Poultry Trading and Farm Biosecurity Status: Introduction of Avian Influenza to Broiler Farms in Chittagong, Bangladesh

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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June 2018

I dedicate this MS thesis

to my beloved parents

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
BBS	Bangladesh Bureau of Statistics
CVASU	Chittagong Veterinary and Animal Sciences University
GDP	Gross Domestic Product
HPAI	Highly Pathogenic Avian Influenza
LBM	Live Bird Market
LPAI	Low Pathogenic Avian Influenza
M-gene	Matrix gene
OIE	Office International des Epizooties (World Organization for Animal Health)
RNA	Ribo Nucleic Acid
rt-PCR	Reverse Transcriptase Polymerase Chain Reaction
WHO	World Health Organization

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Abstract

To meet Bangladesh's ever-growing protein demands, the poultry industry has dramatically increased its intensive farming practices, contributing significantly to its share of the gross domestic product. However, infectious diseases have greatly threatened the stability of the poultry industry. Poultry farmers largely rely on vaccination protocols to prevent and control of infectious diseases and do not consider typically farm hygiene and other biosecurity practices for this purpose. Therefore, the present study was conducted to assess the role of poultry trading and broiler farm biosecurity status in the occurrence of avian influenza on broiler farms in Chittagong, Bangladesh. Biosecurity ***Principle 1 Isolation*** requires farms to be at a certain distance from different objects: Neighbouring poultry farms, backyard poultry farms, live bird markets, residential areas, waste disposal facilities, ponds, water bodies and large trees. However, 46-98% of broiler farms (N=39) did not meet this requirement. Under ***Principle 1***, the majority of the broiler farms did not have a protective fence, main lockable gates, bird proof netting (67%) and a safe dead bird disposal system (67-74%). A risky practice observed during this study was farm personnel visiting other farms, affected during a disease outbreak (23%). Many farms (33-82%) did not have pest management or other animal control systems in place. Biosecurity ***Principle 2 Good Farm Hygiene*** was observed at the majority of farms, such as "used litter" not stored near clean litter (72%), litter removal equipment disinfected properly after each use (85%), sheds swept thoroughly after litter removal (100%), letting the shed to dry $\geq$ 2 weeks after cleaning and final disinfection (84%). Personal hygiene practices on farms were reasonable such as: a) mandated employee washing and changing clothes (54%), b) separate pairs of sandals must be used for each shed (54%) and c) hands must be cleaned before and after use (55%). However, most of the farms did not have foot baths (92%). So, hygienic conditions were not maintained. Biosecurity ***Principle 3 Good Farm Management Practices*** was observed to be lacking on many farms with 56-67% farms not having a structured paper-based record keeping system. However, the all-in-all-out system principle was followed for 92% farms. After-trading had no effect on the introduction of AI to the studied farms. Only H9 subtype was found in the study. Items and/or practices involving biosecurity *Principles 1 and 3* need substantial improvement to prevent the introduction of infectious poultry diseases like avian influenza.

***Keywords*:** Biosecurity, Trading, Avian Influenza, Broiler farm, Chittagong

Chapter 1: Introduction

Livestock holds a key position in the 2030 Agenda for Sustainable Development (UN, 2015). The share of livestock to the agricultural Gross Domestic Product, GDP, is 18.6% in which 3.2% of GDP is from the poultry industry in Bangladesh (MoP, 2015). The poultry industry has grown extensively over several decades. In 2013, there were 77,880 registered commercial live poultry farms in Bangladesh (broiler, layer and duck farms)(MoP, 2015) The total number of registered broiler farms was 53,112 of which 7,819 was in Chittagong division (Hamid et al., 2017). The poultry sector provides significant supply to meet the protein demand for many people in Bangladesh. Infectious disease, its control, and unsanitary poultry trading pose significant challenges to this sector (Sultana et al., 2012).

Common poultry diseases include avian influenza (AI) Newcastle disease, infectious laryngotracheitis, infectious bursal disease, colibacillosis and salmonellosis. Of these, AI – particularly highly pathogenic avian influenza (HPAI) H5N1 –has caused large disease outbreaks with high morbidity and mortality and significant economic losses. Between 2007 and 2015 the number of total outbreaks due to HPAI H5N1 has been estimated to be 583 and 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, 2017a; OIE, 2017b). Moreover, the H5N1 subtype virus has zoonotic potential and may cause human mortality (Heine et al., 2015). In particular, occurrence of AI due to the H9 subtype has been found in different poultry sectors in Bangladesh and has caused production loss and mortality, yet this subtype does not hold zoonotic potential (Hassan et al., 2017).

Transmission of AI,H5and H9,occurs via the following routes: direct contact with poultry or indirectly through exposure to contaminated faecal materials or through aerosols, water, feed and bedding materials and utensils (de Jong and Hien, 2006; Zhou et al.,2016; Fournié et al., 2017).

There are different approaches to controlling HPAI H5N1, such as vaccination, farm hygiene and farm biosecurity. In Bangladesh, vaccination against H5N1 is widely used in commercial poultry farm throughout the country. Therefore, other control and preventive approaches are of utmost importance. However, generally the existing

standard of farm hygiene and biosecurity remains sub-standard in Bangladesh (Rimi et al., 2017) with farmers rarely following a standard farm hygiene and biosecurity guideline. A poultry biosecurity guideline has recently been developed by the Department of Livestock Services of Bangladesh (DLS, 2011), but it needs to be validated and adjusted in a regional context of Bangladesh by conducting a field study in Chittagong. Consequently, this case study aimed to assess the biosecurity status of broiler poultry farms in Chittagong in line with the DLS biosecurity guideline.

Broiler poultry trading predominantly occurs directly at the farm level with middle-men traders, usually purchasing saleable poultry at the farm gate and selling them to varying local or city live bird markets. This study assesses the risk of introduction of AI to farm through poultry trading.

