.0)	7.8(1.2-51.7)		-		-	

Risk factors (listed in risk groups) (N=106)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR (95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR (95% CI)	H9 P value	H5 OR (95% CI)	H5 P value	H9 OR (95% CI)	H9 P value
Farm location or geographical factors													
Total number of broiler farms operating with 0.5 km of the sampled farm	0-2	-	-	-	-	1(1.6)	61(98.4)	Reference	0.065	-	-	-	-
	≥3	-	-	-		5(11.4)	39(88.6)	7.8(0.9-69.5)		-		-	

Of the 344 potential risk factors examined for association with H5 and H9 serpositivity on broiler farms, 335 were excluded based on unconditional associations below the cut-off p-value in the univariate analysis or because of high pairwise correlations. Thus, nine risk factors associated with H5 and nine risk factors associated with H9 seropositivity (**Table 5.1**) were included in the multivariable analysis. Of these 18 risk factors, six risk factors were identical for H5 and H9 seropositivity (**Table 5.1**). Two risk factors were retained in the final multivariable models for H5 and H9 seropositivity, with one common risk factor increasing the odds of H5 and H9 seropositivity (i.e. *workers from another commercial chicken farm visited the sampled farm during the current production cycle*) (**Table 5.1**).

The Hosmer–Lemeshow goodness-of-fit statistics, with p-values of 0.371 for H5 and 0.755 for H9 seropositivity indicated good fitting models. The Area Under the ROC Curve was 0.877 and 0.943 for H5 and H9 models, respectively, indicating excellent predictive power of both models and good ability to discriminate between seropositive and seronegative farms (Hosmer & Lemeshow, 2000).

5.3.3 Farm-level risk factors associated with H5 and H9 flock-level seroprevalence on commercial, unvaccinated layer farms

Table 5.2 Results of the univariate and multivariable analysis for farm-level risk factors associated with H5 and H9 flock-level seroprevalence on unvaccinated layer farms. LBM=Live Bird Markets. DOC=Day Old Chick.

Risk factors (listed in risk groups) (N=100)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Source of the DOC/pullets and feed													
DOC or pullets were obtained from a hatchery or breeding farm	No	30(35.7)	54(64.3)	Reference	0.045	-	-	-	-	Reference	0.003	-	-
	Yes	1(6.3)	15(93.8)	0.1(0.0-1.0)		-	-	-		0.0(0.0-0.3)		-	
Feed and Chick Dealer (FCD) provided feed or feed ingredients	No	-	-	-	-	2(7.4)	25(92.6)	Reference	0.047	-	-	Reference	0.049
	Yes	-	-	-		20(27.4)	53(72.6)	4.7(1.0-21.8)		-		5.9(1.0-33.9)	
Stray dogs													
Access of stray dogs to the sampled farm	No	13(24.5)	40(75.5)	Reference	0.140	8(15.1)	45(84.9)	Reference	0.081	Reference	0.040	Reference	0.039
	Yes	18(38.3)	29(61.7)	1.9(0.8-4.5)		14(29.8)	33(70.2)	2.4(0.9-6.3)		3.1(1.1-9.1)		4.0(1.1-15.3)	
In- and out farm movements													
Farm owner worked or managed another commercial poultry farm	No	-	-	-	-	14(17.7)	65(82.3)	Reference	0.051	-	-	-	-
	Yes	-	-	-		8(38.1)	13(61.9)	2.9(1.0-8.2)		-		-	
Visits of LBMs in the last month by farmers, workers or family members that had access to the sampled farm	No	-	-	-	-	8(15.7)	43(84.3)	Reference	0.124	-	-	-	-
	Yes	-	-	-		14(28.6)	35(71.4)	2.2(0.8-5.7)		-		-	
Frequency of LBM visits in the last month by farmers, workers or family members that had access to the sampled farm	0 times	14(27.5)	37(72.6)	Reference	0.027	-	-	-	-	-	-	-	-
	1 to 10 times	2(11.1)	16(88.9)	0.3(0.1-1.6)		-	-	-		-		-	
	>10 times	15(48.4)	16(51.6)	2.5(1.0-6.3)		-	-	-		-		-	

Risk factors (listed in risk groups) (N=100)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Visits of other commercial poultry farms in the last month by farmers, workers or family members who had access to the sampled farm	No	-	-	-	-	16(18.8)	69(81.2)	Reference	0.076	-	-	Reference	0.039
	Yes	-	-	-		6(40.0)	9(60.0)	2.9(0.9-9.2)		-		4.7(1.1-20.6)	
Feed delivery on sampled farm in the current production cycle	No	20(26.7)	55(73.3)	Reference	0.109	-	-	-	-	-	-	-	-
	Yes	11(44.0)	14(56.0)	2.2(0.8-5.5)		-	-	-		-		-	
Sampled farm used its own vehicle for farm activities/movements	Yes	5(17.2)	24(82.8)	Reference	0.064	3(10.3)	26(89.7)	Reference	0.084	-	-	-	-
	No	26(36.6)	45(63.4)	2.8(0.9-8.1)		19(26.8)	52(73.2)	3.2(0.9-11.7)		-		-	
Vehicles entered the sampled farm (excluding vehicles of traders who purchased chicken or eggs)	No	4(15.4)	22(84.6)	Reference	0.053	-	-	-	-	Reference	0.011	-	-
	Yes	27(36.5)	47(63.5)	3.2(1.0-10.1)		-	-	-		5.8(1.5-22.4)		-	
Total number of workers on the sampled farm	0 to 2	13(22.0)	46(78.0)	Reference	0.062	-	-	-	-	Reference	0.013	-	-
	3 to 4	11(40.7)	16(59.3)	2.4(0.9-6.5)		-	-	-		4.8(1.4-16.3)		-	
	>=5	7(50.0)	7(50.0)	3.5(1.0-11.9)		-	-	-		5.8(1.2-28.2)		-	
Marketing practices													
Total number of spent layers sold in the last batch	0 to ≤950	-	-	-	-	7(13.0)	47(87.0)	Reference	0.044	-	-	Reference	0.004
	>950≤2000	-	-	-		7(26.9)	19(73.1)	2.5(0.8-8.0)		-		5.9(1.2-29.1)	
	>2000	-	-	-		8(40.0)	12(60.0)	4.5(1.4-14.8)		-		24.0(3.7-155.0)	
Frequency of sales of spent layers sold from the last batch	0 to 1 time	15(23.1)	50(76.9)	Reference	0.022	-	-	-	-	-	-	-	-
	≥ 2 times	16(45.7)	19(54.3)	2.8(1.2-6.8)		-	-	-		-		-	

Risk factors (listed in risk groups) (N=100)	Univariate analysis									Multivariable analysis			
	Category	H5 positive (%)	H5 negative (%)	H5 OR(95% CI)	H5 P value	H9 positive (%)	H9 negative (%)	H9 OR(95% CI)	H9 P value	H5 OR(95% CI)	H5 P value	H9 OR(95% CI)	H9 P value
Sale of the last batch of spent layers to a Feed and Chick Dealer (FCD)	No	23(26.7)	63(73.3)	Reference	0.029	16(18.6)	70(81.4)	Reference	0.050	-	-	-	-
	Yes	8(57.1)	642.9)	3.7(1.1-11.7)		6(42.9)	8(57.1)	3.3(1.0-10.8)		-		-	
Minimum number of spent layers sold over the last 24 months	0 to <1700	17(25.0)	51(75.0)	Reference	0.113	-	-	-	-	-	-	-	-
	≥1700≤2000	7(53.9)	6(46.2)	3.5(1.0-11.9)		-	-	-		-		-	
	>2000	7(36.8)	12(63.2)	1.8(0.6-5.2)		-	-	-		-		-	
Minimum number of eggs sold per sale in the last month	0 to 1000	-	-	-	-	4(11.1)	32(88.9)	Reference	0.080	-	-	-	-
	1001 to 5000	-	-	-		12(24.5)	37(75.5)	2.6(0.8-8.8)		-		-	
	>5000	-	-	-		6(40.0)	9(60.0)	5.3(1.2-23.1)		-		-	
Cleaning practices and disposal of dead birds													
Frequency of replacing litter or droppings during the current production cycle	Daily or weekly	-	-	-	-	14(17.5)	66(82.5)	Reference	0.060	-	-	Reference	0.013
	Fortnightly, monthly or >monthly	-	-	-		4(30.8)	9(69.2)	2.1(0.6-7.8)		-		4.6(0.7- 29.0)	
	Not at all	-	-	-		4(57.1)	3(42.9)	6.3(1.3-31.3)		-		28.3(2.8-284.2)	
Sale of litter or droppings to fish farmers	No	27(35.1)	50(64.9)	Reference	0.116	-	-	-	-	-	-	-	-
	Yes	4(17.4)	19(82.6)	0.4(0.1-1.3)		-	-	-		-		-	
Burying of dead birds near sampled farm	No	4(14.8)	23(85.2)	Reference	0.040	-	-	-	-	Reference	0.026	-	-
	Yes	27(37.0)	46(63.0)	3.4(1.1-10.8)		-	-	-		4.6(1.2-17.3)		-	

Risk factors (listed in risk groups) (N=100)	Univariate analysis									Multivariable analysis			
	Category	**H5 positive (%)**	**H5 negative (%)**	**H5 OR(95% CI)**	**H5 P value**	**H9 positive (%)**	**H9 negative (%)**	**H9 OR(95% CI)**	**H9 P value**	**H5 OR(95% CI)**	**H5 P value**	**H9 OR(95% CI)**	**H9 P value**
Garbage piled up near the chicken sheds	No	-	-	-	-	6(14.3)	36(85.7)	Reference	0.119	-	-	-	-
	Yes	-	-	-		16(27.6)	42(72.4)	2.3(0.8-6.5)		-		-	
Farm location or geographical factors													
Total number of layer farms operating with 0.5 km of the sampled farm	0	13(23.6)	42(76.4)	Reference	0.066	-	-	-	-	-	-	-	-
	1	10(32.3)	21(67.7)	1.5(0.6-4.1)		-	-	-		-		-	
	>1	8(57.1)	6(42.9)	4.3(1.3-14.7)		-	-	-		-		-	

Out of 421 potential risk factors associated with H5 and H9 seropositivity on unvaccinated layer farms, 408 risk factors were excluded for H5 and 410 were excluded for H9 seropositivity, resulting in the inclusion of 13 risk factors for H5 and 11 risk factors for H9 seropositivity modelled in the multivariable analysis (**Table 5.2**). Of these 24 risk factors, 3 risk factors were identical for H5 and H9 seropositivity (**Table 5.2**). The final H5 multivariable model included 4 risk and 1 protective factors, whereas 5 risk factors were retained in the final H9 multivariable model (**Table 5.2**). *Access of stray dogs to the sampled farm* was a common risk factor for H5 and H9 seropositivity in the final multivariable models

The Hosmer–Lemeshow goodness-of-fit statistics, with p-values of 0.825 for H5 and 0.520 for H9 indicated good fitting models. Likewise, the discriminatory abilities of both models were excellent with Areas Under the ROC Curve of 0.824 and 0.843 for H5 and H9 models, respectively (Hosmer & Lemeshow, 2000).

5.4 Discussion

Previous research in Bangladesh focused on the identification of risk factors associated with H5N1 epidemics on commercial farms during the major HPAI outbreak period of 2007-2011 (Biswas et al., 2009b; Biswas et al., 2011; Osmani et al., 2014). In contrast, our study conducted in 2017 explored the associations between farm-level risk factors and H5 and H9 serological status of commercial flocks under current endemic conditions. All the chickens in the sampled flocks were apparently healthy and no HPAI outbreaks were observed on the farms within the last 12 month before sampling. Thus, this is the first research study that explored farm-level risk factors associated with H5 and H9 seropositivity on HPAI outbreak-free commercial broiler and layer chicken farms in Bangladesh.

5.4.1 Risk factors for H5 and H9 infection on commercial, unvaccinated broiler farms

We identified that access of ducks from neighbouring backyard farms to commercial broiler farms increased the odds of H5 seropostivity. Free-grazing ducks have been reported to be associated with HPAI outbreak occurrence in Thailand in 2004 (Gilbert et al., 2006). In Bangladesh, many backyard farmers rear ducks along with chickens (Alam, Ali, Das, & Rahman, 2014). During daytime, ducks scavenge for feed around backyard farms, in the villages, on ponds/wetlands or on other agriculture lands (Barua & Yoshimura, 1997), and they might enter other backyard or commercial poultry farms. As commercial broiler chickens are raised in enclosed sheds it is unlikely that roaming ducks are able to enter these sheds. The introduction of virus into broiler flocks might happen through droppings of ducks, which contaminate commercial farm premises and then virus is carried mechanically by workers (via their clothes or shoes etc.) or through farm equipment (e.g. waterer, feeders) into the broiler sheds.

Workers from other commercial chicken farms who visited the sampled broiler farms during the current production cycle increased the odds of both, H5 and H9 seropositivity. This underpins the importance of human movements for H5 and H9 disease spread (Alexander, 1995; Kung et al. 2007). An increased number of employees working on farms was also associated with increased odds of H9 seropositivity on broiler farms. More employees represent more movements and more contacts to potential sources of AIV infection. A case-control study conducted on in Bangladesh also identified an increased number of employees as a risk factor for H5N1 outbreak occurrence on commercial chicken farms (Osmani et al., 2014). Although an increased number (>5) of vehicles (rickshaw van, pick-up, motorized vehicle etc.) used by traders to collect chickens on the sampled farm was

associated with increased odds of H5 and H9 seropositivity in the univariate analysis (and would also represent increased movements), this variable was not significant in the multivariable analysis.

5.4.2. Risk factors for H5 and H9 infection on commercial, unvaccinated layer farms

Stray dogs were associated with increased odds of H5 and H9 seropositivity on unvaccinated layer farms. Previously, a case-control risk factor study conducted in Bangladesh (Biswas et al., 2009b) highlighted that feral and wild animals including dogs were a strong risk factor associated with H5N1 infections on commercial farms. Experimental work conducted by Amirsalehy, Nili, and Mohammadi (2012) found that H9N2 virus isolated from broiler chickens was able to infect dogs, which were then able to shed the virus, while a study in Thailand reported that one dog died following ingestion of an H5N1-infected duck during an outbreak (Songserm et al., 2006). This emphasizes that dogs might play a role in the transmission of AIVs.

In Bangladesh, multiple stakeholders are involved in the poultry production chain which can impact on the spread of H5N1 (FAO, 2011; Sims, 2007). We found that purchases of DOCs or pullets directly from hatcheries or breeding farms reduced the risk of H5 infection and seropositivity in unvaccinated layer chickens compared to purchases of DOC or pullets from FCDs or through middle-men. FCDs do supply DOC, feed, medicine and equipment to commercial farms, but FCDs also conduct regular visits to farms to provide advice on disease management (and might have contact to sick birds). Hatcheries on the other hand focus only on chick production and usually have good biosecurity, thus representing a source of DOC/pullets of lower AIV infection risk. Similarly, farms where chicken feed or feed ingredients were provided through FCD had higher odds ratios for H9 infection compared to farms without FCD involvement in the feed supply.

The disposal of carcasses can be a challenge for commercial chicken farmers (Ritz, 2014). We found that farmers disposing dead birds by burying them near farm premises had higher odds for H5 seropositivity. Disposal of poultry carcasses might include burial, incineration, composting and rendering (Blake & Donald, 1992). However, considering the significant risk for human health and the environment through contamination of ground water with pathogens, some countries do not permit the burial of dead birds (Ritz, 2014). Nevertheless, if burial of dead birds is conducted, carcasses need to be buried deeply so that feral and wild animals are not able to retrieve carcasses (Aravinth & Prakash, 2015). Busquets et al. (2010) showed that HPAI virus remained infectious in carcasses for a duration up to 6 days at temperatures of 22–23^{0}C, thus carcasses of birds that died of

HPAI and were not buried effectively are an important source of infection. In Bangladesh, not properly disposed carcasses might attract dogs, jackals and fox (Rimi et al., 2017; Szabó, Heltai, & Lanszki, 2010).

The frequency of changing litter or droppings in chicken houses during the production cycle was also associated with increased H9 seropositivity in unvaccinated layer chickens. Kurmi et al. (2013) estimated that AIV can survive up to 18 hours at 42 °C, 24 hours at 37 °C, 5 days at 24 °C and 8 weeks at 4 °C in dry and wet faeces, respectively; while survival of AIV in poultry sheds for up to 5 weeks had been reported by others (Webster et al., 1978). Thus, poultry litter can provide a favourable environment for AIV spread.

Farms, where vehicles were entering farm premises to deliver feed or DOCs (except to buy or collect chickens or eggs) or to collect litter/droppings had an increased risk of H5 seropositivity compared to farms without such vehicle visits. Transport vehicles usually move between farms to deliver feed or chicks and thereby might be able to spread AIV from one infected farm to a non-infected farm. In addition, poultry droppings are used by fish farmers as fish feed in Bangladesh (Hoq, Das, & Uddin, 1999) and poultry litter to be used as fish feed is usually collected from multiple poultry farms. Thus transport vehicles that collect or deliver chicken faeces might play an important role in the spread of H5 virus (Duvauchelle, Huneau-Salaün, Balaine, Rose, & Michel, 2013).

Layer farms where farmers, workers or their family members visited other commercial poultry farms were also at higher risk for H9 infection. The purposes of those visits is mainly for informal information exchange or gossiping which is a common cultural practice in Bangladesh and in other developing countries and has been linked to increased risk of HPAI outbreaks (Henning et al., 2009).

Furthermore, we found that farms that sold a larger number of spent layers (>2000) in the last batch had higher odds for H9 seropositivity than farms selling fewer spent layers (>950 to ≤2000, 0 to ≤950). Sales of a larger number of spent layers might involve a larger number of traders or middlemen visiting the farm premises. Moreover, we also explored the possibility of risk of management of multiples batches at the same time within the farm comparing to all-in-all out management or selling practices for H5 and H9 seroprevalence, but, this factor was excluded during the univariate analysis at cut-off p value ≤0.15. Kung et al. (2007), also found that farms visited by more than one person from retail markets was an important risk factor for H5N1 infection in chickens. Similar to H9

infections in broiler chickens, we observed that an increased number of workers on the farms was associated with an increased risk of H5 infections in unvaccinated layer chickens.

5.4.3 Limitations of the study

This study had some limitations. Firstly, some of the information collected on the marketing and production of chickens referred to the last 12 months and therefore relied on the memory recall by farmers. However, we tried to limit recall bias by simplifying the questions and focussed on dichotomised or simple ordinal responses to questions Secondly, due to the cross-sectional nature of the study, we were unable to observe detailed seasonal variation, for example in regards to the number of chickens reared or sold per month. However, by including questions on observations of general seasonality (e.g. *Was there any specific season in the last 12 months when you reared more chickens? or Was there any specific season in the last 12 months when you sold more chickens?*) we were able to include some season-focussed variables as potential risk factors in our analysis.

5.4.4 Recommendations

Although we explored H5 and H9 seropositivity separately, many of the risk factors identified could be grouped under the same themes, with general movement of people in and out of the farms being strongly associated with H5 and/or H9 seropositivity. It is not feasible to restrict the movement of people, such as workers or traders. However, it is recommend farms should have facilities for changing clothes and footwear before entering or leaving the farm as well as hand and foot washing facilities. It is also recommended, that vehicles should be cleaned and disinfected properly before entering and leaving farm premises. If possible, access of vehicles should be restricted to only one entry and exit point to the commercial farm and parking of vehicles should be conducted not within 30 meters from chicken sheds (DLS, 2010). Protective perimeter fencing around the farms is highly advisable to prevent animals such as ducks or dogs entering farm premises. Daily or at least weekly cleaning of litter, and disposal of dead birds as far as possible from the farms (with at least 2 feet deep burial of birds) is recommended to reduce the risk of H5 or H9 spread. Farmers also need to be educated in risk-reducing behaviours such sourcing DOC from suppliers with good biosecurity (e.g. hatcheries). The aforementioned recommendations based on the findings of this study could help policy makers to develop more effective prevention and control strategies to reduce the risk of H5 and H9 infections on commercial broiler and on commercial layer chicken farms.

Submitted manuscripts included in this thesis

A manuscript (stated below) was prepared based on the next **Chapter 6**, and submitted to publish on '*Transboundary and Emerging Diseases'* journal. This paper has been accepted for publication.

Gupta, S. D., Fournié, G., Hoque, M. A., & Henning, J. (2019). Factors influencing chicken farmers' decisions to implement prevention and control measures to reduce avian influenza virus spread under endemic conditions. *Transboundary and Emerging Diseases*. In Press.

Gupta, S. D. (Suman Das Gupta, the candidate) contributed a total of 80% to the conception and design of the study and field data collection, 90% to the analysis and interpretation of the questionnaire data, 85% to the formatting, drafting and editing of the manuscript. Fournié, G. (Dr Guillaume Fournié, the co-supervisor of the candidate) contributed 6% to the conception and design of the study, 1% to the analysis and interpretation of the data and 4% to the manuscript editing. Hoque, M. A. (Dr Md. Ahasanul Hoque, the co-supervisor of the candidate) contributed 2% to the conception and design of the study and field data collection, 3% to the analysis and interpretation of the data and 1% to the manuscript editing. Henning, J. (Dr Joerg Henning, the principal supervisor of the candidate) contributed 12% to the conception and design of the study, 6% to the analysis and interpretation of the data and 10% to the manuscript editing.

