Epidemiology and molecular characterization of avian influenza virus in chickens and ducks at backyard farms in Chattogram, Bangladesh

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Authorization

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THE AUTHOR

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Dedication

I dedicate this MS thesis to my beloved parents and teachers,

Without whom none of my success would be possible

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List of abbreviations

Abbreviations	Elaborations
%	Percentages
95% CI	95% Confidence Interval
AGID	Agar Gel Immunodiffusion
AI	Avian Influenza
AIV	Avian Influenza Virus
BLAST	Basic Local Alignment Tool
BLRI	Bangladesh Livestock Research Institute
CDC	Centers for Disease Control and Prevention
CT	Cycle Threshold
CVASU	Chattogram Veterinary and Animal Sciences University
DFID	Department for International Development
DLS	Department of Livestock Services
E	East
ELISA	Enzyme Linked Immunosorbent Assay
et al.,	And others
FAO	Food and Agriculture Organization
GoB	Government of Bangladesh
HA	Hemagglutinin
HBIS	Hospital-Based Influenza Surveillance
HI	Hemagglutination-inhibition
HPAI	High Pathogenic Avian Influenza
iccdr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
IEDCR	Institute of Epidemiology Disease Control and Research
LBM	Live Bird Market
LPAI	low pathogenic avian influenza
M	Matrix
mm	millimeter
N	North
NA	Neuraminidase
ND	Newcastle Disease
NRL-AI	National References Laboratory for Avian Influenza
NISB	National Influenza Surveillance, Bangladesh
PB1	Polymerase Base 1
PCR	Polymerase Chain Reaction
PRTC	Poultry Research and Training Centre
RNA	Ribonucleic Acid
rRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
SMS	Short Message Service
sqkm	Square Kilometer
UK	United Kingdom
USAID	United States Agency for International Development
VTM	Viral Transport Media

Summary

Bangladesh is an agriculture-based country where large portions of rural households have backyard poultry, which play an important role in their dietary protein needs. Besides that, selling the meat and egg to the local markets to support the additional family expenses. Avian influenza virus has a catastrophic impact on household poultry next to commercial poultry industries by causing high mortality or reducing egg production. Over the past two decades, highly pathogenic avian influenza (HPAI) has triggered serious outbreaks in poultry and has affected humans with causing mortality across the world, including Bangladesh. As the ducks are believed as natural reservoir of avian influenza virus, it can act as reassortment vessel in the transmission of HPAI virus and low pathogenic avian influenza (LPAI) virus among the other domesticated, wild bird species and humans. Most of the epidemiological research on avian influenza have been previously been limited into commercial poultry and live bird markets and to date, studies on apparently healthy poultry at household level are not yet available. The present cross-sectional study was carried out to reveal the epidemiological traits of avian influenza of backyard poultry in coastal (Anowara) and plain land (Rangunia) areas to find out prevalence, associated factors and molecular characterization of the avian influenza virus (AIV). A total of 300 households' poultry (having both chicken and duck) were randomly selected and cloacal swabs of one bird per household were sampled. Structured pre-tested questionnaires were used to collect the information related to risk factors at household level by direct interview of farmers and recorded. Cloacal samples were pooled in small groups and tested first for the matrix gene (M gene) presence by real time reverse transcriptase polymerase chain reaction (rRT-PCR) with reference primers and probes, and then M gene positive swabs pooled were further tested for H5 and H9 subtypes using specific primers and probes by rRT-PCR. All AIV positive samples were subjected to sequencing for the four gene segments (M, PB1, HA and NA gene). We were able to amplified Eight (8) M genes, four (4) for each HA, NA, PB1gene segments and then performed phylogenetic analysis. We detected overall viral RNA, Influenza A (M-gene) prevalence at household level was 6% (95% CI: 3.6 – 9.3; N=300) where this prevalence was 3.6% (95% CI: 1.7 – 6.4; N = 281) in household duck and 3.2% (95% CI: 1.4 – 6.2; N= 251) in household chicken. During the winter season the prevalence was estimated 8.2% (95% CI: 4.5 – 13.3; N= 171 whereas in

the summer it was 3.1% (95% CI: 0.8 – 7.7; N= 129). According to subtype, the prevalence of H5 and H9 in backyard poultry was 2.7% (95% CI: 1.1 – 5.2; N= 300) and 3.3% (95% CI: 1.6 – 6; N= 300), respectively. The phylogenetic analysis of eight partial M gene sequences suggested that the M gene sequences detected in backyard poultry were almost similar to each other and closely related to the previously reported M gene sequences of HPAI and LPAI subtypes in poultry in Bangladesh as well as Southeast Asia. Besides, the phylogenetic analysis of HA, NA and PB1 gene also showed the similarity in sequences with each other and closely related to the gene sequences of previously reported HPAI in different poultry sectors in Bangladesh. Overall results reflect that both H5 and H9 subtypes of avian influenza virus are circulating in the household poultry with or without showing any clinical symptoms. Besides regular surveillance and early detection of avian influenza virus in this area, molecular identification of AIV's subtypes in the study area helps to get clear idea of circulating subtypes of AIV virus in the backyard poultry rearing system of the study areas and take effective control measure to prevent the infection and control the zoonotic transmission.

Key words: avian influenza virus, backyards poultry, prevalence, M gene, viral RNA subtype, molecular characterizations.