1.1 Specific objectives of the study

Targeting the commercial live bird sector in Chittagong, Bangladesh, the specific study objectives were:

1.1.1To *determine the status of broiler farm biosecurity* in the light of DLS guideline

1.1.2To *assess how farm poultry trading affects the introduction of avian influenza to broiler farms in Chittagong district*

1.1.3 To *estimate the proportionate prevalence of avian influenza and its H5 and H9 subtypes at farms*

1.2 Outcomes

1.2.1*Determined broiler farm biosecurity practices in Chittagong district*, compared to the DLS biosecurity guideline (DLS, 2011)

1.2.2 *Identified the role of farm trading in introducing avian influenza to farms* in Chittagong district

1.2.3 *Estimated farm level proportionate prevalence of avian influenza and its selective subtypes* in Chittagong district

Chapter 2: Literature Review

The literature for the current study covers *poultry population*, *poultry rearing challenges*, *common diseases*, with particular reference to AI, *transmission and prevalence of AI and associated risk factors and consequences and control approaches*, that is, vaccination, farm hygiene and biosecurity, and *farm poultry trading practices*. The objectives of this chapter were to discuss the necessary data from previous studies to identify knowledge gaps and justify the present Master's research. The literature was found by searching PubMed, Google Scholar, printed papers and hard copy journal articles. The findings of importance in published and unpublished articles are introduced below.

Livestock holds a key position in the 2030 Agenda for Sustainable Development (UN, 2015). The share of livestock to the agricultural Gross Domestic Product, GDP, is 18.6% (MoP, 2015). The poultry industry has grown extensively over several decades. In 2013, Bangladesh had 77,880 registered commercial live poultry farms (broiler, layer and duck farms). The total number of registered broiler farms in Bangladesh was 53,112, 7,819 of them in Chittagong Division (Hamid et al., 2017).The most common farm types are small-scale farms with 500-2,000 chickens and medium-scale farms with 3,000-20,000 chickens (Hamid et al., 2017). This poultry sector provides significant supply to meet the protein demand for many people in Bangladesh. Infectious disease, its control, and unsanitary poultry trading pose significant challenges to the live bird sector (Sultana et al., 2012; Shamsuddoha, 2015).

Common poultry diseases include avian influenza, Newcastle disease, infectious laryngotracheitis, infectious bursal disease, colibacillosis and salmonellosis (Barua and Yoshimura, 2007; Conan et al., 2012). Of these, AIVs– particularly HPAI H5N1 – belonging to the *Orthomyxoviridae* family, cause large disease outbreaks, high morbidity and mortality and significant economic losses. Between 2007 and 2015 the number of total outbreaks due to HPAI H5N1 has been estimated to be 583 and 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, 2017a; OIE, 2017b). The H5N1 subtype virus has zoonotic potential and may cause human mortality (Heine et al., 2015). In particular, occurrence of AI due to the H9 subtype has been found in different poultry sectors in Bangladesh and has caused production loss – for example

drop in egg production – and mortality, but this subtype does not hold zoonotic potential (Hassan et al., 2017).

Transmission of H5N1 and H9 occurs by different means. Poultry-to-poultry transmission occurs through direct contact or indirectly by exposure to contaminated faecal material or through aerosols, water, feed, 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 live bird markets* (Van Kerkhove et al., 2011). Direct or indirect contact with infected wild poultry resident or migratory birds is suggested as the most likely pathway of exposure of deshi and ducks to AIVs (Alexander, 2000; Fouchier and Munster, 2009; Yee et al., 2009).

Eight human beings have been infected with H5N1 in Bangladesh, children and poultry workers, since 2008. Six H5N1 and 2 H9N2 cases were reported in Dhaka City South. The 2-year old boy infected with H5N1 in 2013 in Comilla district died. The latest H5N1 case in October 2015 in Mymensingh district involved a 60-year old man who survived. The persons infected were exposed to live, sick or dead poultry, slaughtering or meat preparation (WHO, 2008; Brooks et al., 2009; ICDDRB, 2011; WHO, 2011a; WHO, 2011b; IEDCR, 2012a; IEDCR, 2012b; ICDDRB, 2013; WHO, 2013; WHO, 2015; WHO, 2016).

The prevalence of AI and its main subtypes H5 and H9 at broiler farm level has been documented as 5.0-7.5% (AI), 0-0% (H5), 1.9-2.0% (H9) and 3-5.6.0% (Un-type) (Personal Communication, Prof Md. Ahasanul Hoque, National Coordinator of BALZAC Project). Other reported AI subtypes at farm level were 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 (Pant and Selleck, 2007; Negovetich et al., 2011; Gerloff et al., 2014; Biswas et al., 2017). Farm level risk factors associated with AI/H5N1/H9 have been reported as follows: surface water on farm, presence of open water reservoir on farm, dense vegetation around farm, presence of tall trees around farm, husbandry practices, allowing rodents/wild birds to access poultry feed, vaccination of birds by the commercial vaccinator,

allowing middlemen to entrance in the farm premises, presence of free range and water birds on farm (ducks, geese, local chickens, etc.) (Shapiro and Stewart-Brown, 2009; Chowdhury et al., 2015; Alhaji and Yatswako, 2017; Rimi et al., 2017; Singh et al., 2018). Broiler poultry trading predominantly occurs directly at the farm level. Middlemen traders ask their employees to collect poultry in trucks at the farms and then transport them to varying local and city live bird markets.

There are different approaches to controlling HPAI H5N1 such as vaccination, farm hygiene and farm biosecurity. In Bangladesh, vaccination against H5N1 is widely used in commercial farm, because many users doubt the effectiveness of the available vaccines. Therefore, other control and preventive approaches are of utmost importance. However, generally the existing standard of farm hygiene and biosecurity is sub-standard in Bangladesh (Rimi et al., 2017). In spite of mass vaccination against H5N1,in commercial chicken,H5N1 is still circulating in commercial poultry this indicates that current vaccination programme is not properly working to reduce HPAI viruses transmission among poultry population. Therefore proper biosecurity measures are necessary. Farmers rarely follow a standard farm hygiene and biosecurity guideline. Farm hygiene and biosecurity practices are wanting: *lack of fencing, lack of foot bath, workers not using personal protective equipment, no quarantine system in place for sick birds, allowing traders to enter the farm premises, lack of proper disposal of dead birds* and *the use of different vaccine brands in the same flock* (Ibrahim et al., 2016).In Bangladesh, *Vectormune HVT AIV by CEVA* is used by hatchery owners for day old chicks (Personal Communication, Dr. Md. Moynul Hossain, Business Manager, ACI Animal Health of Bangladesh).