CHAPTER 6

FACTORS INFLUENCING CHICKEN FARMERS' DECISIONS TO IMPLEMENT PREVENTION AND CONTROL MEASURES TO REDUCE HPAI VIRUS SPREAD IN BANGLADESH

6.1 Introduction

HPAI H5N1 was first reported in 1959 on a small poultry farm in Scotland, UK (Capua & Alexander, 2007). Since then, several localised outbreaks occurred in different countries across the world. However, in 1996, HPAI H5N1 emerged in southern China, and subsequently spread across Asia, Europe and Africa, resulting in high mortalities of birds, and requiring the culling of many infected and unaffected flocks (Alexander, 2000; OIE, 2019). Moreover, the zoonotic potential of the virus raises public health concerns (Fournie, Hog, Barnett, Pfeiffer, & Mangtani, 2017). Although the combined efforts from national and international communities resulted in the elimination of HPAI H5N1 in a number of countries, the virus remains endemic in Bangladesh, China, Egypt, India, Indonesia and Vietnam (FAO, 2011, 2013; OIE, 2019).

A long-term approach was recommended by FAO/OIE in 2008 to eliminate HPAI H5N1 virus circulation in these endemically infected countries. It includes disease monitoring and surveillance, stamping out, the application of country-adjusted preventive measures (e.g. vaccination) and improved biosecurity measures (FAO, 2011; OIE,2019). Disease monitoring and surveillance are essential for the early detection of HPAI H5N1 in order to trigger a rapid response to reduce the viral load in poultry and in the environment (FAO, 2011, 2013; OIE, 2019). Stamping out of HPAI H5N1 infected flocks has only been partly successful in endemically infected countries, as moving or selling poultry by farmers before culling takes place, and the absence or inadequate compensation mechanisms are major constraints to control and prevention programs (FAO, 2011, 2013; OIE, 2019; USDA, 2017). All endemically infected countries except India are currently using vaccination against HPAI with a focus on commercial poultry, but several factors, including poor vaccine-induced immune response due to antigenic mismatch or inappropriate cold chains, limit the effectiveness of vaccination programmes (FAO, 2011; Kandeil et al., 2018; Kapczynski et al., 2015). Thus, improved biosecurity is the first line of defence in HPAI prevention as it establishes a barrier for the introduction of HPAI virus onto farms (Conan et al., 2012). Improved biosecurity measures include restricting the movement of visitors and vehicles to farms, cleaning and disinfecting of farms and farm equipment and wearing of protective gear while handling of poultry. However, the compliance with recommended biosecurity measures is often poor in HPAI endemically infected countries (Conan et al., 2012; FAO, 2011, 2013; Rimi et al., 2017). Hence, there is a need to understand the factors that influence farmers' decision to implement HPAI preventive or control measures on their farms. Yet, the diversity of husbandry practices, scale of production and livelihood strategies of farmers in HPAI-

endemic countries may mean that factors influencing their decisions vary greatly between poultry production systems (Cui et al., 2017; Cui et al., 2016a; Jemberu et al, 2015).

Qualitative and semi-quantitative methods can be used to provide insights into farmers' perceptions of and the factors influencing their attitudes towards biosecurity measures (Cui & Liu, 2016; Cui et al., 2019b; Oliveira et al., 2018). For example, Knowledge Attitudes and Practices (KAP) approaches have been used to describe knowledge, attitudes and and practices of farmers towards HPAI (Ismail & Ahmed, 2010; Sarker, Sumon, Khan, & Islam, 2016; Xiang et al., 2010; Zhou et al., 2019), but these type of studies do not fully consider the integrated nature of farmer's perceptions and its influence on farmers' behaviours. This limits the applicability of KAP study results in health education or promotion programs (Caldwell, Caldwell, & Quiggin, 1989; Cleland, 1973; Green, 2001; Ratcliffe, 1976; Smith, 1993). A number of psychological or behavioural frameworks (e.g. Protection Motivation Theory, Social Cognitive Theory, Theory of Belief Functions or Dempster–Shafer Theory) have been developed to analyse individual's perceptions or beliefs that influence their decision making (Ajzen, 2011; Bandura, 2001; Rogers, 1975; Shafer, 1992). The Health Belief Model (HBM) framework is a social cognition model that is frequently used in health education and promotion programs. In the HBM framework, behaviours and actions of individuals are explored while their perceptions and attitudes towards diseases and towards negative or positive outcomes of certain actions are considered. Thus, the HBM framework considers that the likelihood of implementing health-protecting behaviours is influenced by individual's perception of their susceptibility to a disease, the consequences of the disease, the benefits of implementing actions, and any constraints or barriers to the implementaiton of those actions. In addition, sources of information that may influence individuals' perceptions are also considered (Champion & Skinner, 2008; Glanz & Bishop, 2010; Glanz et al., 2008; Rosenstock, 1974).

Using the HBM framework, the objectives of our research were 1) to describe biosecurity measures implemented by poultry farmers operating under different production systems in Bangladesh to prevent HPAI infection in their flocks, and 2) to identify factors influencing the implementation of the biosecurity measures by farmers.

6.2 Materials and methods

6.2.1 Theoretical framework

In the HBM framework, multiple aspects of individuals' perceptions of a given topic are assessed and used to describe the individuals' decision-making (Glanz, Marcus Lewis, & Rimer, 1997; Glanz et al., 2008). Following the HBM framework (**Figure 6.1**) we aimed to identify the factors that influence backyard and commercial chicken farmers to implement HPAI preventive and control measures. Six HBM components or constructs were considered as part of a farmer's decision making (Becker, 1974; Champion & Skinner, 2008; Rosenstock, 1974):

i. *Perceived susceptibility*: Perception of the risk of chickens or humans becoming infected with HPAI virus
ii. *Perceived severity*: Perception of the consequences associated with HPAI infection in chickens and humans
iii. *Perceived benefits*: Perception of the positive impacts of HPAI preventive measures on chickens and humans
iv. *Perceived barriers*: Perceptions of constraints that refrain farmers to implement HPAI preventive measures
v. *Cues to action*: Engagement with different sources of information on HPAI preventive and control measures
vi. *Self-efficacy*: Likelihood of farmers to implement HPAI preventive and control measures

We hypothesized that *perceived susceptibility, perceived severity, perceived benefits* and *perceived barriers* had a direct influence on the likelihood of farmers to implement HPAI preventive and control measures (i.e. *self-efficacy*), and that *cues to action* might had: a mediating role on the impact of the four perceptive constructs on *self-efficacy*, and/or (2) a direct influence on *self-efficacy*.

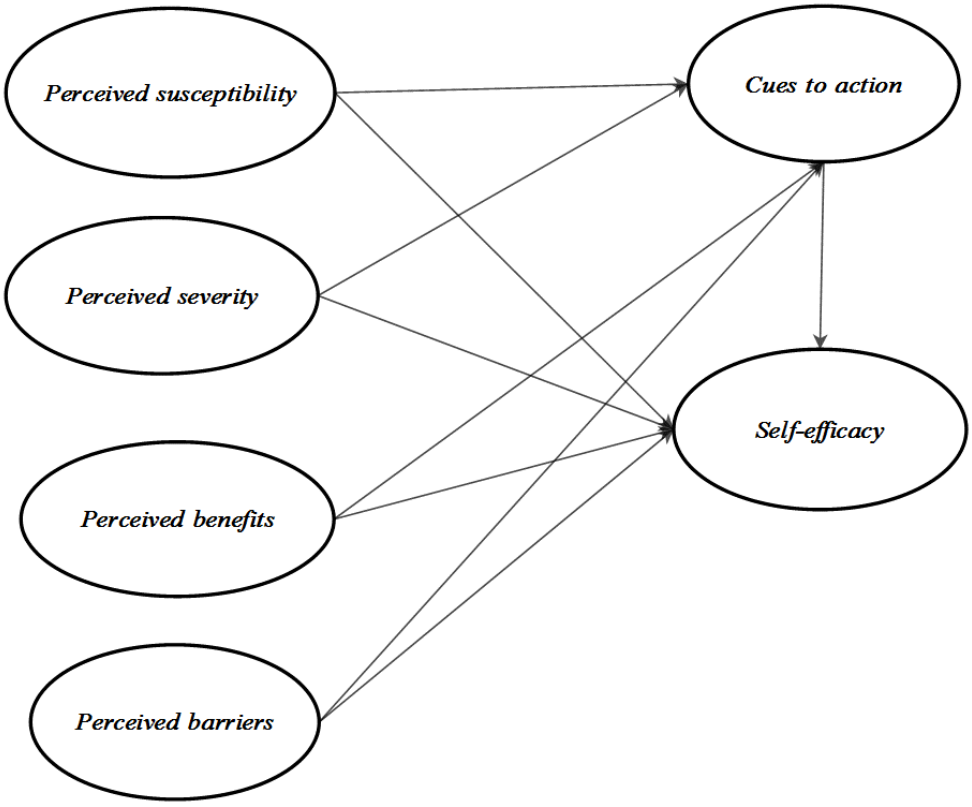

Figure 6.1 Diagram of Conceptual Health Belief Model framework used to explore the drivers that influence chicken farmers' decision to implement Highly Pathogenic Avian Influenza control and prevention measures

6.2.2. Study design

Two cross-sectional studies were conducted in the Chittagong and Cox's Bazaar districts of Bangladesh to explore farmers' perceptions and attitudes towards HPAI prevention and control. A total of 144 backyard chicken farmers were interviewed from February to April 2016, while 106 commercial broiler and 113 layer chicken farmers were interviewed from February to April 2017. Backyard chicken farmers usually raise Deshi (meaning 'indigenous' in Bengali language) chickens under scavenging or free ranging condition (Barua & Yoshimura, 1997; Das et al., 2008; FAO, 2008), whereas commercial farmers raise chickens of mainly exotic strains under confined or intensive systems with provision of supplementary feed (FAO, 2008; Huque et al., 2011). The design of these cross-sectional studies is described in detail in Chapter 3.

6.2.3 Questionnaire

Two questionnaires were designed, one for backyard chicken farmers, and one for commercial broiler and layer chicken farmers. The questionnaires were developed in English and then translated into Bengali language. Each of the HBM constructs were measured in the questionnaire by a set of 6-12 questions and all answers were recorded on a 6-Point Likert scale ('Strongly disagree', 'Disagree', 'Neither agree nor disagree', 'Don't know', 'Agree', 'Strongly agree'). The questionnaires were pilot-tested with 6 backyard chicken, 5 broiler and 5 layer farmers who were not part of the finally interviewed cohort and resulted in minor modifications of 5 questions in the backyard and 3 questions in the commercial chicken farmer questionnaires. The interviews were conducted by one female and one male field veterinarians who were trained in interviewing techniques. Each interview lasted about 25 minutes.

6.2.4 Data analyses

Frequencies of farmers' responses to each question were summarized in STATA 14.1 (Stata Corporation, College Station, Texas, USA). In the subsequent analytical analysis, the categories 'Don't know' and 'Neither agree nor disagree' were combined in a category 'Uncertain'. We then used Structural Equation Modelling (SEM) to identify factors influencing farmers' decisions to implement HPAI preventive or control measures. SEM is a statistical approach used in behavioural sciences (Hox & Bechger, 1998) to explore the theoretical or underlying constructs that cannot be directly observed and therefore are named latent variables. The 6 HBM constructs in our study represented the latent variables in the SEM models. SEM included two parts: a measurement part, in

which latent variables were related to observed variables, and 2) a structural part, in which relationships between latent variables were explored (Wuensch, 2009).

In our study, a conceptual model (Supplementary Figures: Appendices 4-6) was initially developed to visualize the observed or questionnaire variables informing each HBM construct, and the hypothesized causal relationships between the HBM constructs.

We then followed the two-step SEM approach developed by Anderson and Gerbing (1988, 1992): for the measurement part of the SEM, we used one-factor Confirmatory Factor Analysis to identify for each HBM construct the minimum set of observed variables that best represented this constructs. Then, in the structural part of the model, we considered *perceived susceptibility*, *perceived severity*, *perceived benefits*, *perceived barriers* and *cues to action* as independent variables influencing *self-efficacy*, the main dependent variable in the model. We also considered *cues to action* as intervening variable that could mediate the effect of the constructs measuring perceptions on *self-efficacy*. The results of the measurement part of the model were displayed using a path diagram. Results were displayed as direct effects of perceived *susceptibility*, *perceived severity*, *perceived benefits*, *perceived barriers* and *cues to action* on *self-efficacy*, as indirect effects of the four *perceived* constructs via *cues to action* on *self-efficacy*, and as total effects. The effects were measured by standardized regression coefficients (β). Bootstrapping was used to test the significance (p-values) of the effects. Finally, to assess how well the data fitted the final models, we used the Hu and Bentler's Two-Index Presentation Strategy (Hu & Bentler, 1999).

Separate models were developed for backyard, commercial broiler and commercial layer chicken farmers. The SEM analysis was performed using AMOS software version 25.0 (IBM® SPSS® Amos™ 25, IBM Corp., 2017. U.S.A).

6.3 Results

6.3.1 Study populations

The background of interviewed farmers (gender, marital status, religion, educational qualification, age and experiences in chicken farming) is presented in **Table 6.1**. Most (>91%) of backyard chicken farmers were women and married; in contrast, almost all of the commercial chicken farmers were male (>98%), of which more than two-thirds were married. Commercial layer farmers had a higher level of education than backyard and commercial broiler chicken farmers. There was no major

difference in the mean age of farmers across production systems, but backyard chicken farmers were more experienced in raising chickens than commercial farmers.

Frequency of responses of backyard, commercial broiler and layer chicken farmers to the questions on a 6-Point Likert scale are shown in Supplementary Tables (Appendices 1-3).For further analysis, responses were summarized on a 5-Point Likert Scale for the SEM (**Tables 6.2-6.4**).

Table 6.1 Demographic information on the chicken farmers interviewed. †represents 15 days, ‡ represents 90 days

	Backyard chicken farmer	Commercial broiler chicken farmer	Commercial layer chicken farmer
	% (n)	% (n)	% (n)
Gender			
Male	6.3 (9)	98.1 (104)	99.1 (112)
Female	93.7 (135)	1.9 (2)	0.9 (1)
Marital status			
Single	2.1 (3)	31.1 (33)	31.0 (35)
Married	91.7 (132)	68.9 (73)	69.0 (78)
Divorced	0.7 (1)	-	-
Widowed	5.5 (8)	-	-
Religion			
Muslim	90.3 (130)	94.3 (100)	89.4 (101)
Hindu	6.9 (10)	5.7 (6)	9.7 (11)
Buddhist	2.8 (4)	-	0.9 (1)
Education			
Illiterate	12.5 (18)	1.9 (2)	3.5 (4)
Primary	56.2 (81)	22.6 (24)	15.9 (18)
Secondary	25.7 (37)	39.6 (42)	38.1 (43)
Higher Secondary	4.9 (7)	17.0 (18)	16.8 (19)
Tertiary	0.7 (1)	18.9 (20)	25.7 (29)
Mean (Minimum, Maximum)			
Age (in years)	38.2 (17, 70)	36.6 (15, 70)	35.0 (6, 58)
Experience in chicken farming (in years)	20.4 (2, 52)	8.5 (<1†, 23)	9.2 (<1‡, 27)

6.3.2 Backyard chicken farmers

Backyard chicken farmers (**Table 6.2**) were very willing to implement HPAI preventive and control measures, with more than 96% of farmers agreeing or strongly agreeing to conduct actions that would reduce the chance of HPAI virus spread from their properties (e.g. informing livestock officers if they suspected HPAI outbreaks in their flocks). However, backyard chicken farmers were often concerned about constraints to implement these measures on their farms. For example, about a third of backyard farmers indicated that washing of hands after handling chickens was not practicable. Backyard farmers were strongly influenced by social pressures. For example, almost 30% of them would not apply hygienic measures if their neighbours did not use them. However, almost 90% of backyard farmers were open to learn more about HPAI and biosecurity if they were provided with information through the media or via other sources.

Table 6.2 Summary statistics (percentage, number of responses) of variables associated with Health Belief Model constructs retained in the final Structural Equation Model for backyard chicken farmers

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier % (n)				
		Strongly disagree (Very low perception or belief or barrier)	Disagree (Low perception or belief or barrier)	Uncertain	Agree (High perception or belief or barrier)	Strongly agree (Very high perception or belief or barrier)
Self-efficacy	It is a good idea to clean poultry house/equipment regularly **(SEff2)**	0.0 (0)	1.4 (2)	0.0 (0)	41.7 (60)	56.9 (82)
	I would be able to identify signs of the disease, if my chickens were infected with avian influenza/bird flu **(SEff3)**	0.7 (1)	0.7 (1)	0.7 (1)	46.5 (67)	51.4 (74)
	I will inform the local livestock related personnel, when I suspect that my chickens have avian influenza/bird flu **(SEff4)**	1.4 (2)	1.4 (2)	0.7 (1)	49.3 (71)	47.2 (68)
	I could wash my hands with soap before and after handling poultry, even if my neighbours are not**(SEff7)**	1.4 (2)	0.0 (0)	1.4 (2)	46.5 (67)	50.7 (73)
Perceived barriers	Regular cleaning of poultry house/equipment is time consuming and not practical for me, because my family/I have to do many other things**(PBar3)**	40.3 (58)	35.4 (51)	0.0 (0)	21.5 (31)	2.8 (4)
	Washing hands before and after handling poultry is not practical for me, because my family/I have to do many other things**(PBar4)**	38.9 (56)	28.5 (41)	0.0 (0)	28.5 (41)	4.2 (6)
	I can't cover my mouth and nose with cloths during handling chickens, because they are not conducive for work**(PBar5)**	37.5 (54)	25.0 (36)	4.2 (6)	29.9 (43)	3.5 (5)
	I don't cover my mouth and nose with cloths during handling chickens, because my neighbour do not**(PBar6)**	37.5 (54)	32.6 (47)	0.0 (0)	25.7 (37)	4.2 (6)

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier % (n)				
		Strongly disagree (Very low perception or belief or barrier)	**Disagree (Low perception or belief or barrier)**	**Uncertain**	**Agree (High perception or belief or barrier)**	**Strongly agree (Very high perception or belief or barrier)**
Cues to action	If I find a program on TV about avian influenza/bird flu and other aspects of poultry rearing, then I would watch it**(Cue2)**	1.4 (2)	2.8 (4)	0.0 (0)	38.2 (55)	57.6 (83)
	If I find a program on the radio about avian influenza/bird flu and other aspects of poultry rearing, then I would listen to it**(Cue3)**	1.4 (2)	2.8 (4)	0.0 (0)	38.9 (56)	56.9 (82)
	If I get invited to a meeting or campaign, etc. about avian influenza/bird flu and other aspects of poultry rearing, then I would attend it**(Cue4)**	2.1 (3)	6.9 (10)	0.0 (0)	41.0 (59)	50.0 (72)

The final SEM for backyard chicken farmers (**Figure 6.2**) highlighted that the likelihood of farmers to implement HPAI preventive measures on their farms was strongly reduced by *perceived barriers* (β=-0.52, $p<0.001$). However, information provided on HPAI marginally reduced this negative impact of *perceived barriers* (β=-0.13, $p<0.072$), and had a direct positive impact on the likelihood of farmers implementing HPAI prevention and control measures (β=0.26, $p<0.01$). Surprisingly, the risk and consequences associated with HPAI infection in chickens and people, and the advantages of implementing preventive actions did not influence backyard chicken farmers to implement HPAI prevention and control measures.

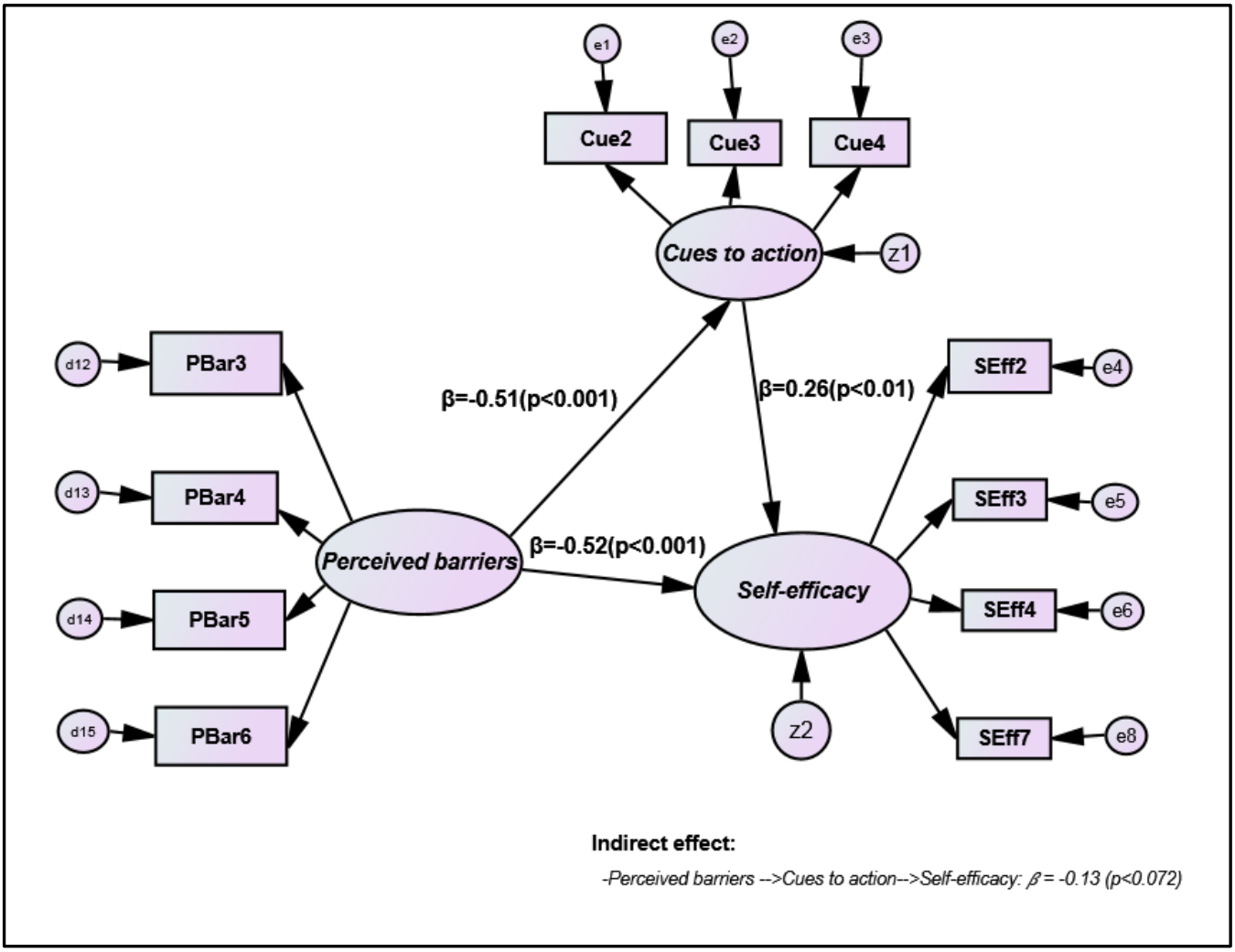

Figure 6.2 Final Structural Equation Model for backyard chicken farmers. The total effect for *Perceived barriers*→*Cues to action*→*Self-efficacy* was β=-0.66 (p<0.01).