Chapter-1: Introduction

The poultry sector, the subsector of livestock is an integral part of the agricultural production system, which has created both direct and indirect employment opportunity, improving food security and enhancing supply of protein to humans. This sector contributes to the economic development and decreasing poverty rate in rural and urban areas of Bangladesh (Hamid *et al.*, 2017). Considerable employment has been created with the development of the poultry sector through the production of poultry and poultry products in Bangladesh (Da Silva and Ranking, 2013). The progress of poultry industry in Bangladesh is mainly dependent on the private sector. Besides the private sector, a portion of this progress depends on backyard poultry which is raised by rural communities of Bangladesh(Mack *et al.*, 2005). In Bangladesh, approximately 64% of the populations live in rural villages and almost 71% of them raise backyard poultry (UNICEF, 2007). The practice of backyard poultry rising makes a pivotally important contribution to the livelihood of rural families, and also to the national economy (Sultana *et al.*, 2012a). Chickens and Ducks are the most common poultry species reared in rural areas of Bangladesh in which ducks are reared mostly in nomadic and household rearing system where household ducks are kept overnight near or within the farmer's house and travel only over short distance (Henning *et al.*, 2009; Ghosh *et al.*, 2012). Besides, almost every rural community keeps small flocks of indigenous chickens under a backyard production system (Aini, 1990). These backyard poultry commonly raised close contact with human which is high risk to transmit infection through indirectly by their daily rearing practices including poultry sheds management, feeding and slaughtering of sick poultry. (Sultana *et al.*, 2012a). The setting of close living with poultry put them in a high risk of zoonotic diseases transmission (Rimi *et al.*, 2019).

Avian influenza (AI) or "Bird Flu" is a viral infectious disease caused by Type A influenza virus under the family of *Orthomyxoviridae.* Influenza A type virus is a negative sense, single stranded ribonucleic acid (RNA) virus of which general form of disease establishment by bird to bird transmission (Capua and Alexander, 2007). Besides birds, it has crossed the species barrier and has the potential to infect avian and mammalian species including humans, pigs, horses, dogs and sea mammals (Webster and Hulse, 2004; Zhu *et al.*, 2018a). Most of the pathogenic avian influenza virus (AIV)

strains are considered non-pathogenic or cause only mild diseases (Parvin *et al.*, 2018). AIV are categorized as highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) viruses in two classes, depending on their ability to cause disease (Duvauchelle *et al.*, 2013). HPAI (H5N1 and H7N9) is characterized by the mild to severe form of illness with the high mortality rate up to 100% deaths (Swayne, 2008). LPAI (H9N2) can produce swelling of periorbital tissues and sinuses and typical respiratory discharge and reduced rate of egg production in chicken (Capua and Terregino, 2009; Akhter *et al.*, 2017). Waterfowls including ducks are considered as a natural reservoir for all subtypes of influenza A viruses (Hinshaw *et al.*, 1985; Marchenko *et al.*, 2012). H5N1 viruses in domestic ducks may results in asymptomatic, subclinical, or clinical infections where asymptomatic ducks often shed the viruses through feces and respiratory droplets (Hulse-Post *et al.*, 2005; Sturm-Ramirez *et al.*, 2005). Ducks in different rearing system including household, nomadic and free range are considered to play a vital role in the maintenance and transmission of viruses among commercial and wild bird populations in South East Asian regions (Bi *et al.*, 2016; Jiang *et al.*, 2017).

The HPAI virus has concerned demolishing economic and social effects on the poultry industry both for Bangladesh and other countries, specially in international trade on agriculture and rural households in different continents such as Asia, Europe and Africa (McLeod *et al.*, 2005; Otte *et al.*, 2008). The economic losses of the 2007 outbreak in Bangladesh has been estimated at US$746 million in poultry sectors as well as negative social effects (Chakma and Rushton, 2008). The first HPAI H5N1 virus was detected in waterfowl, A/Goose/Guangdong/1/1996 was identified in 1996 in Guangdong, China considering the progenitor of current panzootic H5N1 viruses (Xu *et al.*, 1999) and the first reported case was in Government of Bangladesh in 2007 in domestic poultry (Biswas *et al.*, 2008; Gilbert *et al.*, 2010). From January 2013–May 2018, 68 countries and territories have been reported of HPAI in domestic birds with a total of 7122 outbreaks (OIE, 2018). In Bangladesh, about 550 outbreaks have been reported in the poultry sector including wild species (house crow) since the first outbreak in 2007 (Khan *et al.*, 2014; Haider *et al.*, 2017). There were 861 confirmed HPAI H5N1 human cases including 455 fatality reported in the world since 2003 of which 8 cases of H5N1 and 3 cases of H9N2 including one fatality in humans were reported in Bangladesh (WHO, 2020). The different levels of AI sero-prevalence have been recorded in

different backyard poultry and wild bird in Bangladesh (Hassan *et al.*, 2020b). In the case of household chicken, this prevalence has been recorded as 14-38.6% in different parts of Bangladesh (Alam *et al.*, 2003), 15% in layer poultry, 12.5% in broiler poultry and 0 - 3.3% in household chickens (Rahman *et al.*, 2012). The RNA prevalence of avian influenza in chickens recorded in the range of 23-35% in Bangladesh (Thuy *et al.*, 2016; Turner *et al.*, 2017). The sero-subtypes of circulating AI have been reported as 1.3-4.1% for H5 and 6.8-87% for H9 in household chicken in Bangladesh (Turner *et al.*, 2017; Kim *et al.*, 2018). On the other side the sero-prevalence in domestic ducks has been documented as 30-90.2% in different regions of Bangladesh (Khatun *et al.*, 2013; Hassan *et al.*, 2015; Sarkar *et al.*, 2017), where the viral prevalence has been recorded in between 24% to 89% (Haider *et al.*, 2017; Sarkar *et al.*, 2017; Turner *et al.*, 2017). Limited studies have been investigated the associated risk factors of AI in Bangladesh (Biswas *et al.*, 2009; Loth *et al.*, 2010; Ahmed *et al.*, 2012a; Osmani *et al.*, 2014). However, in Chattogram, Bangladesh, a detailed review of the status and distribution of AI and its sero sub-types in apparently healthy household poultry has not been thoroughly studied.

