Characteristics of Deshi chicken and Duck Trading Patterns and their Impact on Zoonotic Disease Transmission

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This is to certify that we have examined the above Master's thesis and have found that is satisfactory in all respects for evaluation

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Dedication

I dedicate this MS thesis to my beloved Late Father, Mother and the BALZAC Project

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List of Abbreviations

Abbreviation	Elaboration
AI	Avian Influenza
AIV	Avian Influenza Virus
BALZAC	Behavioural Adaptations in Live Poultry Trading and Farming Systems and Zoonosis Control in Bangladesh
CCC	Chittagong City Corporation
CDA	Chittagong Development Authority
CVASU	Chittagong Veterinary and Animal Sciences University
DLS	Department of Livestock Services
GDP	Gross Domestic Product
FAO	Food and Agriculture Organization of the United Nations
HPAI	Highly Pathogenic Avian Influenza
LBM	Live Bird Market
LPAI	Low Pathogenic Avian Influenza
M-gene	Matrix gene
MM	Middleman
OIE	Office International des Epizooties (World Organization for Animal Health)
PB	Pahartali Bazar
RB	Reazuddin Bazar
RVC	Royal Veterinary College
rRT-PCR	Real time Reverse Transcriptase Polymerase Chain Reaction
SH	Stallholder
WS	Wholesaler
UK	United Kingdom
VTM	Viral Transport Medium
WHO	World Health Organization
%	Percentage

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Abstract

Live bird markets (LBMs) may function as hubs for the dissemination of pathogens and thus a potential source of avian influenza. Under a cross-sectional study 22 stallholders and 40 middlemen were selected and interviewed from two wholesale LBMs in Chittagong City Corporation (CCC), in order to describe the flow of deshi chickens and ducks and to assess the risk of release of avian influenza viruses (AIVs) in 2 LBMs at CCC through deshi chickens and ducks trade. A total of 44 environmental pooled samples were collected from middleman storage facilities from outside of 6 LBMs in CCC. Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) was used to detect M gene then followed by H5 and H9 subtypes in order to determine whether deshi chickens and ducks were infected by AIVs prior to their arrival at CCC LBMs. In the present cross-sectional study we found that most of the deshi chickens were handled by 2 to 3 middleman and 1 or 2 either wholesaler or retailer. It was rarely found the involvement of 2 wholesalers in the desi chickens transaction chain. In case of ducks transaction chain between farms and end customer is more likely that 1 to 2 middlemen and 1 wholesaler or retailer were involved but did not find the involvement of 2 wholesalers in the transaction chain. Two main pathways (**P1**and **P2***)* of release assessment were identified for AIVs transmission. In pathway1 (**P1**) deshi chickens or ducks sold by farmers being infected at the farm gate, risk of release of AIVs to the susceptible poultry at LBMs in CCC was high, whereas in the pathway 2 (**P2**) of deshi chickens or ducks sold by farmers being not infected at the farm, but infected any step of the pathway (storage or transport) and arrives as infectious at CCC LBMs, risk of release of AIVs to the susceptible poultry at CCC LBMs was low, medium and high depending upon when and at which step of the pathway susceptible deshi chickens and ducks become getting infected in the transaction chain and dissemination of AIVs to the susceptible poultry at CCC LBMs. We found overall prevalence of AIV in the environmental sample sites was 26.3% for M gene, 19.5% for H5 and 12.8% for H9 (95% CI: N=44). The proportion of positive samples in the present study was identical (p- 0.81) to the results obtained from the BALZAC project cross sectional study. It was therefore likely that the poultry entering the LBMs may be already contaminated with AIVs. Knowledge from this study could provide a new understanding of the deshi chickens and ducks value chain and release of avian influenza through LBMs at CCC in Bangladesh. These findings could be used to develop a programme concerning biosecurity and hygienic management to reduce the risk of release of AIVs and spreading of avian influenza through LBMs in Bangladesh via deshi chickens and ducks trade.

Keywords: Live bird market, deshi chickens, ducks, transaction chain, risk pathway, avian influenza

Chapter 1: Introduction

The poultry sector is one of the promising sectors in Bangladesh which provides animal protein for human consumption that generates income and contributes to the Gross Domestic Product (GDP).Agricultural sector contribute 18.6% of the Bangladeshi GDP in which 3.2% of GDP comes from the poultry industry (MoP, 2015).The poultry population in Bangladesh was about 338 million in 2017-2018 in which 282.1 million chickens and 55.9 million ducks (DLS, 2018). A large section of marginalized rural people earns their living through this industry. Backyard poultry are mostly reared by women or children for subsistence income and family consumption (Hamid et al., 2017).

Meat of backyard poultry is tasty, widely accepted and sold at a very good market price. Therefore, traders involved with the backyard poultry business collect backyard poultry from different sources and supply them to different city LBMs in Bangladesh. Since Chittagong city has a population of over 6.5 million (BBS, 2014), and there is a huge poultry demand in this city, traders choose this city to sell backyard poultry collected from different sources across the country. The transaction chain and trading patterns of backyard poultry is very complex and there is no systematic scientific study to describe this system along with associated business and disease risk.

Disease is one of the main challenges in backyard poultry rearing and trading. Common poultry diseases include Newcastle disease, salmonellosis, colibacillosis and avian influenza (Morishita, 2004; Barua and Yoshimura, 2007; Conan et al., 2012). These diseases may cause mortality of poultry at household farms, storage and during transportation. Avian influenza, particularly highly pathogenic avian influenza (HPAI) H5N1, has been causing many outbreaks in backyard poultry since 2007 in Bangladesh (Biswas et al., 2009). This disease has zoonotic importance for public health risk. HPAI H5N1 is now considered as endemic along with seasonal epidemic in different poultry sectors in Bangladesh (Ahmed et al., 2012; Osmani et al., 2014).

Several studies have been conducted on avian influenza in Bangladeshi chicken LBMs (Negovetich et al., 2011; Chowdhury, 2013; Nasreen et al., 2015; Sayeed et al., 2017; Turner et al., 2017; Hassan et al., 2018). However none of this study considered poultry upon arrival, before entering the different poultry stalls, to

investigate avian influenza viruses, AIVs, and their potential role in introducing viruses to LBMs. Moreover risk analysis of HPAI H5N1 introduction to different big city LBMs through backyard poultry trading has previously not been studied in Bangladesh.

Considering the above background the present study was therefore designed with the following specific objectives:

1. Map the deshi chickens and ducks transaction chains between backyard farms and supplying end consumers at LBMs in Chittagong Corporation City Corporation (CCC).
2. Assess the risk of release pathway of AIVs (HPAI) in CCC LBMs through deshi chickens and ducks trade.
3. Determine if the environment through which deshi chicken chicken and ducks transit prior to their arrival at CCC LBMs was contaminated with AIVs.
4. If the environment is proven to be AIV contaminated, then determine whether the virus present is H5 or H9.

Outcome of the project

An improved understanding of the backyard poultry transaction chain and HPAI H5N1 release to CCC LBMs and other AIV environment contamination at arrival of poultry in the environment of CCC LBMs were which will help to control the transmission of infectious diseases like AI and implementation of preventive strategies to control them. .

Chapter 2: Literature Review

The literature for the current study, including the role and characteristics of traders involved in LBMs, deshi chickens and ducks rearing systems, presents the status of production and requirement of eggs, and poultry meat. It also contains information on possible disease transmission, outbreaks, zoonotic significance, prevalence of avian influenza viruses and their subtypes and diagnostic approaches. The objectives of this part were to collect the necessary data from previous studies in order to identify knowledge gaps and justify the present Master's research. The literature was found by searching Pub Med and Google Scholar. The findings of importance in published and unpublished articles are introduced under the accompanying headings below.

2.1 Poultry Population and Rearing Systems of deshi chickens and ducks

By 2015, the total poultry population in Bangladesh was about 313 million (261.8 million chickens and 50.5 million ducks) (MoP, 2015). The current poultry production in Bangladesh can be divided into four categories: 1) traditional rural backyard scavenging system; 2) semi-scavenging system; 3) commercial farming system; 4) contract farming or integrated system (Saleque, 2009). There are four main poultry keeping standards: In the traditional rural backyard scavenging system, poultry are housed at night, but allowed to free-range during the day. They are usually fed a handful of grain or leftover food in the morning and evening to supplement their scavenging. In the semi scavenging system, poultry are raised in a combination of extensive and intensive systems, where the birds are confined to a certain area with access to shelter at night. They are mainly found in rural areas and also in urban and peri-urban locations. In Bangladesh, the majority of backyard farmers raise their deshi chickens and ducks in open small-scale yards and they do not pay much attention to biosecurity. The flocks are raised for family consumption and for selling to local village, union and upazila markets and to neighbours. In the commercial farming system, poultry are only reared for commercial purposes and they are reared in a controlled environment. In the contract farming or integrated farming system, the entire production system is integrated from producers, feed suppliers, marketers and/or farmers (Begum, 2005; Begum, 2006; Saleque, 2009).

2.2 Poultry Meat and Egg Consumption

The meat and egg consumption by the people in Bangladesh is one of the lowest in the world. The average meat and egg requirement is 43.3 kg and 104 eggs per person per year, respectively, but the availability for Bangladeshi chicken people is only 1.9 kg and 70 eggs per year per person (Hamid et al., 2017). The current egg and meat production can only meet 64% and 68% of the national demand. People prefer to eat deshi chicken and duck meat and eggs in both urban and rural areas, rather than other poultry types, due to their unique taste and flavour, as well as being allergen free (Das, 1995). As a result, the high demand for deshi chickens and ducks leads to higher market prices (Islam and Nishibori, 2009). Deshi chickens and ducks are also an additional source of income for household farmers, particularly for women, in addition to their use as the main source of meat and eggs for family consumption.

2.3 Related Definitions

A live bird market is a place where poultry traders, middlemen and end consumers come to buy live or slaughtered birds for their consumption or trading purposes. 'Live poultry' indicates mature birds intended to be slaughtered, either by the market seller in front of the consumer, or at home by the consumer (Fournié et al., 2012). A stall-holder is a person who owns or runs a stall at a market, at least once a week, from which he sells poultry. A stallholder can be a retailer, who sells live or dressed poultry to end users, or a wholesaler, who sells at least some live birds to other traders. A middleman is a person, who buys his poultry directly from the producer or another middleman or wholesalers in order to sell to other middlemen or stallholders. Local names for middlemen include *aratdar*, *faria*, *bepari* and *paiker*, operating in different locations. Thus, middlemen are responsible for transporting the birds between farms and markets. A poultry farmer is someone who raises his own poultry, whether it is his main source of income or not, and who comes less than once a week to the market to sell his own poultry.

2.4 Live Bird Markets in Bangladesh

Live bird markets, also known as 'wet markets', are commonly found in many parts of the world (Cardona et al., 2009; Samaan et al., 2011; Fournié et al., 2012), including Bangladesh. Fifty-five LBMs were identified in Chittagong city Corporation (CCC, 2015). LBMs, acting as hubs in the poultry trading system, play an important

role in the circulation and also dissemination of avian influenza viruses (Biswas et al., 2017). Indeed, LBMs bring together a mix of different poultry species, produced by multiple farmers in different parts of the country, in a variety of poultry production systems (Cardona et al., 2009). LBMs are also considered to be man-made reservoirs of AIVs, as deshi chickens and ducks play significant role in the transmission of AIVs through the exchange of contaminated equipment and vehicles between LBMs and poultry farms (Biswas et al., 2008).