In conclusion, the literature suggests scientific information gaps about hygienic and biosecurity practices in the commercial poultry farming systems in Bangladesh. This includes the lack of scientific knowledge about farm level trading practices and associated roles in introducing avian influenza to farms. Therefore, this case study aimed to assess the biosecurity status at broiler poultry farms in Chittagong, in line with the DLS biosecurity guideline (DLS, 2011).We examined whether these farm trading activities may cause the introduction of avian influenza to broiler farms.

Chapter 3: Materials and Methods

3.1 Broiler Poultry Farm Selection Plan for Biosecurity Assessment

Broiler farms from *Sitakundo, Raozan* and *Patiya,* three Chittagong sub-districts, and one Chittagong metro Thana, *Chandgoan,* corresponding to the previous BALZAC AI study, were considered for this study between March and May 2018. These study areas were selected because of their close proximity to Chittagong City and for the fact that most of the broilers supplied here originate from the local region.

A complete list of farms, including farm size, is not available. The list developed by the previous BALZAC study (N=100 farms) was therefore used to randomly select the required number of farms. A broiler farm consisting of at least 500 birds was considered as the smallest epidemiological unit. A total of 50 farms were needed for the study. However, we were only able to recruit 39 farms due to the non-response of 11 farmers. The following formula and assumptions were used to calculate the sample size:

Sample size $\boldsymbol{n = [DEFF*Np\ (1-p)]/\ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]}$

Assumptions were:

Population size (for finite population correction factor)(*N*): 100

Hypothesized % frequency of outcome factor in the population (*p*): 50% ±10%

Confidence limits as % of 100(absolute +/- %) (*d*): 10%

Source: (OpenEpi, 2013)

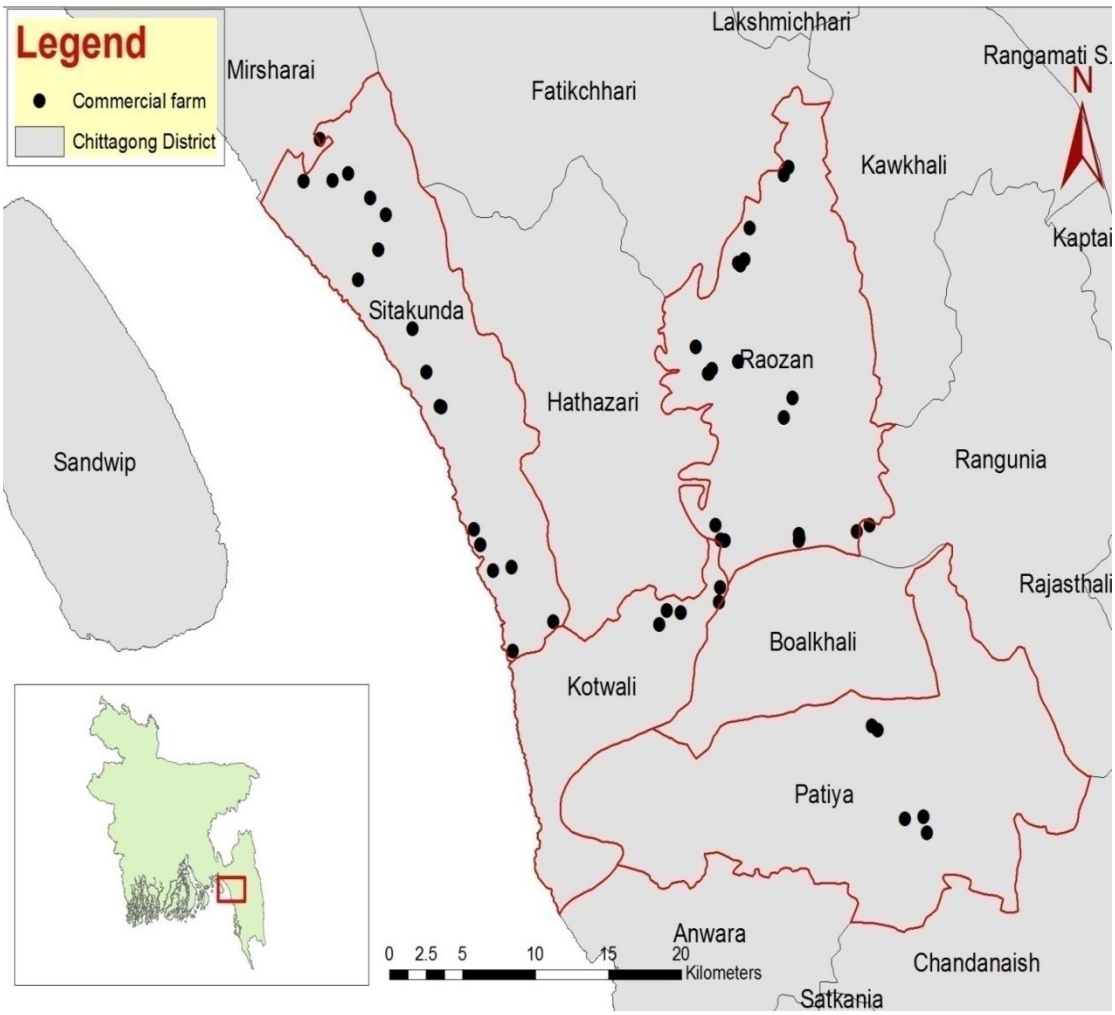

Figure 3.1: Farm locations in the study areas

3.2 Data Collection

A questionnaire was developed based on the DLS biosecurity guideline (DLS, 2011). The questionnaire contained 4 different sections: **Section A**: Contact information, **Section B**: Farm Type and Composition, **Section C**: Demographic information and **Section D**: Biosecurity assessment questions for scoring. The biosecurity section had three different principles:

Principle 1 Isolation in terms of farm location and characteristics, traffic on and off the farm and pest and another animal management

Principle 2Good Farm Hygiene, including apparel cleaning, personal hygiene, and house cleaning and disinfection

Principle 3 Good Farm Management Practices, such as a paper recording system

Both closed and open questions were included in the questionnaire. The questionnaire was pre-tested and refined using 3 randomly selected farms before the start of the main study. The pilot farms were not considered for the main study. The detailed questionnaire is given as Appendix 1.