For the last two decades, H5N1 viruses have been expressed massively; deviated hemagglutinin (HA) genes into 40 clades but currently only of them 2.2.1.2, 2.3.2.1a, 2.3.2.1c and 2.3.4.4 are circulating in the world. Since the first detection of HPAI H5N1 viruses, various clades including 2.2.2, 2.3.2, 2.3.2.1a, 2.3.4.2, and 2.3.4.4 have been identified in Bangladesh (Barman *et al.*, 2017; Islam *et al.*, 2020). Among them, multiple HPAI outbreaks were also changed over time by the molecular assessment mechanism for viruses (Gerloff *et al.*, 2016). At the same time, LPAI H9N2 were circulating in different poultry species in Bangladesh, which have been firstly reported in 2006 (Parvin *et al.*, 2014a). While H9N2 and the other subtypes of LPAI viruses co-circulate with H5N1 virus subtype in Bangladesh (Gerloff *et al.*, 2016), reassortment between HPAI H5N1 and LPAI viruses is quite uncommon (Barman *et al.*, 2017). Containing the matrix (M) gene from the H9N2 viruses of Chinese lineage (Marinova-Petkova *et al.*, 2014) or the polymerase base 1(PB1) gene from the Bangladeshi lineage (Monne *et al.*, 2013b; Gerloff *et al.*, 2014; Marinova-Petkova *et al.*, 2014), reassortant H5N1 have been isolated from the live bird markets (LBMs) in Bangladesh though those viruses did not stable and evanished quickly (Barman *et al.*, 2017). Several genetic analysis showed that the LPAI H9N2 isolated from the LBMs

between 2009 and 2011 had obtained three internal genes (nonstructural, PB1, polymerase) from the HPAI H7N3 viruses by reassortment (Shanmuganatham *et al.*, 2013). Backyard poultry are reared in free scavenging system in Bangladesh sharing the open wetlands with large numbers of migratory waterfowl, wild bird and other resident birds, transmission of HPAI H5N1 and LPAI H9N2 may occur easily where the migratory birds are considered one of the potential course of introducing new clades of HPAI H5N1 in Bangladesh (Parvin *et al.*, 2014b). Besides this, LBMs are also a dynamic source of LPAI H9N2 viruses where the backyard poultry are kept for sell with other poultry species. That is why; a continuous monitoring of the evolutionary pattern of avian influenza in household poultry is needed.

Bangladesh is a densely populated country with 1077 populations per square kilometer (sqkm) (BBS, 2017),where 90% of total rural households raise poultry (Sultana *et al.*, 2012a) with the sharing of living places with household (Hassan *et al.*, 2015). Low awareness of AIV among the backyard poultry farmers and biosecurity measures are occasionally observed in several studies (UNICEF, 2007; Sultana *et al.*, 2012a; Shanta *et al.*, 2017) that pose risks for spreading of AIV. In those circumstances, poultry and humans could easily be co-infected with AIV and the fatality in humans may also rise in future as susceptibility increases due to prior exposure of pandemic influenza virus strain (Saunders-Hastings and Krewski, 2016).Therefore, it is important to find out the potential ways of introducing HPAI and LPAI viruses into the backyard poultry and figure out the risk factors of developing and transmission of the viruses and continuous monitoring to making better preparedness, early detection and quick response.

Depending on the above backgrounds, the current study was conducted in poultry (Ducks and Chickens) at household level to evaluate epidemiological facts of AIV in Chattogram, Bangladesh. The specific objectives of the present study were:

1. Determine the viral RNA prevalence of AIV, H5 and H9 in chickens and ducks at backyard poultry farms
2. To find out the potential factors of AIV, H5 and H9 prevalence in household poultry in the study areas
3. To characterize the molecular nature of the matrix (M) gene, HA gene, NA gene and PB1 gene segments of AIV.

Chapter-2: Review of Literature

The goal of this chapter is to give an overview for the designed research on epidemiological study of AIV in backyard poultry in Chattogram district of Bangladesh by reviewing the related previously published studies. Relevant literature on backyard poultry, contribution on national economy, epidemiology of AI, surveillance, public health significance, diagnosis, prevention and control have been reviewed, and presented precisely in this chapter. The review outcomes of important articles have been described under the concurrent heading as follows.

2.1 Backyard Poultry

Backyard poultry raising is common in the rural communities, which provides a source of income for them in many low income countries (Shanta *et al.*, 2017). Production system of backyard poultry demarks as a low input business which is characterized by night shelter system followed locally, scavenging system, natural hatching of chicks, poor productivity of birds, with less supplementary feeding, marketing locally and less or absent of treatment practice (Singh *et al.*, 2017). Backyard poultry rearing in rural households is a longstanding practice in Bangladesh (Barua and Yoshimura, 1997), mainly owned and maintained by women to earn money for giving support to their children education and other household expenditure (Guèye, 2005; Mack *et al.*, 2005). In Bangladesh, the backyard system of poultry rearing is a low-input and low-output system, principally comprising local genetic resources and crossbreds birds housed with minimum facilities (Alam *et al.*, 2014) . This system allows the birds for free movement for scavenging food sources, leftovers of household food and self-produced food grains by the farmers (Sonaiya, 2007). Households who cannot afford to sustain the stock of large animals like cattle or goats can easily rear a few stocks of backyard poultry (chicken, duck and/or pigeon) (Singh *et al.*, 2017).

2.2 Backyard Poultry Species

The most common backyard poultry species are chicken, ducks and pigeon however quail, and goose guinea fowl and turkey are also kept by the rural people nowadays mainly as the hobby (Dolberg, 2008).