2.5 Overall Poultry Marketing and Trading Practices

In Bangladesh, LBMs are the primary hub for poultry marketing throughout the country (Hassan et al., 2018). Deshi chicken and ducks are mostly traded alive, because of cultural and religious preference for consuming freshly slaughtered poultry and the lack of refrigeration, particularly among rural households (Giasuddin et al., 2002). Deshi chicken and duck middlemen operate at several levels: backyards, rural LBMs, semi-urban LBMs, districts, city middlemen and city LBMs. They purchase poultry from backyard farms and rural LBMs to sell them to the semi-urban LBMs. From there, poultry is transported to middlemen or stallholders in districts or cities, where they are, in turn, purchased by end-users or other middlemen or stallholders who will sell to end-users. The middlemen need to collect poultry from many backyard farms to supply city LBMs. At the local markets, villagers sell their deshi chickens and ducks to local consumers or to poultry traders, who collect them to sell them at larger city markets, but these trading patterns of deshi chickens, ducks and their epidemiological consequences are not clearly understood and have not been submitted to epidemiological scrutiny.

2.6 Prevalent Zoonotic Diseases

Multiple AIV subtypes, including H5N1, H5H7 and H5H9 have been identified in Hong Kong, China, Indonesia, South Korea, Vietnam, Thailand, and Egypt (Pepin et al., 2014). H5N1 is most frequently reported in poultry. Evidence shows that H9 may occur more often in humans in China than H5 or H7 (Jia et al., 2009). H5N1 is endemic in the context of Bangladesh (Chen et al., 2006; Osmani et al., 2014; Nasreen et al., 2015; Hassan et al., 2017). In LBMs in southern China, H9 is the most prevalent subtype, while H5 is relatively rare. Thus, in southern China, H9 and H5 present the largest risk of spill-over infections to humans. While many influenza A subtypes

have a strong host preference for ducks, H9 is well-adapted to chicken and quail, and H5 is adapted to all three of these hosts (Pepin et al., 2014).

Since LBMs were shown to be heavily contaminated with AIVs, we set out to test the environment in which poultry transit prior to their arrival at the LBM to understand whether the entire deshi chicken and duck transaction chain and these kinds of birds that enter LBMs are already excreting AIVs on arrival or whether they are more likely to be contaminated in the LBMs.

2.6.1 Avian Influenza

Avian Influenza is a viral disease caused by single-stranded and segmented RNA virus of the family Orthomyxoviridae (Capua and Alexander, 2006; Kraidi, 2017). This RNA virus has 8 gene segments (Haemagglutinin: H, Neuraminidase: N, Matrix: M1 and M2, Nucleoprotein: NP, Polybasic: PB1 and PB2, Poly acidic: PA and Non-structural: NS) which encode 10 different proteins (surface proteins and internal proteins) (Swayne and Suarez, 2000; Bouvier and Palese, 2008; Kraidi, 2017; Nagy et al., 2017). In Bangladesh, a pandemic of AI could start, due to its dense human population, widespread live poultry production and endemic HPAI,(H5N1) in poultry (Sultana et al., 2012). Most consumers are interested in purchasing live birds instead of processed birds due to lack of trust, whether they were slaughtered using the halal method, or whether the processed poultry stem from dead or diseased animals (Das and Raha, 1998). Poor LBM hygienic conditions, like mixing of different poultry types, leftover poultry of the same poultry type in the same storage area, mixing different poultry type in the same cage, slaughtering within the stall, lack of waste disposal facilities, lack of disposal of dead poultry, lack of disinfectant supply, and lack of regular investigation by the public health authorities are very common in Bangladesh, similar to the situations in Myanmar, Pakistan and India (Sheikh, 2012). Identifying the probability of AIVs to be released in the LBM environment through deshi chickens and ducks trade, as we did in this study, can help justify the development of interventions to reduce the risk of AI transmission.

2.6.2 Transmission of Avian Influenza

AIVs can be transmitted from poultry to poultry through direct contact or indirectly by exposure to contaminated faecal material or through aerosols, water, feed and bedding materials and utensils (de Jong and Hien, 2006; Zhou et al., 2016; Fournié et al., 2017). The most commonly identified factors associated with H5N1 virus infection in humans include exposure to infected blood or bodily fluids of infected poultry via food preparation practices; touching and caring for infected poultry, consuming uncooked poultry products and exposure to HPAI H5N1 at LBMs (Van Kerkhove et al., 2011). Direct or indirect contact with infected wild poultry – resident or migratory – is suggested as the most likely pathway of exposure of deshi chickens and ducks to AIVs (Alexander, 2000; Fouchier and Munster, 2009; Yee et al., 2009). All domestic and captive poultry worldwide can be infected by AIVs. However, the frequency of primary infections in birds is dependent on the level of contact with infected ones (Alexander, 2000). Although primary introduction can be due to wild birds, the spread and maintenance of viruses are largely influenced by human activities. For example, if poultry traders trade infected poultry or share contaminated equipment, then the virus can easily be transmitted to poultry and contaminate LBM environments (Fournié et al., 2013). Earlier studies reported that multiple poultry species from backyard and commercial production systems are sold together in LBMs, which may increase the risk of cross-species AIV transmission (Woo et al., 2006; Wisedchanwet et al., 2011). The transportation of poultry or working utensils may facilitate the transmission and spread of avian diseases (Paul et al., 2011). In particular, transitive LBMs are believed to have played an instrumental role in several outbreaks of avian diseases that have caused significant economic loss in India (Biswas et al., 2009). AIVs that have been isolated from backyard flocks and free range poultry are usually associated with the transmission of the lowly pathogenic AIV from wild birds (Terregino et al., 2007).

2.6.3 Outbreaks of Avian Influenza

In Bangladesh, AI was first reported in 2007 (Biswas et al., 2008) and later spread to at least 51 of 64 districts of the country. By 16 April 2016, 583 outbreaks had been reported. Since then, only 6 outbreaks have been reported, in 2016 and 2017, of which 98.1% were in domestic poultry chickens, pigeons, quail, and ducks (OIE, 2016; OIE,

2017b; OIE, 2017c). Only 0.3% of the AI outbreaks were found in crows, 0.2% in domestic swine and 1.4% in unspecified poultry species (FAO, 2016).

In India, the initial evidence of rapid spread of HPAI H5N1 outbreaks among the different states was found in 2008, where market poultry constituted the major proportion of infected birds followed by domestic and wild birds (Rao, 2008). Until 16 April 2016, the number of recorded outbreaks in India, Bhutan and Nepal was 159, 21 and 16, respectively (Chaudhary and Pahwa, 2013; OIE, 2017a) and in Nepal, the first serologic evidence of AI was found in October 2005 (Pant and Selleck, 2007). According to FAO, H5N1 is endemic in poultry in Bangladesh, China, Egypt, India, Indonesia and Vietnam. Along with HPAI H5N1, a total number of 1,660 H7 outbreaks, including high and low pathogenic forms of H7N1, H7N2, H7N3, H7N6, H7N7, H7N8 and H7N9, and 1,619 H9 outbreaks, including LPAI H9N1 and H9N2, have been reported throughout the world (FAO, 2016).

2.6.4 Avian Influenza at LBM and Associated Factors

Several studies have shown that LBMs act as a hub for the circulation of AIV in Asian and African countries including China, Vietnam, and Indonesia (Indriani et al., 2010; Soares Magalhães et al., 2010; Fournié et al., 2012). During an outbreak, farmers instinctively intend to sell their birds, regardless of healthy or non-healthy as quickly as possible to minimize its negative economic impact (Sultana et al., 2012). AIVs may spread to LBM stalls, contaminating the market environment and newly arrived unaffected poultry, as well as previously contaminated environment unaffected poultry and pose a potential threat for human populations. If AIVs establish in a LBM, then they can easily mutate and produce new strains (Webster, 1998). Through a spread-back process, when the traders go back and forth with their vehicles between farms and markets without applying routine disinfection practices and then enter farm premises, the traders thereby may spread viruses to commercial and backyard farms. In recent years, a number of AI outbreaks have been reported in Europe and North America, which were associated with rare infections among humans exposed to infected poultry (Fouchier et al., 2004; Herlocher et al., 2004; Hirst et al., 2004; Koopmans et al., 2004; Tweed et al., 2004). This suggests that markets could be a potential source for H5N1 infection among poultry and humans. This phenomenon has been studied in Vietnam, Thailand, Indonesia, Egypt, and Cambodia (Nguyen et

al., 2005; Amonsin et al., 2008; Abdelwhab et al., 2010; Indriani et al., 2010; Wan et al., 2011; Leung et al., 2012). Both HPAI (H5N1) and LPAI – H5N1, H5N2, H5N3, H5N4, H5N5, H5N6, H5N7, H5N8, and H5N9, H9N1, H9N2, H9N3, H9N4, H9N5, H9N6, H9N7, H9N8, and H9N9 subtypes have been identified from LBMs in Bangladesh (Pant and Selleck, 2007; Negovetich et al., 2011; Gerloff et al., 2014; Biswas et al., 2017).

Earlier studies conducted in Bangladesh suggested the overall estimated AI and subtype specific AI prevalence at stall level to be 26% avian influenza , 4% H5 and 14% H9 (Sayeed et al., 2017). However, in another study conducted in Dhaka and Chittagong, the prevalence of AI at market level was 13.5%, 9.4% H5 and 1.6% H9 (Biswas et al., 2017). The stall level prevalence of LPAI H9N2 was reported to be 16.5% in Bangladesh (Negovetich et al., 2011).

In contrast, high prevalence has been reported in Indonesian live bird markets: Avian influenza Type A 47% and H5 32% (Indriani et al., 2010; Nguyen et al., 2014). Other studies reported prevalence in other regional countries: 32.2% H5 in Vietnam, 10% AI and 6.8% H9N2 in South Korea (Leung et al., 2012), 4.4% H9N2 in Hong Kong (Shortridge, 1999), and 12.4-40.8% H5 in Egypt (Abdelwhab et al., 2010).

2.7 Consequences of Avian Influenza

Sometimes, the virus can travel back to the farms from LBMs, which may lead to devastating culling of poultry. This leads to high economic loss for farmers (Trock et al., 1997; Trampuz et al., 2004). If a LBM environment is constantly contaminated, then this may lead to mutation and re-assortment of AIVs. Therefore, a novel AIV is likely to emerge, which may pose a potential pandemic threat. Such a pandemic could have direct consequences for the poultry sector and public health. The role played by deshi chickens and ducks for the transmission and spreading of AIVs has not yet been clearly quantified and assessed.

2.8 Conclusion

There is little to no information on the deshi chickens and ducks trading systems in Bangladesh. As this specific trading system is not well known, adapted and targeted prevention measures cannot be implemented. In order to address the lack of information on the deshi chickens and ducks trading systems and the potential consequence of an AI outbreak in such a system and to assess the circulation of AIVs in deshi chickens and duck transaction chains, prior to their arrival in CCC LBMs, the present cross-sectional survey was carried out.

Chapter 3: Materials and Methods

3.1 Selection of Live Poultry Markets

Two main LBMs in CCC were selected for the questionnaire survey for the present study: Reazuddin Bazar(RB) and Pahartali Bazar(PB) Stallholder(SH) were interviewed and environmental sampling were collected from both these LBMs. In addition to RB and PB, Bahaddarhat Bazar, Kazir Dauri Kacha Bazar, CDA Karnafully Market and Karnafully Complex were chosen for environmental sampling. These LBMs were chosen based on the highest volume of daily trading of deshi chicken chicken and ducks, as evidenced by the ongoing study under the UK funded project *Behavioural Adaptations in Live Poultry Trading and Farming Systems and Zoonoses Control in Bangladesh* (BALZAC) and the previous study conducted by FAO and DLS (FAOSTAT, 2015; Moyen et al., 2015).

3.2 Study Type and Period

A cross-sectional study was conducted in the aforementioned markets of CCC from (Questionnaire survey, March 2017 to July 2017 and Environmental sampling in January 2018)

3.3 Study population

All poultry stallholders traded deshi chickens and/or ducks with or without other poultry types and Deshi chickens and duck middleman(s) supply poultry at the two selected LBMs were included in the study.