A survey team comprising 3 members was formed to collect data. Each member visited 3 farms per day with each interview taking approximately 45 minutes. A total of 5 working days were required to complete the survey.

3.3 Sampling Plan

Biological sampling was collected from 30 broiler farms used in the biosecurity assessment study. The farms were selected from Sitakundo, Raozan and Patiya, three sub-districts of Chittagong in August and September 2018.The farms sampled had at least 500 birds per farm at the end of a production cycle. The farms were also required to have sold out its entire flock of chickens at least twice before sampling. Farms were sampled twice during the study: the first session *before trading* and the second session *at least 48 hours after trading*. We took cloacal and oropharyngeal swab samples from ten birds and collected five faecal swabs from environment during each session at each of the farms. Samples were then pooled by swab type and farm type with each pool consisting of 5 swabs. Accordingly, there were 5 pools per farm and 150 pools for 30 farms per session and 300 pools for the first and the second sessions combined.

For this study, a four-member team collected samples and basic farm and trading information. Communication with selected farmers via phone was used for sample collection prior to poultry trading. The middlemen were also approached to prepare a calendar containing specific dates and times of poultry trading. The second field visit to a particular farm happened, when we assured a minimum difference of 48 hours between the first and the second trading dates.

Since most of the trading happened before dawn and after dusk, sampling was performed from one farm during each field visit. We therefore needed 60 working days

to complete all the sampling (first and second sampling sessions). A monetary incentive was given to each farmer to participate in our study.

Cloacal and oropharyngeal swab samples were taken from birds by inserting and swabbing sterile cotton tipped applicator sticks deeply into the vent or oropharynx of each individual chicken. Fisher brand® Sterile Swabs (Catalogue No. 14-959-97B) were used for swabbing.

Environmental swabs were collected via a sterile swab moistened with VTM. The moist swab was then rolled over the sample surface, and excess matter was removed by shaking before placing it into the VTM.

Each pool – cloacal, oropharyngeal and environmental was placed in a vial containing 3 ml VTM labelled with a unique identification number. The samples were then stored in an insulated container with ice packs until being transferred within 3-4 hours to a -80°C freezer at the CVASU laboratory. VTM consisted of phosphate-buffered saline (PBS) (Scarab S. L. GatoParez, 33-P.I. Masad'Encia. 08181 Sebtmenat, Spain) with penicillin (4000000/l) and gentamicin sulphate (500 mg/l) following the guideline of WHO(WHO, 2006).

For a pool size of 5, a minimum of 4 pools must be tested to provide 95% probability of detecting a prevalence of 0.2, assuming a test sensitivity of 0.9 for all pool sizes. However, all samples were tested at the end.

3.4 Laboratory Evaluation

For molecular testing, RNA was extracted from pooled swab samples using MagMAX™-96 Viral RNA Isolation Kit (robotic), Catalogue Numbers AM1836, AMB-1836-5, Publication Number 1836M and Revision H (amnion®, life technologies™). RNA extracts were then used in One Step Real Time Reverse Transcriptase Polymerase Chain Reaction (rt-PCR) directed at the Matrix (M) gene followed by H5 and H9 genes on M-gene positive samples in a Fast Real Time PCR machine (ABI 7500) (Monne et al., 2008; Heine et al., 2015). Specific primers and probes and AgPath-IDTM reaction kits (Catalogue no lot AM1005) were used for testing.

3.5 Statistical Evaluation

Field and laboratory data were entered into Microsoft Excel 2007, cleaned for errors and inconsistencies, sorted, coded and checked for integrity. Data were then exported to STATA-IC-13 *(StataCorp, 4905, Lakeway Drive, College Station, Texas 77845, USA)* for analysis.

Descriptive analysis– *frequency number*, *percentages*, *mean*, *median*, *minimum* and *maximum* was conducted to express the items associated with farm biosecurity principles.

The AI and its sub-type specific proportionate prevalence based on rRT-PCR were estimated at farm level (first and second sampling sessions) and pool level (first and second sampling sessions). The proportion of AI positive between first and second sessions at both farm and pool levels were compared using Fisher's exact test ($p \leq 0.05$).

Chapter 4: Results

4.1 Assessment of Biosecurity Status in Broiler Farms

4.1.1Biosecurity Principle 1 in Broiler Farms: Isolation

Biosecurity ***Principle 1***, farms maintaining a proper distance from certain objects, was not met by 46-98% of broiler farms (N=39). 3-54% maintained the recommended distance (see Table 4.1). The majority of the broiler farms did not have protective fencing (74%), main lockable gates, bird proof nettings (67%) or a safe dead bird disposal system (69.2%).

62% of the farmers did not have their own vehicles. Among the rest of the farmers, 13% of the farm vehicles were taken off the farm for other uses (such as transporting poultry for other farms). At 49% of the farms, trader vehicles were allowed to drive right up to the poultry sheds without using antiseptic spray. Vehicles leaving the farm to be cleaned and disinfected regularly occurred only at 5% of the farms. Farm workers were permitted in other areas of the farm besides their assigned work houses at 28% of the farms. Farm managers and employees at 21% of the farms visited other farms. It was recorded that farm managers and employees at 23% of the farms visited other farms during a disease outbreak (see Table 4.3).

Up to 33-82% farms did not have a pest management or other animal control system in place, whereas 18-68% farms did have such control systems (see Table 4.4).