2.2.1 Household Chickens

Most of the backyard poultry raising households raise chickens. Household chickens, non-descriptive indigenous breeds, are known as "Deshi chickens" (Bhuiyan *et al.*, 2005) and traced across the country (Huque and Khan, 2017). This backyard chickens consist of the Aseel, Frizzled Plumage, Naked Neck, Native Dwarf, Red Jungle Fowl, Tiger birds and Yasin (Bhuiyan *et al.*, 2005; Das *et al.*, 2008; Siddiky, 2017). Backyard chicken act as an investment and source of security for households in addition as sources of meat and eggs for consumption (Muchadeyi *et al.*, 2007) and generating some surplus which is sold to generate income (Siddiky, 2017). In rural areas women are the owner and key person to rear the backyard chicken (Okitoi *et al.*, 2007). Currently the total chicken population of Bangladesh is 296.6 million (DLS, 2020) and backyard chicken has a great contribution in this population. 94% of total backyard poultry households rear chicken (Shanta *et al.*, 2017). In backyard chickens flock size is less than 20 (Chowdhury, 2013) in average 8.1 to 10.4(1-20) (Alam *et al.*, 2014; FAO, 2015). Most of the rural households keep their poultry inside their bedroom at night where 48% of total households have no separate poultry shed and some use a separate cage on the front yard (Sultana *et al.*, 2012a; Shanta *et al.*, 2017). Chickens are scavenged both inside and outside in the backyard poultry rearing system. They are observed most of the time in kitchen, bedrooms, yard, cattle shed, nearby bushes, inside the neighboring house and paddy field (Sultana *et al.*, 2012a). Minimal nutritional requirement is needed for deshi chicken growth (Magala *et al.*, 2012). Rural households generally offered less supplementary feed including boiled rice, rice polish and broken rice. During scavenging backyard chickens take earthworms, insects, seeds, green leaves and other plant materials as feed source from the household yard. The average meat production of chicken in the backyard poultry rearing system is 1 kg – 4.5 kg per chicken and annual egg production per hen is 33 – 55 on average in this rearing system (Siddiky, 2017). This type of chicken has contributed 19.8% of meat and 25.1% of egg in annual total production in Bangladesh (Dutta *et al.*, 2013). In Bangladesh, backyard chicken's meats and eggs are more fascinating to the people of both urban and rural areas (Das, 1995). Consumers of Bangladesh prefer backyard chickens due to good taste, firmness, pigmentation, leanness, suitability for special dishes even in higher market price than exotic replication (Islam and Nishibori, 2009) and as bird of choice for special occasions (Hamid *et al.*, 2017).

In rural areas, veterinary services and livestock extension works are limited due to widespread area and lack of resources and infrastructure. Mortality of the backyard chickens depends on some factors like diseases, predators, bad weathers and many other factors (Singh *et al.*, 2017). Newcastle disease (ND) is the most common disease of backyard chickens in Bangladesh where 51% of total backyard chicken deaths happen by this disease (Siddiky, 2017) where vaccination uptake is very low (Mori *et al.*, 1994). That's why ND is one of the most challenging reasons for rearing backyard chicken in rural households of Bangladesh (Das *et al.*, 2008). However, about 40 percent of overall mortality is caused by Fowl cholera and Fowl pox, (Siddiky, 2017) and other diseases also have a poor effect on backyard chicken such as salmonella, avian influenza, etc. (Alam *et al.*, 2003).

2.2.2 Domestic ducks

Duck ranks 2nd highest poultry species in Bangladesh after the chicken in producing of poultry meat and eggs (Islam *et al.*, 2016). At present the duck population is 59.72 million in Bangladesh (DLS, 2020). One-ninth of total land is low land containing natural water bodies include north eastern regions, southern regions, coastal areas, marshy lands, haors, rivers, ponds and cannels in Bangladesh which is very much favorable for duck rearing (Hoque *et al.*, 2011; Islam *et al.*, 2016; Bhuiyan *et al.*, 2017). More than half of total household poultry raiser (51%) rear ducks in Bangladesh (Shanta *et al.*, 2017). Deshi indigenous, Khakhi Campbell, Indian Runner, Jinding, Muscovy, Pekin, Nageswari, Shylet Mete are the common duck breeds that are reared in different parts of the Bangladesh (Das *et al.*, 2008; Alam *et al.*, 2012; Morduzzaman *et al.*, 2015; Bhuiyan *et al.*, 2017). About 95% of the reared duck populations are of the indigenous type in Bangladesh (Huque and Hossain, 1991). In Bangladesh ducks are mainly raised as for egg production (Das *et al.*, 2008). Prices of commercial poultry meat and eggs are beyond rural people's purchasing capacity, which is why ducks are deemed the most valuable asset both for poverty elevation and food production besides vital source of income specially for the rural women (Khanum *et al.*, 2005; Islam *et al.*, 2016). Household ducks are those ducks which are reared in a semi-scavenging system as for family consumption of meat and eggs and also for selling to generate income sources. Household ducks are reared in two different subsystems as presence or absence of large water bodies. In presence of large water bodies, large scale ducks are reared (Khanum *et al.*, 2005) where at the absence of such large water bodies small scale ducks are

reared with domestic chickens (Rahaman, 2003). In rural household, generally 2-10 ducks are kept in flock (Das *et al.*, 2008) somewhere it up to 69 ducks in number with the average number of 11 ducks per household (Islam *et al.*, 2016). In backyard rearing, adult ducks gain 1.4 – 2.0 kg body weight and annual egg production in 80-200 eggs per hen (Islam *et al.*, 2016). Ducks are exploit in low lying area around large water bodies including marshy lands, rivers, canals, haors, ponds and take weeds, small fishes, insects, snails and fallen grains as feeds though household farmers use a mass variety of subsidiary feed for their ducks such as rice, broken rice, rice polish, wheat bran and commercial feed (Islam *et al.*, 2016). Rural people are less concerned about bio safety and hygiene of duck rearing. Mortality of household ducks range from 0 to 35% (Islam *et al.*, 2016). Infectious diseases are the most important barrier for the household duck rearing including Duck cholera, duck viral enteritis, duck viral hepatitis (Hoque, 2011; Rahman *et al.*, 2019), duck plague (Islam *et al.*, 2016; Siddiky, 2017) and avian influenza which becoming endemic in Bangladesh (Yamamoto *et al.*, 2008).

2.3. Contribution of Backyard poultry rearing system

Rearing of backyard poultry makes a definite contribution to the livelihood of rural households and also to the national economy of Bangladesh (Sultana *et al.*, 2012a). This is a very common practice to the rural women for many decades in Bangladesh where women are the predominant owners of the family poultry (Dolberg, 2008). This kind of rearing system serves as a small scale business for 50% of total rural women (Sultana *et al.*, 2012a), which can be generated in average 53.23 working man days per year as employment (Islam *et al.*, 2015). Selling eggs and birds is a significant way of cash income in rural areas by which they meet their financial gap in emergency needs and fulfill daily necessities like paying school fee of children, purchasing of household equipment, buying food and medicine and costing in social activities (Siddiky, 2017). From the household poultry women earn an income of 3705.95 taka per year as net return from this rearing system (Islam *et al.*, 2015). Backyard poultry production has a great contribution in total poultry product demand in Bangladesh. 2 kg of the 4.6 kg per capita poultry meat consumption comes from backyard poultry production system in Bangladesh besides this, 67% of total egg production of Bangladesh comes from this system which is estimated to be 4.4 billion eggs in a year (Dolberg, 2008).