3.4 Market Description

Among the 2 wholesale LBMs Reazuddin Bazar is located in the centre and Pahartoli Bazar is located in the north-western part of the city. In Reazuddin Bazar, there were 24 retail stalls, selling poultry directly to end customers and 8 wholesale stalls, selling poultry to other retail stalls within the same market or other markets, as well as to other customers, for example hotels, restaurants, community centres and hospitals. At Pahartoli there were 22 retail stalls and 4 wholesale stalls. Environmental samples were collected from four other markets: Bahaddarhat Bazar, Kazirdauri Kacha Bazar, CDA Karnafully Market and Karnafully Complex, all located in CCC. The

geographic locations of the LBMs included in this study are shown on the map below (Figure 3.1).

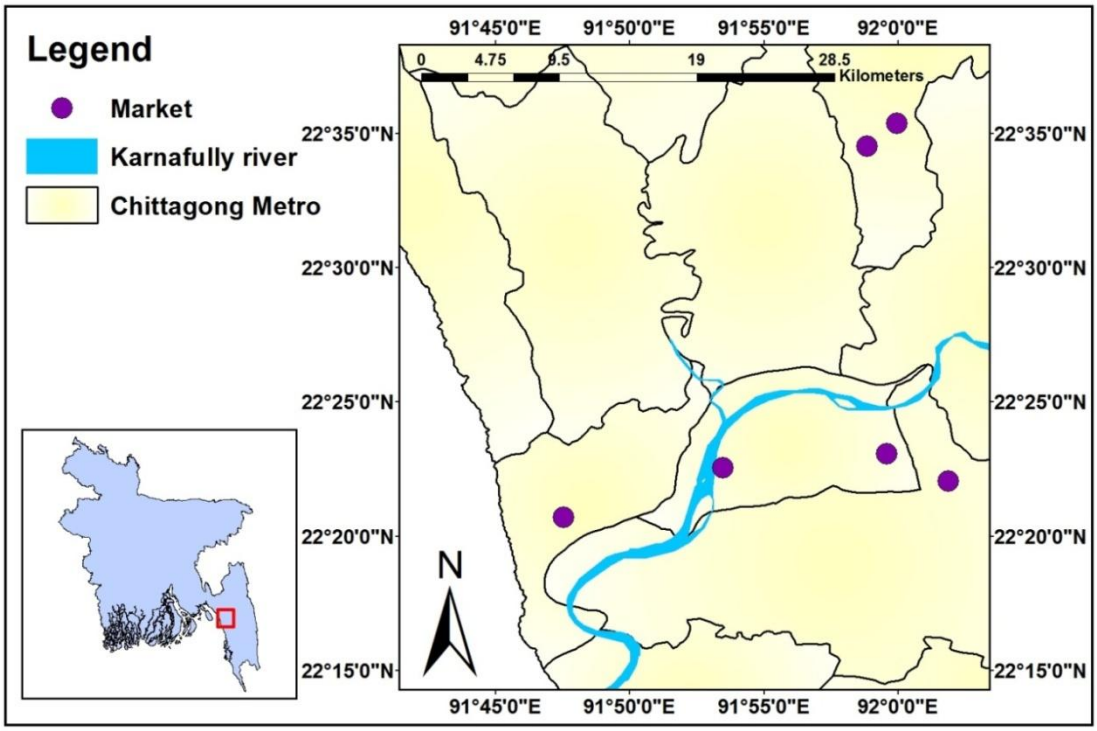

Figure 3.1: Geographic Locations of the Study Live Bird Markets

3.5 Identification of Poultry Traders for the Study

All poultry stallholders at the two LBMs which traded deshi chicken and/or ducks with or without other poultry types were included in the study: 13 SHs from Reazuddin and 8 SHs from Pahartoli were identified. The SHs identified 45 middlemen as deshi chicken and/or duck suppliers. Four of the 45 middlemen were interviewed during our pilot study. Then, 50% of the remaining 41 middlemen were selected as follows:

1. All 11 duck middlemen were included as they were few of them in total number.
2. All the middlemen (5) named by both Pahartoli and Reazuddin stallholders were included as they trade a large number of deshi chicken daily,which would represent a significant proportion of deshi chicken entering Chittagong City.
3. The last 4 middlemen were randomly selected from the list of all middlemen.

This group of middlemen (20) identified 41 other middlemen as their deshi chicken chicken and/or duck suppliers (26 deshi chicken middlemen and 15 duck middlemen). We then set out to interview 50% of deshi chicken middlemen and 50% of duck middlemen in a second round of interviews. These middlemen were selected as follows:

- **Deshi chicken middlemen**: 1 middleman was randomly selected per district. This process yielded a list of 11 middlemen, the 2 other middlemen needed to meet the target of 13 were randomly selected. If a middleman refused to participate, then he was replaced by another middleman (by random selection).
- **Duck middlemen**: The same process was used to achieve sample of 8 middlemen.

3.5.1 Ethical Approval

This study obtained ethical approval from the Royal Veterinary College (under the code URN 2015 1318) and Chittagong Veterinary and Animal Sciences University Ethics Committee (Memo no.CVASU/Dir(R&E)AEEC/2015/04, Date:11/08/2015). Verbal consent was obtained from all interviewees before they participated in the study.

3.6 Data Collection

Trader specific structured questionnaires were used to record data from the selected poultry traders through face-to-face and/or phone interviews. The first questionnaire was designed for the middlemen, with questions on their buying and selling practices over the last two days, including information about the date, time, number of poultry bought and sold, poultry origins, storage, means, duration and cost of transportation, surplus poultry, and storehouse, duration of collection, storage and transportation means to LBMs (**Appendix A**). The second questionnaire was designed for the poultry stallholders and included questions about their buying and selling practices over the last two days, including information about date, time, number of poultry bought and sold, origins of poultry, storage, transportation (means, vehicle used, duration and cost), surplus poultry, storehouse and storage time of leftover poultry,

storage and transportation (**Appendix B**). We also inquired about the distance covered for the collection of sufficient numbers of deshi chickens and ducks for 1 shipment to LBMs in accordance to the demand of the trader(s). In both questionnaires, we collected data on hygiene practices on market stalls and middlemen's storage facilities and vehicles. Before the start of the study, the questionnaires were pre-tested in a pilot study on 4 deshi chicken traders (2 stallholders and 2 middlemen) and 3 duck traders (2 stallholders and 1 middleman), purposively chosen. These traders were chosen randomly from LBMs, which were not included in the present study. We checked the quality of the questions, including gaps and proper timing. The questionnaires were then revised accordingly.

Questionnaires were in English and the interviews were only conducted by the author in Bengali. The interview schedule was organised through consultation with the traders. Stallholder interviews lasted 45-60 minutes, whereas middleman interviews lasted 1.5 hours. We provided an incentive for participating in our study, compensating for the time invested, in the form of 150 BDT phone credit to each informant.

All stallholder interviews were conducted directly without previous appointment. On the other hand, we needed to contact the middlemen in advance, made an appointment, as they were constantly on the move. It was impossible to meet some of the middlemen in person, since they lived in other districts. Thus, the middlemen interviews were conducted either on the phone or face-to-face.

3.6.1 Transaction chains

Our objective was to re-construct the chains of transactions through which poultry purchased by LBM end customers in each LBM transited. We started from the end, sequentially estimating the number of poultry purchased by each actor from any possible supplier according to their hierarchical position in the chain. Indeed, categories were defined so that there was no transaction among actors within the same category. The transactions were unidirectional between any two categories. At the first step, we estimated the number of poultry purchased by end-users. At each following step, we estimated the number of poultry purchased by a category of middlemen or stallholders for whom the sales had already been estimated in the previous step(s). In this way, for any given group of middlemen or stallholders, their purchases would be equal to their sales. For example, we started by determining the total number of poultry sold to end-

users by retailers and we could then determine the number of poultry they had purchased from their suppliers. We then sequentially estimated the purchases of actors classified as W3, W2, W1, MM3, MM2, MM1, and lastly farms (**Table 3.1)**.

Table 3.1 Summarises and actor purchased and sold poultry. Note that not all these categories of middlemen and stallholders were present in each transaction chain.

Table 3.1: Definitions of Stallholder and Middlemen Categories

Stall-holders	Retailer	R	A stallholder who sold all his poultry to end consumers and restaurants.
	Wholesalers	W1	'The first wholesaler that poultry meets'. W1s buy all their poultry from farms, through the of middlemen or not. None of the poultry they purchased were handled by another stallholder.
		W2	A wholesaler who bought poultry at least once from W1, either directly or via the of middleman (defined as MM2 here below).
		W3	A wholesaler who bought poultry at least once from W2.
	Middlemen	MM1	A middleman who bought all his poultry directly from farms.
		MM2	A middleman who bought at least some poultry from MM1. Did not buy from a wholesaler.
		MM3	A middleman who bought at least some poultry from wholesaler. Did not buy from MM2 or MM3

**A source of poultry is either a farm, a backyard farm (household), an upazilla or union LBM, a group of wholesalers of a given category in a LBM, or middlemen (category defined in Table 3.1).

The steps described here below were repeated twice, first with the minimum number of poultry sold by stallholders to end-users, second with the maximum number of poultry sold by the middlemen. The method was continued until numbers purchased and sold had been determined for each category of middlemen and stallholders.

STEP 1	Stallholders and middlemen were classified as explained in Table 3.1.
STEP 2	As stallholders or middlemen did not declare selling as much as they purchased, numbers purchased and sold were adjusted as follows: • The minimum number of deshi chickens traded was the minimum between the minimum numbers of deshi chickens sold or purchased (as reported in the interview). • The maximum number of deshi chickens traded was the maximum between the maximum numbers of deshi chickens sold or purchased (as reported in the interview). For the sum of purchases (sales) from each source (to each purchaser) to remain equal to the total number purchased (sold), numbers of deshi chickens purchased from (and sold to) each source (purchaser) were adjusted by multiplying the number of poultry traded by the proportion of purchases (sales) represented by each source of poultry (purchaser).
	We now have, for each interviewed stallholder and middleman Sum of sales = Sum of purchases = Total number traded
STEP 3	The minimum (maximum) number of poultry sold by all the interviewed retailers to end-users was calculated by summing the number of poultry each retailer sold to end-users.
STEP 4	The proportion Po of poultry purchased by retailers from each source of poultry was calculated: Po = (Number of poultry purchased by retailers from source)/ (Total number of poultry retailers purchased from all sources) There are as many Po as there are sources of poultry The number of poultry retailers purchased from each source was then: Po * Total number of poultry retailers sold to end-users.
STEP 5	Wholesalers W3 did not exist
STEP 6	Wholesalers W2: • Their sales are now defined as the sum of what retailers purchased from them (calculated at STEP 4) and what they sold to end-users. ▪ Purchases from each source were calculated by: ▪ Po * Total number of poultry W2 sold. Po = (Number of poultry purchased by W2 from source o)/(Total number of poultry W2 purchased from all their sources of poultry)

3.6.2 Release Assessments

We assumed that there were two main pathways via which AIVs could enter the LBM in CCC through deshi chickens and ducks trade **(Figure 3.2**).