6.3.3 Broiler chicken farmers

All (100%) commercial broiler chicken farmers (**Table 6.3)** either agreed or strongly agreed to implement actions that would reduce the chances of HPAI virus spread, such as the proper disposal of dead birds or litter.

Broiler farmers also strongly acknowledged the risk of chickens to become infected by HPAI if biosecurity is not properly maintained. For example, 95% of broiler farmers believed that chickens have an increased risk of becoming sick if the farm and farm equipment are not regularly cleaned and disinfected. However, they were somewhat concerned about constraints to implement these measures on their farms, with for example about 8% of farmers indicating that wearing protective gear was not conducive for work with chickens. On the other hand, broiler farmers were also aware of the advantages of adopting HPAI prevention and control measures, with, for example, more than 85% farmers agreeing or strongly agreeing that fewer chickens and farmers will become sick if good biosecurity is maintained on farms. Social pressures were reported to have a lesser impact than for backyard farmers, with only a small number of broiler farmers (10%) agreeing or strongly agreeing that they would not use HPAI vaccine because neighbouring farmers did not do so. Commercial broiler farmers also showed a strong interest in being informed about HPAI, with almost all farmers (99%) strongly agreeing or agreeing to be interested in receiving information about HPAI.

Table 6.3 Summary statistics (percentage, number of responses) of variables associated with Health Belief Model constructs retained in the final Structural Equation Model for commercial broiler chicken farmers

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier %(n)				
		Strongly disagree (Very low perception or belief or barrier)	Disagree (Low perception or belief or barrier)	Uncertain	Agree (High perception or belief or barrier)	Strongly agree (Very high perception or belief or barrier)
Self-efficacy	I could dispose dead birds/litter/waste properly **(SEff5)**	0.0 (0)	0.0 (0)	0.0 (0)	23.6 (25)	76.4 (81)
	I could clean & disinfect poultry house/equipment regularly **(SEff6)**	0.0 (0)	0.0 (0)	0.0 (0)	25.5 (27)	74.5 (79)
	I could wear protective wear, even if my neighbouring poultry farmers are not **(SEff7)**	0.0 (0)	0.0 (0)	0.0 (0)	31.1 (33)	68.9 (73)
Perceived susceptibility	My chickens have an increased risk of getting avian influenza/bird flu: when I don't regularly clean and disinfect my farm and farm equipment**(PSus3)**	0.0 (0)	4.7 (5)	0.0 (0)	31.1 (33)	64.2 (68)
	My chickens have an increased risk of getting avian influenza/bird flu: when I don't control wild birds/backyard poultry from entering into my poultry shed/house **(PSus4)**	0.0 (0)	5.7 (6)	1.9 (2)	28.3 (30)	64.2 (68)
	My chickens have an increased risk of getting avian influenza/bird flu: when my workers don't wash their hands/feet/change clothes before entering poultry shed/house **(PSus5)**	0.0 (0)	5.7 (6)	0.9 (1)	27.4 (29)	66.0 (70)
	My chickens have an increased risk of getting avian influenza/bird flu: when I don't clean and disinfect vehicles, egg trays, cages, de-beaking machine, vaccination gun, etc. before entering into my farm **(PSus6)**	0.0 (0)	4.7 (5)	0.0 (0)	33.0 (35)	62.3 (66)
Perceived benefits	If I maintain biosecurity (proper prevention & control measures) in my poultry farm, then my chickens will : not get sick from avian influenza and the possibility of disease outbreaks in my locality will reduce**(PBen2)**	0.0 (0)	7.6 (8)	0.0 (0)	24.5 (26)	67.9 (72)

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier %(n)				
		Strongly disagree (Very low perception or belief or barrier)	**Disagree (Low perception or belief or barrier)**	**Uncertain**	**Agree (High perception or belief or barrier)**	**Strongly agree (Very high perception or belief or barrier)**
	If I maintain biosecurity(proper prevention & control measures) in my poultry farm, then my chickens will : not get sick from AI as well as my family members and I will not get sick from AI **(PBen3)**	0.0 (0)	8.5 (9)	5.7 (6)	28.3 (30)	57.6 (61)
Perceived barriers	My neighbouring farmer doesn't use avian influenza vaccine, so I don't use avian influenza vaccine**(PBar8)**	67.0 (71)	22.6 (24)	0.0 (0)	8.5 (9)	1.9 (2)
	I can't wear protective gear, because they are not conducive for work**(PBar9)**	68.9 (73)	23.6 (25)	0.0 (0)	6.6 (7)	0.9 (1)
	I don't wear protective wear because my neighbouring poultry farmers do not**(PBar10)**	72.6 (77)	18.9 (20)	0.0 (0)	6.6 (7)	1.9 (2)
Cues to action	If I find a program on TV about avian influenza, then I would watch it **(Cue3)**	0.0 (0)	0.9 (1)	0.0 (0)	15.1 (16)	84.0 (89)
	If I find a program on the radio about avian influenza, then I would listen to it**(Cue4)**	0.0 (0)	0.9 (1)	0.0 (0)	14.2 (15)	84.9 (90)
	If I find information about avian influenza in leaflet/brochure/billboard, etc., then I would read it**(Cue5)**	0.0 (0)	0.0 (0)	0.9 (1)	16.0 (17)	83.0 (88)

The final SEM for broiler farmers (**Figure 6.3**) highlighted that the likelihood to implement HPAI preventive or control measures was strongly reduced by *perceived barriers* to implement these measures (β=-0.41, $p<0.001$), but strongly increased by *perceived benefits* (β=0.44, $p<0.001$) and *perceived susceptibility* (β=0.16, $p<0.046$). Information provided on HPAI (i.e. *cues to action*) also had a direct impact on the implementation of measures (β=0.12, $p<0.067$), but not a mediating effect. Consequences associated with HPAI infection did not influence broiler farmers' decision to implement HPAI preventive measures.

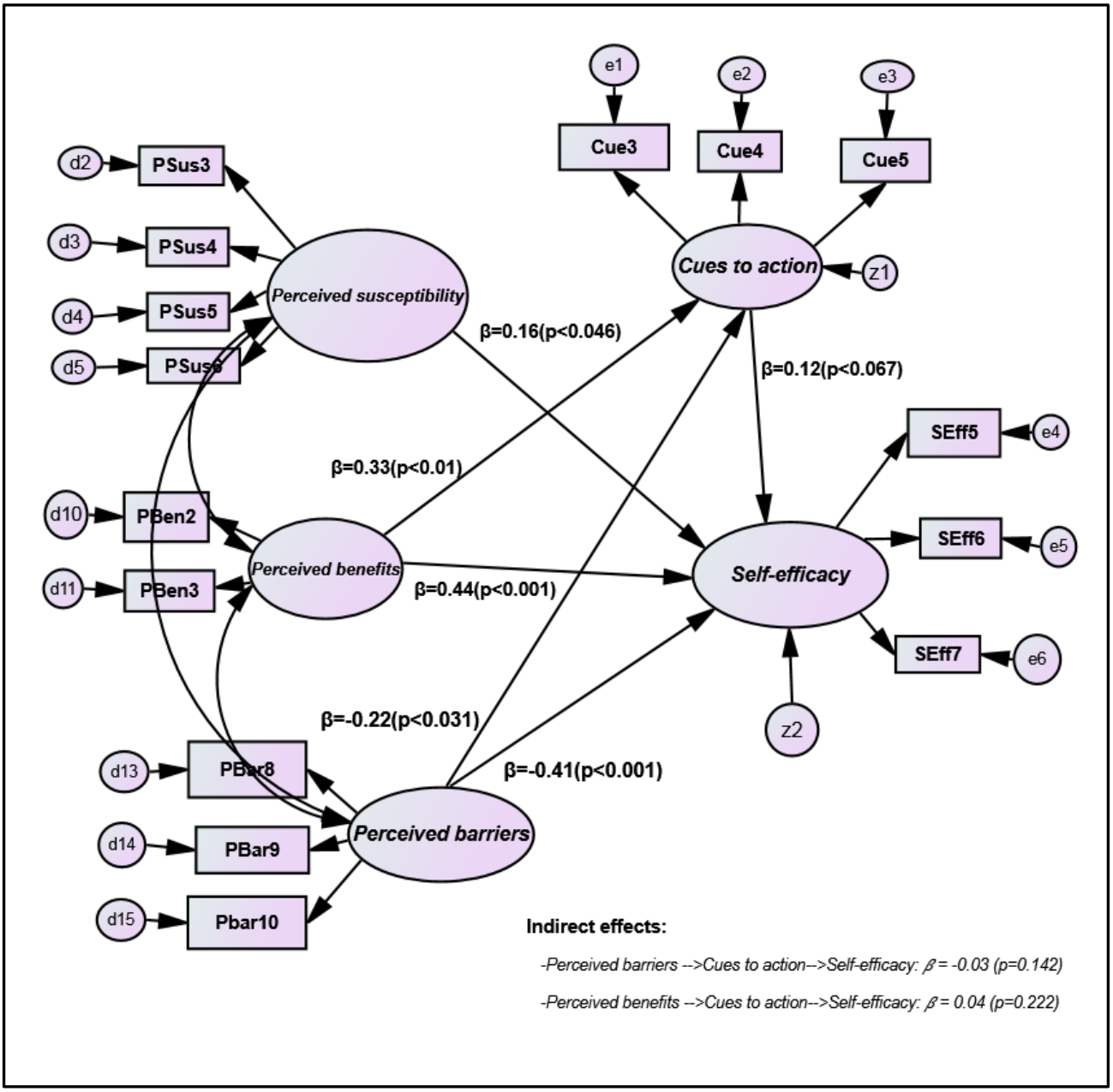

Figure 6.3 Final Structural Equation Model for commercial broiler chicken farmers. The total effect for *Perceived barriers→Cues to action→Self-efficacy* was β= -0.43 (p<0.01), and for *Perceived benefits→Cues to action→Self-efficacy* was β= 0.48 (p<0.01).

6.3.4 Layer farmers

Similarly to backyard and broiler farmers, almost all commercial layer farmers (>98%) agreed or strongly agreed to follow recommended actions to avoid HPAI infection and spread (e.g. wearing protective equipment even if neighbouring poultry farmers do not) (**Table 6.4**). Most striking was that although layer farmers were aware of the obstacles to implement HPAI preventive measures, much fewer (compared to backyard and broiler farmers) highlighted that these obstacles negatively influenced their decision-making. They were also less likely to be influenced by social pressures. For instance, only 9% would not use HPAI vaccine if their neighbouring farmers did not use it.

Layer farmers were strongly convinced about the advantages of maintaining good biosecurity on their farms, with more than 80% farmers agreeing or strongly agreeing that good maintenance of biosecurity measures would results in less HPAI cases in chickens and humans. Once again, almost 98% of layer farmers were interested in receiving additional information about HPAI and biosecurity measures.

Table 6.4 Summary statistics (percentage, number of responses) of variables associated with Health Belief Model constructs retained in the final Structural Equation Model for commercial layer chicken farmers

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier %(n)				
		Strongly disagree (Very low perception or belief or barrier)	Disagree (Low perception or belief or barrier)	Uncertain	Agree (High perception or belief or barrier)	Strongly agree (Very high perception or belief or barrier)
Self-efficacy	I could wear protective wear, even if my neighbouring poultry farmers are not **(SEff7)**	0.9 (1)	0.0 (0)	0.0 (0)	35.4 (40)	63.7 (72)
	I could wash my hands with soap before and after handling chickens even if my neighbouring poultry farmers are not **(SEff8)**	0.9 (1)	0.9 (1)	0.0 (0)	33.6 (38)	64.6 (73)
Perceived benefits	If I maintain biosecurity (proper prevention & control measures) in my poultry farm, then my chickens will not get sick from avian influenza, and I will not lose income**(PBen1)**	0.0 (0)	6.2 (7)	1.8 (2)	28.3 (32)	63.7 (72)
	If I maintain biosecurity(proper prevention & control measures) in my poultry farm, then my chickens will not get sick from avian influenza and the possibility of disease outbreaks in my locality will reduce**(PBen2)**	0.9 (1)	8.0 (9)	2.7 (3)	24.8 (28)	63.7 (72)
	If I maintain biosecurity(proper prevention & control measures) in my poultry farm, then my chickens will : not get sick from AI as well as my family members and I will not get sick from AI **(PBen3)**	0.9 (1)	15.0 (17)	3.5 (4)	23.0 (26)	57.5 (65)
	If my chickens receive avian influenza vaccine, then they will not get sick and die and I will not lose income**(PBen4)**	0.9 (1)	7.1 (8)	0.9 (1)	24.8 (28)	66.4 (75)
Perceived barriers	Washing hands all the time is not practical for me, because I have to do many other things**(PBar7)**	62.0 (70)	25.7 (29)	0.0 (0)	12.4 (14)	0.0 (0)
	My neighbouring farmer doesn't use avian influenza vaccine, so I don't use avian influenza vaccine**(PBar8)**	62.8 (71)	28.3 (32)	0.0 (0)	8.9 (10)	0.0 (0)
	I don't wear protective wear because my neighbouring poultry farmers do not**(PBar10)**	65.5 (74)	28.3 (32)	0.0 (0)	5.3 (6)	0.9 (1)

Constructs retained in the final model	Observed variables measured the constructs (ID used to represent the variable in the model)	Farmer's responses or perceptions or belief or barrier %(n)				
		Strongly disagree (Very low perception or belief or barrier)	**Disagree (Low perception or belief or barrier)**	**Uncertain**	**Agree (High perception or belief or barrier)**	**Strongly agree (Very high perception or belief or barrier)**
Cues to action	If I find a program on TV about avian influenza, then I would watch it **(Cue3)**	0.0 (0)	0.9 (1)	0.0 (0)	23.9 (27)	75.2 (85)
	If I find a program on the radio about avian influenza, then I would listen to it**(Cue4)**	0.0 (0)	1.8 (2)	0.0 (0)	23.0 (26)	75.2 (85)
	If I get invited to a meeting or campaign, etc. about avian influenza, then I would attend it**(Cue6)**	0.0 (0)	1.8 (2)	0.9 (1)	23.9 (27)	73.5 (83)

In the final SEM for layer farmers (**Figure 6.4**), the likelihood that farmers implement HPAI preventive and control measures on their farms was strongly increased by the *perceived benefits* (β=0.68, $p<0.001$) and, to a lesser extent, by the information provided on HPAI (i.e. *cues to action*) (β=0.15, $p<0.065$). Interestingly, *perceived barriers* did not seem to influence the implementation of HPAI preventive measures. *Cues to action* had no significant mediating effect on preventive measures. Likewise, consequences associated with HPAI infection and risk of chickens and humans to become infected did not influence layer farmers' decisions to implement HPAI preventive or control measures.

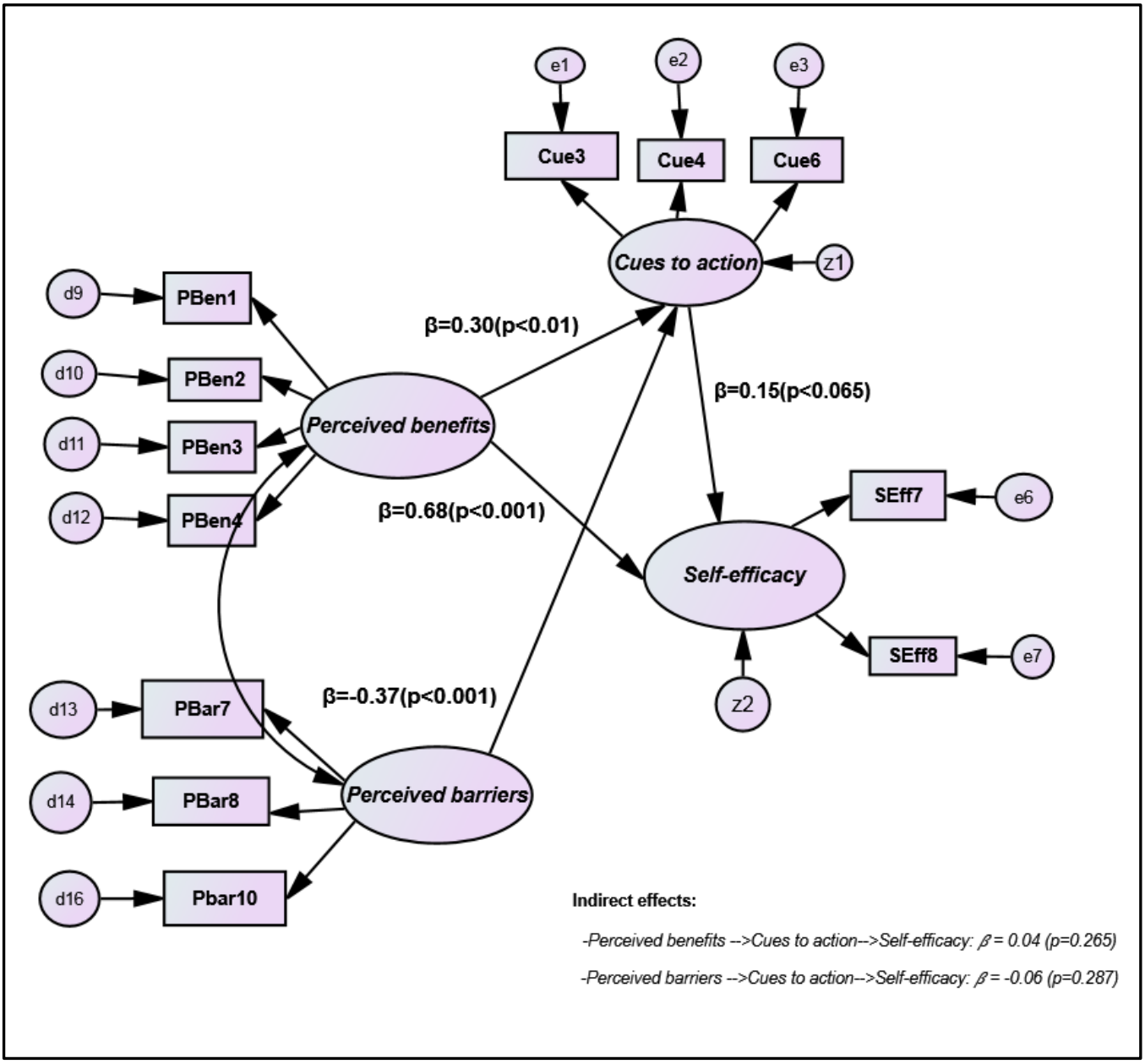

Figure 6.4 Final Structural Equation Model for commercial layer chicken farmers. The total effect for *Perceived benefits*→*Cues to action*→*Self-efficacy* was β= 0.72 (p<0.01).

6.4 Discussion

To our knowledge, this is the first study that used the HBM framework to explore the perceptions of farmers across different chicken production systems (backyard, commercial broiler and layer farmers) on the implementation of HPAI prevention and control measures. Our research provided new insights about factors influencing poultry farmers' decision-making processes in regards to improved biosecurity which can be used to guide the design of more effective preventive behaviour-change interventions (Glanz et al., 2008).

Farmers showed different perceptions on HPAI prevention and control depending on the practiced poultry management, reflecting different contexts, needs, and experiences. This is consistent with findings by Jemberu et al. (2015) who identified that farmers' perception on FMD control measures differed by cattle production systems, such as crop-livestock, pastoral and market-oriented systems. The HBM constructs in our study (*perceived severity*, *perceived susceptibility*, *perceived barriers*, *perceived benefits* and *cues to action*) had a different impact on the likelihood of implementing HPAI preventive measures (*self-efficacy*) in different poultry production systems. For example, *perceived barriers* refrained broiler and backyard farmers to implement HPAI preventive actions, but did not influence commercial layer farmers' decision-making. One possible explanation for this finding is that commercial layer farmers raise flocks over longer periods, manage larger flock size, with comparatively larger capital investment, which might make them more conscious of the need to plan preventive and control measures in the long term, enabling them to overcome *perceived barriers.*

Nevertheless, *perceived barriers* were the most influential construct affecting poultry farmers' behaviors. A meta-analysis of the effectiveness of HBM variables in predicting human actions conducted by Carpenter (2010) and a critical review carried by Janz and Becker (1984) of 46 HBM-related studies highlighted that *perceived barriers* were the HBM construct with the strongest influence on individuals' health-related behaviours. Similary, focussing on preventive medical interventions, Tanner-Smith and Brown (2010) indentified that conducting a pap smear, which was considered by women as embarassing and time consuming, was a signficant *perceived barrier* for the involvement of these women in cervical cancer prevention programs. Jemberu et al. (2015) also found that the cost of vaccination was a strong *perceived barrier* impacting on farmers' intentions to vaccinate their animals against FMD.