Table 4.1: Estimated distance of broiler farms to different objects (N=39)

Distance of different objects from the study farm	Recom. distance (meter)	Mean distance	Median Distance	Min-Max	Distance maintained (n, %)	Distance violated (n, %)
Neighbouring poultry farm	200	400.7	274	20-3,000	21 (53.9%)	18 (46.2%)
Backyard poultry farm	200	59.1	30	3-500	2 (5.1%)	37 (94.9%)
Live bird market	1,000	852.7	800	6-2,000	21 (53.6%)	18 (46.2%)
Residential area	200	60.9	30	3-500	3 (7.7%)	36 (92.3%)
Nearest waste disposal facilities	1,000	102.0	36	1.5-1,000	1 (2.6%)	38 (97.4%)
Pond/Ditch	200	22.5	6	1.2-500	1 (2.7%)	38 (97.4%)
Lake/River	1,000	809.4	500	3-10,000	12 (30.7%)	27 (69.2%)
Large trees for roosting wild birds	100	17.8	3	0.6-200	5 (12.8%)	34 (87.2%)

Table 4.2: Overview of characteristics of the study farms (N=39)

Characteristics	Present	Absent
Protective fence	10 (25.6%)	29 (74.4%)
Main lockable gate	13 (33.3%)	26 (66.7%)
Minimum distance between sheds is maintained (double the width of shed)	14 (50%)	14 (50%)
Bird proof netting	13 (33.3%)	26 (66.7%)
Different species on the same farm	3 (7.7)	36 (92.3%)
Birds of different ages allowed in the same shed	6 (15.4%)	33 (84.6%)
Safe dead bird disposal system (pit, incinerator etc.)	12 (30.8%)	27 (69.2%)

Table 4.3: Traffic on and off farm (N=39)

Traffic activities	Yes	No
Farm vehicles taken off the farm	2 (13.3%)	13 (86.7)
Visitors permitted besides authorized personnel	13 (33.3%)	26 (66.7%)
Ensure no previous contact with poultry over the last 24 hours	19 (48.7%)	20 (51.3%)
Vehicle allowed to drive from outside into the poultry sheds area	19 (48.7%)	20 (51.3%)
Load-out crews permitted to go anywhere on the farm including the house for which they were assigned to work	11 (28.2%)	28 (71.8%)
Farm manager and employees sometimes visit other farms	8 (20.5%)	31 (79.5%)
Farm manager and employees sometimes visit other farms during a disease outbreak	9 (23.1%)	30 (76.9%)
Vehicles that leave the farm be cleaned and disinfected regularly	2 (5.3%)	36 (94.7%)

Table 4.4: Pest management and other animal control systems of farm (N=39)

Items	Yes	No
Rodent control plan	26 (68.4%)	12 (32.6%)
Bait boxes and traps checked regularly	16 (45.7%)	19 (54.3%)
Rodent activity checked regularly	19 (52.8%)	17 (47.2%)
Trash and junk allowed to pile up within 30 meters	18 (47.4%)	20 (52.6%)
Grass and weeds must be trimmed	20 (51.3%)	19 (48.7%)
Outside feed spills must be cleaned promptly	25 (64.1%)	14 (35.9%)
The screening in the poultry house eaves must be checked regularly to prevent wild birds from roosting	10 (25.6%)	29 (74.4%)
Shed must have a concrete floor	23 (58.9%)	16 (41.3%)
Regular spraying for insect control	18 (46.2%)	21 (53.9%)
Stray dog and cat control plan	14 (35.9%)	25 (64.1%)
Pet dogs, cats and birds allowed on farm	8 (20.5%)	31 (79.5%)
Farm animals like cattle, goats, etc. allowed on the farm	7 (17.95%)	32 (82.05%)
Farm animals allowed within 30 meters of poultry sheds	25 (64.1%)	14 (35.9%)

4.1.2 Biosecurity Principle 2 in Broiler Farms: Good Farm Hygiene

The majority of the farms had good farm hygienic practices in the following areas:

1)	Litter covered with plastic sheet after removal	57%
2)	Used litter not stored near clean litter	72%
3)	Litter removal equipment disinfected properly after each use	85%
4)	Sweeping of sheds thoroughly after litter removal	100%
5)	A designated sprayer for washing and disinfecting	64%
6)	Feed pans emptied prior to cleaning	87%
7)	Feed pans and drinkers scrubbed and disinfected as needed	90%
8)	Regular cleaning and disinfection of feed bin	51%
9)	Regular cleaning of disinfection of sandals	55%
10)	Letting the shed to dry $\geq$ 2 weeks after cleaning and final disinfection	84%
11)	Feeders and drinkers cleaned and disinfected prior to refilling	84%

(see Table 4.5)

Measurement of personal hygiene practices at farms was measured as follows:

1) Employees must wash and change clothes 54%
2) A separate pair of sandals must be used for each shed 54%
3) Hands must be cleaned before and after use 55%
4) Most of the farms did not have foot baths 92%
5) Most farms did not require visitors to wear clean apparel 80%

(see Table 4.6)

Table 4.5: Housecleaning and disinfection in broiler farms (N=39)

Activities	Yes (%)	No (%)
Litter covered with plastic sheet after removal	22 (56.6%)	17 (43.4)
Used litter treated properly prior to selling	7 (17.95%)	32 (82.05)
Used litter stored near clean litter	11 (28.2%)	28 (71.8%)
Litter removal equipment disinfected properly after each use	33 (84.6%)	6 (15.4%)
Sweeping of sheds thoroughly after litter removal	39 (100%)	0
A sprayer for washing and disinfection	25 (64.1%)	14 (35.9%)
Feed pans emptied prior to cleaning	34 (87.2%)	5 (12.8%)
Feed pans and drinkers scrubbed and disinfected as per needed	35 (89.7%)	4 (10.3%)
Regular cleaning and disinfection of feed bin	20 (51.3%)	19 (48.7%)
Regular cleaning of disinfection of sandals	21 (55.3)	17 (44.7)
Letting the shed to dry ≥ 2 weeks after cleaning and final disinfection	31 (83.8)	6 (16.2%)
Feeders or drinkers cleaned and disinfected prior to refilling	31 (83.8)	6 (16.2%)

Table 4.6: Personal hygiene measures exercised in broiler farms (N=39)

Rule	Followed	Not followed
Employees must wash and change clothes	21 (53.8%)	18 (46.2%)
Separate pair of sandals must be used for each shed	21 (53.8%)	18 (46.2%)
Sandals must be cleaned before and after use	6 (28.6%)	15 (71.4%)
Hand must be cleaned and disinfected often and when move between units	21 (55.3%)	17 (44.7%)
Farm must have a foot bath	3 (7.7%)	36 (92.3%)
Visitor who wishes to enter must wear clean apparel	8 (20.5%)	31 (79.5%)

4.1.3 Biosecurity Principal 3 in Broiler Farms: Good Farm Management Practices

Many farms did not have a record keeping system: 56-67% of the farms did not have isolated area for dead birds stocked piled overnight. Dead birds were left inside the shed for ≥2-5 hours. Up to 92% of farms incorporated an all-in-all-out system. Good food storage was kept with 72% of farms by having feed stored with no rodent access and free of fungus (see Table 4.7).