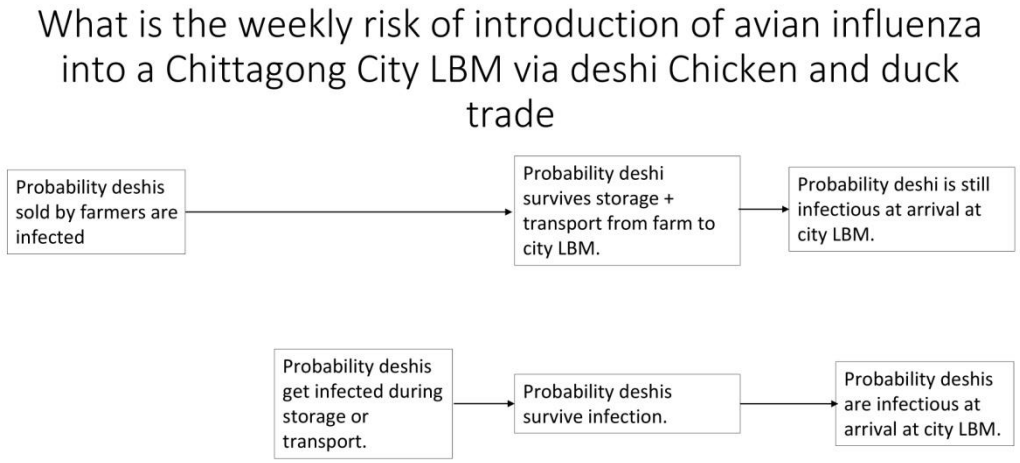

Figure 3.2: Risk of Weekly Introduction of AI into Chittagong City LBMs

We assessed the risk of each step to occur using available literature or data collected during this cross-sectional survey. The probability of each step to occur was qualified as Negligible, Very Low, and Medium and High, the definitions for these terms are described in (**Table 3.2)**

Table 3.2: Definitions of Risk Levels

Negligible	Risk or frequency/consequence is so low as to not merit consideration
Very Low	Risk or frequency/consequence is almost negligible, but due to uncertainty or other extenuating circumstances cannot be excluded from consideration.
Low	Risk or frequency/consequence is small/ infrequent, but still worth considering intervention/mitigation.
Medium	Occurs frequently, or event associated with a modest consequence
High	Event occurs often, and/or is associated with a significant consequence
	Event occurs almost certainly, and/or is associated with a serious consequence

3.7 Sample Collection, Preservation and Transportation

Each market was visited only once for sampling. Samples were collected between late night and early morning (3:00-9:00 AM). The time of our visit was adapted to be able to collect sample an empty environment, before the arrival of new deshi chickens and ducks. We did this investigative exercise upon the arrival of deshi chickens and ducks at a LBM from different environmental sites in which the deshi chickens and ducks had arrived. These samples were taken from sites before they reached the LBM environment, such as water cages, baskets, vehicles in which the deshi chickens and ducks were transported before their entrance into different poultry stalls at the selected markets. Sampling was done between 11 and 25 January 2018 from the selected study sites. Five pooled environmental swab samples were collected from each of the selected markets and middlemen store houses before entrance into the stalls within the LBMs. Accordingly, 44 pooled samples were obtained. Each pool consisted of five items as follows:

1. Poultry drinking water
2. Poultry basket/cage floor (where droppings were seen)
3. Poultry basket/cage wall
4. Floor of rickshaw van (where the poultry baskets are stored)
5. Floor of middleman store house: the poultry storing place/stall, where poultry were kept before delivery to the stallholder, and underneath floor of the vehicle used to carry poultry through to the store house – where feathers and droppings can be seen

Sterilized individual cotton-tipped swab sticks were used to swab each item of environmental samples and placed together in a vial containing Viral Transport Medium, VTM, labelled with unique identity number [[**LMR] – [Market code]-[Origin]-[site type]-[Pool reference]]**. A sterile swab was moistened with VTM before the swabbing sample from any of the environmental samples except for the water sample. The moist swab was rolled over the surface to sample and we removed excess matter by shaking before placing it into the VTM. For each type of site the focus was given to possible visibly dirty and moist surfaces.

Samples collected from the selected markets of CCC were transported immediately to CVASU's laboratory with insulated cool boxes and stored at (-)80°C freezer until further analysis. For sampling sites and LBM codes of CCC, **(Appendix C**).

3.8 Laboratory Evaluation

Viral RNA from pooled samples was extracted by using the Mag MAX RNA isolation kit (Thermo fisher, Scientific ,US), and reverse transcribed and amplified by using the AgPath-IDTM One-Step RT-PCR. Pools were screened for AIVs using real-time reverse transcription-polymerase chain reaction (rRT-PCR) and specific primers and probes (Monne et al., 2008; Heine et al., 2015) and reverse transcribed and amplified by using the AgPath-IDTM One-Step RT-PCR (Catalogue no lot AM1005). A pool with a cycle threshold value CT<40 for the AIV Matrix gene was then screened for the H5 and H9 genes and said to be rRT-PCR-positive if CT<38 and 40, respectively (Monne et al., 2008; Heine et al., 2015).

3.9 Data Management and Statistical Evaluation

Data were recorded on paper-based questionnaires at the time of the interview. Data obtained were entered into Epi Data Software (EpiData, 2018). Validation of data and consistency checks were carried out before exporting the dataset to R.3.5.1 for epidemiological analysis and to STATA-13 for statistical analyses.

Biological testing data produced from the present study was compared with the data produced through an earlier cross-sectional study conducted by BALZAC research group on the same LBMs in Chittagong. A Fisher's exact test was performed to assess the proportion of RT-PCR AI results (M or H5 or H9) in environmental samples between two studies (the present MSc study versus BALZAC cross-sectional study). The results were expressed in frequency number, percentage and p value. The cut off probability value of less than or equal 0.05 was considered as significant.

Chapter 4: Results

4.1 Deshi chicken and Duck Transaction Chains

In the present study, deshi chickens transaction chain were handled by a maximum of 3 middlemen and 1-2 stallholders, either wholesaler or retailer, in CCC LBMs. The middlemen who purchased deshi chickens from farms or union and upazila LBMs were keys to the chain as they handled all the poultry and distributed it to stallholders or another middleman in the 2 LBMs surveyed. Wholesalers were the second most important actors of the chain in terms of proportion of poultry purchased by the retailer or middlemen and end consumers that they handled (64.1%). Most (55.8%) of the deshi chickens purchased by the end consumers was sold by retailers **(Figure 4.1)**. Only 16.2% of the poultry that was purchased by end customers were handled by two middlemen and only 3% of the poultry ultimately purchased by customers were handled by two wholesalers, in addition to 1 or 2 middlemen.

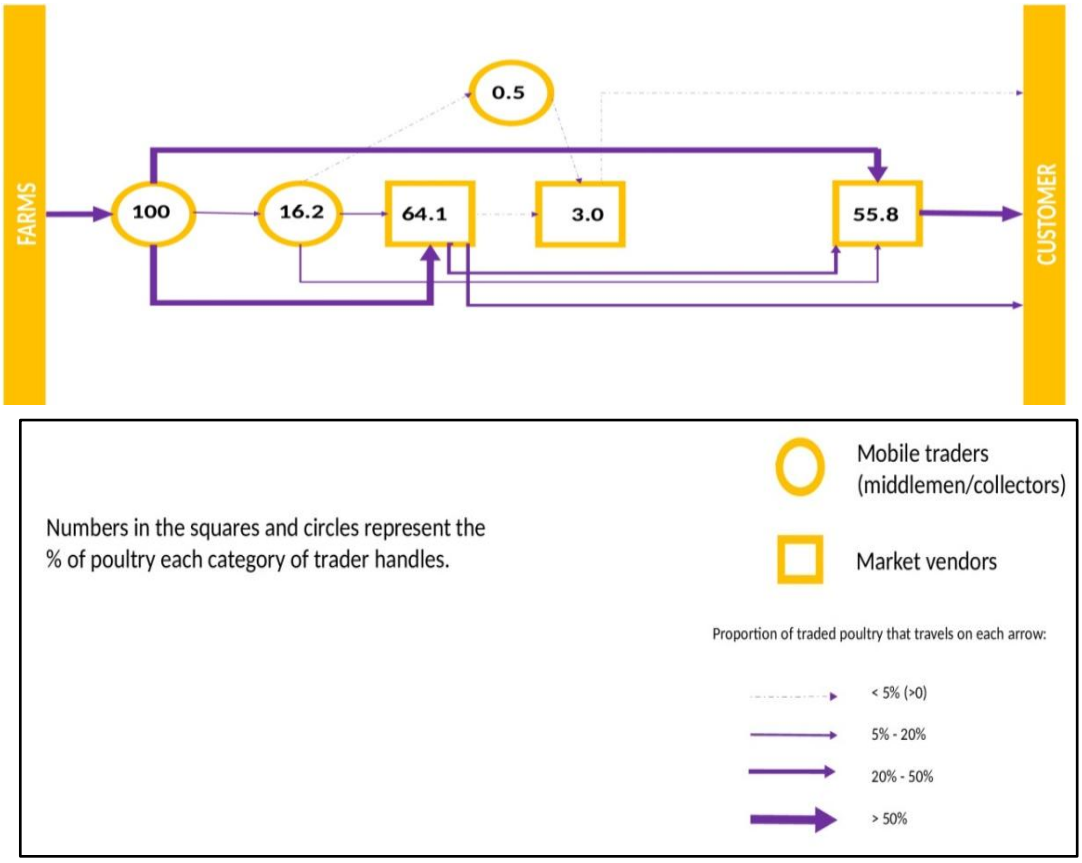

Figure 4.1: Deshi chickens Transaction Chain between Farms and Customers. In the figure, farms represent union, village and upazila LBMs as well as backyard farms (households)

The ducks transaction chain was similar to the deshi chickens transaction chain, but there were potentially fewer actors involved, than in the deshi chickens transaction chain. In ducks transaction chain between farms and end customer is more likely that 1 to 2 middlemen and 1 wholesaler or retailer were involved but did not found the involvement of 2 wholesalers in the transaction chain. Most (68.8%) of the ducks purchased by the end customers was handled by retailers, who were the type of stallholder from whom end customers bought the most **(Figure 4.2).** Contrary to the deshi chicken transaction chain, there was no main route through which ducks were transported from farm to the end customer. As for the deshi chickens transaction chain, the first middleman was the key factor in the chain, as he collected and supplied the ducks to stallholders in the 2 surveyed LBMs at CCC.