Our study further highlighted that *perceived benefits* of preventive and control measures only influenced broiler and layer farmers' decisions, most likely as the potential financial losses due to

HPAI outbreaks are more substantial for commercial farmers compared to backyard farmers, with backyard poultry raising being usually conducted only for supplementary income (Henning, Pym, Hla, Kyaw, & Meers, 2007). This is supported by research conducted in China and Kenya, which highlighted that farmers with larger flock sizes were more aware of the advantages of improved biosecurity (Cui et al., 2019a; Tiongco et al., 2012).

P*erceived susceptibility* of HPAI infection only influenced broiler farmers to implement HPAI preventive measures, but it did not influence backyard and layer farmers. A possible reason for this finding might be that as the production cycle for backyard and layer chickens is longer, farmers might believe that birds develop immunity over time, making them less susceptible to HPAI virus infection.

Surprisingly, the *perceived severity* of HPAI infection in chickens and people did not influence backyard, broiler and layer farmers' likelihood to implement HPAI prevention and control measures. Poultry farmers might have developed lesser concerns about the impact of HPAI, as there are fewer official and media reports on HPAI outbreaks and human infections in endemically infected countries like Bangladesh (DLS, 2019; WHO, 2019), or because farmers reduced potential economic consequences by conducting rapid sales of their chickens when an HPAI outbreak is experienced (Høg et al., 2018).

Usually little attention is been paid in animal health research to farmers' willingness to seek information (Valeeva et al., 2011). We identified that the availability information on HPAI played an important role in the farmers' decision-making to implement HPAI prevention and control measures for all three chicken production systems. Similarly, Toma, Stott, Heffernan, Ringrose, and Gunn (2013) found that the provision of biosecurity information had a positive impact on farmers' biosecurity behaviour while Cui et al. (2019b) also observed that information on AI disseminated through TV, web news and chats and via conversations between chicken farmers influenced the implementation of HPAI preventive measures. Unfortunately, farmers with different levels of intensification are often provided with similar advice on disease management. In our study, farmers of different chicken production systems had different perceptions on HPAI prevention and control, highlighting that information and extension messages need to be tailored to the respective audiences. A study conducted in the UK by Heffernan et al. (2008) found that bio-security behaviours by cattle and sheep farmers did not improve despite the provision of information through multiple sources (e.g. TV, radio, newspapers, Government agencies, private actors like feed representatives etc.), and the authors speculated that the communication of the information might have been viewed negatively by

some farmers. The researchers highlighted the importance of reframing biosecurity messages by paying attention to farmers' perceptions and to the way in which information is delivered to farmers. Thus, to communicate advice succesfully, appropriate comunciation methods need to be considered that account for the cultural environment, education level and experience of farmers (Henning, Hla, & Meers, 2014). Furthermore, behavior change communication through education programs need to be interactive and innovative and could include tools like documentaries, docu-drama, social marketing campaigns and puppet plays (Jones, Waters, Holland, Bevins, & Iverson, 2010).

Our study had some limitations. Firstly, we explored farmers' likelihood to implement HPAI prevention and control measures, but if these measures were actually implemented by farmers was beyond the scope of our study. Measuring the continuing implementation of measures would require a longitudinal study and such a study would be resource intensive to conduct. Secondly, we hypothesized and analyzed causal relationships between perceptions and the implementation of HPAI preventive measures, but validating these causal relationships was not possible in our cross-sectional study design. Finally, the framework used in this research paid more attention to the subjective state of an individual rather than other contextual factors, such as social acceptability, which would need to be explored through more qualitative approaches.

Overall, the results of our research can assist policy makers to tailor specific education programs to different types of poultry farmers and will thereby support the establishment of a more effective strategy to control and prevent HPAI virus spread.

CHAPTER 7

GENERAL DISCUSSION

7.1 Discussion of the key findings

Research question 1 (Chapter 3): How do the patterns of avian influenza A (H5) and A (H9) virus infection differ between backyard, commercial broiler and layer chicken farms in Bangladesh?

Overall, viral prevalence was low. Although all sampled backyard, commercial broiler and layer chicken farms tested negative for H5 viral subtype, H5 seropositive birds were found in all three systems. There were more unvaccinated commercial farms positive for H9 than backyard farms, and the proportions of H9-positive commercial broiler and unvaccinated layer farms were similar. Interestingly, no major disease or mortalities were reported by farmers, which raised questions about the virulence of the circulating H5 and H9 viruses and their impact on poultry health. It might be possible that endmicity of H5 and H9 infection might resulted in reduced pathogenicity of viruses due to viral evoloution or that birds became less susceptible to showing disease symtoms due to the dvelopment of cell-induced immunity to Influenza A viruses (Kapczynski, 2008; Wang et al., 2016). Furthermore, the H5 and H9 prevalence might differed due to the different epidemiology of each subtypes, including varying mortalities (Ducatez, Webster, & Webby, 2008).

The similar Influenza A and H9 viral prevalence in broiler and layer farms suggests similar levels of exposure of both farming systems to AIVs. This finding suggests the importance of considering the inclusion of broilers in AI vaccination program, although a detailed cost-benefit analysis and input from poultry industry stakeholders would be required before such a prevention strategy can be recommended.

Significant clustering was observed for H5 seroprevalence in backyard ducks, indicating that if one duck was H5 seropositive, other ducks in the same flock were also likely to be H5 seropositive. This is in contrast to backyard and commercial broiler and layer chickens, where usually only single birds out of the sampled chickens had H5 antibodies. This highlights that ducks remain an important source of H5 infection as this virus subtype seem to present in a large proportion of the backyard duck population (Henning et al., 2011; Hulse-Post et al., 2005; Kishida et al., 2005; Sarkar et al., 2017).

Furthermore, the occurrence of H5 antibodies in unvaccinated flocks resulting from a cross-contamination with HVT-vector vaccine that was used in vaccinated flocks has been considered.

However, such a cross-contamination between vaccinated and unvaccinated flocks is unlikely. Yasuda et al. (2016) found that neither HVT-H5 vaccine nor parental HVT vaccine spread from vaccinated chickens to non-vaccinated in-contact chickens. The authors vaccinated one-day-old Specific Pathogen Free chickens in ovo with HVT-H5 vector or parental HVT, and then raised these chickens with unvaccinated in-contact chickens. At 10, 14, and 21 days of the age birds were sampled, but no virus could be isolated from in-contact chickens, although virus was isolated from the vaccinated chickens.

Finally, the detection of only low antibody titres in H5 vaccinated layer flocks was surprising. This poor immune response might have been a result of misapplication of the vaccine or of vaccine selection pressure (Zihadi & Vahlenkamp, 2017).

Research question 2 (Chapter 4): What are the village and farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of backyard chicken farms in Bangladesh?

The risk factors associated with H5 and H9 virus spread were related to the following categories: (1) environmental or ecological features in the village, (2) garbage management, (3) trading practices, and (4) interspecies transmission.

The abundance of crows around village garbage places was associated with increased odds of backyard farms being seropositive for H5 and H9. An investigation conducted in Bangladesh to investigate unusual crow mortality in 2011 speculated about the potential for crows to act as vectors of infection for backyard poultry when they were scavenging near backyard poultry farms (Khan et al., 2014) (although crows becoming contaminated when near infected poultry might be equally important). Alternatively, crows may just be an indicator of abundant and mismanaged garbage, with garbage actually being a source of AIV infection as it could contain infected poultry ‘material’ such as carcasses of dead birds or intestines of slaughtered poultry (Walz et al., 2018). This interpretation is supported by the observation, that garbage piled up around farms was a risk factor for both H5 and H9 seroprevalence in the risk factor studies reported in this thesis. In fact, dead poultry, but also poultry waste are often disposed into domestic garbage (Cointreau, 2007). A study conducted by Sheta et al. (2014) in Egypt found that about 42% of surveyed backyard poultry farms disposed poultry faeces and 60% dead poultry into garbage, and this practice was highly correlated with the

occurrence of H5N1 outbreaks.

Trading practices, in particular visiting LBMs to purchase poultry for inclusion into backyard flocks and the purchase of poultry for household consumption were associated with increased odds of H5 and H9 infection. Several studies have previously highlighted the role of LBMs and poultry trading practices as a source of AIV infection for chicken farms (Henning et al., 2019; Sealy et al., 2019; Turner et al., 2017).

The free-roaming nature of raising backyard poultry means that there are frequent contacts between species, including chickens, domestic ducks, wild waterfowl and other domestic poultry and other animal species, increasing the likelihood of spreading AIV between infected reservoirs and susceptible birds (Gilbert et al., 2006; Henning et al., 2013; Henning et al., 2011; Henning et al., 2010; Sarkar et al., 2017). Indeed, using the same equipment to feed multiple species of poultry, and the presence of migratory birds in villages were risk factors for H5 and/or H9 infection in the research studies reported in this thesis.

Research question 3 (Chapter 5): What are the farm-level risk factors associated with avian influenza A (H5) and A (H9) seropositivity of commercial broiler and layer farms in Bangladesh?

Movements in and out farms, for instance, involving visitors, contractors, service personnel and farm workers are known to be a major route of AIV transmission between farms (Alexander, 1995; Duvauchelle et al., 2013; Henning et al., 2019; Kung et al., 2007; Scott et al., 2018). The research presented in this thesis, identified visits of other poultry farms by farm workers and movements of vehicles supplying production inputs or collecting waste as risk factors for H5 and/or H9 infection in broiler and/or layer farms. Other risk factors associated with H5 and H9 virus spread in layer farms were related to the following categories: (1) origin of production inputs, (2) stray dogs, (3) marketing practices, (4) cleaning practices and disposal of dead birds.

It has been suggested that the risk of viral transmission may increases as the number of intermediaries involved in poultry production increases (FAO, 2011; Sims, 2007). This is supported by the findings of the risk factor studies reported in this thesis, where layer farms purchasing DOC or pullets directly

from the hatcheries or breeding farms had lower odds of being H5 seropositive compared to farms purchasing DOC or pullets from FCDs or middlemen.

Presence of stray dogs were associated with increased odds of H5 and H9 infection in layer farms, suggesting that domestic animal species other than poultry might also play a role in the transmission of AIVs (Amirsalehy et al., 2012; Biswas et al. 2009b; Songserm et al., 2006).

Regarding specific marketing practices, farms selling larger numbers of spent layers experienced higher odds of H9 infection than farms selling less spent layers. Sales of a large numbers of spent layers might involve multiple traders, with several vehicles visiting the farm premises, increasing the risk of introduction of AIVs into the farms.

Layer farms of which the litter had not been changed during the production cycle were associated with higher odds of being seropositive for H9. Likewise, farms disposing dead birds by burying them near the premise had higher odds of being seropositive for H5, as observed in former studies (Busquets et al., 2010; Ritz, 2014).

Research question 4 (Chapter 6): What drives or hinders backyard, commercial broiler and layer chicken farmers to implement HPAI prevention and control measures on their farms?

The decision-making process of farmers about the implementation of prevention and control measures differed according to the farming system in which they operated. These findings are consistent with other studies, e.g. Jemberu et al. (2015), who found that farmers' perception towards implementation of FMD control measures varied by different cattle production systems (crop-livestock, pastoral and market-oriented systems), for instance, most of the farmers of pastoral and market-oriented systems.

While perceived barriers to the implementation of prevention and control measures (e.g. wearing protective equipment when handling chickens) refrained both broiler and backyard farmers to adopt interventions, perceived benefits of measures (e.g. maintaining high biosecurity to reduce the risk of birds becoming sick) strongly influenced broiler and layer farmers', but not backyard farmers'

decisions. Information provided on HPAI through media, meetings or via information campaigns played an important role in farmers' decision making in all production systems (Toma et al., 2013).

7.2 Significance of the research

The co-circulation of HPAI H5N1 and LPAI H9N2 in Bangladesh has a severe impact on poultry production, and raises concerns that it could lead to re-assortments and the emergence of a new virus variant of significant public health concern (Marinova-Petkova et al., 2016; Parvin et al., 2018). In order to control and prevent the spread of H5N1 and H9N2 viruses, a detailed understanding of the factors influencing AIV epidemiology in farms is paramount.

Unfortunately, previous research on AIV circulation in Bangladesh focussed predominately on LBMs (Kim et al., 2018; Sayeed et al., 2017), but rarely on poultry farms. The ease of sampling, as birds raised under different production systems are brought together in a single location at this markets, and the high expected prevalence of infection in marketed poultry, partly explains the preference for LBM research. In contrast, sampling of farmed poultry pose several challenges, including potential farmers' reluctance to have their birds sampled (in particular the collection of blood) and the time and resources required to visit farms across large geographical areas. Furthermore, studies conducted on farms generally focused on only one production system (Biswas et al., 2009a; Khatun et al., 2013; Nooruddin et al., 2006), limiting the understanding of virus circulation across the whole domestic chicken population and did not describe the concurrent circulation of different virus subtypes. In addition, all studies aiming to assess risk factor for HPAI H5N1 infection in farms in Bangladesh were based on outbreak reports (Biswas et al., 2009b, 2011; Loth et al., 2010; Osmani et al., 2014) and once again, focussed only on one chicken production system. Finally, although biosecurity plays an important role in AI prevention (Conan et al., 2012), compliance with recommended biosecurity measures is often poor in HPAI-endemic countries like Bangladesh, (Conan et al., 2012; FAO, 2011, 2013; Rimi et al., 2017) and no research has studied jointly risk factors for infection and farmers' perceptions on implementing biosecurity measures.

To address the aforementioned research gaps, this thesis includes four studies, presented in four research Chapters (Chapter 3, Chapter 4, Chapter 5, Chapter 6):

Chapter 3 concurrently researched endemicity of H5 and H9 viral circulation in different poultry production systems and different age groups of chickens. The results obtained provide a deeper

understanding of the patterns of H5 and H9 viral circulation in clinically healthy populations of backyard, commercial broiler and layer chickens. The research findings will also support the prioritisation of implementing control measures across chicken production systems, and do provide important parameters for mathematical models exploring the infection dynamics of AIVs in endemic settings. Chapter 4 and Chapter 5 explored farm- and village-level risk factors associated with the H5 and H9 serological status of backyard chicken farms and of commercial broiler and layer farms. The specific risk factors identified will guide policy makers to develop more specific and practical biosecurity measures aiming to mitigate the risk of AIV infection. Finally, Chapter 6 used the HBM framework to explored farmers' perceptions on the implementation of HPAI prevention and control measures across all chicken farming systems (backyard, commercial broiler and layer chickens). Outcomes of this research can be used to tailor messages on HPAI control and prevention for different poultry farming groups by accounting for specific factors influencing their decision-making, instead of using one-size-fit-all communication approach. Overall, this thesis provides a comprehensive picture of the factors influencing the epidemiology of AIV across all chicken farming systems in Bangladesh, by describing AIV infection patterns, risk factors of infection, and farmer's perceptions related to HPAI prevention and control.

So what is the future for HPAI in Bangladesh? Will it be possible to further reduce AIV prevalence or even eradicate HAPI from Bangladesh? Unfortunately, Bangladesh is facing a number of ecological, climatic and economic challenges that make it difficult to control the spread of AIV. Bangladesh is located in a broad deltaic plain which is prone to frequent flooding of two major rivers, the Jamuna (Brahmaputra) and Padma (Ganges). These flood areas and the country's shallow coastal waters attracts large populations of migratory birds, coming from Northern and Central Asia to overwinter in Bangladesh (Lepage, 2014), providing many opportunities for mingling of wild birds with domestic water birds. Furthermore chickens and ducks are often reared together on backyard farms (Alam et al., 2014), and left to scavenge for food during the day (Barua & Yoshimura, 1997), promoting contacts between domestic ducks and chickens with wild birds (Terregino et al., 2007). Furthermore, disease control activities are expensive and with a Gross Domestic Product of only 274.0 billion US dollars (WB, 2019a), Bangladesh financial resources are limited to provide compensation to poultry farmers when infected flocks need to be culled, or to establish a national surveillance and reporting system for HPAI or even support disinfection or vaccination programs. Nevertheless, Bangladesh strongly supports collaborative research to identify solutions for AI control (UKRI, 2019).

Nevertheless, the research presented here identified some interesting infection patterns. "Traditionally", if HPAI H5N1 virus is present, it would be expected to result in the death of all infected chickens, and, therefore, the absence of seropositive chickens which could have survived infection. Thus, the H5 antibodies detected in the research presented here may have been caused by a low pathogenic AIV strain, which would have then not resulted in the death of these chickens. Similarly, ducks could have been also infected with a LPAI H5 virus strain. Indeed, multiple H5 strains have been identified on Bangladeshi LBMs (Yang et al., 2019) and might well be circulating on farms as well. Alternatively, the severity of an H5 infection may be reduced due to the development of cell-mediated host resistance (Wang et al., 2016) or through cross-protective immunity, resulting in the survival of the infected birds. Cross-protective immunity might have resulted from the co-circulation of H5 with H9 subtypes. A study conducted by Khalenkov, Perk, Panshin, Golender, and Webster (2009) found that 90%-100% chickens previously inoculated with H9N2 virus survived subsequent inoculation by HPAI H5N1 viruses 1 to 35 days later. This suggests that previous infection by H9N2 viruses, and one may speculate, by other AIVs, may confer cross-immune protection against infection by HPAI H5N1 viruses.

Furthermore, it has been speculated that local chicken breeds may be resistant to HPAI H5N1 (GRAIN, 2006), pointing towards a genetic component of reduced host susceptibility. For example, a study conducted by Boonyanuwat, Thummabutra, Sookmanee, Vatchavalkhu, and Siripholvat (2006) suggested that the B21 haplotype in the major histocompatibility complex (MHC) class I molecule may explain the survival of some Thai indigenous breed chickens during an HPAI H5N1 outbreak. However, evidence supporting this hypothesis is limited, with experimental trials highlighting that B21 induced only partial protection against H5N1 (Hunt, Jadhao, & Swayne, 2010).

Considering the biological and evolutionary changes that come with AIV being endemic in Bangladesh, the potential natural reservoir for AIV in the specific ecological environment of Bangladesh, and the complex poultry production and marketing system, inadequate veterinary capacity, and farmers' unwillingness to report outbreaks to the authorities in Bangladesh (Rimi et al., 2019), raises the question if AI might remain an endemic poultry disease among others, such as Newcastle Disease, in Bangladesh with poultry producers needing to learn or already learning "how to live with it" (Spradbrow, 1996).

7.3 Recommendations for AI control in Bangladesh

The specific recommendations provided in individual Chapters 3-6 could guide policy makers to modify current or develop new approaches to control and prevent AIV spread in Bangladesh. In the following, further advice on integrating specific suggestions in an overarching policy framework are provided.

Firstly, the current AI vaccination program of Bangladesh mostly focus on hatcheries, breeder and commercial layer farms. While including broiler farms in the vaccination program could be epidemiologically relevant, the cost of the vaccine might refrain farmers from vaccinating their birds. Hence, the Government of Bangladesh could provide incentives or motivate broiler farmers to join such a vaccination program.

Secondly, although some recommendations need to be tailored to specific production types, others can be communicated to all types of poultry producers. For example, while backyard farmers should be encouraged to not pile up garbage around poultry houses and discouraged to purchase DOCs and pullets from "unreliable" neighbouring backyard farms or markets, commercial farms should have facilities for changing clothes and footwear before entering or leaving the farm as well as footbaths and facilities to wash hands. However, across all production types, risk factors for AIV spread relating to marketing and contact patterns were identified, highlighting the need for a system approach that not just focusses on the point of production or point of sale, but includes all linkages and networks where AIV could multiply across the poultry chain.

Similarly, messages on HPAI control and prevention for different poultry farming groups have to account for their different decision-making process. For example, while barriers to implement control measures were important for broiler and backyard farmers, benefits of control measures were only important for commercial farmers. On the other hand, the willingness of farmers to learn more about biosecurity and HPAI control and the impact of education programs on farmers' perceptions was present across all production systems.

Most importantly, this PhD research has already made some impact by 1) providing advice to two Chatham House roundtable policy discussions on AI prevention in Bangladesh (ChatamHouse, 2018; ChathamHouse, 2016; Chattopadhyay et al., 2018), 2) guiding the development of the current multidisciplinary GCRF One Health Poultry Hub project (UKRI, 2019), and 3) by informing a multi-

country FAO expert discussion on HPAI in endemically infected countries on the research presented in this thesis (FAO, 2019a). Last but not least, we are proud that we chose a truly participatory approach and provided detailed feedback on our research outcomes to all farmers involved in the research. Thus, we provided a certificate summarizing the test results obtained on each farm and information leaflet providing specific recommendations based on our study results to all farmers that were part of this research.

7.4 Limitations of the research and recommendations for future investigations

The research presented here had a number of limitations.

Firstly, some questions posed to farmers required recall of information. However, recall bias was limited by interviewing the person who actually worked with the poultry flocks and by taking care of the way the questions were asked. For example, farmers might have not remembered details of an outbreak (number of birds that died, dates of the outbreak etc.), but they would certainly remembered an outbreak occurrence as they are excellent in monitoring the health of their birds (Katcher & Beck, 1987). Thus the questions asked in the cross-sectional survey focussed not on the number of mortalities, but on the existence of mortality events or events with clinical symptoms typical of AIV infection over the past 12 months. Similarly, dichotomised or ordinal responses were requested when information about marketing and production of chickens was collected.

Secondly, some farmers were unable to provide detailed information on AI vaccinations (i.e. dates of vaccinations, name of AI vaccine used), as written records are rarely made by farmers. Therefore is was not possible to explore the association between antibody titres and the dates of vaccination. However, farmers were able to specify if birds were vaccinated in the past 12 months and this was considered in the data analysis. Nevertheless, only a small group of layer farmers vaccinated actually vaccinated against AI.