2.4. Avian Influenza

AI is an infectious viral disease of poultry species which is caused by AIV Type A. This virus belongs to the *Orthomyxoviridiae* family characterized by eight segmented, negative-sense, single-stranded, enveloped RNA viruses (Capua and Alexander, 2007; Choi *et al.*, 2008). The eight segments of this virus are Haemagglutinin: H, Neuraminidase: N, Matrix: M1 and M2, Nucleoprotein: NP, Polybasic: PB1 and PB2, Polyacidic: PA and Non-structural: NS which can encode 10 different proteins (surface protein and internal protein) (Swayne and Suarez, 2000). On the basis of the two surface glycoprotein there are 18 different H subtypes and 11 different N subtypes (Tong *et al.*, 2012; Kraidi *et al.*, 2017) where 16 H subtypes and 9 N subtypes have been isolated from aquatic birds (Fouchier and Munster, 2009) and two H and N subtypes (H17N10 and H18N11) have recently been detected in bat(Tong *et al.*, 2013). The H protein is very important for transmission of virus and also a major determinant of host range (Neumann and Kawaoka, 2006).

The frequent variation in H antigens modify the pathogenicity and host ranges of the disease (Webster *et al.*, 1992). Most of the strains of the avian influenza virus are non-pathogenic and cause only mild clinical diseases (Parvin *et al.*, 2018). Based on the ability to cause systemic disease, AIV are broadly classified into two groups, LPAI virus and HPAI virus (Alexander, 2000). Some strains like H5N1, H7N9 are in the HPAI group where H9N2, H6N5 strains are in the LPAI group (Duvauchelle *et al.*, 2013).

Waterfowls are the natural reservoir of all subtypes of AI type A virus where no avert clinical manifestation in those birds that are affected by AIVs (Henning *et al.*, 2010; Khatun *et al.*, 2013). The H5N1 subtype (HPAI) can causes clinical manifestation ranging from mild to severe respiratory, nervous, reproductive and gastrointestinal system disorder, up to 100% mortality in domestic poultry and scattered incidence in humans (Chmielewski and Swayne, 2011; Chatziprodromidou *et al.*, 2018). Subtype H9N2 (LPAI) shows milder clinical signs in poultry like swelling of periorbital sinuses and tissues, typical respiratory discharge and reduced production of egg in domestic chickens (Hira *et al.*, 2017) while this subtype causes inflammatory lesions in the respiratory tract including trachea, bronchus and para-bronchous in some cases (Zhu *et al.*, 2018b) and also milder disease in humans which is less well identified (Uyeki *et al.*, 2012). Other subtypes of the AIV including H1, H2, H3, H4, H6, H7 and H11N3

have been isolated from apparently healthy birds from live bird markets in Bangladesh (Chowdhury, 2019). Some AIV subtypes like as H5, H7 and H9 can spread rapidly to the domestic poultry and other species and cause large scale outbreaks resulting severe damage to poultry industry (Alexander, 2007; WHO, 2016)by causing severe clinical signs and even death (Webster *et al.*, 2005).

2.5. Outbreak history of avian influenza

HPAI subtype H7 was first visualized in 1878 in Italian poultry causing highly lethal disease (Lupiani and Reddy, 2009). Primary outbreak of HPAI in poultry have been reported during 1959 to 1990 where eight outbreaks were happened in poultry worldwide where the first isolation of AIV (HPAI H5N3) was recorded in 1961in South Africa from feral bird(common terns) (Becker, 1966; Alexander, 2000). After that 3 major outbreaks happened in between 1991-1995 in the USA and Mexico in chickens and turkeys (Horimoto *et al.,* 1995; Halvorson *et al.,* 2003). From 1995 the outbreaks of HPAI virus have been reported with increased pattern at various places of the world (Alexander, 2000). The first isolated HPAI H5N1 in waterfowl (Geese) was recorded in 1996 in Guangdong, China (Xu *et al.,* 1999) and in domestic duck in 1997 from live bird market of Hong Kong (Shortridge *et al.,* 1998). After that year, HPAI H5N1 was reported frequently in live poultry markets and farms in the following years (1999, 2000, 2001 and 2002) (Sims *et al.*, 2003). Outbreaks of different subtypes of HPAI have been reported in poultry in many countries in recent years. During January 2005 – December 2012, 8345 outbreaks of HPAI were reported in domestic birds in 65 countries and territories where 4 subtypes were identified. From January 2013 – August 2018, 7122 outbreaks were reported in domestic birds in 68 countries and territories (OIE, 2018). South Asian countries, Pakistan, India and Myanmar have experienced HPAI outbreak in domestic poultry in early 2006 (Alam *et al.,* 2010). In March 2007, the first reported outbreak of HPAI H5N1 in domestic poultry in its territory (Biswas *et al.*, 2008) which was diagnosed and confirmed the presence of H5 subtype by the National References Laboratory for Avian Influenza (NRL-AI) at Bangladesh Livestock Research Institute (BLRI) (Alam *et al.*, 2010). After that, NRL-AI has traced 323 H5 and 3 H9 positive cases up to 22 March 2009 (Alam *et al.*, 2010). This disease gradually spread to at least 51 (80%) districts of Bangladesh (Giasuddin *et al.*, 2009), where 556 outbreaks of H5 were reported till December 2012 including 499 were in

commercial chicken and rest of the 57 in backyard chicken. During this outbreak, higher number of outbreaks started from the month of February followed by March (Giasuddin *et al.*, 2012), causing an estimation of US$746 million in financial loss to the poultry industry in Bangladesh (Chakma and Rushton, 2008).