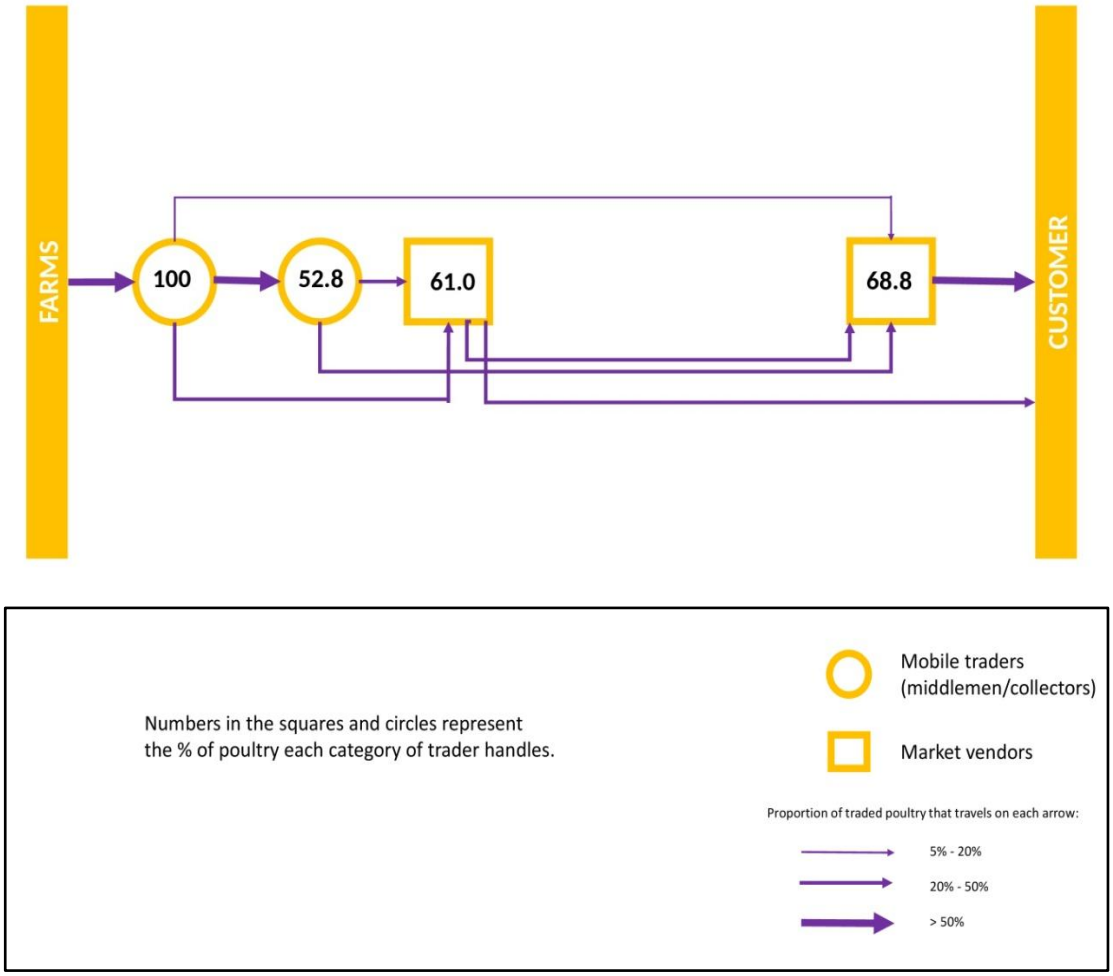

Figure 4.2: Duck Transaction Chain between Farms and Customers

In the figure, farms represent union, village and upazila LBMs as well as backyard farms (households)

Mixing of deshi chickens at storage and during transportation and at poultry stalls wholesalers and retailers from multiple sources were common, whereas mixing of deshi chickens and ducks at storage and during transportation and at poultry stalls from multiple sources was less common. Most of the deshi chickens and ducks middlemen used common vehicles and shared equipment.

The present study also explored that there had not been any biosecurity or hygienic measure undertaken during the whole transaction chain of deshi chickens and ducks from its farm gate to the end consumers.

The present study also found that no proper disinfection were used for cages and vehicles used while transportation deshi chickens and ducks and traders visiting several farms or markets and trade birds of different origins.

4.2 Release Assessment

In the present study, there were 2 pathways through which AIVs (HPAI) could be released into the LBM environments. The first pathway (P_1) of release assessment that depended on deshi chickens and ducks being infected at the farm and remained infected at entrance showed that the probability of release of AI particularly HPAI infection or AIVs to the selected surveyed LBMs in Chittagong City was high at the time of entry. Contrarily, the second pathway (P_2) of release assessment that depended on deshi chicken and ducks not being infected at the farm, but becoming infected during storage and transportation and arriving infected at the LBM determined that the probability of release of AI (HPAI) infection or AIVs to the selected surveyed market was low, medium and high. The overall risk of release of AIVs is shown in **Tables, 4.1** and **4.2.**

Table 4.1: Risk of AIVs Release Assessment along the deshi chickens and ducks Transaction Chain or Risk of AIVs Release into the LMBs of CCC **(Pathway 1)**

Step on the pathway	Data	Risk category
Probability that deshi chicken chicken or ducks sold by farmers are infected at the farm gate	Prevalence of AI in backyard farms in the earlier study found 9.6% in Bangladesh (Turner et al., 2017). Time for collection, storage and transport of deshi chicken chicken between farm and Chittagong City Corporation LBMs was calculated to be a mean value of 37.3 (95% CI: 33.1-41.6) hours in the present study	High
Probability of deshi chicken chicken is still infectious at arrival at CCC, LBM	The present study found 37.3 hours (95% CI: 33.1-41.6)) hours needed for storage and transportation	High

Table 4.2: Risk of AIVs Release Assessment along the deshi chickens and ducks Transaction Chain or Risk of AIVs Release into the LMBs of CCC **(Pathway 2)**

Step on the pathway	Data	Risk category
Probability that deshi chicken chicken or ducks sold by farmers are not infected at the farm gate, but infected during any point of storage or transportation	Average transport time of deshi chicken between farm gate and CCC was 6.8 (95% CI: 2.9-10.8) hours. The current study found the probability of mortality that deshi chicken chicken become infectious during storage or transportation on their way to LBMs is less than1%	High
Probability deshi chicken that survive in spite of infection during storage and transport between farm gate and CCC,LBMs	We found mortality rate is less than 1% in the current study and AIVs incubation period is about 83.7 (95% CI: 76.4-90.9) hours (Bouma et al., 2009)	Medium
Mortality rates according to time how likely is a deshi chicken and duck chicken likely to survive	Less than 1% mortality	Low
Probability deshi chicken are infectious and arrival at city LBM	The present study found 37.3 hours (95% CI: 33.1-41.6) hours needed for storage and transportation which is able to transmit infection to the LBMs	High

4.3 Environmental Sampling

The present study found 100% of the LBMs studied (6 out of 6) affected with AIVs in the environmental sample outside of the LBMs. The overall prevalence of AIV in the environmental sample in the present study was 26.3% for M gene, 19.5% for H5 and 12.8% for H9 (95% CI: N=44).26.3% environmental samples taken from different sites at entry of the LBMs in CCC had AIVs positives (M-gene) in the present study suggest that poultry get infected by AIVs before entering into LBMs. No significant difference of the proportion of M gene or H5 or H9 was observed between 2 studies, the present study versus the previously performed BALZAC cross-sectional study. Sample sites (Appendix C) of the 2 studies for M gene or H5 or H9 did not vary significantly (Table 4.3). It is therefore likely that the poultry entering the LBMs may be already contaminated with AIVs and H5, H9 subtypes.

Table 4.3: Univariate Association between RT-PCR Results of Avian Influenza (M/H5/H9) and Different Factors

		M Gene			**H5**			**H9**		
Variable	**Category**	**+ (%)**	**-(%)**	**p**	**+ (%)**	**-(%)**	**p**	**+ (%)**	**-(%)**	**p**
Study	BALZAC CS	5 (100)	0	0.81	2 (40)	0	0.93	3 (60)	0	0.74
	Present Study	4 (80)	1 (20)		4 (80)	0		3 (60)	0	
SH1	BALZAC CS	2 (40)	3 (60)	0.42		5 (100)	1	0	5 (100)	1
	Present Study	1 (16.7)	5 (83.3)		1 (16.7)	5 (83.3)		1 (16.7)	5 (83.3)	
SH2	BALZAC CS	2 (40)	3 (60)	0.65	0	5 (100)	1	0	5 (100)	1
	Present Study	2 (33.3)	4 (66.7)		1 (16.7)	5 (83.3)		0	6 (100)	
SH3	BALZAC CS	4 (66.7)	2 (33.3)	0.28	2 (33.3)	4 (66.7)	0.73	2 (33.3)	4 (66.7)	0.73
	Present Study	2 (33.3)	4 (66.7)		2 (33.3)	4 (66.7)		2 (33.3)	4 (66.7)	
SH4	BALZAC CS	2 (100)		0.21	0	2 (100)	1	0	2 (100)	1
	Present Study	2 (33.3)	4 (66.7)		2 (33.3)	4 (66.7)		0	6 (100)	
SH5	BALZAC CS	2 (50)	2 (50)	0.53	0	4 (100)	1	0	4 (100)	1
	Present Study	1 (16.7)	5 (83.3)		0	6 (100)		1 (16.7)	5 (83.3)	

Chapter 5: Discussion

Live bird markets are considered hubs for the dissemination of infectious disease pathogens (Meyer et al., 2018) and thus potential sources of AI in Bangladesh. Live birds are coming to consumers through complex transaction chains (Desvaux et al., 2014). Therefore, it is more likely that the birds can spread pathogens in various environments through the transaction chain. The present study was conducted to describe the transaction chains of deshi chickens and ducks and assess the risk release of AIVs in LBM of CCC. This chapter discusses significant findings of the current study with implications and limitations.

5.1 Transaction Chains

Regardless of poultry species, a maximum of 3 middlemen were identified to be involved in the deshi chickens or ducks transaction chain in the present study. This number of middlemen is higher than number of middlemen in the transaction chain of industrial poultry (broiler, sonali and spent hens) in Bangladesh with a maximum of 2 middlemen. These findings are sensible, as deshi chickens and ducks trading ends up at long-distance destination LBMs, whereas industrial broilers usually end up at short-distance destination LBMs. This explanation is supported by previous studies (Fournié et al., 2012; Begum et al., 2013; Moyen et al., 2018). This result also suggests that the deshi chickens and ducks transaction chains can increase the risk of transmission of AIVs along the chain more than that of industrial poultry transaction chain due to its involvement of multiple actors (MM1, MM2, MM3, W1, W2, SH, etc.) from the farm gate to the LBMs, mixing with the different types of poultry as well as travelling long distance before arriving at CCC LBMs.

Middleman1 (MM1), who buys poultry directly from different household farms or other sources, was identified as an important player in supplying deshi chickens, due to his continuous movement to the households and/or local villages and upazilla markets for the collection of deshi chickens and ducks, before selling them to retailers and W1. M2 could also purchase from MM1 and farms. In this sense, poultry brought by the MM2 was necessarily handled by an MM1.

In the transaction chain of the surveyed LBMs, we found that there are many intermediaries involved before poultry are received by the first group of traders (wholesaler(s) and or retailer(s).

Between W1 and W2, W1 was identified as an important player in the trading chain due to the fact that W1 received poultry from more than one middleman type (MM1, MM2 and MM3) at the same time. W1 also traded poultry to W2 at the same or at different markets, or to retailers, at the same or different markets, or even to MM3 or mobile vendors. Therefore, this creates a complex poultry business, connected with multiple traders and end consumers that can lead to the introduction and spread of AIVs to different places and between traders and consumers.

Regardless of poultry species, end consumers mostly prefer retailers over wholesalers to purchase birds which could be due to the unavailability of slaughter facilities at wholesaler stalls. End consumers usually buy live poultry and get them slaughtered at poultry stalls in front of them. Therefore, any zoonotic disease transmission like AI can occur more likely at retailer poultry stalls.

Mixing of deshi chickens at storage and during transportation and at poultry stalls (wholesalers and retailers) from multiple sources is common. However, it depends on seasons. For example, during the rainy season deshi chicken and duck mixing might be more common than during other seasons. Similar practices were reported in Indonesia (Indriani et al., 2010). The general notion is that mixing different poultry at different places and times can increase the risk of infection and spreading of AIVs.

The present study also explored that there had not been any biosecurity or hygienic measure undertaken during the dash chicken and duck transaction chains from the farm gate to the end consumers. This has previously been reported in Vietnam and Cambodia (Fournié et al., 2012). Lack of implementation of biosecurity or hygienic measures can lead to the transmission of AIVs at the different points of the complex chain which may lead to human transmission of AI.

The present study also found that there was no proper disinfection of the cages or of the vehicles used to transport deshi chickens and ducks. Moreover, traders visit several farms or markets and trade birds of different origins. A similar scenario was found in earlier studies in Vietnam (Fournié et al., 2012; Desvaux et al., 2014).

Infected birds could transmit the infectious agent to the healthy birds and assume a part in the spread of infection (Fournié et al., 2012) through this transaction chain.