Thirdly, the antigen used in the HI test might have impacted on the serological results. The HI test is considered as a ‘gold standard’ for AI antibody subtyping because of its very high sensitivity (98·8%) and specificity (99·5%), and it is recommended by both EU and OIE for subtype specific AI diagnosis (Comin et al., 2013). However, the performance of HI tests might depend on the use of the country specific antigen. In the research presented here, the antigen used was prepared from field virus isolated from different countries. However, a study conducted by Yamamoto et al. (2007) estimated

that the sensitivity and specificity of the HI test were 99% and 90% respectively, even when different antigens were used, thus highlighting that number of potentially false positives will be minimal.

Fourthly, due to the cross-sectional nature of the study, it was not possible to describe detailed seasonal variations, for example, in regards to the number of chickens reared or sold per month. However, by including some questions about general seasonal patterns, it was possible to include these variables in the risk factor analysis. Nevertheless, a year-long longitudinal study with monthly or bi-monthly data collection would be recommended.

Fifthly, although causal relationships between perceptions and the implementation of HPAI preventive measures were hypothesized, validating these presumed causal relationships was not possible in the used cross-sectional study design. Furthermore, the HBM framework used in this research paid more attention to the subjective state of an individual rather than other contextual factors, such as social acceptability, which would need to be explored through more qualitative approaches.

Finally, although separate risk factor analyses were conducted for H5 and H9 seroprevalence, commonalities in risk factors were identified (for example, risk factors related to environmental or ecological features, trading practices, poultry movements and sources of the Day Old Chicks, pullets and feed) indicting likely similar transmission dynamics for both viruses. Nevertheless, certain farm management practices might be associated with a higher probability of either H5 or H9 infection, but this could not be confirmed with the study design used.

Future research could explore in a longitudinal framework the continuing implementation of HPAI prevention and control measures by farmers and their direct impact on AIV circulation. Alternatively, data of the research presented here could be used to generate a follow-up study by revisiting farms and exploring in separated datasets whether farmers' perceptions and attitudes were predictors of the seropositivity status of their flocks, and of the management practices reported by them.

Furthermore, the research presented here estimated that the exposure of broiler chickens to AIVs was comparable to layer farms, suggesting that broiler farms may play a substantial role in the spread of AIVs, and consequently that broiler farms may need to be considered in AI vaccination program. A detailed cost-benefit analysis of the feasibility of AI vaccination in broiler chicken farms would be highly recommended.

In Bangladesh, most of the molecular studies on H9 relied on LBM sampling (Negovetich et al., 2011; Turner et al., 2017). However, the H9 virus isolated from farms in this research could be further processed for sequencing and might provide further insights into the molecular evolution of LPAI viruses across different chicken production systems in Bangladesh.

Finally, mathematical transmission modelling, value chain analysis and risk based mapping could be additional studies that could be conducted and informed by the data generated in the thesis.

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APPENDICES

Appendix 1: Supplementary Table 1: Descriptive statistics of original responses collected from backyard chicken farmers

SD= Strongly Disagree, D=Disagree, N= Neither agree nor disagree, DK= Do not Know, A=Agree, SA= Strongly Agree

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Perceived susceptibility						
My chickens have an increased risk of getting avian influenza/bird flu						
When I rear different species of poultry together(PSus1)	13.2(19)	16.0(23)	1.4(2)	15.3(22)	17.4(25)	36.8(53)
When I keep chickens and ducks in the same house(PSus2)	13.2(19)	15.3(22)	1.4(2)	11.8(17)	22.2(32)	36.1(52)
When I don't regularly clean poultry house/equipment(PSus3)	4.2(6)	9.7(14)	0.0(0)	4.2(6)	30.6(44)	51.4(74)
When my chickens mix with neighbour sick poultry during scavenging(PSus4)	1.4(2)	4.2(6)	2.1(3)	0.0(0)	37.5(54)	54.9(79)
When my chickens mix with wild birds(PSus5)	14.5(21)	6.3(9)	0.0(0)	24.3(35)	27.1(39)	27.8(40)
When my family members or I bring back unsold poultry from LBM & put together with other poultry(PSus6)	4.9(7)	4.9(7)	0.0(0)	5.6(8)	27.8(40)	56.9(82)
I am at increased risk of getting avian influenza/bird flu						
Because of my poultry rearing(PSus7)	19.4(28)	20.1(29)	0.0(0)	34.0(49)	15.3(22)	11.1(16)
When I handle sick poultry(PSus8)	19.4(28)	18.1(26)	0.0(0)	34.0(49)	13.9(20)	14.6(21)
When I don't cover my mouth and nose with cloths during handling poultry(PSus9)	16.7(24)	17.4(25)	0.0(0)	31.9(46)	14.6(21)	19.4(28)
When I don't wash my hands with soap water after handling poultry(PSus10)	16.0(23)	17.4(25)	0.7(1)	31.9(46)	13.2(19)	20.8(30)
My family members are at increased risk of getting avian influenza/bird flu						
Because of my poultry rearing(PSus11)	22.9(33)	18.8(27)	0.0(0)	33.3(48)	13.9(20)	11.1(16)
Uncooked poultry meat doesn't pose risk for getting avian influenza/bird flu(PSus12)	31.3(45)	17.4(25)	1.4(2)	21.5(31)	22.9(33)	5.6(8)
Perceived severity						
If my chickens get sick from avian influenza/bird flu						

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Then the illness would be very bad, and the chickens will most likely die(PSev1)	0.7(1)	2.8(4)	0.0(0)	0.7(1)	39.6(57)	56.3(81)
Then avian influenza/bird flu could be passed to other poultry in my locality(PSev2)	0.0(0)	0.7(1)	0.0(0)	1.4(2)	43.8(63)	54.2(78)
Then avian influenza/bird flu could be passed on to me(PSev3)	18.8(27)	18.1(26)	0.0(0)	33.3(48)	13.9(20)	16.0(23)
If my chickens get sick and die from avian influenza/bird flu						
Then I will lose income and family consumption(PSev4)	1.4(2)	1.4(2)	0.0(0)	0.0(0)	44.4(64)	52.8(76)
If I get sick from avian influenza/bird flu						
Then other members in my home will get sick(PSev5)	20.8(30)	19.4(28)	0.0(0)	33.3(48)	13.2(19)	13.2(19)
Then I will die(PSev6)	27.1(39)	18.8(27)	1.4(2)	39.6(57)	7.6(11)	5.6(8)
If my family members get sick from avian influenza/bird flu						
Then they will die(PSev7)	27.8(40)	18.8(27)	1.4(2)	39.6(57)	9.0(13)	3.5(5)
Chickens that catch avian influenza/bird flu cannot be treated(PSev8)	37.5(54)	11.1(16)	4.2(6)	21.5(31)	15.3(22)	10.4(15)
Perceived benefits						
My chickens will not get sick from avian influenza/bird flu						
If I don't rear different species of poultry together(PBen1)	13.2(19)	16.0(23)	1.4(2)	15.3(22)	17.4(25)	36.8(53)
If I don't keep chickens and ducks together in same house(PBen2)	13.2(19)	15.3(22)	1.4(2)	11.8(17)	22.2(32)	36.1(52)
If I regularly clean poultry house/equipment(PBen3)	4.2(6)	9.7(14)	0.0(0)	4.2(6)	30.6(44)	51.4(74)
If my family members or I don't bring unsold poultry from LBM/don't put unsold poultry with other poultry after bring back from LBM(PBen4)	4.9(7)	4.9(7)	0.0(0)	5.6(8)	27.1(39)	57.6(83)
If my chickens will not get sick from avian influenza/bird flu						
I will not lose income and family consumption(PBen5)	1.4(2)	1.4(2)	0.0(0)	0.0(0)	44.4(64)	52.8(76)
The possibility of disease outbreaks in my locality will reduce(PBen6)	0.7(1)	0.7(1)	0.0(0)	1.4(2)	43.1(62)	54.2(78)
My family members and I will not get sick from avian influenza/bird flu(PBen7)	18.8(27)	18.8(27)	0.0(0)	34.0(49)	13.9(20)	14.6(21)
Perceived barriers						
Construction of separate house to keep chickens and ducks separately is expensive and required more spaces which I don't have (PBar1)	13.2(19)	16.0(23)	0.0(0)	0.0(0)	36.8(53)	34.0(49)

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
It's not worth to protect my chickens from avian influenza/bird flu, because I don't earn sufficient money from rearing chickens (PBar2)	52.1(75)	37.5(54)	0.0(0)	0.0(0)	9.0(13)	1.4(2)
Regular cleaning of poultry house/equipment is time consuming and not practical for me, because my family/I have to do many other things (PBar3)	40.3(58)	35.4(51)	0.0(0)	0.0(0)	21.5(31)	2.8(4)
Washing hands before and after handling poultry is not practical for me, because my family/I have to do many other things (PBar4)	38.9(56)	28.5(41)	0.0(0)	0.0(0)	28.5(41)	4.2(6)
I can't cover my mouth and nose with cloths during handling chickens, because they are not conducive for work (PBar5)	37.5(54)	25.0(36)	2.8(4)	1.4(2)	29.9(43)	3.5(5)
I don't cover my mouth and nose with cloths during handling chickens, because my neighbour do not (PBar6)	37.5(54)	32.6(47)	0.0(0)	0.0(0)	25.7(37)	4.2(6)
Cues to action						
I would receive training regarding avian influenza/bird flu prevention & control and other aspects of poultry rearing, if DLS or any other organization would provide it (Cue1)	2.8(4)	9.0(13)	0.0(0)	0.0(0)	36.1(52)	52.1(75)
If I						
Find a program on TV about avian influenza/bird flu and other aspects of poultry rearing, then I would watch it(Cue2)	1.4(2)	2.8(4)	0.0(0)	0.0(0)	38.2(55)	57.6(83)
Find a program on the radio about avian influenza/bird flu and other aspects of poultry rearing, then I would listen to it(Cue3)	1.4(2)	2.8(4)	0.0(0)	0.0(0)	38.9(56)	56.9(82)
Get invited to a meeting or campaign, etc. about avian influenza/bird flu and other aspects of poultry rearing, then I would attend it(Cue4)	2.1(3)	6.9(10)	0.0(0)	0.0(0)	41.0(59)	50.0(72)
It is a good idea for me to talk						
With local livestock related personnel about risks of avian influenza/bird flu disease transmission between chickens(Cue5)	0.7(1)	5.6(8)	0.0(0)	0.0(0)	53.5(77)	40.3(58)
With community health workers or nearby hospital doctor about risks of disease transmission between chickens and humans(Cue6)	3.5(5)	5.6(8)	0.0(0)	0.0(0)	46.5(67)	44.4(64)
Self-efficacy						
It is a good idea						

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
To invest in separate houses for chicken and duck(SEff1)	1.4(2)	0.0(0)	0.0(0)	0.0(0)	43.8(63)	54.9(79)
To clean poultry house/equipment regularly(SEff2)	0.0(0)	1.4(2)	0.0(0)	0.0(0)	41.7(60)	56.9(82)
I would be able to identify signs of the disease, if my chickens were infected with avian influenza/bird flu(SEff3)	0.7(1)	0.7(1)	0.7(1)	0.0(0)	46.5(67)	51.4(74)
I will inform the local livestock related personnel, when I suspect that my chickens have avian influenza/bird flu (SEff4)	1.4(2)	1.4(2)	0.7(1)	0.0(0)	49.3(71)	47.2(68)
I could						
Dispose dead birds properly(bury them)(SEff5)	0.7(1)	1.4(2)	1.4(2)	0.0(0)	39.6(57)	56.9(82)
Cover my mouth and nose with cloths during handling poultry, even if my neighbours are not(SEff6)	0.7(1)	0.7(1)	0.7(1)	0.7(1)	46.5(67)	50.7(73)
Wash my hands with soap before and after handling poultry, even if my neighbours are not(SEff7)	1.4(2)	0.0(0)	0.7(1)	0.7(1)	46.5(67)	50.7(73)

Appendix 2: Supplementary Table 2: Descriptive statistics of original responses collected from commercial broiler chicken farmers

SD= Strongly Disagree, D=Disagree, N= Neither agree nor disagree, DK= Do not Know, A=Agree, SA= Strongly Agree

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Perceived susceptibility						
My chickens have an increased risk of getting avian influenza/bird flu						
When I don't vaccinate them (PSus1)	2.8(3)	7.6(8)	0.9(1)	5.7(6)	34.0(36)	49.1(52)
When I don't restrict who comes onto my farm(PSus2)	0.9(1)	8.5(9)	0.9(1)	0.0(0)	35.9(38)	53.8(57)
When I don't regularly clean and disinfect my farm and farm equipment(PSus3)	0.0(0)	4.7(5)	0.0(0)	0.0(0)	31.1(33)	64.2(68)
When I don't control wild birds/backyard poultry from entering into my poultry shed/house(PSus4)	0.0(0)	5.7(6)	0.0(0)	1.9(2)	28.3(30)	64.2(68)
When my workers don't wash their hands/feet/change clothes before entering poultry shed/house(PSus5)	0.0(0)	5.7(6)	0.0(0)	0.9(1)	27.4(29)	66.0(70)
When I don't clean and disinfect vehicles, egg trays, cages, de-beaking machine, vaccination gun, etc. before entering into my farm(PSus6)	0.0(0)	4.7(5)	0.0(0)	0.0(0)	33.0(35)	62.3(66)
I am at increased risk of getting avian influenza/bird flu						
Because of my poultry business(PSus7)	7.6(8)	17.9(19)	0.0(0)	23.6(25)	34.0(36)	17.0(18)
When I don't wear protective equipment (mask, gloves, dedicated sandals/shoes, apron, etc.) during handling chickens(PSus8)	0.9(1)	13.2(14)	0.0(0)	18.9(20)	36.8(39)	30.2(32)
When I don't wash my hands with soap water after handling chickens(PSus9)	0.9(1)	13.2(14)	0.0(0)	18.9(20)	31.1(33)	35.9(38)
My family members are at increased risk of getting avian influenza/bird flu						
Because of my poultry business(PSus10)	9.4(10)	21.7(23)	0.0(0)	22.6(24)	28.3(30)	17.9(19)
Uncooked poultry meat doesn't pose risk for getting avian influenza/bird flu(PSus11)	31.1(33)	47.2(50)	0.9(1)	10.4(11)	7.6(8)	2.8(3)
Perceived severity						
If my chickens get sick from avian influenza/bird flu						

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Then the illness would be very bad, and the chickens will most likely die(PSev1)	0.0(0)	6.6(7)	0.0(0)	5.7(6)	35.9(38)	51.9(55)
Then avian influenza could be passed to other poultry farms in my locality(PSev2)	0.0(0)	3.8(4)	0.0(0)	6.6(7)	30.2(32)	59.4(63)
Then avian influenza could be passed on to me(PSev3)	5.7(6)	12.3(13)	0.0(0)	23.6(25)	34.9(37)	23.6(25)
If my chickens get sick and die from avian influenza/bird flu						
Then I will lose income (PSev4)	0.0(0)	0.9(1)	0.0(0)	2.8(3)	14.2(15)	82.1(87)
If I get sick from avian influenza/bird flu						
Then other members in my family will get sick(PSev5)	8.5(9)	22.6(24)	0.0(0)	22.6(24)	33.0(35)	13.2(14)
Then I will die(PSev6)	17.9(19)	24.5(26)	1.9(2)	31.1(33)	18.9(20)	5.7(6)
If my family members get sick from avian influenza/bird flu						
Then they will die(PSev7)	17.9(19)	23.6(25)	1.9(2)	32.1(34)	18.9(20)	5.7(6)
Chickens that catch avian influenza/bird flu cannot be treated(PSev8)	9.4(10)	50.0(53)	0.0(0)	7.6(8)	19.8(21)	13.2(14)
Perceived benefits						
If I maintain biosecurity(proper prevention & control measures) in my poultry farm, then my chickens will :						
Not get sick from avian influenza, and I will not lose income(PBen1)	0.0(0)	5.7(6)	0.0(0)	0.9(1)	26.4(28)	67.0(71)
Not get sick from avian influenza and the possibility of disease outbreaks in my locality will reduce(PBen2)	0.0(0)	7.6(8)	0.0(0)	0.0(0)	24.5(26)	67.9(72)
Not get sick from AI as well as my family members and I will not get sick from AI(PBen3)	0.0(0)	8.5(9)	0.0(0)	5.7(6)	28.3(30)	57.6(61)
If my chickens receive avian influenza vaccine, then they will not get sick :						
And die and I will not lose income(PBen4)	0.0(0)	6.6(7)	0.0(0)	1.9(2)	22.6(24)	68.9(73)
And the possibility of disease outbreaks in my locality will reduce(PBen5)	0.0(0)	9.4(10)	0.0(0)	1.9(2)	27.4(29)	61.3(65)
From AI as well as my family members and I will not get sick from AI(PBen6)	1.9(2)	14.2(15)	0.0(0)	9.4(10)	33.0(35)	41.5(44)
Perceived barriers						
Maintaining biosecurity (proper prevention & control measures) is expensive (PBar1)	41.5(44)	19.8(21)	0.0(0)	1.9(2)	32.1(34)	4.7(5)

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Vaccination of chickens for avian influenza is expensive (PBar2)	36.8(39)	21.7(23)	0.0(0)	16.0(17)	21.7(23)	3.8(4)
There is a shortage of quality avian influenza vaccine for chickens in Bangladesh (PBar3)	1.9(2)	23.6(25)	1.9(2)	46.2(49)	24.5(26)	1.9(2)
Vaccine can't protect chickens from getting avian influenza (PBar4)	3.8(4)	44.3(47)	0.0(0)	17.9(19)	27.4(29)	6.6(7)
My chickens may get sick from the avian influenza vaccine (PBar5)	8.5(9)	17.9(19)	0.0(0)	12.3(13)	57.6(61)	3.8(4)
Cooking meat thoroughly takes so much time (PBar6)	42.5(45)	50.0(53)	0.0(0)	0.0(0)	5.7(6)	1.9(2)
Washing hands all the time is not practical for me, because I have to do many other things(PBar7)	64.2(68)	24.5(26)	0.0(0)	0.0(0)	10.4(11)	0.9(1)
My neighbouring farmer doesn't use avian influenza vaccine, so I don't use avian influenza vaccine(PBar8)	67.0(71)	22.6(24)	0.0(0)	0.0(0)	8.5(9)	1.9(2)
I can't wear protective gear, because they are not conducive for work(PBar9)	68.8(73)	23.6(25)	0.0(0)	0.0(0)	6.6(7)	0.9(1)
I don't wear protective gear because my neighbouring poultry farmers do not(PBar10)	72.6(77)	18.9(20)	0.0(0)	0.0(0)	6.6(7)	1.9(2)
Cues to action						
I would receive training regarding avian influenza prevention and control, if DLS or any other organization would provide it(Cue1)	0.9(1)	3.8(4)	0.9(1)	0.0(0)	14.2(15)	80.2(85)
If I						
See an article in a newspaper about avian influenza, then I would read it(Cue2)	0.0(0)	0.0(0)	0.0(0)	0.9(1)	14.2(15)	84.9(90)
Find a program on TV about avian influenza, then I would watch it(Cue3)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	15.1(16)	84.0(89)
Find a program on the radio about avian influenza, then I would listen to it(Cue4)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	14.2(15)	84.9(90)
Find information about avian influenza – leaflet/brochure/billboard, etc., then I would read it(Cue5)	0.0(0)	0.0(0)	0.0(0)	0.9(1)	16.0(17)	83.0(88)
Get invited to a meeting or campaign, etc. about avian influenza, then I would attend it(Cue6)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	15.1(16)	84.9(90)
It is a good idea for me to talk						
With local livestock officers about risks of avian influenza disease transmission between birds(Cue7)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	16.0(17)	83.0(88)

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
With my family doctor about risks of disease transmission between birds and humans(Cue8)	0.0(0)	0.9(1)	0.0(0)	0.9(1)	20.8(22)	77.4(82)
Self-efficacy						
It is a good idea						
To invest in biosecurity (proper prevention & control measures) at my farm(SEff1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	23.6(25)	76.4(81)
To invest in avian influenza vaccination of my chickens(SEff2)	0.0(0)	0.0(0)	0.0(0)	0.9(1)	24.5(26)	74.5(79)
I would be able to identify signs of the disease, if my chickens were infected with avian influenza(SEff3)	7.6(8)	10.4(11)	4.7(5)	9.4(10)	27.4(29)	40.6(43)
I will inform the local livestock office, when I suspect that my chickens have avian influenza (SEff4)	0.9(1)	2.8(3)	10.4(11)	2.8(3)	35.9(38)	47.2(50)
I could						
Dispose dead birds/litter/waste properly(SEff5)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	23.6(25)	76.4(81)
Clean & disinfect poultry house/equipment regularly(SEff6)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	25.5(27)	74.5(79)
Wear protective gear, even if my neighbouring poultry farmers are not (SEff7)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	31.1(33)	68.9(73)
Wash my hands with soap before and after handling chickens even if my neighbouring poultry farmers are not(SEff8)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	25.5(27)	73.6(78)

Appendix 3: Supplementary Table 3: Descriptive statistics of original responses collected from commercial layer chicken farmers

SD= Strongly Disagree, D=Disagree, N= Neither agree nor disagree, DK= Do not Know, A=Agree, SA= Strongly Agree