Table 4.7: Status of good farm management system practicing in broiler farms (N=39)

Considering variables	Practiced	Not practiced
Keeping records (at least one)	17 (43.6)	22 (56.4)
All-in-All-out	36 (92.3)	3 (7.7)
Dead bird stocked piled overnight	16 (41.0)	23 (59.0)
Dead bird left inside shed ≥ 2-5 hours	13 (33.3)	26 (66.7)
Feed stored with no access to rodents and free of fungus	28 (71.8)	11 (28.2)

4.2Avian Influenza Virus Assessment at Broiler Farms

4.2.1 Assessment of Avian Influenza Introduction through Poultry Traders

From the second sampling session, after trading, only 3 farms were positive for AI M-gene (single positive pool per farm considered as farm positive) (N=30) of which 2 farms were subtype H9 positive, and 1 farm was un-typed AIV positive. None of the 3 farms had H5 positive. These 3 M-gene positive farms were negative at the first session of sampling (before trading).

Five farms (18.2%) were AI M-gene positive at the first session of sampling (N=30), but none were positive at the second session of sampling. Among the AI M-gene positive farms 4 had H9, and 1 had unsubtyped AIV, but none of the farms were H5 positive.

Regardless of sampling session, 22 farms were AI M-gene negative.

4.2.2 Prevalence of Avian Influenza

The estimated proportionate prevalence (PP) of AI at the farm level in this study was not equal but difference may not be statistically significant in both sessions (16.6%; 95% CI 5.6-34.7%; N=30 farms versus 10%; 95% CI 2.1-26.5%; N=30 farms) ($p>0.05$). The PP of AI at the pool level was statistically identical in both sessions (6%; 95% CI 2.8-11.1%; N=150; N=30 farms versus 6.7%; 95% CI 3.2-11.9; N=150, N=30 farms) ($p>0.05$).

Chapter 5: Discussion

Poultry farm biosecurity is a crucial aspect requiring strict maintenance to prevent the introduction of infectious diseases to the farm premises. However, the current biosecurity status of broiler farms in Chittagong, Bangladesh has not previously been assessed against the DLS biosecurity guideline (DLS, 2011). This chapter discusses important findings, implications, limitations, conclusions, recommendations and future directions.

5.1 Farm Biosecurity Principle-1: *Isolation*

The present study determined that the majority of the broiler farms did not have a protective fence, main lockable gates, bird proof netting and a safe dead bird disposal system (67-74% of farms) which suggests poor biosecurity standards through which infectious agents like AIVs can gain access to farms causing outbreaks, morbidity and mortality (Rao, 2008; OIE, 2018). Using an all-in-all-out system, which ensures the same strain and age of the broilers (85-92% farm), is one of the indication of good farm biosecurity principle by reducing the introduction of poultry diseases like AI and Newcastle disease (OIE, 2018).

More than 50% of the farmers did not have their own vehicles, because they are usually poor. It remains a financial constraint to purchase their own vehicles. Farmers also purchase feed and medicines on credit from the trader (e.g., feed dealer), which means that those farmers heavily rely on trader decisions, when they sell their birds, and also for the fact that the farmers do not sell their entire flock in a single day. Thus, farmers hire vehicles to transport their poultry to the LBMs. The vehicles could act as fomites, as they are usually used by multiple farmers and are typically not disinfected after each use (Islam and Nishibori, 2009; Ibrahim et al., 2016). Moreover, farmers are not interested in investing in farm biosecurity to reduce their production costs. In fact, they have little to no knowledge about biosecurity. Therefore, each farm should have its own poultry transport vehicle and a regular disinfection plan. In this case, soft loan credit can be provisioned for farmers.

Risky practices observed in the study included allowing trader vehicles to drive from outside into the poultry sheds without antiseptic spray use (49% farms) and farm man-

agers and employees' visiting other farms (21-23% farms) during disease outbreaks. These practices can easily disperse infectious disease from one farm to another (Paul et al., 2011; Fournié et al., 2013).

An alarming proportion of farms did not have a pest management or other animal control system (33-82%). Therefore, carrier or vector-borne diseases can easily be introduced to farms causing disease outbreaks (Naughton et al., 1996). Hence, compulsory pest and other animal control systems should be used on every broiler farm.

5.2 Farm Biosecurity Principle-2: *Good Farm Hygiene*

The present study explored farm hygienic practices including litter management and cleanliness, sheds, floors, feed pans, drinkers, and feed bin disinfection (Table 4.5). The percentages seen indicate broiler rearing with improved biosecurity in Chittagong providing disease free broiler meat to end consumers. This findings is supported by the scoring system to measure and quantify the level of biosecurity on broiler farms and their technical performance conducted in earlier study (Gelaude et al., 2014). However, most of the farms did not have foot baths (92%), a common scenario in Bangladesh (Biswas et al., 2008).

5.3 Biosecurity Principal 3 of Broiler Farm: *Good Farm Management Practices*

In general, farm record keeping systems were poor at the studied farms, and none of the farms had a computer-based system, although a few of them have manual registration books. This poor standard of record keeping is common throughout the country. The exception is that some big broiler farms (more than 0.1 million capacity) have been established in many parts of the country by local and international companies in the poultry sector, where they have developed computer based record keeping systems (Personal communication, General Manager, Kazi farms group, Bangladesh). However, all-in-all-out systems were followed by most of the farms, which is essential for profitable broiler rearing.

5.4 Assessment of Avian Influenza Introduction through Poultry Traders

Ten percent of the farms were AI positive at the second sampling session (after trading), but the same farms were negative at the first sampling session. Therefore,

this result indicates that trading might have been responsible for introducing AI infection. This theory is supported by other studies elsewhere in the world (Indriani et al., 2010; Leung et al., 2012; Chaudhary and Pahwa, 2013). However, this interpretation should be considered cautiously, as after-trading at 90% of the studied farms had no effect. No difference of PP prevalence of AI (farm or pool level) was found between sampling sessions in the current study indicative of no significant effect of after-trading in introducing AI to the studied farms.