2.6. Transmission of Avian Influenza virus

The viruses are shed at high frequency through fecal and oral route having ability to be transmitted to other avian and occasionally mammalian hosts including humans (Hinshaw *et al.*, 1980; Fouchier *et al.*, 2005; Sturm-Ramirez *et al.*, 2005). AIV can be transmitted from infected bird to healthy bird through direct contact and also by contaminated fecal materials, aerosols, water, feed, bedding materials and utensils (De Jong *et al.*, 2005). In rural areas, many households keep ducks and chickens in the same poultry sheds and in some cases people allow dwelling places as the sharing place for both household poultries and humans (Hassan *et al.*, 2015). These cultures of rearing systems can accelerate the AIV co-infection between domestic birds and humans (Biswas *et al.*, 2009; Sultana *et al.*, 2012a). Because of the free range rearing system, backyard chickens are more vulnerable to the HPAI H5N1 subtype viral infection and can transmit to the other backyard chickens and domestic ducks (Biswas *et al.*, 2009). Infected ducks can be the potential source of AIVs virus transmission to susceptible birds and humans while they graze on harvested rice fields and irrigation canals (Kim *et al.*, 2009; Pawar *et al.*, 2012; Prosser *et al.*, 2015). The contact between domestic ducks with wild waterfowl and other poultry species and human poses risk for spreading of AIVs (Gilbert *et al.*, 2006; Henning *et al.*, 2010; Henning *et al.*, 2011; Henning *et al.*, 2013). During winter in Bangladesh, there are a huge number of migratory birds come and share the open water bodies such as haors, rivers, canals with domestic ducks and these landscapes act as an important site for interaction between wild waterfowl and domestic ducks. The risk of AIV circulation increases prior to the arrival and departure of the migratory birds where domestic ducks graze in the same location(Cappelle *et al.*, 2014; Hassan *et al.*, 2018).

LBMs play an important role in epidemiology of AIV (Shortridge, 1999; Liu *et al.*, 2003). In LBMs with a mixture of wholesalers and retailers birds are at higher risk for viral infection rather than LBMs with only retail poultry business (Kim *et al.*, 2018). Indirect contacts of domestic poultry into LBMs through contaminated fecal materials, aerosols, water, feed, bedding and utensils are significantly associated with AIV

transmission (Katz, 2003; Dinh *et al.*, 2006; Chmielewski and Swayne, 2011). Sharing of equipment between poultry traders and selling of infected ducks with other poultry species increases the risk of virus transmission (Fournié *et al.*, 2013). Besides slaughtering of infected poultry for consumption can increase the risk of virus transmission (WHO, 2005).

2.7. Risk factors associated with Avian Influenza

The viral prevalence of AIV from cloacal samples in Backyard poultry have been recorded in variable level in different studies in between 2.2% - 51% (Alam *et al.*, 2010; Haider *et al.*, 2017; Turner *et al.*, 2017; Khan *et al.*, 2018b).The viral prevalence of household chicken was 8.3-40% (Negovetich *et al.*, 2011; Haider *et al.*, 2017) and incase of marketed household chicken subtype of AIV was recorded 4.1-6.3% H5 and 34.6-87% H9 in Bangladesh (Turner *et al.*, 2017; Kim *et al.*, 2018).Different level of viral prevalence in clocal sample of ducks in different parts of the Bangladesh: 0% in Shylet, 0.5% Mymenshing (Sarker *et al.*, 2017), 3.3% in Dinajpur, 4% in Rajshahi, 4.9-80% in Netrokona, 13.3% in Kishoreganj(Haider *et al.*, 2017; Khan *et al.*, 2018b), 18.3-26.9% in Hakalukihaor, Tanguarhaor and Jahangir lake during three winter season from 2009 -2011(Khatun *et al.*, 2013).The differences in AI viral prevalence can be seen in worldwide and it ranges from 0.05-13.2% in different location of the world whereas it is 0.05-3.4% in Asia, 2.5% in Africa, 1.7% in North America, 2.6-13.2% in Northern Europe and 5.1% in South Europe (Fereidouni *et al.*, 2010; Henning *et al.*, 2010; Karmacharya *et al.*, 2015; Kayali *et al.*, 2016). The subtype prevalence of AI viral prevalence in domestic ducks was recorded as 9% H9 and 34-56% H5 from cloacal sample in Bangladesh (Haider *et al.*, 2017; Turner *et al.*, 2017).

Based on the above-mentioned literature review, it is evident that the viral prevalence of avian influenza has previously been observed in poultry, independent of backyard or commercial poultry, at individual levels in Bangladesh and other countries of the world. Therefore, this present study has attempted to explore the status of AI in backyard poultry in Chattogram, Bangladesh. There are no available published literatures on risk factors associated with AI status in backyard poultry in Bangladesh. Therefore, only few studies conducted in Bangladesh that has explored the risk factors of AIV in the country including poultry health seeking behavior, farm biosecurity, socio demographic status of farmers, farming and trading practices (Biswas *et al.*, 2009; Loth *et al.*, 2010; Osmani *et al.*, 2014). Besides this, some global studies have been

conducted to find out the risk factors. In Indonesia, risk factors for spreading of HPAI is included movements of poultry, contact between ducks and other poultry and animal species, poor poultry husbandry practice, inadequate handling of sick birds and dead ducks by owners of flocks, and low knowledge among poultry farmers of control strategies (Henning *et al.*, 2010).

Above review revealed that, risk factors for AI RNA prevalence in backyard poultry are non-existent or limited in Bangladesh and elsewhere in the world. So, the current study to consider risk factor expeditions involved with AIV in backyard poultry in Bangladesh is needed to make reorganization to support policy-making decisions to control deadly AIV disease.