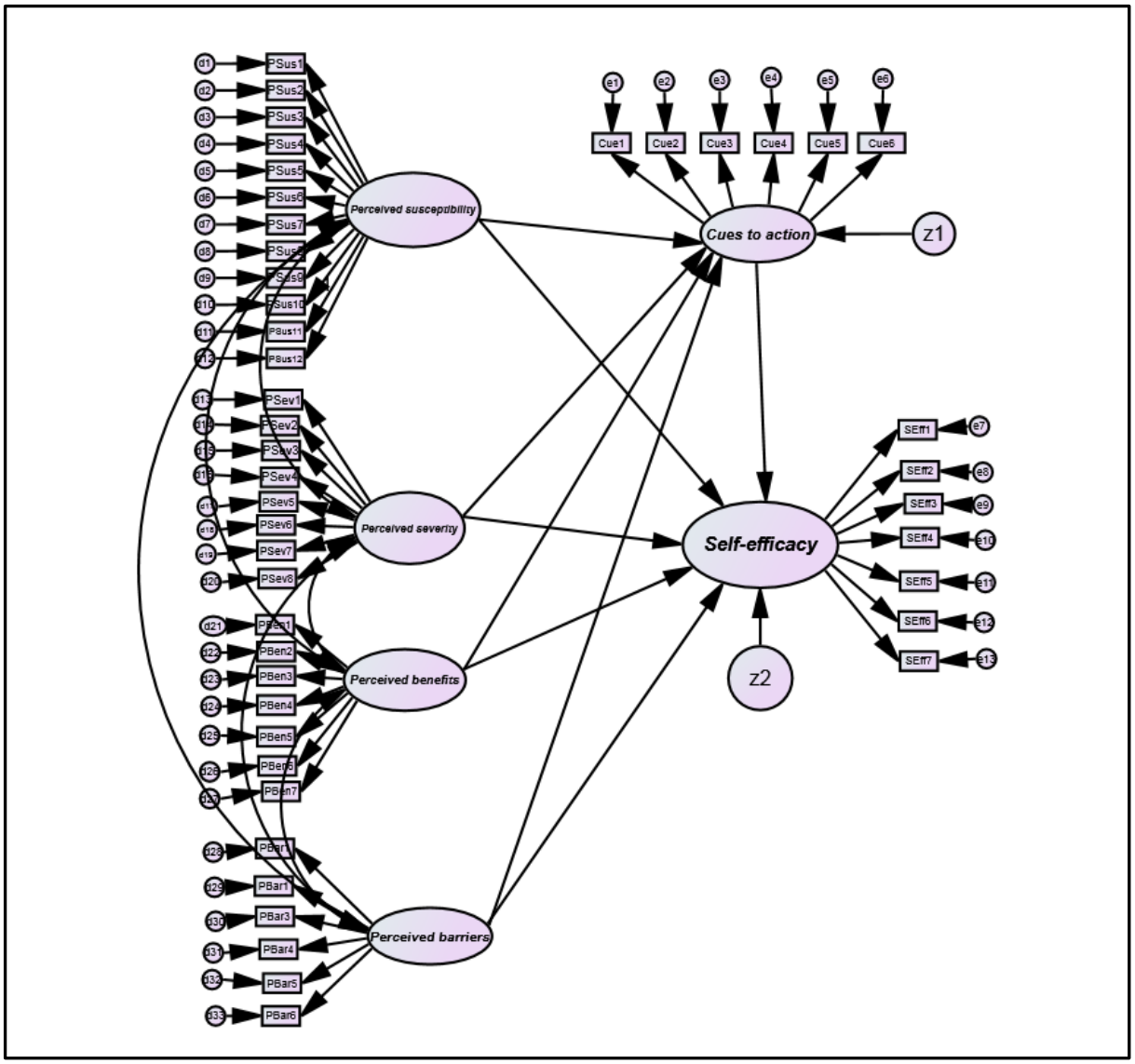
Perceived susceptibility						
	SD	**D**	**N**	**DK**	**A**	**SA**
	%(n)	**%(n)**	**%(n)**	**%(n)**	**%(n)**	**%(n)**
My chickens have an increased risk of getting avian influenza/bird flu						
When I don't vaccinate them (PSus1)	0.9(1)	6.2(7)	0.9(1)	2.7(3)	42.5(48)	46.9(53)
When I don't restrict who comes onto my farm(PSus2)	0.0(0)	2.7(3)	0.0(0)	1.8(2)	36.3(41)	59.3(67)
When I don't regularly clean and disinfect my farm and farm equipment(PSus3)	0.0(0)	0.9(1)	0.0(0)	0.9(1)	33.6(38)	64.6(73)
When I don't control wild birds/backyard poultry from entering into my poultry shed/house(PSus4)	0.0(0)	1.8(2)	0.0(0)	1.8(2)	34.5(39)	62.0(70)
When my workers don't wash their hands/feet/change clothes before entering poultry shed/house(PSus5)	0.0(0)	0.9(1)	0.0(0)	2.7(3)	32.7(37)	63.7(72)
When I don't clean and disinfect vehicles, egg trays, cages, de-beaking machine, vaccination gun, etc. before entering into my farm(PSus6)	0.0(0)	2.7(3)	0.0(0)	1.8(2)	31.0(35)	64.6(73)
I am at increased risk of getting avian influenza/bird flu						
Because of my poultry business(PSus7)	9.7(11)	14.2(16)	0.9(1)	23.0(26)	32.7(37)	19.5(22)
When I don't wear protective equipment (mask, gloves, dedicated sandals/shoes, apron, etc.) during handling chickens(PSus8)	1.8(2)	9.7(11)	0.0(0)	17.7(20)	42.5(48)	28.3(32)
When I don't wash my hands with soap water after handling chickens(PSus9)	0.9(1)	9.7(11)	0.0(0)	17.7(20)	26.6(30)	45.1(51)
My family members are at increased risk of getting avian influenza/bird flu						
Because of my poultry business(PSus10)	12.4(14)	23.0(26)	0.9(1)	24.8(28)	26.6(30)	12.4(14)
Uncooked poultry meat doesn't pose risk for getting avian influenza/bird flu(PSus11)	29.2(33)	47.8(54)	0.0(0)	13.3(15)	8.0(9)	1.8(2)

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Perceived severity						
If my chickens get sick from avian influenza/bird flu						
Then the illness would be very bad, and the chickens will most likely die(PSev1)	0.0(0)	5.3(6)	0.0(0)	10.6(12)	42.5(48)	41.6(47)
Then avian influenza could be passed to other poultry farms in my locality(PSev2)	0.0(0)	4.4(5)	0.0(0)	9.7(11)	34.5(39)	51.3(58)
Then avian influenza could be passed on to me(PSev3)	7.1(8)	11.5(13)	0.0(0)	24.8(28)	29.2(33)	27.4(31)
If my chickens get sick and die from avian influenza/bird flu						
Then I will lose income (PSev4)	0.0(0)	2.7(3)	0.0(0)	4.4(5)	15.0(17)	77.9(88)
If I get sick from avian influenza/bird flu						
Then other members in my family will get sick(PSev5)	11.5(13)	23.0(26)	0.9(1)	23.9(27)	22.1(25)	18.6(21)
Then I will die(PSev6)	18.6(21)	29.2(33)	2.7(3)	31.0(35)	14.2(16)	4.4(5)
If my family members get sick from avian influenza/bird flu						
Then they will die(PSev7)	17.7(20)	29.2(33)	2.7(3)	31.9(36)	14.2(16)	4.4(5)
Chickens that catch avian influenza/bird flu cannot be treated(PSev8)	10.6(12)	49.6(56)	0.0(0)	8.9(10)	18.6(21)	12.4(14)
Perceived benefits						
If I maintain biosecurity(proper prevention & control measures) in my poultry farm, then my chickens will :						
Not get sick from avian influenza, and I will not lose income(PBen1)	0.0(0)	6.2(7)	0.0(0)	1.8(2)	28.3(32)	63.7(72)
Not get sick from avian influenza and the possibility of disease outbreaks in my locality will reduce(PBen2)	0.9(1)	8.0(9)	0.0(0)	2.7(3)	24.8(28)	63.7(72)
Not get sick from AI as well as my family members and I will not get sick from AI(PBen3)	0.9(1)	15.0(17)	0.9(1)	2.7(3)	23.0(26)	57.5(65)
If my chickens receive avian influenza vaccine, then they will not get sick :						
And die and I will not lose income(PBen4)	0.9(1)	7.1(8)	0.0(0)	0.9(1)	24.8(28)	66.4(75)
And the possibility of disease outbreaks in my locality will reduce(PBen5)	1.8(2)	7.1(8)	0.9(1)	0.9(1)	25.7(29)	63.7(72)
From AI as well as my family members and I will not get sick from AI(PBen6)	3.5(4)	12.4(14)	0.9(1)	6.2(7)	33.6(38)	43.4(49)
Perceived barriers						

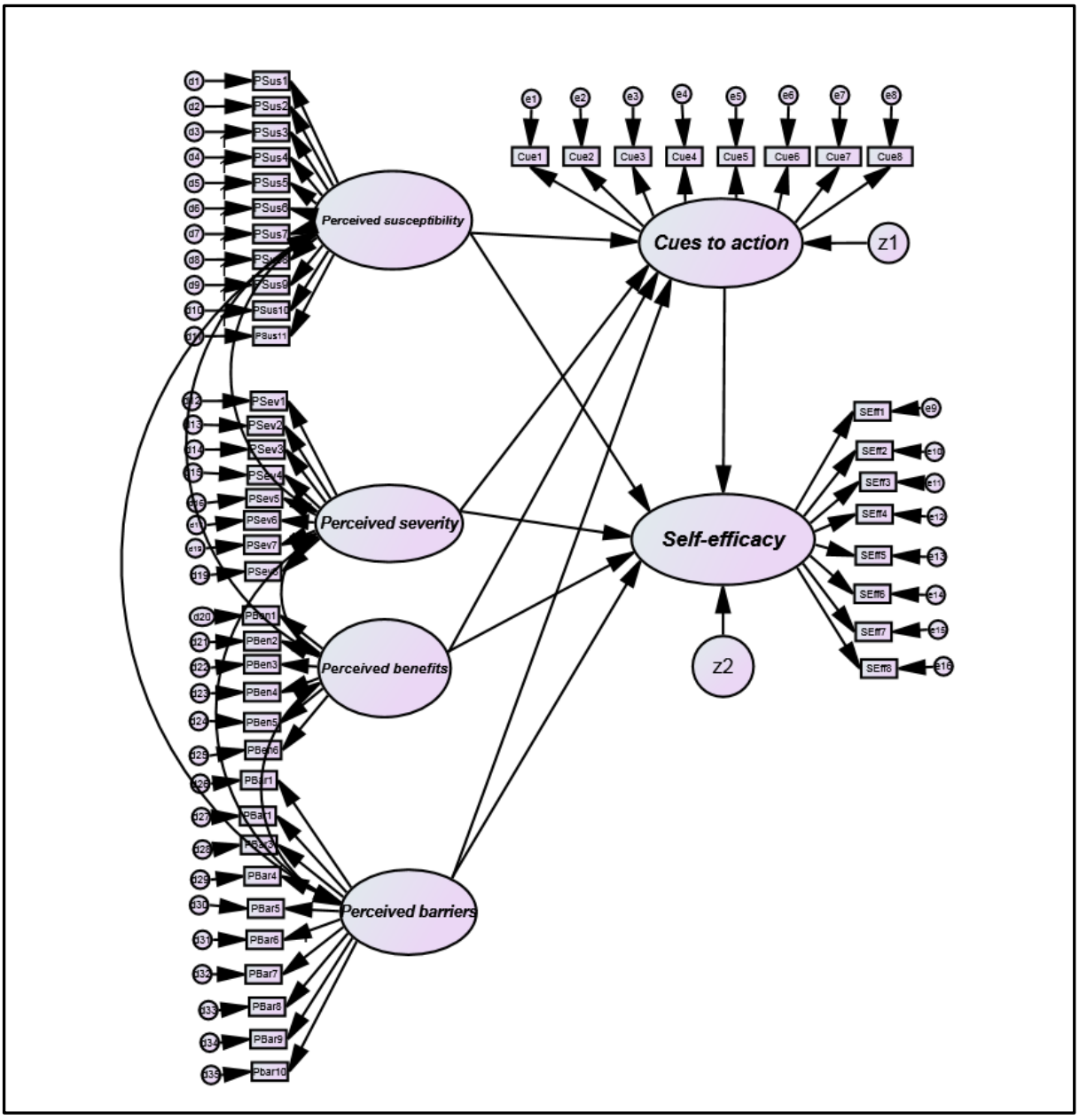
	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
Maintaining biosecurity (proper prevention & control measures) is expensive (PBar1)	36.3(41)	24.8(28)	0.0(0)	0.0(0)	30.1(34)	8.9(10)
Vaccination of chickens for avian influenza is expensive (PBar2)	31.0(35)	20.4(23)	0.9(1)	12.4(14)	27.4(31)	8.0(9)
There is a shortage of quality avian influenza vaccine for chickens in Bangladesh (PBar3)	0.9(1)	21.2(24)	0.9(1)	38.9(44)	31.9(36)	6.2(7)
Vaccine can't protect chickens from getting avian influenza (PBar4)	3.5(4)	42.5(48)	1.8(2)	8.0(9)	31.9(36)	12.4(14)
My chickens may get sick from the avian influenza vaccine (PBar5)	6.2(7)	18.6(21)	0.9(1)	8.9(10)	60.2(68)	5.3(6)
Cooking meat thoroughly takes so much time (PBar6)	34.5(39)	59.3(67)	0.0(0)	0.0(0)	5.3(6)	0.9(1)
Washing hands all the time is not practical for me, because I have to do many other things(PBar7)	62.0(70)	25.7(29)	0.0(0)	0.0(0)	12.4(14)	0.0(0)
My neighbouring farmer doesn't use avian influenza vaccine, so I don't use avian influenza vaccine(PBar8)	62.8(71)	28.3(32)	0.0(0)	0.0(0)	8.9(10)	0.0(0)
I can't wear protective gear, because they are not conducive for work(PBar9)	66.4(75)	23.9(27)	0.0(0)	0.0(0)	9.7(11)	0.0(0)
I don't wear protective gear because my neighbouring poultry farmers do not(PBar10)	65.5(74)	28.3(32)	0.0(0)	0.0(0)	5.3(6)	0.9(1)
Cues to action						
I would receive training regarding avian influenza prevention and control, if DLS or any other organization would provide it(Cue1)	0.0(0)	10.6(12)	0.0(0)	0.0(0)	19.5(22)	69.9(79)
If I						
See an article in a newspaper about avian influenza, then I would read it(Cue2)	0.0(0)	3.5(4)	0.9(1)	1.8(2)	20.4(23)	73.5(83)
Find a program on TV about avian influenza, then I would watch it(Cue3)	0.0(0)	0.9(1)	0.0(0)	0.0(0)	23.9(27)	75.2(85)
Find a program on the radio about avian influenza, then I would listen to it(Cue4)	0.0(0)	1.8(2)	0.0(0)	0.0(0)	23.01(26)	75.2(85)
Find information about avian influenza – leaflet/brochure/billboard, etc., then I would read it(Cue5)	0.0(0)	2.7(3)	0.9(1)	1.8(2)	20.4(23)	74.3(84)
Get invited to a meeting or campaign, etc. about avian influenza, then I would attend it(Cue6)	0.0(0)	1.8(2)	0.0(0)	0.9(1)	23.9(27)	73.5(83)
It is a good idea for me to talk						

	SD	D	N	DK	A	SA
	%(n)	%(n)	%(n)	%(n)	%(n)	%(n)
With local livestock officers about risks of avian influenza disease transmission between birds(Cue7)	0.0(0)	0.0(0)	0.9(1)	0.0(0)	28.3(32)	70.8(80)
With my family doctor about risks of disease transmission between birds and humans(Cue8)	0.9(1)	0.0(0)	0.0(0)	0.0(0)	34.5(39)	64.6(73)
Self-efficacy						
It is a good idea						
To invest in biosecurity (proper prevention & control measures) at my farm(SEff1)	0.9(1)	0.0(0)	0.0(0)	0.0(0)	32.7(37)	66.4(75)
To invest in avian influenza vaccination of my chickens(SEff2)	0.9(1)	0.9(1)	0.0(0)	0.9(1)	31.9(36)	65.5(74)
I would be able to identify signs of the disease, if my chickens were infected with avian influenza(SEff3)	10.6(12)	4.4(5)	0.9(1)	5.3(6)	40.7(46)	38.1(43)
I will inform the local livestock office, when I suspect that my chickens have avian influenza (SEff4)	0.0(0)	6.2(7)	3.5(4)	3.5(4)	45.1(51)	41.6(47)
I could						
Dispose dead birds/litter/waste properly(SEff5)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	31.0(35)	69.0(78)
Clean & disinfect poultry house/equipment regularly(SEff6)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	34.5(39)	65.5(74)
Wear protective gear, even if my neighbouring poultry farmers are not(SEff7)	0.9(1)	0.0(0)	0.0(0)	0.0(0)	35.4(40)	63.7(72)
Wash my hands with soap before and after handling chickens even if my neighbouring poultry farmers are not(SEff8)	0.9(1)	0.9(1)	0.0(0)	0.0(0)	33.6(38)	64.6(73)

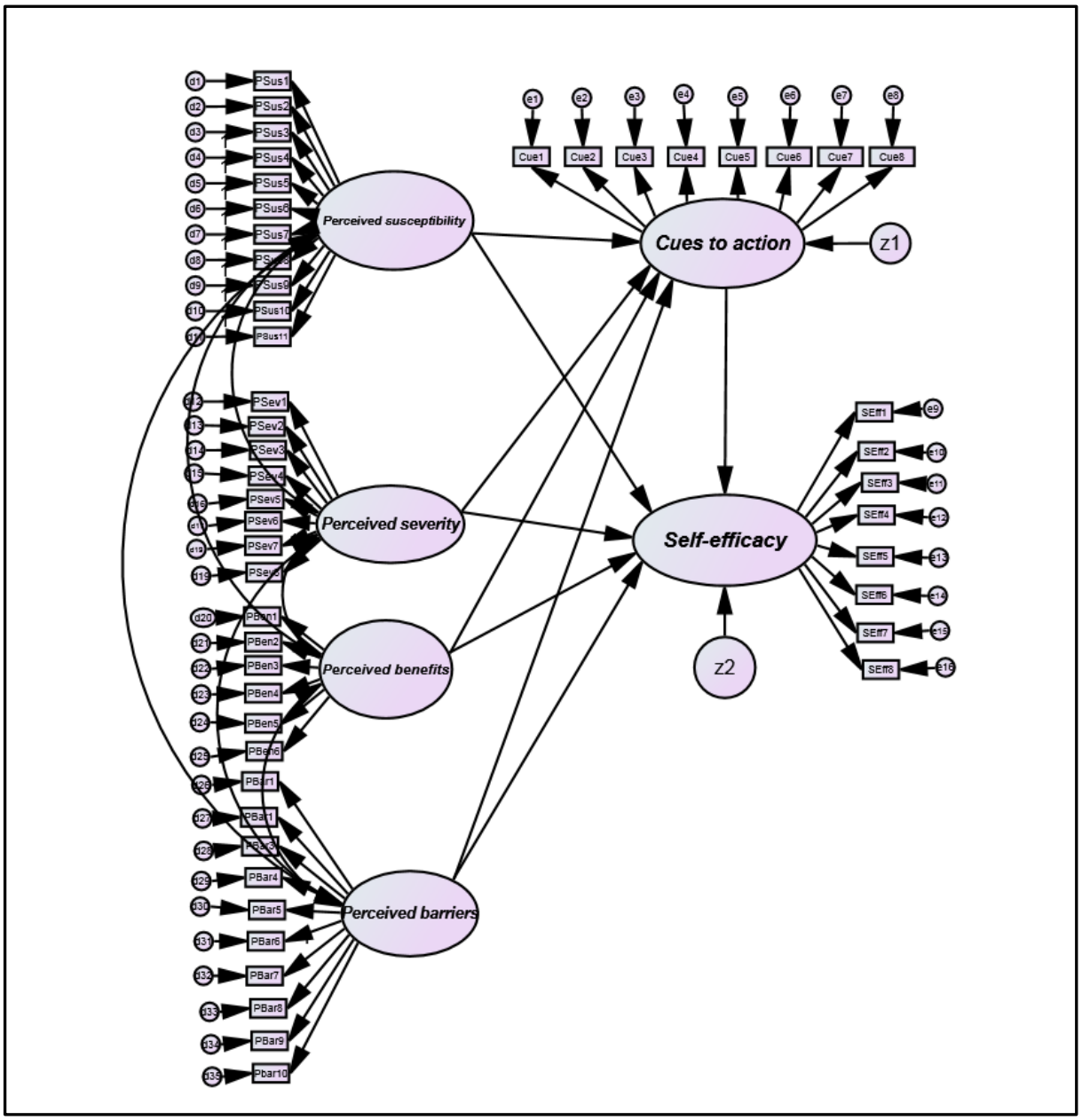
Appendix 4: Supplementary Figure 1: Conceptualization of a Structural Equation Model using the Health Belief Model framework to explore drivers influencing backyard chicken farmers' decision to implement Highly Pathogenic Avian Influenza control and prevention measures



Appendix 5: Supplementary Figure 2: Conceptualization of a Structural Equation Model using the Health Belief Model framework to explore drivers influencing commercial broiler farmers' decision to implement Highly Pathogenic Avian Influenza control and prevention measures



Appendix 6: Supplementary Figure 3: Conceptualization of a Structural Equation Model using the Health Belief Model framework to explore drivers influencing commercial layer farmers' decision to implement Highly Pathogenic Avian Influenza control and prevention measures



Appendix 7: Human Ethical approval by Behavioural & Social sciences Ethical review committee

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	Infection Dynamics for Newcastle Disease and Avian Influenza Virus Circulation Along the Poultry Market Chain in Bangladesh
Chief Investigator:	Mr Suman Das Gupta
Supervisor:	Dr Joerg Henning
Co-Investigator(s):	None
School(s):	Veterinary Science, UQ;
Approval Number:	2015001703
Granting Agency/Degree:	Research Biotechnology and Biological Sciences Research Council; Royal Veterinary College
Duration:	31st December 2019

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 9/11/2015

Appendix 8: Animal Ethical approval by Animal Welfare Unit, UQ Research and Innovation, The University of Queensland.

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

UQ Research and Innovation
Director, Research Management Office
Nicole Thompson

Animal Ethics Approval Certificate

02-Mar-2016

Please check all details below and inform the Animal Welfare Unit within 10 working days if anything is incorrect.