5.2 Release Assessment

The first pathway P1 **(Table 4.1)** based on poultry being infected at the farm suggested that the probability of risk of transmission of AIVs infection to the LBMs in CCC was high. In the P1 poultry becomes infected by AI at farm can survives during storage, transportation and remain infectious and shedding virus to the susceptible healthy birds by the time it arrives at the CCC LBMs. Published literature suggests that poultry, particularly indigenous chickens infected by AIVs can survive more than 24 hours (Negovetich et al., 2011; Turner et al., 2017).In the present study we found the time required to collect, storage and transport poultry from the source of origin (farms) to the CCC, LBMs (including collection, storage and transportation time) is 37.3 hours (95% CI: 33.1-41.6),thus infected poultry may have the probability of risk of AIVs transmission was considered as high. Infected ducks may however escape from dying, as this species is considered as a natural reservoir of AIVs (Kim et al., 2009). Moreover, there might be newly infected poultry getting infection from affected deshi chicken and ducks at storage and or during transportation due to the mixing with different poultry types and contaminated vehicle can also enter as infectious poultry at CCC, LBMs and spread infection. Even infected deshi chicken in the farm along with newly infected chicken remain infectious upon arrival at CCC, LBMs, which could increase a high risk of transmission of AI infection to the healthy poultry at CCC LMBs. Not many similar literatures in the earlier study were found to be compared with the present findings.

The second pathway P2 **(Table 4.2)** based on poultry not being infected at the farm, but becoming infected during storage and transportation due to MM collect deshi chickens from diverse origins and all are mix together and store chickens for long periods meaning chickens have time to exchange viruses and middlemen also did not clean storing areas thus the storing areas become great places for viral re-assortment. In addition, mixing of chickens may expose susceptible chickens to viruses have not seen before, therefore chicken are more susceptible and acquire AIVs more easily and shed more virus after at arrival at CCC LBMs. The present study found time required to collect, storage and transport poultry from the source of origin to the LBMs

(including collection, storage and transportation) is 37.3 hours (95% CI: 33.1-41.6) suggested that the probability of risk of transmission of AIVs in the ***P2 a***t CCC LBMs was low, medium and high **(Appendix D)** depending upon when and where and which steps in the ***P2*** the susceptible deshi chickens become infected and how likely is a deshi chickens and ducks to survive after getting infection and release AIVs to the susceptible poultry at CCC LBMs.

Among those the probability of risk of transmission of AI (HPAI) infection to the destination LBMs in CCC has a considerable chance of humans and susceptible poultry and poultry stall environment to be exposed to AIVs infection (Tantawiwattananon et al., 2017; Singh et al., 2018). As a result, bird-to-bird transmission, poultry mortality, bird-to-human transmission and rarely human-to-human transmission can occur (Hayden and Croisier, 2005; Hsieh et al., 2014), and novel or pandemic avian influenza can develop (Fuller et al., 2013; Monne et al., 2013; Gerloff et al., 2014; Parvin et al., 2014).

5.3 Environmental Sampling

Environmental sample result suggests that 100% LBMs that is 6 identical LBMs in both studies (The present study and BALZAC cross sectional study) were found positive. 26.3% of the environmental samples taken from different sites (**Appendix-C)** at entry of the LBMs in CCC were found to be AIVs positive in the present study. The overall prevalence of AIV in the environmental sample was 26.3% for M gene, 19.5% for H5 and 12.8% for H9 (95% CI: N=44). This suggests that poultry get infected by AIVs before entering the LBMs. This finding corresponds to earlier studies, with a variable prevalence of AIV infection: 37% (M-gene) in Indonesia (Indriani et al., 2010), 32.2% (H5) in Vietnam, 10% (M) and 6.8% (H9) in South Korea (Leung et al., 2012), 4.4% (H9) in Hong Kong (Shortridge, 1999), and 12.4% (H5) in Egypt (Abdelwhab et al., 2010). The environmental sample of the present study found an rt-PCR result AIVs subtype H5 (19.8%) and H9 (12.5%) positive was supported by an earlier study in Chittagong, which was conducted in the stall within LBMs (Sayeed et al., 2017).

When AIVs prevalence and the subtypes in the present study were compared with the findings of the previously conducted cross sectional study by the BALZAC research group in the identical LBMs, where sampling was performed from the environmental

sides inside of the LBMs, There was no statistically significant difference, comparing the results of the two studies (Fisher's exact test yields p-0.81). This comparison indicates that poultry might have been infected by AIVs at storage and during transportation. However, this conclusion will cautiously be considered, as the studies were conducted at different time periods and the sample size was small.

5.4 Limitations of the Study

Only 2 LBMs were chosen for the present study, which does not represent all LBMs in CCC. However, these two markets were selected based on the highest buying and selling record of deshi chicken and ducks (FAO, 2015).

The majority of the interviews (>65%) were conducted distantly by cell phone, because the MM and SH were very busy during their working hours. Therefore, cell phone-based interviews might have produced information bias such as recall bias and record bias of the informants, misclassification, etc., though necessary measures were taken to have their full cooperation such as providing cell phone credit equivalent to BDT 150 per participant as an incentive.

Even though random technique was followed to participant for the study some selection bias may have occurred in selecting middlemen, because this was only done by getting their information from retailers and wholesalers.

In risk analysis, exposure and consequence assessment was not performed due to time and money constraints, which were one of the limitations of the present study.

Diagnostic error is a problem in epidemiological studies. However, in this study a high-quality RT-PCR, Sensitivity: 99.5% and specificity: 88.2% (Monne et al., 2008) and experienced laboratory technician was used to minimize the diagnostic error.

A small sample size to evaluate environmental AIV contamination was another limitation of the study which is due to financial constraints. Comparison of the result of environmental contamination in the present study (January-2018) with the previous study conducted by BALZAC research group (February to March-2016) may have been confounded by the time. Therefore, interpretation of the comparative results should be considered cautiously.

5.5 Conclusion

Regardless of poultry species, maximum 3 middlemen were identified to be involved in transaction chain in the present study. MM1 and W1 were identified as important players in supplying poultry to the markets. End consumers at LBMs mostly prefer retailers to purchase birds. Mixing of deshi chickens and duck at storage and during transportation and at poultry stalls from multiple sources was common. The present study found that there had not been any biosecurity or hygienic measure undertaken for cages and vehicles during the whole poultry transaction chain.

The probability of release of AI (HPAI) infection to the LBMs in CCC was high (based on the first pathway) and low, medium and high depending (based on the different step on the second pathway).100% of the study LBMs and 26.3% environmental samples taken from different sites at entry of CCC LBMs had AIVs positives. No significant difference of the proportion of M gene or H5 or H9 was observed between the current study and an earlier BALZAC study. It is therefore likely that environment through which deshi chickens and ducks transit prior to their arrival at CCC LBMs was contaminated with AIVs and its subtype of H5 and H9.

5.6 Recommendations

To prevent AI (HPAI) infection at different points, at farms, storage and during transportation, necessary hygienic and biosecurity measures should strictly be taken at those places including the vehicles used.

Storing poultry collected from different sources in common places might not be preventable, but poultry species specific storage and transportation to the destination LBMs might be possible, which could reduce the risk of AI infection and the release of AI infection to the LBMs in Chittagong. If poultry died at farms, storage and during transportation to the destination LBMs, then those dead birds should be properly disposed.

Avoidance of mixing different poultry species in retailer or wholesaler poultry stalls in LBMs, strictly maintaining hygienic and biosecurity measures and quarantine facilities for sick poultry and proper disposal of dead birds at poultry stalls or LBMs as a whole could reduce the chance of infecting LBM environment, susceptible poultry and humans.

Personal hygiene and protection can also reduce the probability of spreading AI infection from one place to another place during trading poultry.

In conclusion, control interventions can be effective. Future studies should aim to be complemented by a reinforcement of biosecurity measures at farm level and also during poultry transportation from farms to LBMs, with the aim to reduce the viral load introduced into LBMs. Biosecurity is also needed to be reinforced by the stall owner in order to prevent further transmission to human.

5.7 Future Directions

Future studies are recommended with bigger sample sizes covering wider LBMs for assessing the stability of the transaction chains and infection levels over time. Again, the author wants to provide the following directions for the future:

1. Improving the sample strategy should be considered in order to get detailed flow diagrams to show the possible risk of transmission of AIVs.
2. A longitudinal or repeated cross-sectional study with a bigger sample size should be conducted to assess the variability of the transaction chain over time
3. Carry out an assessment of comprehensive risk analysis of AIV in the entire deshi chickens and ducks network
4. The future study should may be conducted by isolating and disinfecting LBMs and examine them before carrying out birds for selling. The birds will be tested during their introduction to the market. The market should be monitored at regular intervals, so that it would be possible to identify the actual risk factor.

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Short Biography

Rashed Mahmud passed the Secondary School Certificate Examination (SSC) in 1993 obtaining first class with star marks (82%) and then Higher Secondary Certificate Examination (HSC) in 1995 obtaining first class with star marks (78%). Mr. Mahmud obtained his Doctor of Veterinary Medicine Degree in 2001 (held in 2004) from Chittagong Government Veterinary College (CGVC) obtaining GPA 3.64 (In the scale of 4) now; he is a candidate for the degree of MSc in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU. He is immensely interested in conducting research on avian influenza epidemiology and live bird trading networks and Anthropology

Appendix A: Stallholder Questionnaire

Characteristics of Deshi chicken Chicken and Duck Trading Patterns and their Impact on Zoonotic Disease Transmission

Date of interview:

Market of interview:

City: Thana:

Create questionnaire ID as follows:

SH				
SH	2 letters	4 characters	3 letters	3 numbers
Stall-holder	Interviewer initials	Interviewee code,	Month	Incremental number of interviews carried out by this interviewer,

Informed consent / *INSTRUCTIONS:*
For this questionnaire, it is very important that we interview the owner or manager of the poultry business so please first ask the poultry vendor at a stall if you can speak to the owner or manager at their business. Please explain to him/her that we are carrying out a survey in order to understand deshi chicken chicken and duck trading practices and networks. To do so, we will them questions about the deshi chicken chickens and ducks they bought and sold over the past few days. We are working at Chittagong Veterinary and Animal Science University and the Royal Veterinary College (London). Their answers will remain anonymous and their personal details will not be shared with anybody else without their consent, neither will they appear in a report. They are free to refuse to answer this questionnaire.

Instructions for the questionnaire: Write down the day of the week the interview relates to at the top of each page. Contact details (below) to be removed from the paper questionnaire as soon as the interviewee code has been devised.

If the person refuses to participate or drops out please explain why here below.
Ask the questions first about the TWO last times the interviewee bought deshi chicken chickens and ducks.

Is this interview taking place (circle one option): face to face /over the phone

Name of the interviewee:
Phone numbers of interviewee:

SECTION A

A1: What time does the stall-holder start selling poultry?

_____

A2: What time does the stall-holder stop selling poultry?

_____

A3: How many days a week is your stall open?

_____

A4: How many of each poultry type did you sell per day in the past 7 days? *(Fill-in table below. Every cell should be filled, if a poultry type is not sold write "0")*:

Broilers	Sonali	deshi chicken	Ducks	Spent hens	Cockerels	

SECTION B/DESHI CHICKEN

B1: How many days a week do you buy deshi chicken?

_____

B2: How many days a week do you sell deshi chicken?