5.5 Avian Influenza Subtype Distribution

Only AIV H9 subtype was found in the present study. Previous published and unpublished studies also found more H9 subtypes over H5 at broiler farms in Bangladesh (Sultana et al., 2012; Chaudhary and Pahwa, 2013; OIE, 2017a; Hassan et al., 2018).

5.6 Limitations of the study

The explanatory power of this study decreased, because the sample size of 50 broiler farms was not reached due to farmer non-participation. However, the study recruited 39 farms, which is still sufficient to produce meaningful data. Farmers were often in a hurry to finish the survey, which may have produced information bias, when recording their answers.

To better understand the effects of after-trading in introducing AI to the studied farms, a larger sample size for biological sampling would be needed. Although diagnostic tests with high-quality rRT-PCR, Sensitivity: 99.5% and specificity: 88.2% (Monne et al., 2008) were used in this study, some technical errors could not be ruled out.

5.7 Conclusions

Overall, farm biosecurity ***Principle1 Isolation*** was poor in this study. However, maintaining the all-in-all-out system with the same broiler strain and age structure in most of the farms was an indication of excellent farm biosecurity *Principle 1*. This supports the prevention and introduction of poultry diseases like AI. ***Principle 2 Good Farm Hygiene*** was good in all aspects, except the missing foot baths. ***Principle 3 Good Farm Management Practices*** was mixed, as many farms did not have a record

keeping system. However, most farmers followed an all-in-all-out system, which is essential for profitable broiler rearing. After-trading had no effect on the introduction of AI to the studied farms. Only the H9 subtype was found in the study.

5.8. Recommendations

Several simple implementations can be used to greatly increase biosecurity at broiler farms. Fences, main lockable gates, bird proof netting, safe dead bird disposal systems, individual farm poultry transport vehicles, vehicle disinfectant spray, decreased employee cross-farm movement during outbreaks, and pest control systems should be implemented to prevent the spread of infectious diseases.

Following biosecurity ***Principle 2 Good Farm Hygiene***, foot baths should be used on all broiler farms.

Farm record keeping systems (paper-based or computer-based) should be established to improve farm management systems. An all-in-all-out system was maintained by most of the farms and should be continued.

The estimated farm AI prevalence, in particular H9, should be reduced with improved farm biosecurity standards.

5.9 Future Directions

Country-wide assessment of poultry farm biosecurity status should be conducted in the future to develop a benchmarking guideline in the Bangladeshi context to identify gap in the biosecurity system for further improvement.

Advanced statistical analysis should be applied in the future to explore potential risk and protective factors, as well as the farmers' behavioural factors, associated with overall farm biosecurity.

As this small study has not been able to determine the effect of after-trading properly in introducing AI to farm, a further study with a larger sample size should therefore be needed to identify the role of trading.

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Appendix 1: Biosecurity Assessment of Commercial Poultry Farms

Section A: Contact information

Interview details			
1. Form ID:		2. Date of interview: _____/_____/2017	
3. Interviewer name:		4. Signature:	
Contact details of interviewee			
5. Name of the person interviewed:		6. Phone number :	
7. Position of the interviewee...	☐ Owner	☐Worker	☐Other (specify).....
8. Farm location details			
a) HoldingNo:	b) Road No:	c) Village name:	
d) Union/city corporation:		e) Upazila:	
f) Zila/District:		g) Division:	
h) GPS Coordinates: Northern (N):		Eastern (E):	

Section B: Farm Type and Composition

10. Type of Farm: ☐ Broiler ☐Layer

11. Size of the farm: ☐Small(1-500 birds)☐ Medium (500-5000 birds) ☐ Large (≥5000 birds)

12.Specify the actual number of birds: …….

Section C: Demographic information:

13. Sex and age of the farmer: ☐ Male ☐ Female; Age: ……

14. Any other occupation of the farmer than poultry farming:☐ Yes ☐ No

15. If yes, specify it and why they do that (does the farming is not profitable?):
……
……

15. How many years have the farmer beendoing poultry farming? ……

15. Highest degree or level of school the farmer has completed:
☐No Schooling☐Primary☐ Secondary☐ Higher Secondary☐ Graduation

16. Annual Income of the farmer (BDT): …….

17. How many workers do the farm has: …….

18. Sex and age of the workers: i) ☐ Male ☐ Female; Age: ……

ii)☐ Male ☐ Female; Age: ……
iii) ☐ Male ☐ Female; Age: ……
iv)☐ Male ☐ Female; Age: ……

19. Education status of the workers:
i) ☐ No Schooling ☐Primary ☐ Secondary ☐ Higher Secondary☐ Graduation

ii) ☐ No Schooling ☐Primary ☐ Secondary ☐ Higher Secondary ☐ Graduation

iii) ☐ No Schooling☐Primary ☐ Secondary ☐ Higher Secondary ☐ Graduation

iv)☐ No Schooling☐Primary ☐ Secondary ☐ Higher Secondary ☐ Graduation

20. Does the farmer have any idea about the term "biosecurity": ☐ Yes ☐No

21. Does the worker have any idea about the term "biosecurity":☐ Yes ☐No

22. Does the farmer have any training on biosecurity: ☐ Yes ☐No

23. Does the worker have any training on biosecurity: ☐ Yes ☐No

24. Specify the training, duration and year:
……

25. Are you satisfied with the current biosecurity status of your farm?☐ Yes ☐ No

26. Does it cost more to maintain biosecurity?☐ Yes ☐ No

27. Do you feel that you are making the highest profit from the existing biosecurity status of yourfarm?☐ Yes ☐ No

Section D: Biosecurity assessment questions for scoring

Principle 1: ISOLATION
Helps to maintain a safe distance between poultry and potential disease threats

A. Location

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. Distance between a poultry farm to the neighbour poultry farm ≥ 200m			
2. Distance allowed of backyard poultry to a poultry farm ≥ 200m			
4. Distance between poultry farm to Live Bird Market ≥ 1km			
5. Distance between poultry farm and residential area ≥ 500m			
6. Distance between a poultry farm and the nearest urban or village waste disposal facilities ≥ 1km			
7. Distance between a poultry farm and the nearest standing body of water (pond, lake, dam) ≥ 200m			
8. Distance between a poultry farm and the nearest river/lake with the possibility of flooding ≥ 1km			
9. Distance between farm fences and a poultry shed ≥ 2m			
10. Distance between a poultry house and large trees that host wild birds ≥ 100m			
11. Distance between shower/ washroom/ toilet for the farm workers and the poultry house ≥ 10m			