2.8. Public health significance

Avian influenza is a devastating disease for birds as well as for humans (Alam *et al.*, 2010). Some subtypes of AIV such as H5, H6, H7, H9, and H10 have caused infection in humans (Short *et al.*, 2015; Nathanson, 2016). The HPAI H5N1 is a major concern of public health. The ability of AIV to cause humans disease, low immunity in the population, transmissibility of the virus are the base of its pandemic potential (Capua and Alexander, 2004; Tanner *et al.*, 2015). In particular, the goose/Guangdong(gs/GD) lineage HPAI H5N1 is highly lethal in humans besides birds (Parvin *et al.*, 2018) which was first detected in humans in 1997 in Hong Kong (Claas *et al.*, 1998) .From 2003 to till now (2020) 861 human cases of HPAI H5N1 have been reported worldwide from them 455 patients died (WHO, 2020). As of present, eight H5N1 and three H9N2 infected human cases have been reported with one fatality in Bangladesh since 2003 (WHO, 2019) having recent history of poultry exposure including 4 of them were workers of live bird market in Bangladesh. There is a link between direct or close exposure with infected or dead poultry and the onset of the disease symptoms in H5N1 infected humans infection in endemic countries like Bangladesh (CDC, 2020). A recent study on backyard poultry raisers in Bangladesh reported, poultry raisers frequently come close contact with backyard poultry through daily rearing practices, feeding of sick birds, handling of sick and dead birds by hand, caring and slaughtering of sick birds inside their houses (Sultana *et al.*, 2012a). This close living system with poultry and low awareness of AIV drag them at high risk of transmitting zoonotic diseases (Sultana *et al.*, 2012b; Shanta *et al.*, 2017).

2.9. Surveillance

Currently there is no constant surveillance system to monitor the outbreak and status of the AI in backyard poultry in rural areas of Bangladesh. In cooperation with and funded by Sweden, the Department of Livestock Services (DLS), the United States Agency for International Development (USAID), the World Bank and the Food and Agriculture Organization (FAO) established active surveillance as part of the influenza preparedness, and response plan in 2008.The project aimed to gathering data and reporting on morbidity and mortality in poultry using the Short Message Service (SMS) gateway system, which was continued until 2013 (Rimi *et al.*, 2019). This system is found useful for farmers, especially for chicken and mixed backyard farming by reduction of the outbreak response time 4.8 days to 1.4 days and captured 86% of the outbreak (Shaman and Kohn, 2009; FAO, 2016). Furthermore, to strengthen the government surveillance system, the icddr,b, the US Centers for Disease Control and Prevention (CDC) in collaboration with DLS have been performing LBMs based sentinel surveillance for AIV in poultry since 2007 (Rimi *et al.*, 2019). This surveillance program is ongoing and has reported year-round detection of AIV in waterfowl, commercial chickens, backyard chickens, and pool environmental swabs (Khan *et al.*, 2018b). In 2016, (Government of Bangladesh) GoB's Animal and Humans Health Service developed 'sink surveillance' in partnership with FAO to detect AIV from Dhaka and Chittagong LBMs from environmental samples of LBMs, where surveillance recorded 87.9% positive LBMs for influenza A, 39.4% positive H5 and 21.2% positive H9 subtype (Rimi *et al.*, 2019). During 2010 – 2012 icddr,b in collaboration with EcoHealth Alliance and in between 2012 to 2015, two different study were conducted to assess the prevalence of AIV on both migratory wild birds and domestic ducks , where both studies have suggested wild birds and domestic ducks are the important reservoir of influenza A virus and can also shed virus (Islam *et al.*, 2013; Hassan *et al.*, 2017).

In 2010, for detecting humans' infection by AIV, the platform of National Influenza Surveillance, Bangladesh (NISB) was initiated by Institute of Epidemiology Disease Control and Research (IEDCR) to identify strains of the influenza virus circulating in Bangladesh. Prior to this, in 2007, icddr,b set up a hospital-based influenza surveillance (HBIS) in 12 hospitals across Bangladesh in coordination with the IEDCR and funded by the US CDC to classify individuals and clusters of infected individuals with life-threatening AIV (Zaman *et al.*, 2009; IEDCR, 2012). As many

studies show wild birds and domestic ducks play an important source of transmission of AIV into backyard poultry and also public health significantly, continuous surveillance is necessary among backyard poultry for early detection, response and management of avian influenza in Bangladesh.

2.10. Clades of avian influenza

The HA genes of H5N1 expressed extensively for last 20 years changing the genetic variance into 40 clades but currently only four clades 2.2.1.2, 2.3.2.1a, 2.3.2.1c and 2.3.4.4are currently circulating in worldwide (Barman *et al.*, 2017). In Bangladesh HPAI H5N1 was first introduced in Bangladesh in February 2007 and identified as Qinghai-like lineage, clade 2.2.2 (Ahmed *et al.*, 2012b) which is circulating until early 2011 in Bangladesh (Marinova-Petkova *et al.*, 2014) causing hundreds of poultry outbreak and two confirmed human cases (Brooks *et al.*, 2009). At the beginning of the 2011 two new H5N1 clades 2.3.2.1 and 2.3.4.2 have been introduced were detected in chickens, quails, crows and migratory bird (Islam *et al.*, 2012; Haque *et al.*, 2014; Parvin *et al.*, 2014b). Multiple outbreaks of H5N1 in crows reported during January-February 2011 where 2.3.2.1a clades of H5N1 were identified from central and southern districts of Bangladesh (Khan *et al.*, 2014). Besides this in 2013 clade 2.3.2.1a virus was noticed in LBMs of Bangladesh where it was detected from apparently health ducks (Haider *et al.*, 2017) and completely replaced into the previous predominant clade 2.2.2 (Marinova-Petkova *et al.*, 2014). On the other hand clade 2.3.4.2 was detected in March 2011 in eastern part of Bangladesh and successfully eradicated after the outbreak (Marinova-Petkova *et al.*, 2016).During January- February 2011, 61% of mortality in duck caused by clade 2.3.2.1a virus was reported on one duck breeding farm of India (Nagarajan *et al.*, 2012). From the outcome of (Shanmuganatham *et al.*, 2013) in Bangladesh, H9N2 viruses are of GI clade which consists of 2 branches differentiated by host species, but the viruses in Bangladesh were divergent from the prototype GI virus. Three lineages of H9N2 virus are currently circulating in poultry worldwide; i) Chicken/Beijing/1/94, ii) Quail/Hong Kong/G1/97, and iii) Duck/Hong Kong/Y439/97 (Nang *et al.*, 2013).