_____

<u>BUYING-last time</u>

B3: When was the last time you bought deshi chicken? Date: _____

B4: The last time you bought deshi chicken, where/from who did you buy your poultry? (fill-in table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi chicken and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Time at which the deshi chicken were purchased	Number of deshi chicken purchased

<u>BUYING- time before the last</u>

B3: When was the time before the last when you bought deshi chicken ? Date: _____

B.4 The time before the last time when you bought deshi chicken, where/from who did you buy your poultry? (fill-in table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi chicken and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Time at which the deshi chicken were purchased	Number of deshi chicken purchased

SURPLUS

B7.1: When you bought deshi for the last 2 times, how many times did you still have deshi left unsold? _____

B7.2: If B7.1 is not 0: how many deshi did you have left unsold? _____

B8: When you have unsold deshi at the end of the day where do they spend the night? _____

SELLING-last day

B9: When was the last day you sold deshi? Date: _____

B10: On that day, how many deshi did you sell to local consumers, hotels and restaurants *(not taken independently)?*_____

B10: On that day, how many deshi did you sell to other traders?_____
Trader = a person who sells poultry as his main activity, can be a broker, a wholesaler, a retailer, a middleman, a stall-holder, etc.

B11: If the interviewee sold to other traders (i.e., if the answer to question B10 is NOT 0), then fill in the table below:

Trader to whom the interviewee sold (write name, address, contact details) If the buyers were stall-holders in other markets write "SH in MARKET NAME"	Number of deshi the interviewee sold to the trader

SELLING-day before the last

B9: When was the day before the last that you sold deshi on? Date: _____

B10: On that day, how many deshi did you sell to local consumers, hotels and restaurants *(not taken independently)?*_____

B10: On that day, how many deshi did you sell to other traders? _____

Trader = a person who sells poultry as his main activity, can be a broker, a wholesaler, a retailer, a middleman, a stall-holder, etc.

B11: If the interviewee sold to other traders (i.e., if the answer to question B10 is NOT 0) fill-in the table below:

Trader to whom the interviewee sold (write name, address, contact details) If the buyers were stall-holders in other markets write "SH in MARKET NAME"	Number of deshi the interviewee sold to the trader

OTHER

B12: In the past month did you buy ducks and or deshi from other middlemen than the ones named above?

If yes please give the name and mobile no of the middlemen with whom you did business, as well as the frequency (on average) at which you do business with them.

Name and address	Phone number	Ducks or deshi	Buying frequency (how many times a week or a month does this interviewee buy from the MM)

SECTION C/DUCKS

C1: How many days a week do you buy ducks? _____

C2: How many days a week do you sell ducks? _____

BUYING-last time

C3: When was the last time you bought ducks? Date: _____

C4: The last time you bought ducks, where/from who did you buy your poultry? (fill-in table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Time at which the ducks were purchased	Number of ducks purchased

BUYING- time before the last

C3: When was the time before the last when you bought ducks? Date: _____

C4: The time before the last time when you bought ducks, where/from who did you buy your poultry? (fill-in table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Time at which the ducks were purchased	Number of ducks purchased

SURPLUS

C7.1: When you bought ducks for the last 2 times, how many times did you still have ducks left unsold? _____

C7.2: If C7.1 is not 0: how many ducks did you have left unsold? _____

C8: When you have unsold ducks at the end of the day where do they spend the night? _____

SELLING-last day

C9: When was the last day you sold ducks? Date: _____

C10: On that day, how many ducks did you sell to local consumers, hotels and restaurants *(not taken independently)?*_____

C10: On that day, how many ducks did you sell to other traders?_____
Trader = a person who sells poultry as his main activity, can be a broker, a wholesaler, a retailer, a middleman, a stall-holder, etc.

C11: If the interviewee sold to other traders (i.e., if the answer to question C10 is NOT 0) fill-in the table below:

Trader to whom the interviewee sold (write name, address, contact details) If the buyers were stall-holders in other markets write "SH in MARKET NAME"	Number of ducks the interviewee sold to the trader

SELLING-day before the last

C9: When was the day before the last that you sold ducks on? Date: _____

C10: On that day, how many ducks did you sell to local consumers, hotels and restaurants *(not taken independently)?*_____

C10: On that day, how many ducks did you sell to other traders? _____
Trader = a person who sells poultry as his main activity, can be a broker, a wholesaler, a retailer, a middleman, a stall-holder, etc.

C11: If the interviewee sold to other traders (i.e. if the answer to question C10 is NOT 0) fill-in the table below:

Trader to whom the interviewee sold (write name, address, contact details) If the buyers were stall-holders in other markets write "SH in MARKET NAME"	Number of ducks the interviewee sold to the trader

OTHER

C12: In the past month did you buy ducks and or ducks from other middlemen than the ones named above?

If yes please give the name and mobile no of the middlemen with whom you did business, as well as the frequency (on average) at which you do business with them.

Name and address	Phone number	Ducks or ducks	Buying frequency (how many times a week or a month does this interviewee buy from the MM)

SECTION D/RISK ASSESSMENT

D1.1: Were you responsible for transporting the deshi and ducks at any point during the last 2 times you bought deshi and ducks? *(Circle one option)* Yes No

D1.2: If D1.1 is YES, fill-in the table below

Means of transport used	Duration of transport	Was the vehicle cleaned before loading the deshi/ducks? (Y or N)	Was the vehicle cleaned after loading the deshi/ducks? (Y or N)

D1.3: If the interviewee said "YES" to one of the 2 questions in the last 2 columns of the table, how was the vehicle cleaned?

D1.3: If the interviewee said "YES" to one of the 2 questions in the last 2 columns of the table, how often is the vehicle cleaned?

D2: How many ducks do you transport at once (as a "batch")?

D3: How many deshi do you transport at once?

	Always	Almost every time	Almost never	Never	If not "Never", with how many other poultry are ducks and deshi mixed
Would you say that you mix ducks and deshi in the same vehicle?					
Are these deshi and ducks transported with other poultry types (in the same vehicle)					
Would you say that you carry same types of poultry from different sources (Farm /Middleman/wholesaler) in the same vehicle?					

	Are the poultry you buy always taken directly from where you pick them up to your stall? (Y/N)	If .NO, where do you store them first?	Which proportion do you store between purchase and sell?			How long do you store them for on average?				
			Less than 30%	30 to 60%	60 to 100%	Less than 6 hours	6 to 12 hours	12 to 24 hours	1 to 2 days	More, specify.
deshi										
Ducks										

D4: How many ducks die in transport on average (per day)? _____

D5: How many deshi die in transport on average (per day)? _____

D6: If any type of poultry dies during transport what do with it?

D7: If any type of poultry dies at your stall, what do you do with it?

D8: How many birds die at your stall per day on average? _____

D9: If any type of poultry arrives or becomes sick at your stall, what do you do with it?

D10: How many poultry show signs of disease per day on average? _____

D11: How many workers are working at this stall today? _____

D12: Among the workers present at this stall today, how many work:

Less than 3 hours a day	3 to 6 hours a day	6 to 9 hours a day	9 to 12 hours a day	More than 12 hours a day

D13: Among the workers present today, how many are involved in culling and preparing and cleaning? _____

Appendix B: Middleman Questionnaire

Characteristics of Deshi Chicken and Duck Trading Patterns and their Impact on Zoonotic Disease Transmission

Date of interview:
Market of interview:

City:
Thana:

Create questionnaire ID as follows:

MM				
MM	2 letters	4 characters	3 letters	3 numbers
Middleman	Interviewer initials	Interviewee code,	Month	Incremental number of interviews carried out by this interviewer,

Informed consent / *INSTRUCTIONS:*
For this questionnaire, it is very important that we interview the owner or manager of the poultry business so please first ask the poultry vendor at a stall if you can speak to the owner or manager at their business. Please explain to him/her that we are carrying out a survey in order to understand deshi chicken and duck trading practices and networks. To do so, we will them questions about the deshi chickens and ducks they bought and sold over the past few days. We are working at Chittagong Veterinary and Animal Science University and the Royal Veterinary College (London). Their answers will remain anonymous and their personal details will not be shared with anybody else without their consent, neither will they appear in a report. They are free to refuse to answer this questionnaire.

Instructions for the questionnaire: Contact details (below) to be removed from the paper questionnaire as soon as the interviewee code has been devised.

If the person refuses to participate or drops out please explain why here below.
Ask the questions first about the TWO last times the interviewee bought deshi chickens and ducks.

Is this interview taking place (circle one option): face to face / over the phone?

Name of the interviewee:

Phone numbers of interviewee:

SECTION A

A1: What time does the middleman start selling poultry?

_____

A2: What time does the middleman stop selling poultry?

_____

A3: In the past 7 days, how many days did you sell poultry (all types considered)? *(Fill-in table below. Every cell should be filled, if a poultry type is not sold write "0")*:

Broilers	Sonali	deshi	Ducks	Spent hens	Cockerels	

A4: In the past 7 days, how many days did you buy poultry (all types considered)? *(Fill-in table below. Every cell should be filled, if a poultry type is not sold write "0")*:

Broilers	Sonali	deshi	Ducks	Spent hens	Cockerels	

A5: How many of each poultry type did you sell per day in the past 7 days? *(Fill-in table below. Every cell should be filled, if a poultry type is not sold write "0")*:

Broilers	Sonali	deshi	Ducks	Spent hens	Cockerels	

SECTION B/DESHI

SELLING-last time

B1: When was the last time you sold deshi? Date: _____

B2: Where did you sell deshi on that day and at what price?

To whom the interviewee sold (write name, address, and contact details). For shops, hotels or restaurants write "shop", "hotel" or "restaurant" respectively and address, For markets indicate if the deshi were sold to stall-holders or middlemen, write the market name and address For private parties write "function" + city name	Number of deshi the interviewee sold to that client	Time at which the deshi were sold	Price at which they were sold

B3: Where did the deshi sold above come from and how much did they cost? (Fill-in the table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Date at which the deshi were purchased (=the date on which they were picked up at the farm/market NOT the date on which they arrived where they are sold)	Time at which the deshi were purchased (=the time at which they were picked up at the farm/market NOT the time at which they arrived where they are sold)	Number of deshi purchased	Price at which deshi were bought.

CHECK THAT THE SUM IN THIS TABLE IS = SUM IN PREVIOUS TABLE

B4: could you say exactly which origin supplied which selling place named in B2?
(Circle one option) Yes No
B4.1: If Yes, please explain:

B5: At the end of that selling day, did you have deshi unsold that you were hoping to sell? *(Circle one option)*
Yes No

B5.1: If Yes, how many did you have left unsold? _____
B5.2: If Yes, where did they stay? _____

<u>SELLING- time before the last</u>
B6: When was the time before the last when you sold deshi? Date_____

B7: Where did you sell deshi on that day and at what price?

To whom the interviewee sold (write name, address, and contact details). For shops, hotels or restaurants write "shop", "hotel" or "restaurant" respectively and address, For markets indicate if the deshi were sold to stall-holders or middlemen, write the market name and address For private parties write "function" + city name	Number of deshi the interviewee sold to that client	Time at which the deshi were sold	Price at which they were sold.

B8: Where did the deshi sold above come from? (Fill-in the table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" *If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included* *In this table write down the name and phone number of the suppliers identified.*	Date at which the deshi were purchased (=the date on which they were picked up at the farm/market NOT the date on which they arrived where they are sold)	Time at which the deshi were purchased (=the time at which they were picked up at the farm/market NOT the time at which they arrived where they are sold)	Number of deshi purchased	Price at which deshi were bought.

CHECK THAT THE SUM IN THIS TABLE IS = SUM IN PREVIOUS TABLE

B9: Could you say exactly which origin supplied which selling place named in B2? *(Circle one option)* Yes No

B9.1: If Yes, please explain:

B10: At the end of that selling day, did you have deshi unsold that you were hoping to sell? *(Circle one option)* Yes No

B10.1: If Yes, how many did you have left unsold? _____
B10.2: If Yes, where did they stay? _____

OTHER

B11.1: In the past month did you buy deshi from other suppliers than the ones named above?

If yes please give the name and mobile no of those suppliers with whom you did business, as well as the frequency (on average) at which you do business with them.

Name and address	Phone number	Buying frequency (how many times a week or a month does this interviewee buy from this supplier)

B11.2: In the past month did you sell deshi to the other market/ middleman/places than the ones named above?