Activity Details

Chief Investigator: Dr Joerg Henning, Veterinary Science

Title: Infection dynamics for Newcastle & AI virus circulation along the poultry market chain in Bangladesh

AEC Approval Number: SVS/465/15/RVC

Previous AEC Number:

Approval Duration: 03-Mar-2016 to 03-Mar-2019

Funding Body:

Group: Production and Companion Animal

Other Staff/Students: Suman Gupta, Md. Billal Uddin, Ahasanul Hoque, Kazi Mohammed Rokan Uddin, Rashed Mahmud, Abdul Ahad

Location(s): Other International Location

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Poultry	Chicken	Adults	Unknown	Commercial breeding colony	1200	1200

Permits

Provisos

Overseas Proviso:
i) The CI is required to ensure that all documentation required overseas is obtained.
ii) That the CI withdraws from the project if the welfare of the animals falls below an equivalent acceptable standard to that which the Committee would normally approve.

Approval Details

Description	Amount	Balance
Poultry (Chicken, Unknown, Adults, Commercial breeding colony)		
17 Feb 2016 Initial approval	1200	1200

Animal Welfare Unit
UQ Research and Innovation
The University of Queensland

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+61 7 336 52713 (Coordinator)

animalwelfare@research.uq.edu.au
uq.edu.au/research

Please note the animal numbers supplied on this certificate are the total allocated for the approval duration

Please use this Approval Number:

1. When ordering animals from Animal Breeding Houses
2. For labelling of all animal cages or holding areas. In addition please include on the label, Chief Investigator's name and contact phone number.
3. When you need to communicate with this office about the project.

It is a condition of this approval that all project animal details be made available to Animal House OIC.
(UAEC Ruling 14/12/2001)

The Chief Investigator takes responsibility for ensuring all legislative, regulatory and compliance objectives are satisfied for this project.

This certificate supercedes all preceeding certificates for this project (i.e. those certificates dated before 02-Mar-2016)

Animal Welfare Unit
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Appendix 9: Questionnaire used to collect information on backyards farms

PART 1: INTERVIEWEE DETAILS AND FARM LOCATIONS

[Tick appropriate box(s) or write in the blank space/cell as appropriate]

Interview details	
1.Date of interview: _____/_____/2016	**2.Form ID[1]: ...../BQ/COX.....**

Details of the interviewee

3.Position of the interviewee in household(NB: Please select the person as interviewee who actually deal with poultry):
□ **1=** Farm owner □**2=** Son of the owner □3= Daughter of the owner □ **4=** Spouse of the owner
□ **5=**Other(specify).....

4.Name:

5.Age: .....Years.....Months

6.Gender: □ **1=** Male □ **2=** Female

7.Educational qualification:
□ **1=** Illiterate □**2=** Primary □**3=** Secondary □**4=** Higher Secondary □**5=** Tertiary (i.e. graduate & above)
□ **6=**Other (specify).....

8.Marital status: □ **1=**Single/Never married □**2=**Married □ **3=**Divorced/Separated □**4=**Widowed □**5=**Don't response

9.Religion: □ **1=**Muslim □**2=**Hindu □ **3=** Buddhist □**4=** Christian □**5=**Don't response

10. How long have you been in poultry farming?.....

11. Mobile number(at least on request number):

12. What is the source of income of the family? [***Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency of SOURCES in the blank spaces (highest frequency=1)***]

1=Poultry rearing	**2=**Livestock (cattle/goat/ sheep etc.) rearing	**3=**Agricultural crop production	**4=**Fishing	**5=**Family business (other than poultry, livestock, agricultural crop and fishing)	**6=**Daily labor	**7=**GO/ NGO Job	**8=**Other (specify)
□.....	□.....	□.....	□.....	□.....	□.....	□.....	□.....

Location details of farm

13.1 House/Bari/Para(if applicable):	
13.2 Village:	**13.3**Union:
13.4 Upazilla:	**13.5** District:
13.6 Latitude(N):	**13.7** Longitude (E):

[1]**Form ID: Serial no. (continuous)/BQ/village code e.g. 01/BQ/COX01**

PART 2: POULTRY FLOCK

14. What species, breed, ages, sex and number of poultry do you have TODAY?

Species	Breed	Age	Sex: M/F/DK(Don't Know)	Number
Chicken		.....weeks/months/years		
		.....weeks/months/years		
Duck		.....weeks/months/years		
		.....weeks/months/years		
Pigeon		.....weeks/months/years		
		.....weeks/months/years		
Goose		.....weeks/months/years		
		.....weeks/months/years		
Other(Specify).....		.....weeks/months/years		

15. Where did you get your poultry in the LAST 12 MONTHS? How many and how often did you get poultry from the source(s)? For source: *Please tick(✓) the appropriate box(s)* **'□'. For how many?:** *Put the number of poultry (if farmer could provide no.)* ***OR*** *RANK the frequency (if farmer couldn't provide no.) in the blank space* **'.....'***(highest frequency=1 for individual species).* **For how often?:** *Write(eg. one time/two times/three times etc.) within the bracket* **'[..... time(s)]'**

	1=Hatched in own farm	2=Local Market	3=Middlmen/bepari	4=Neighbour	5=Relatives	6=Others (specify)
Chicken	□..... [.....time(s)]	□..... [.....time(s)]	□..... [.....time(s)]	□..... [.....time(s)]	□.....[.... .....time(s)]	□.....[.. .....time(s)]
Duck	□..... [.....time(s)]	□..... [.....time(s)]	□..... [.....time(s)]	□..... [.....time(s)]	□.....[. .....time(s)]	□.....[.. .....time(s)]

If the source is '2=Local Market', please ask:
15.1 What is the name, address (only village name/location) and distance of local market/LBM where you get poultry?

Name of the LBM	Name of the village/location where market located	Distance between your HH & LBM
		..... Feet/Meter/Km

PART 3: HOUSING, FEEDING, WATERING AND OTHERS

16.Where did your poultry scavenge in the LAST 12 MONTHS? [***Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency in the blank spaces (highest frequency=1 for individual species)***]

	1=Household premises	2=Rice paddies	3=Rivers/wetlands/ ponds	4=Vegetable land	5= No scavenging	6=Others(specify)
Chicken	□.....	□.....	□.....	□.....	□.....	□.....
Duck	□.....	□.....	□.....	□.....	□.....	□.....

If answer is 'No Scavenging', go to Q 18
17. Do your poultry mix/come into contact with neighboring backyard waterfowls during scavenging?

[*Please tick(✓) the appropriate box*]

1=YES	2=NO	3=Don't Know
□	□	□

If answer is 'No', please ask
17.1 Why your poultry do not mix with other scavenging backyard waterfowls?.....

18. What is the estimated distance between your farm & nearest standing body of water (pond, lake, dam, river etc.)?

Type of the nearest standing body of water	Estimated distance between your farm & nearest standing body of water	If possible, coordinates of the body of water	
		Latitude (N)	Longitude (E)
□ Pond	.....……. Feet/Meter/Km		
□ Lake/River/Canal	.....…….Feet/Meter/Km		

19. What is the estimated distance between your farm and the nearest commercial poultry farm?

Estimated distance between your farm & nearest commercial poultry farm	Name of the location/village	If possible, coordinates of the nearest farms	
		Latitude (N)	Longitude (E)
...……. Feet/Meter/Km			
...……. Feet/Meter/Km			

20. Do you provide any house to the poultry? □ 1=YES □2= NO

If answer is 'NO', please ask following question:

20.1 Where do you keep your poultry at night?…….

If answer is 'YES', please ask following questions(20.2, 203, 20.4):

20.2 What kind of house do you have? [*Please tick(✓) the appropriate box(s)*] (observed by the interviewer, if possible please take picture**)**

1=Wooden	2=Bamboo	3= Muddy	4= Concrete	5=Metallic	6=Other (Specify)
□	□	□	□	□	□.....

20.3 Do you keep different species of poultry together in the same house? □ 1=YES □2= NO □3= NA

20.4 Is there any unwanted holes/openings in the house that can allow rats, snakes, wild animals etc.?
(observed by the interviewer, if possible please take picture**) □1= YES □2=NO**

20.5 Do you provide any litter in the house/space where poultry keep? □1=YES □2= NO

If answer is 'YES', please observe:

20.5.1 What kind of litter do they use? [*Please tick(✓) the appropriate box(s)*]

1=Rice husk	2=Saw dust	3=Wood shavings	4=Sand	5=Straw pieces	6=Ash	7=Other (Specify)
□	□	□	□	□	□	□.....

21. Do you provide NEST BOX for the poultry? □ 1=YES □2= NO
If answer is 'YES', please **OBSERVE** (if possible take picture):

21.1What kind of NEST BOX do they provide to poultry? [*Please tick(✓) the appropriate box(s)*]

1=Wooden	2=Bamboo	3=Concrete	4=Plastic	5=Metallic	6=Other (Specify)
☐	☐	☐	☐	☐	☐.....

21.2 What do they use in the NEST BOX? [*Please tick(✓) the appropriate box(s)*]

1=Ash	2=Straw	3=Rice husk	4=Saw dust	5= Wood shavings	6= Sand	7=Other(Specify)....
☐	☐	☐	☐	☐	☐	☐.....

22. Do you keep newly introduced poultry separate from other poultry in a safe and separate place/house before introducing into the existing poultry flock?

☐ 1= YES ☐ 2=NO ☐ 3= NA

If yes, please ask following questions:

22.1 Where do you keep?	**22.2** How many days do you keep separate?

23. What do you feed your poultry? [***Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency in the blank spaces (highest frequency=1 for individual species)***]

	1= Rice bran	2=Rice polish	3=Paddy	4=Whole rice	5=Cooked rice	6=Scraps from the family food	7=Commercial feed	8=Grain	9=Slaughter remnants of purchased chickens	10=Nothing other than what they find outside	11=Other (Specify)
Chicken	□.....	□.....	□.....	□.....	□.....	□.....	□.....	□.....	□.....	□.....	□....
Duck	□.....	□.....	□.....	□....	□.....	□.....	□.....	□.....	□.....	□.....	□...

If answer is **'10=Nothing other than.....outside'**, please **GO TO Q 26**

If answer is **'7=Commercial feed'**, please ask following question:

23.1 Where do you get your feed? [*Please tick(✓) the appropriate box(s)*]

1=Local market	2=Feed dealer	3=Other(please specify)
☐	☐	☐.....

24. How do you feed your poultry? [*Please tick(✓) the appropriate box(s)*]

1=Use separate feeder	2=Use same trough for both feeding and watering	3=Scattered on the yard	4=Other(please specify)
☐	☐	☐	☐.....

25. Do you feed different species of poultry in a same feeder/trough/space?

1=YES	2=NO	3=NA (if rear only one species)
☐	☐	☐

26. Do you provide water to the poultry?
☐ 1=YES ☐2=NO

If answer is **'NO'**, please **GO TO Q 27**

26.1 How do you provide water to poultry? [*Please tick(✓) the appropriate box(s)*]

1=Use separate waterer	2=Use same trough for both watering and feeding	3=Other(please specify)
☐	☐	☐.....

26.2 What is the source of water? [*Please tick(✓) the appropriate box(s)*]

1= Tube-well	2= Deep tube-well	3= Pond	4= River/lake	5= Supply water by govt.authority	6=Other (Specify)
☐	☐	☐	☐	☐	☐.....

26.3 Do you provide water to different species of poultry in a same WATERER/trough?

1=YES	2=NO	3=NA (if rear only one species/don't have waterer or trough)
☐	☐	☐

PART 4: FAMILY, VISITORS AND THEIR MOVEMENT

27. Please fill-in the following table:

27.1 Who take care(feeding, watering, cleaning etc) of the poultry?	**27.2** What type of care the person usually takes?	**27.3** Did the person receive any training on poultry?
☐ **Interviewee**	☐Feeding ☐ Watering ☐ Cleaning of poultry house/equipment ☐ Other(specify).....	☐ Yes ☐ No
☐ **Husband/Wife** ☐ **Daughter** ☐ **Son** ☐ **Other (specify)...**	☐Feeding ☐ Watering ☐ Cleaning of poultry house/equipment ☐ Other(specify).....	☐ Yes ☐ No

28. Do you or your family members WORK at commercial poultry farms?

☐ **1=YES** ☐**2=NO**

If answer is **'YES'**, please ask following questions:

28.1 Name & address (only village name/location) of the farm(s) where you/they work	**28.2** Type of work you/they do on that farm	**28.3** How Frequently you/they go to the farm?	**28.4** When visited last time to the farm?
			..... days/weeks ago

29. Did you or your family members VISIT commercial poultry farms within the last 12 months for which they do not work ?

☐ **1=YES** ☐ **2=NO**

If answer is **'YES'**, please ask following questions:

29.1 Purpose	**29.2** How Frequently?	**29.3** When visited last time to the farm?
		..... days/weeks/months ago

30. Did you/family members VISIT homes of relatives/friends within the last 12 months who own poultry farms?

☐ **1=YES** ☐**2= NO**

If answer is **'YES'**, please ask following questions:

30.1 How Frequently?	**30.2** When visited last time?
	..... days/weeks/months ago

31. Stakeholders (other than trader/collector/bepari/middlemen) movement or access to the HH/farm: Please fill-in the following table:

31.1 Which type of stakeholders visit your HH/farm?	**31.2** How frequent they visit your HH/farm?	**31.3** When they visited last time to your HH/ farm?	**31.4** Do you allow them within less than 1 meter of the poultry house area?
□ Poultry vaccinator		..... days/weeks/months ago	□ YES □ NO
□ Veterinarian		..... days/weeks/months ago	□ YES □ NO
□ Village quack		..... days/weeks/months ago	□ YES □ NO
□ Paravet/Vet.Field Assistant(VFA)		..... days/weeks/months ago	□ YES □ NO
□ Community Workers (NGO)		..... days/weeks/months ago	□ YES □ NO
□ Others(specify).....		..... days/weeks/months ago	□ YES □ NO

PART 5: CLEANING AND DISINFECTION PRACTICE

32. Please fill-in the following table as appropriate:

	POULTRY HOUSE	**FEEDER**	**WATERER**	**NEST BOX**
32.1 Do you clean & disinfect...?	□**1**=Clean only □**2**=Disinfect only □3=Both clean & disinfect □**4**=Don't clean & disinfect □**5**=NA □**6**=Others(specify)…	□**1**=Clean only □**2**=Disinfect only □3=Both clean & disinfect □**4**=Don't clean & disinfect □**5**=NA □**6**=Others(specify)……	□**1**=Clean only □**2**=Disinfect only □3=Both clean & disinfect □**4**=Don't clean & disinfect □**5**=NA □**6**=Others(specify)…..	□**1**=Clean only □**2**=Disinfect only □3=Both clean & disinfect □**4**=Don't clean & disinfect □**5**=NA □**6**=Others(specify)……
32.2 How frequently do you clean...?	□**1**=Daily □**2**=Once a week □3=Twice a week □**4**=Once a month □**5**=NA □**6**=Others(specify)……	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……
32.3 How frequently do you disinfect..?	□**1**=Daily □**2**=Once a week □3=Twice a week □**4**=Once a month □**5**=NA □**6**=Others(specify)…	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……	□**1**=Every time after use □**2**=Once a day □**3**=Once a week □**4**=Once a month □**5**=NA □**6**= Others(specify)……
32.4 How & what do you use for cleaning?				
32.5 How & what doyou use for disinf.?				

33. Do you or your family member wash hands and feet with water before handling poultry/poultry house/equipment?

1=Always	2=Often	3=Sometimes	4=Never
☐	☐	☐	☐

If answer is '**Never**',please **GO TO Q 34:**

33.1 Do you or your family member use soap to wash hands and feet?

1=Always	2=Often	3=Sometimes	4=Never
☐	☐	☐	☐

PART 6: POULTRY DROPPINGS/WASTE/LITTER AND DEAD BIRDS MANAGEMENT

34. How frequently do you change or clean the litter/ droppings/waste?

1= Once a day	2= Once a week	3= Twice a week	4= Once a month	5= Not at all	6= Other (specify)
☐	☐	☐	☐	☐	☐.....

35. How do you dispose litter/droppings/waste? [*Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency in the blank spaces (highest frequency=1*]

1=Spread on your fields	2=Compost it & then spread it on your fields	3=Bury them	4=Burn them	5=Throw in the nearby pond	6=Throw in the nearby river/lake/canal	7=Left in the yard	8=Throw on nearby bushes/jungle	9=Throw on roadside	10=Other (specify)
☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....

36. How do you dispose dead birds? [*Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency in the blank spaces (highest frequency=1*]

1=Bury them	2= Feed to dogs/fox/other animals	3=Throw in the nearby pond	4=Throw in the nearby lake/river/canal	5=Throw on nearby bushes/jungle	6=Throw on roadside	7=Burn them	8=Other (specify)
☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....	☐.....

PART7: PEST MANAGEMENT AND CONTROL OF WILD BIRDS & OTHER ANIMALS

37. Do you have rodents in your household?
☐ 1=**Yes** ☐ **2=No**

If answer is **'YES'**, please ask following question:

37.1 How do you control rodent? ……

38. Do they allow trash and junk to pile up around the poultry house? (Observed by the interviewer)

☐ 1=**Yes** ☐ **2=No** ☐ **3=NA**

39. Do they trim grass and weeds around poultry house regularly? (Observed by the interviewer)

☐ 1=**Yes** ☐ **2= No** ☐ **3=NA**

40. How do you keep feed/feed ingredients? (Observed by the interviewer)

1= Keep in closed container	2=Keep open	3=Other, please specify
☐	☐	☐.....

41. Can stray dogs and cats enter into your household area? ☐ 1=Yes ☐ 2=No

If answer is **'YES'**, please ask following question:

41.1 How do you control stray dogs and cats?……....

42. Do you rear wild birds within the household/farm premises? ☐ 1=YES ☐2=NO

If answer is **'YES'**, please ask following questions:

42.1 What species and number?		**42.2** Do wild birds **that you rear** come in contact with the poultry?	**42.3** Do you **share** the same **feeder** use for wild birds with poultry?	**42.4** Do you **share** the same **waterer** use for wild birds with poultry?
Species	No.			
		□ YES □ NO	**□ YES □ NO**	**□ YES □ NO**

43. Information on mixing or contact of migratory & non-migratory wild birds(which aren't reared) with domestic poultry: Please ask following questions

43.1 Do your poultry mix/come into contact with migratory and/or non-migratory wild birds in the last 12 months? [*Please tick(✓) the appropriate box(s)*]

1=Mix/contact with migratory wild birds	2= Mix/contact with non-migratory wild birds	3=Don't mix/contact with either migratory or non-migratory wild birds	4=Don't know
□	□	□	□

43.2 Do you limit contacts between domestic and wild birds? □ 1=YES □2=NO

43.2.1 If YES, how?.....

PART 8: HEALTH HISTORY, VACCINATION

44. What kind of problem(s)/constraint(s) do you have with management of the poultry? [Please tick the appropriate box(s) and/or **RANK** (if answere is multiple) the frequency in the blank spaces (highest frequency=1)**]**

1=Disease	2=Predators	3=High price of feed	4=Shortage of quality vaccine	5=Irregular/insufficient Govt. vaccination campaign	6=High price of vaccine	7=Marketing of the farm products	8=Others (specify)
□.....	□.....	□.....	□.....	□.....	□.....	□.....	□.....

45. Morbidity and mortality details: please fill-in the following table

Species	Describe diseases (if confirmed) or clinical signs/symptoms you have seen in your poultry during the **LAST 12 MONTHS**?	How many birds were sick during the **LAST 12 MONTHS**?	How many birds were died during the **LAST 12 MONTHS**?
Chicken			
Duck			

46. Was there any specific season/month in the LAST 12 MONTHS when poultry died more?
□ 1=YES □2=NO

If answer is **'YES'**, please ask following questions:

46.1 Which season/month poultry died more?

Name of the season	Name of the month

47. Please tick the appropriate boxes and RANK the frequency for individual column(47.1, 47.2, 47.3). For each column highest frequency=1.

	When your farm affected by disease, what do you do with the.....?		**47.3** When your birds are not sick, but some birds around your farm/within the village are sick; what do you do WITH YOUR BIRDS
	47.1 SICK BIRDS	**47.2 BIRDS WHICH ARE NOT SICK YET**	
Sell to the local market	□.....	□.....	□.....
Slaughter and eat	□.....	□.....	□.....
Go to the local DLS office with sick poultry	□.....	□.....	□.....
Buy medicine from local vet. pharmacy & treat poultry	□.....	□.....	□.....
Contact with village quack	□.....	□.....	□.....
Do nothing	□.....	□.....	□.....
Others(specify).....	□.....	□.....	□.....

48. Had there been any unusual death (sudden increased number of bird mortality within a short period of time) of birds around your farms/within the village in the LAST 12 MONTHS? □ 1=YES □2=NO

48.1 Have you heard about....?

Avian Influenza/Bird Flu	**□ YES □ NO**
Disease that cause high number of bird mortality	**□ YES □ NO**

49. Do you vaccinate your poultry? □ 1=YES □2=NO

If answer is 'YES,

49.1 Please fill-in the following table:

Species	Against what diseases birds in the present flock have already been vaccinated in the last 12 months?	At what age?	Route of administration	Name of the vaccine	Source of the vaccine	Next vaccination plan		
						Against what diseases	At what age	Name of the vaccine (if known)
Chicken								
Duck								

49.2 Who vaccinate your birds?

1= Private poultry vaccinator	2= Government poultry vaccinator	3= Village quack	4= Others(specify)
□	□	□	□.....

49.3 How do you dispose of vaccine vial/bottle, used needle & syringes etc. after vaccination? [*Please tick(✓) the appropriate box(s) and/or RANK(if answer is multiple) the frequency in the blank spaces (highest frequency=1*]

1=Burn in a pit or above ground of HH premises	2=Bury at HH premises	3=Throw on the ground of HH premises	4=Throw on roadside	5=Throw in the river	6=Other (specify)
□.....	□.....	□.....	□.....	□.....	□.....