B. Farm Characteristics

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. Farm must be surrounded by a protective fence (if yes, height ≥ 2m)			
2. Farm must have only one main lockable gate entrance, in and out of the farm			
3. A minimum distance between sheds on farm is maintained (Yes –how much or No) (if yes, double the width of the shed)			
4. All sheds must have bird proof nettings			
5. Birds of different species (chickens and ducks) are not allowed on the same farm			
6. Birds of different ages are allowed in the same shed(No)			
7. A safe dead birds' and manure disposal system (pit, incinerator, composter) present at the farm			

C. Traffic On and Off the Farm

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. Farm vehicles never to be taken off the farm			
2. No visitors are permitted on the premises except authorized personnel			
3. All visitors must be asked where they have been prior to coming on the farm, to ensure no previous contact with poultry over the last hours (Yes – how many hours ago ≥ 24h)			
4. All vehicles coming onto the farm must be checked to see if they are clean			
5. All vehicles must be washed with detergent and then disinfected (wheels, undercarriage, driver cabin, etc.) before entering the farm			
6. No vehicles allowed to drive from outside the farm into the poultry sheds area			
7. Vehicles must park no less than 30 meters of poultry sheds			
8. Farm employees not allowed to ride between two or more units or farms in the feed or egg truck			
9. Employees are assigned to their own sheds and don't visit other sheds unless they showered and change clothing (in case of broiler,			

if farm capacity is over 5000 birds)			
10. Load-out crews are not permitted to go anywhere else on the farm except for house they are assigned to work in			
11. Farm manager and employees never visit other farms			
12. Farm manager and employees never visit other farms during any disease outbreak			

D. Pest Management and other animals

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. Farm must have a rodent control plan			
2. Bait boxes and traps must be regularly checked to be sure that the bait is fresh and to remove dead rodents			
3. Rodent activity must be regularly checked, e.g., active holes near the foundations, chewed curtains and insulation, rodent droppings on sills and in entry-rooms			
4. No trash and junk are allowed to pile up for 30 meters around the sheds and in the entry- room			
5. Grass and weeds must be trimmed around the house			
6. Outside feed spills must be cleaned promptly			
7. The screening in the eaves of poultry houses must be weekly checked and repaired to prevent wild bird			
8. Sheds must have concrete floor or slates and pads long the walls and at every entrance to the house			
9. Regular spray for insect control, using approved insecticides must be applied at the farm			
10. Farm must have stray dogs and cats control plan			
11. Pet dogs, cats and birds (parrots/ pigeons) are not allowed on farm or to enter any sheds			
12. Other farm animals like cattle, goats, etc., are not allowed on farm			
13. Other farms animals like cattle, goats, etc., are not allowed within 30 meters of poultry sheds			

Principle 2: GOOD FARM HYGIENE
Prevents dissemination of infectious agents by reducing their numbers or eliminating them from the environment

A. House Cleaning and Disinfection

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. Litter that is removed from poultry houses must be stored in a covered shed or covered with plastic sheets			
2. Used litter must be treated in an approved, properly managed composting facility before prior to being sold out			
3. Used litter must not be stored near clean litter			
4. All equipment used during litter removal must be properly cleaned and disinfected after each use			
5. After litter removal the poultry house must be thoroughly swept and clean from top to bottom			
6. A suitable sprayer should be used for washing and disinfection			
7. All feed pans, feed lines, and hoppers should be emptied prior to cleaning			
8. All feed pans, cones, hoppers, and drinkers should be scraped, scrubbed, washed, and disinfected as per need			
9. The feed bin and boot must be regularly cleaned and disinfected			
10. After cleaning and final disinfection the shed should be allowed at least 2 weeks to dry out and remain empty			
11. Removable equipment like feed trays and drinkers should be cleaned and disinfected just prior to setting the house up for new birds			
12. Any equipment such as vaccinators, debeakers, egg trays, etc., brought into the farm must be cleaned and disinfected			

B. Personal Hygiene and Apparel

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1.Vehicles that leave the farm must be cleaned and disinfected regularly, including the cabs and beds			
2. All employees must wash, and change cloths and footwear before entering to work in the farm or poultry houses			
3. Separate pair of boots or sandals must be used for each house and / or brooder and finisher unit			
4. Boots or sandals must be cleaned and disinfected before and after use			
5. Hands must be cleaned and disinfected (disinfectant cream/soap can be used) often and when move between units			
6. Dip pans (foot bath) with a daily refreshed solution should be placed at every poultry house entrance (According to manufacturer's instruction and condition of usage)			
7. All visitors who wish to enter poultry houses must wear clean, sanitized caps, coveralls, gloves, and footwear			

Principle 3: GOOD FARM MANAGEMENT PRACTICES
Make for a healthy environment

Assessment questions (Tick appropriate answers)	Yes	No	Remarks
1. A flock record must be kept for each shed. The record includes information of DOC origin, number of chicks placed, daily mortality (numbers and %), daily feed consumption, daily water consumption, weekly body weight, daily egg production, vaccinations, medications, vitamin administration, laboratory results, etc.			
2. All farms should maintain all in & all out system(Broiler-Yes, Layer-No)			
3. Dead birds are never to be stocked piled overnight before disposal and exposed to pests			
4. Dead birds are never to be left inside the shed longer then 2-5 hours			
5. Feed must be stored on farm in sealed and dry containers with no access to rodents or wild birds and free of fungus and mold			

Brief Biography

Md. Harun Rashid passed the Secondary School Certificate Examination (SSC) in 2007 obtaining GPA 5.00 (A+) and then Higher Secondary Certificate Examination (HSC) in 2009 obtaining GPA 4.80 (A). Mr. Rashid obtained his Doctor of Veterinary Medicine Degree in 2014 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU. He has immense interest to work in Avian Influenza epidemiology and anthropology.