If yes please give the name and mobile no of those with whom you did business with, as well as the frequency (on average) at which you do business with them

Name and address	Phone number	Selling frequency (how many times a week or a month does this interviewee buy from this supplier)

B12.1: How were the deshi transported to the selling points mentioned above, in table B13.2?

From	To	Means of transport used	Duration of transport	Was the vehicle cleaned before loading the deshi? (Y or N)	Was the vehicle cleaned after unloading the deshi? (Y or N)	Who was the owner of the transport vehicle? (you/other)	Transportation cost

B12.2: If “bus” is not mentioned as a means of transport in the table above:

B12.2.1: Do you sometimes send deshi by bus?

B12.2.2: If yes, how much do you pay for each shipment and to who?

B12.3: Are deshi from different origins mixed together during transport? (Y/N)_____

B12.4: Are deshi mixed with other poultry during transport? (Y/N) _____

B12.4.2: If yes, how frequently? _____

B12.4.3: If yes, with which poultry type? _____

B13: IN GENERAL, how many villages to you visit to collect enough deshi for one typical shipment? _____

B14: IN GENERAL, how many days does it take to collect enough deshi for one typical shipment? _____

B14.1: Therefore, in general, how many days do you estimate a deshi spends in stock with you between the moment it arrives and the moment it is sent off? _____

B14.2: Where do the deshi spend this "stocking time"? _____

B14.3: Are all the deshi (from different origins) mixed together during "stocking time"? (Y/N) _____

B15.1: How far apart from one another are the villages that contribute to one shipment? _____

B15.2: How far (minimum and maximum distances) from your office are the villages that contribute to one shipment? _____

B15.3: Overall, how big is the area you cover to collect deshi? _____

B15.4: Please name the upazilas you or your staff visit to collect deshi in the last month:

Name of the Upazila	District	Frequency of visit

SECTION C/DUCKS

SELLING-last time

C1: When was the last time you sold ducks? Date: _____

C2: Where did you sell ducks on that day and at what price?

To whom the interviewee sold (write name, address, and contact details). For shops, hotels or restaurants write "shop", "hotel" or "restaurant" respectively and address, For markets indicate if the ducks were sold to stall-holders or middlemen, write the market name and address For private parties write "function" + city name	Number of ducks the interviewee sold to that client	Time at which the ducks were sold	Price at which they were sold.

C3: Where did the ducks sold above come from and how much did they cost? (Fill-in the table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included *In this table write down the name and phone number of the suppliers identified.*	Date at which the ducks were purchased (=the date on which they were picked up at the farm/market NOT the date on which they arrived where they are sold)	Time at which the ducks were purchased (=the time at which they were picked up at the farm/market NOT the time at which they arrived where they are sold)	Number of ducks purchased	Price at which ducks were bought.

CHECK THAT THE SUM IN THIS TABLE IS = SUM IN PREVIOUS TABLE

C4: could you say exactly which origin supplied which selling place named in B2?

(Circle one option) Yes No

C4.1: If Yes, please explain:

C5: At the end of that selling day, did you have ducks unsold that you were hoping to sell? *(Circle one option)* Yes No

C5.1: If Yes, how many did you have left unsold? _____

C5.2: If Yes, where did they stay? _____

<u>SELLING- time before the last</u>

C6: When was the time before the last when you sold ducks? Date:_____

C7: Where did you sell ducks on that day and at what price?

To whom the interviewee sold (write name, address, and contact details). For shops, hotels or restaurants write "shop", "hotel" or "restaurant" respectively and address, For markets indicate if the ducks were sold to stall-holders or middlemen, write the market name and address For private parties write "function" + city name	Number of ducks the interviewee sold to that client	Time at which the ducks were sold	Price at which they were sold.

C8: Where did the ducks sold above come from? (Fill-in the table below)

Indicate place name (city, district, Upazila, Union, Ward and village). If the name of the village or city is unknown please give indications such as "10km north of city X" or "just next to city B" If poultry farm, indicate "PF" instead of place name. If market, indicate the market's name, if it is not known write "M". If the interviewee bought in the market of interview write "here". Be as precise as possible with the geographical location, so one line of the table = one union. Deshi and duck suppliers' names and phone numbers will be included *In this table write down the name and phone number of the suppliers identified.*	Date at which the ducks were purchased (=the date on which they were picked up at the farm/market NOT the date on which they arrived where they are sold)	Time at which the ducks were purchased (=the time at which they were picked up at the farm/market NOT the time at which they arrived where they are sold)	Number of ducks purchased	Price at which ducks were bought.

CHECK THAT THE SUM IN THIS TABLE IS = SUM IN PREVIOUS TABLE

C9: could you say exactly which origin supplied which selling place named in B2? *(Circle one option)* Yes No

C9.1: If Yes, please explain:

C10: At the end of that selling day, did you have ducks unsold that you were hoping to sell? *(Circle one option)* Yes No

C10.1: If Yes, how many did you have left unsold? _____

C10.2: If Yes, where did they stay? _____

OTHER

C11.1: In the past month did you buy ducks from other suppliers than the ones named above?

If yes please give the name and mobile no of those suppliers with whom you did business, as well as the frequency (on average) at which you do business with them.

Name and address	Phone number	Buying frequency (how many times a week or a month does this interviewee buy from this supplier)

C11.2: In the past month did you sell ducks to the other market/ middleman/places than the ones named above?

If yes please give the name and mobile no of those with whom you did business with, as well as the frequency (on average) at which you do business with them

Name and address	Phone number	Selling frequency (how many times a week or a month does this interviewee buy from this supplier)

C12.1: How were the ducks transported to the selling points mentioned above, in Table B13.2?

From	To	Means of transport used	Duration of transport	Was the vehicle cleaned before loading the ducks? (Y or N)	Was the vehicle cleaned after unloading the ducks? (Y or N)	Who was the owner of the transport vehicle? (you/other)	Transportation cost

C12.2: If "bus" is not mentioned as a means of transport in the table above:

C12.2.1: Do you sometimes send ducks by bus?

C12.2.2: If yes, how much do you pay for each shipment and to who?

C12.3: Are ducks from different origins mixed together during transport? (Y/N) ____

C12.4: Are ducks mixed with other poultry during transport? (Y/N)_____

C12.4.2: If yes, how frequently? _____

C12.4.3: If yes, with which poultry type? _____

C13: IN GENERAL, how many villages to you visit to collect enough ducks for one typical shipment? _____

C14: IN GENERAL, how many days does it take to collect enough ducks for one typical shipment? _____

C14.1: Therefore, in general, how many days do you estimate a deshi spends in stock with you between the moment it arrives and the moment it is sent off? _____

C14.2: Where do the ducks spend this "stocking time"? _____

C14.3: Are all the ducks (from different origins) mixed together during "stocking time"? (Y/N) _____

C15.1: How far apart from one another are the villages that contribute to one shipment? _____

C15.2: How far (minimum and maximum distances) from your office are the villages that contribute to one shipment? _____

C15.3: Overall, how big is the area you cover to collect ducks? _____

C15.4: Please name the upazilas you or your staff visit to collect ducks in the last month:

Name of the Upazila	District	Frequency of visit

SECTION D/BIOSECURITY

D1: How many deshi die in transport on average (per day)? _____

D2: How many ducks die in transport on average (per day)? _____

D3: If poultry dies during transport what do you do with it?

D4: If poultry dies at your storehouse, what do you do with it?

D5: How many poultry die at your storehouse per day on average? _____

D6: If poultry arrives or becomes sick at your storehouse, what do you do with it?

D7: How many poultry show signs of disease per day on average?

_____

D8: Do you or your employees wear any of the following when handling poultry? *Fill in the table below. Tick all the options that apply*

Gloves	Facial mask	Closed shoes	Apron	Other PPE, explain

OBSERVATIONS (STALL LEVEL)

1. Density of the poultry
    1.1. How many poultry stalls are adjacent to this stall (include the ones at the back of the stall) or across the aisle?

None	1 to 3	5 to 10	> 10

1.2. How many poultry, in total, are there on this stall? *(tick one option)*

1 to 10	10 to 50	50 to 100	> 100

2. Poultry housing
    2.1. How are the poultry kept? *(describe the housing per poultry type. Indicate if poultry are free roaming, in cages, etc. If poultry is kept in cages indicate what the cage/basket is made of)*
    2.2. Are different species of poultry kept together, in the same cages? *(Circle one option)* YES / NO

3. What is the flooring of the stall made of? *(tick all that apply)*

Tiles	Concrete	Earth	Other:

4. Is there solid waste present on the stall? *(Circle one option)* YES/NO
    if yes, roughly describe where (e.g. table, ground, bucket) and try to estimate how many buckets full
5. Is cleaning equipment visible? *(Circle one option)* YES/NO
6. Can you see sick poultry on the stall? *(Circle one option)* YES/NO
    6.1. If yes, how many sick birds can you see? _____
7. Does the stall have access to running water? *(Circle one option)*
    YES/NO
8. Are the people at the stall wearing any PPE? *Fill in the table below per category of worker. Tick all the options that apply*

	Gloves	Facial mask	Closed shoes	Apron	Another PPE, explain
Person selling/receiving the money from the client**					
Person slaughtering					
Person preparing					
Person feeding/giving water to the poultry					

9. Do stall workers wash their hands? Fill in the table below per category of worker. Tick all the options that apply

	Was not observed	Washed with water from a bucket	Washed with tap water	Used soap/or other detergent	Other, explain
Before selling poultry**					
After having sold poultry**					
Before slaughtering					
After slaughtering					
Before preparing					
After preparing					
After having fed and/or given water to the poultry					

** This excludes the slaughtering/preparing activities

10. Is poultry slaughtered at the stall? *(Circle one option)*

YES / NO

If no, skip this question

if yes fill-in the following tables

	Was not observed	On top of other poultry's cages	On the ground	In a bucket	On a specific table	Other, explain
Poultry slaughtered						
Poultry prepared						

	Was not observed	Blood/offal visible where poultry… (Y/N)	Materiel cleaning after slaughtering or preparing *(tick all that apply)*					
			Materiel not cleaned	Cleaned with tap water	Cleaned with water from a bucket	Cleaned with soap/other detergent	Wiped with cloth/towel	Other, explain
Poultry slaughtered								
Poultry prepared								

Appendix C: Sampling Sites and LBM Codes

Market Code (ID number)

Riazuddin Kasha Bazaar: 038

Pahartoli Bazaar: 036

Bahaddarhat Bazar: 006

Kazirdauri Kacha Bazaar: 032

CDA Karnafully: 018

Karnafully Complex: 030

MM store house: M01, M02, M03.....

Origin:

Environment	E

Site type:

Poultry drinking water	SH1
Poultry basket/cage floor (where droppings can be seen)	SH2 (if no cages: SH0)
Poultry basket/cage walls	SH3
Floor of rickshaw van, where poultry basket was standing	SH4
Poultry storing place/stall where poultry were kept before delivery to the Stall holder	SH5
Floor of the store house	SH6
Vehicle used to bring poultry to the store house (floor, where feathers and droppings can be seen)	SH7

Pool reference: the number of the pool collected for a given type of environmental site

Appendix D: Definitions of Qualitative Risk

Negligible	Risk or frequency/consequence is so low as to not merit consideration
Very Low	Risk or frequency/consequence is almost negligible, but due to uncertainty or other extenuating circumstances cannot be excluded from consideration
Low	Risk or frequency/consequence is small/infrequent, but still worth considering intervention / mitigation
Medium	Occurs frequently, or event associated with a modest consequence
High	Event occurs often, and/ or is associated with a significant consequence
Very High	Event occurs almost certainly, and/ or is associated with a serious consequence