PART 9: PRODUCTION AND MARKETING

50. Information on production: Please ask following questions in the table and fill-in the blank cells(Note: A batch = a group of eggs produced in the laying period of a hen)

	Chicken				Duck		
	Ave.	**Min.**	**Max.**		**Ave**	**Min.**	**Max.**
50.1 How many batches are produced by EACH hen per year?							
50.2 How many eggs does A hen produce in a batch?							
50.3 How many eggs are set under EACH hen to be hatched?							
50.4 How many chicks/ducklings are hatched PER BATCH?							

51. Of these …….....(write the AVERAGE number from the question **50.4) AVERAGE number of chicks/ducklings hatched PER BATCH, specify the total number of losses and the cause-specific number of losses per age group:**

	Chicken				Duck		
	Chicks (< 2months)	Growers (2-5months)	Adults (> 5 months)		Ducklings (< 2 months)	Growers (2-5 months)	Adults (> 5 months)
TOTAL LOSSES							
Diseases							
Predators							
Theft							
Exposure to climate (rain/wind/heat/cold)							
Unknown							
Other (specify) …….							

52. Do you sell EGGS? □ 1=YES □2=NO
If answer is 'YES',

52.1 Please ask following questions:

	52.1.1 How many times did you sale eggs within the LAST 12 MONTHS?	**52.1.2** Number of eggs sold per sale		
		Average	Min	Max
Chicken				
Duck				

52.2 Where did you sell your eggs in the LAST 12 MONTHS? [Please tick the appropriate box(s) and/or **RANK** (if answere is multiple) the frequency in the blank spaces (highest frequency=1)**]**

	1= At local LBM	2=To trader(s) /local collector/bepari visiting village/HH	3= To traders/local collector/bepari on the roadside/elsewhere	4=To neighbor	5=Other, specify
Chicken	□.....	□.....	□.....	□.....	□.....
Duck	□.....	□.....	□.....	□.....	□.....

If EGGS sell to traders/local collector/bepari visiting the village, please ask following questions(52.2.1-52.2.6):

52.2.1 How do you deal with the traders/local collector/bepari?

1=Call them to let know that you have eggs to sell	2=They come regularly to see whether eggs are offered for sale	3=Other(specify)
□	□	□.....

52.2.2 How often a trader/local collector/bepari comes to your farm?

1=Once a month	2=Twice a month	3=Thrice a month	4=Other(specify)
□	□	□	□.....

52.2.3 HOW MANY different traders/local collector/bepari visit your HH/farm in average <u>PER MONTH</u>?

52.2.4 Do you work with:

1=Always the same traders/local collector/bepari	2=Mostly the same but sometimes new or different traders/local collector/bepari	3=Neverthe same traders/local collector/bepari
□	□	□

52.2.5 When trader/local collector/bepari visited your HH last time?

… …..days/weeks/months ago

52.2.6 Do you allow trader/bepari/local collector/middlemen within less than 1 meter of the poultry house area?

□ 1=YES □ 2= NO

<u>If EGGS sell at LBM, please ask following questions (52.2.7-52.2.10):</u>

52.2.7 What is the name, address(only village name/location) and distance of LBM where you sell eggs?

Name of the LBM	Name of the village/location where market located	Distance between your HH & LBM
		..... Feet/Meter/Km

52.2.8 How frequently do you go to LBM for selling eggs?

1=Once a week	2=Once a month	3= Twice a month	4= Thrice a month	5=Other(specify)
□	□	□	□	□.....

52.2.9 When you visited LBM last time for selling eggs?

……. …..days/weeks/months ago

52.2.10 How many eggs do you sell when go to LBM?

Average	Min.	Max.
.....eggs sold/visit	*.....eggs sold/visit*	*.....eggs sold/visit*

53. Do you sell your POULTRY? □ 1=YES □ 2=NO

<u>If answer is 'YES',</u>

53.1 Please ask following questions:

	53.1.1 At what age do you usually sell poultry?			**53.1.2** How many times did you sell poultry in the LAST 12 MONTHS?	**53.1.3** Number of birds sold per sale		
	Male	Female			Average	Min.	Max.
		Grower/Adult	Spent Hen/Duck				
Chicken	.....weeks/months	.....weeks/months	.....months				
Duck	.....weeks/months	.....weeks/months	.....months				

53.2 Was there any specific season or festival within the <u>LAST 12 MONTHS</u> when you sold more POULTRY?

□ 1=YES □2=NO

53.2.1 If YES, please fill in the table below:

	SEASON					**FESTIVAL**			
	Name of the season	Month	How many times did you sell during that season?	Average no. of poultry sold per sell		Name of the festival	Month	How many times did you sell during that festival?	Average no. of birds sold per sell
Chicken									
Duck									

53.3 Where did you sell poultry in the <u>LAST 12 MONTHS</u>? *[Please tick the appropriate box(s) and/or RANK (if answere is multiple) the frequency in the blank spaces (highest frequency=1 for individual species)]*

	1=At local LBM	2=To trader(s) /local collector/bepari visiting the village	3=Totraders/localcollector/bepari on the roadside/elsewhere	3=To neighbor	4=Other, specify
Chicken	**□.....**	**□.....**	**□.....**	**□.....**	**□.....**
Duck	**□.....**	**□.....**	**□.....**	**□.....**	**□.....**

<u>If POULTRY sell to traders/local collector/bepari VISITING THE VILLAGE, please ask following questions(53.3.1-53.3.6):</u>

53.3.1 How do you deal with the traders/local collector/bepari?

1=Call them to let know that you have poultry to sell	2=They come regularly to see whether birds are offered for sale	3=Other(specify)
□	□	**□.....**

53.3.2 How often a trader/local collector/bepari comes to your HH/farm?

1=Once a month	2=Twice a month	3=Thrice a month	4=Other(specify)
□	□	□	**□.....**

53.3.3 HOW MANY different traders/local collector/bepari visit your HH/farm in average <u>PER MONTH?</u>

53.3.4 Do you work with:

1=Always the same traders/local collector/bepari	2=Mostly the same but sometimes new or different traders/local collector/bepari	3=Neverthe same traders/local collector/bepari
□	□	□

53.3.5 When trader/local collector/bepari visited your farm last time?

… …..days/weeks/months ago

53.3.6 Do you allow trader/bepari/local collector/middlemen within less than 1 meter of the poultry house area?

□ 1=YES □ 2= NO

<u>If POULTRY sell at LBM, please ask following questions(53.3.7-53.3.13):</u>

53.3.7 What is the name, address(only village name/location) and distance of LBM where you sell poultry?

Name of the LBM	Name of the village/location where market located	Distance between your HH & LBM
		..... Feet/Meter/Km

53.3.8 How frequently do you go to LBM for selling poultry?

1=Once a month	2=Twice a month	3= Every after two months	4= Every after three months	5=Other(specify)
□	□	□	□	□.....

53.3.9 When you visited LBM last time for selling poultry?

……. …..days/weeks/months ago

53.3.10 How many poultry do you sell when go to LBM?

Average	Min.	Max.
.....birds sold/visit	*.....birds sold/visit*	*.....birds sold/visit*

53.3.11 How often do you have unsold POULTRY in the last 12 months?.....

53.3.12 Doyou or your family bring back any unsold POULTRY from a market? □ 1=YES □2=NO □3=NA

53.3.12.1 <u>If YES,</u> What do you do with the unsold POULTRY after bringing them back from market? [Tick the appropriate box(s) and/or RANK (if answer is multiple) the frequency in the blank spaces]

1= Put together with other poultry in the same house/coop	2= Keep separate before mixing with other poultry	3= Slaughter and eat	4= Give it to friend	5=Other(specify)
□.....	□.....	□.....	□.....	□.....

53.3.13 Do you bring back any baskets, crates or other equipment back to your poultry houses/coop after trading at live bird market?

□ 1=YES 2=NO

53.3.13.1 <u>If YES,</u> What do you do with the baskets, crates or other equipment?

1= Clean before using again	2= Clean & disinfect before using again	3= Don't clean & disinfect	4=Other(specify)
□	□	□	□.....

<u>If answer is '3=Don't clean & disinfect, please **GO TO Q 54**</u>

53.3.13.1.1 If answer is 1/2/4, please fill-in the following table as appropriate:

How and what do you use for cleaning?	How and what do you use for disinfection?

54. Do you or your family membersVISIT live bird markets for other purpose? □ 1=YES □2=NO

54.1 <u>If YES,</u> please fill-in the following table:

Purpose to visit	How Frequently?	When visited last time?
		.....days/weeks/months ago

55. Do you or your family members buy poultry from LBM for consumption? □ 1= YES □ 2= NO

55.1 <u>If YES,</u> please ask, Where do you slaughter and process it?

1=Only slaughter at LBM	2=Both slaughter & process at LBM	3=Process at home	4=Both slaughter & process at home	5=Other(specify)
□	□	□	□	□.....

56. Do you or family slaughter & process POULTRY (that you rear) at home for consumption?

□ 1=YES □2=NO

56.1 <u>If YES,</u> please fill-in the following table:

Species	Breed	Age at which birds are slaughtered		
		Male	Female	
			Grower/Adult	Spent Hen/Duck
Chicken		*.....weeks/months*	*.....weeks/months*	*.....months*
Duck		*.....weeks/months*	*.....weeks/months*	*.....months*

56.2 How many poultry(that you rear) did your family consume in the LAST 12 MONTHS?

	Average
Chicken	
Duck	

57.Was there any specific season or festival in the LAST 12 MONTHS when your family CONSUMED more POULTRY?
1=□ YES 2=□ NO

57.1 <u>If YES,</u> please fill-in the following table:

	SPECIFIC SEASON				SPECIFIC FESTIVAL		
	Name of the season	Month	Average no. of poultry consumed/month during that specific season		Name of the festival	Month	Average no. of poultry consumed/month during that specific festival
Chicken							
Duck							

58. Please fill-in the following table considering period of LAST 12 MONTHS. [Tick the appropriate box(s) and/or **RANK** (if answer is multiple) the frequency in the blank spaces]

Species	EGGS (Rank the frequency, highest frequency=1)						POULTRY (Rank the frequency, highest frequency=1)			
	1=Consume	2=Own reproduction	3= Sale	4=Give to neighbor/ relatives	5=Other, specify...		1=Consume	2=Sale	3=Give to neighbor/ relatives	4=Other, specify
Chicken	□.....	□.....	□.....	□.....	□.....		□.....	□.....	□.....	□.....
Duck	□.....	□.....	□.....	□.....	□.....		□.....	□.....	□.....	□.....

Name of the interviewer:.....

Appendix 10: Questionnaire used to collect information on village

1.Village address:

Name of the village:	Union:	Upazilla:	District:

PART 1: OBSERVATION (Q2-Q15)

[When necessary, please also cross-check during interview with headman/key informants**]**

2. Distribution of HH within the village: [*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

Colony type (>1 HH grouped together)	Isolated	Other(specify
☐	☐	☐ **.....**

3. The structure of the HHs is made of:[*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

Mud	Bamboo	Bricks, cement, concrete etc	Tin	Other(specify
☐	☐	☐	☐	☐ **.....**

4. Agricultural enterprises prevail within the village: [*Tick the appropriate boxes and* RANK *the frequency in the blank space (highest frequency=1)***]**

Cattle/buffalo/goat/ sheep rearing	Backyard poultry rearing	Commercial poultry rearing	Agricultural crop production	Fishing	Other(specify)
☐**....**	☐**....**	☐**....**	☐**....**	☐**...**	☐ .....

5. Agricultural crop land/production prevails within the village: [*Tick the appropriate boxes and* RANK *the frequency in the blank space(highest frequency=1)***]**

Rice	Wheat	Betel leaf	Sugarcane	Vegetables	Other(specify)
☐**....**	☐**....**	☐**...**	☐**...**	☐**...**	☐ .....

6. Kinds of available wetland/water body prevail within the village: [*Tick the appropriate box(s) and collect GPS coordinates***]**

□ Pond			□Canal/River/Lake			□No wetland/water body	□Other (specify)..... ..	
How many ponds?	**N**	**E**	How many canals/rivers/lakes?	**N**	**E**		**N**	**E**

7. Presence of any forest/hill/jungle in the village: □ YES □ NO

8. Presence of commercial poultry farm in the village: □ YES □ NO
8.1 If YES, Type: □ Layer □ Broiler □ Sonali/Fayoumi □ Other (specify).....

9. Separation of one HH/colony from the other HH/colony: [*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

By crops field	By lake/river/pond etc.	By forest/hill/jungle	By road	Other(specify
□	□	□	□	□ .....

10. Kind of road passed through the village? [*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

Kacha/Muddy	Pacca/Concrete,brick etc	Other(specify)
□	□	□

11. Estimated distance between the village and highway road: ..... Feet/Meter/km

12. Kind of transports people usually use to move from one village to another village: [*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

By rickshaw/rickshaw van	By motorized vehicle	CNG/taxi	By boat	Other(specify)
☐	☐	☐	☐	☐

13. Presence of any kind of following stoppage in the village: [*Tick the appropriate box(s) and/or* RANK *the frequency in the blank space (highest frequency=1)***]**

Bus	Train	CNG/Taxi	Absence of any kind of stoppage	Other(specify)
☐	☐	☐	☐	☐**.....**

14. Presence of any garbage dumping place in the village where people dispose wastage:
☐ YES ☐ NO

If YES, **14.1 Crow abundance around the garbage dumping place: ☐ YES ☐ NO**

15. How frequent crows observe in the village?

Always	Often	Sometimes	Never
☐	☐	☐	☐

PART 2: INFORMATION TO BE COLLECTED FROM VILLAGE HEADMAN/ KEY INFORMANTS (Q16-Q26)

16. What is the family size of the HH?	Average:	Min.:	Max.:

17. What is the educational background of the village people? [*Tick the appropriate boxes and* RANK *the frequency in the blank space (highest frequency=1)***]**

1= Illiterate	**2=** Primary	**3=** Secondary	**4=** Higher Secondary	**5=** Tertiary (i.e. graduate & above)	**6=**Other(specify)
☐**.....**	☐**.....**	☐**.....**	☐**.....**	☐**.....**	☐**.....**

18. Which ethnic group is more common within the village? [*Tick the appropriate box(s)***]**

☐ **1=**Muslim ☐ **2=**Hindu ☐ **3=** Buddhist ☐ **4=** Christian ☐ **5=** Other (specify)..... ☐ **6=** Don't response

19. Information on HHs with or without backyard poultry:

19.1 How many HHs have in the village?	**19.2** How many HHs have backyard poultry?	**19.3** How many HHs have both chicken & duck?	**19.4** How many HHs have chicken only?	**19.5** How many HHs have duck only?

20. Which species of backyard poultry are reared by HHs? [*Tick the appropriate box(x) and* **RANK** *the frequency in the blank space (highest frequency=1)***]**

Chicken	Duck	Pigeon	Goose	Other(specify)
☐	☐	☐	☐	☐.....

21. How many backyard poultry are reared by EACH HH?

	Average	Min.	Max.
Chicken			
Duck			

22. Was there any poultry disease outbreak within the village in the last 12 months? ☐ YES ☐ NO
If YES, please ask

22.1 How long ago?	.....days/weeks/months ago	
22.2 What type of farm affected?	☐ Commercial ☐ Backyard	
22.3 Do you know which disease outbreak happened?	☐ **YES**, if yes please ask name of the disease:	☐ **NO**, if no please ask what clinical signs/abnormality observed: .....

23. Was there any village vaccination campaign on Ranikhet/ND in the last 12 months within the village?
☐ **YES** ☐ **NO** If YES, please ask

23.1 How long ago?.....days/weeks/months ago

23.2 Who organized the campaign? ☐ DLS ☐ NGO ☐ Other (specify).....

24. Is there any village market within the village where trading of poultry takes place?
□ YES □ NO
If YES, please ask,

24.1 Name of the market (If possible take GPS coordinates**).....N:.....E:.....**

24.2 Business day of the market:

Daily	Once a week	Twice a week	Other (please specify)
□	□	□	□

25. What is the name and estimated distance of nearest LBM (outside the village) where villagers go to buy/sell poultry?

Name of the LBM:	Estimated distance(If possible take GPS coordinates): .....m/km N: E:

26. Do migratory wild birds visit village? □ YES □ NO

If YES, **25.1 Are they mix with domestic poultry?**

YES	No	Don't know
□	□	□

GPS COORDINATES, PICTURE AND LAYPOUT MAP OF THE VILLAGE

- Take GPS coordinates(at least 4) at different points on the edges of the village:

Latitude(N)	Longitude(E)

- Take pictures of the village
- Sketch a simple map of the layout of the village(indicating **main roads**, **river/wetland**, **HH density**, **place where wild bird come**, **market where trading of poultry take place**, **any stoppage**, **any commercial poultry farm**, **type of agricultural land**)

Name of the village headman/key informant:.....

Designation:.....

Mobile No:.....

Name of the interviewer:.....

Appendix 11: Health Belief Model (HBM) Questionnaire

Backyard Poultry Farmers Health Belief Model (HBM) Questionnaire													
P E R C E I V E D S U S C E P T I B I L I T Y													
A = Strongly disagree	**B = Disagree**	**C = Agree**	**D = Strongly agree**	**E = Neither agree nor disagree**						**F=Don't Know**			
My chickens have an increased risk of getting avian influenza/bird flu													
When I rear different species of poultry together	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When I keep chickens and ducks in the same house	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When I don't regularly clean poultry house/equipment	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When my chickens mix with neighbour sick poultry during scavenging	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When my chickens mix with wild birds	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When my family members or I bring back unsold poultry from LBM & put together with other poultry	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
I am at increased risk of getting avian influenza/bird flu													
Because of my poultry rearing	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When I handle sick poultry	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When I don't cover my mouth and nose with cloths during handling poultry	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
When I don't wash my hands with soap water after handling poultry	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
My family members are at increased risk of getting avian influenza/bird flu													
Because of my poultry rearing	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
Uncooked poultry meat doesn't pose risk for getting avian influenza/bird flu	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
P E R C E I V E D S E V E R I T Y													
A = Strongly disagree	**B = Disagree**	**C = Agree**	**D = Strongly agree**	**E = Neither agree nor disagree**						**F=Don't Know**			
If my chickens get sick from avian influenza/bird flu													
Then the illness would be very bad, and the chickens will most likely die	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
Then avian influenza/bird flu could be passed to other poultry in my locality	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
Then avian influenza/bird flu could be passed on to me	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If my chickens get sick and die from avian influenza/bird flu													
Then I will lose income and family consumption	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If I get sick from avian influenza/bird flu													
Then other members in my home will get sick	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
Then I will die	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If my family members get sick from avian influenza/bird flu													
Then they will die	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
Chickens that catch avian influenza/bird flu cannot be treated	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
P E R C E I V E D B E N E F I T S													
A = Strongly disagree	**B = Disagree**	**C = Agree**	**D = Strongly agree**	**E = Neither agree nor disagree**						**F=Don't Know**			
My chickens will not get sick from avian influenza/bird flu													
If I don't rear different species of poultry together	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If I don't keep chickens and ducks together in same house	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If I regularly clean poultry house/equipment	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If my family members or I don't bring unsold poultry from LBM/don't put unsold poultry with other poultry after bring back from LBM	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	
If my chickens will not get sick from avian influenza/bird flu													
I will not lose income and family consumption	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐	

The possibility of disease outbreaks in my locality will reduce	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
My family members and I will not get sick from avian influenza/bird flu	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
P E R C E I V E D B A R R I E R S																								
A = Strongly disagree	**B = Disagree**		**C = Agree**		**D = Strongly agree**		**E = Neither agree nor disagree**						**F=Don't Know**											
Construction of separate house to keep chickens and ducks separately is expensive and required more spaces which I don't have	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
It's not worth to protect my chickens from avian influenza/bird flu, because I don't earn sufficient money from rearing chickens	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Regular cleaning of poultry house/equipment is time consuming and not practical for me, because my family/I have to do many other things	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Washing hands before and after handling poultry is not practical for me, because my family/I have to do many other things	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
I can't cover my mouth and nose with cloths during handling chickens, because they are not conducive for work	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
I don't cover my mouth and nose with cloths during handling chickens, because my neighbour do not	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
C U E T O A C T I O N																								
A = Strongly disagree	**B = Disagree**		**C = Agree**		**D = Strongly agree**		**E = Neither agree nor disagree**						**F=Don't Know**											
I would receive training regarding avian influenza/bird flu prevention & control and other aspects of poultry rearing, if DLS or any other organization would provide it	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
If I																								
Find a program on TV about avian influenza/bird flu and other aspects of poultry rearing, then I would watch it	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Find a program on the radio about avian influenza/bird flu and other aspects of poultry rearing, then I would listen to it	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Get invited to a meeting or campaign, etc. about avian influenza/bird flu and other aspects of poultry rearing, then I would attend it	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
It is a good idea for me to talk																								
With local livestock related personnel about risks of avian influenza/bird flu disease transmission between chickens	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
With community health workers or nearby hospital doctor about risks of disease transmission between chickens and humans	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
S E L F - E F F I C A C Y																								
A = Strongly disagree	**B = Disagree**		**C = Agree**		**D = Strongly agree**		**E = Neither agree nor disagree**						**F=Don't Know**											
It is a good idea																								
To invest in separate houses for chicken and duck	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
To clean poultry house/equipment regularly	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
I would be able to identify signs of the disease, if my chickens were infected with avian influenza/bird flu	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
I will inform the local livestock related personnel, when I suspect that my chickens have avian influenza/bird flu	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
I could																								
Dispose dead birds properly(bury them)	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Cover my mouth and nose with cloths during handling poultry, even if my neighbours are not	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐
Wash my hands with soap before and after handling poultry, even if my neighbours are not	A	☐	B	☐	C	☐	D	☐	E	☐	F	☐