2.11. Laboratory diagnosis of avian influenza

Different types of diagnostic tests such as serological and molecular tests are available for the detection of AIV in the laboratory from different specimens. In

serological tests Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) having sensitivity 86% and specificity 88%, Agar Gel Immunodiffusion (AGID) test having 97% sensitivity and 99.8% specificity (Swayne and Suarez, 2000; Song *et al.*, 2009; Arnold *et al.*, 2018) and Hemagglutination-inhibition (HI) test having 98.8% sensitivity and 99.5% specificity are commonly used to detect AIV antibody from serum sample (Comin *et al.*, 2013).For molecular detection, nucleic acid magnification has been established as most sensitive and rapid process for detecting the virus where Polymerase Chain Reaction (PCR) is more sensitive and time saving method than other traditional methods of virus isolation process (Fouchier *et al.*, 2000; Pasick, 2008).For convenience and high sensitivity, Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is used to detect viral RNA and various influenza virus genes from samples (Liu, 2014; Bird and Mazet, 2018). The sensitivity of real time RT-PCR was reported as 93-99% and specificity was reported as 99.8-99.9% (Beck *et al.*, 2010; Li *et al.*, 2016).Furthermore, to diagnosis AIV, viral isolation in cell culture, immunofluorescence assays, nucleic acid amplification studies, rapid diagnostic tests focused on immune chromatography and nucleic acid sequencing methods are often used (Vemula *et al.*, 2016).

2.12. Prevention and Control

Farm biosecurity and hygiene is the effective approach to prevent and control infectious diseases in poultry. It can play an important role in preventing AIV and thus reduce the potential risk of zoonotic transmission to humans (Kelly *et al.*, 2008). Segregation, cleaning and disinfection are the three principle elements of biosecurity (FAO, 2008). Bangladesh farm biosecurity level varies across the different poultry production systems and it is very poor or non-existence in domestic poultry (Rimi *et al.*, 2017; Rahman *et al.*, 2019).Vaccination program against HPAI H5N1 has been practicing in limited scale in commercial; poultry in H5N1 endemic countries including Asian countries (Magalhães *et al.*, 2010; Tarigan *et al.*, 2018; Durr *et al.*, 2019). In Bangladesh vaccination against HPAI H5N1 has been available for commercial layers and breeders farms (Islam *et al.*, 2017) since 2014 authorized by the drug administration authority of the GoB (Rimi *et al.*, 2019). AI vaccine was introduced for the first time in Bangladesh in 2012 by the GoB and breeder association of Bangladesh in commercial poultry of two districts in Bangladesh (Rimi *et al.*, 2019). Currently some AIV vaccines

are available in Bangladesh; Cevac FLU H9 K (ACI pharmaceuticals), TROVACAIVH5, GALLIMUNE Flu and FLUVAC (Merial pharmaceuticals) and Nobilis Influenza H9N2 (Intervet) (Rahman, 2019). The vaccine of HPAI H5N1 is provided at the age of 4, 9 and 18 weeks of age for commercial birds (FAO, 2009; Tarigan *et al.*, 2018). H9N2 infection is not notifiable and vaccination against H9N2 is not permitted in Bangladesh (Parvin *et al.*, 2018). However, AI vaccination is not practiced in backyard poultry in Bangladesh though backyard poultry are susceptible to AIV where ducks are considered a natural reservoir as well as an important source of spreading infection to other poultry species and also humans. Awareness and educational campaigns among the backyard poultry raiser is also required to prevent and control infectious diseases like AI in poultry (Ansari *et al.*, 2016; Rimi *et al.*, 2019; Zhou *et al.*, 2019). Lack of awareness and lack of knowledge and practice of bio security has also contributed to continuing AI infection in Bangladesh (Parvin *et al.*, 2018). However, backyard poultry farmers in Bangladesh are lacking in gathering awareness and education about disease control measures (Rimi *et al.*, 2018).To prevent the risk of disease and its spread, backyard poultry farmers need to take measures such as avoiding fed remnants of slaughtered birds from markets or mobile poultry vendors to chicken and duck (Rahman, 2019). Law enforcement related to poultry trade helps in preventing and controlling AI as practice in different parts of the world (Guan *et al.*, 2007; Biswas *et al.*, 2009; Brooks-Moizer *et al.*, 2009). In Bangladesh, veterinary laws and regulation on trade are absent or poorly maintained in poultry sectors.

2.13. Conclusion:

From the above reviewed literature, we can suggest that a good number of studies have been previously conducted on the AIV on commercial poultry and LBMs in Bangladesh. However, some studies have been found on domestic ducks in Bangladesh. Nevertheless, epidemiological studies of AI in backyard poultry are absent or very limited in Bangladesh and particularly in the Chattogram region. Therefore, the present study on Epidemiological investigation of AI on backyard poultry in Chattogram district will help to enrich our understanding on the epidemiology of AIV of this region on backyard poultry. Besides this, our findings are believed to serve as a baseline for future researchers and making policy for public health intervention to control AI.

Chapter-3: Materials and Methods

3.1 Description of Study sites

Chattogram district is situated in the southeastern part of Bangladesh having 22°0'45'' North (N) latitude and 92°6'1'' East (E) longitude. This district is subdivided into 18 administrative areas called upazila or thana (smallest administrative boundary) (Anon, 2019). The present study was conducted in two different Anowara upazila and Rangunia upazila of Chattogram district. Anowara upazila is a coastal area, located between 22.1° and 22.2° N and between 91.5° and 91.6° E where Rangunia upazila is inland, residing between 22.2° and 22.4° N and between 91.6° and 92.1° E. These two areas have both low and high land, hills and rivers (Hossain *et al.*, 2016). The climate characteristics of these areas vary from 13° to 32°C in environmental temperature and humidity is 70 to 85% where average rainfall is in 5.6mm to 727.0 mm range (Anon, 2019). The Anowara and Rangunia regions are 164.1 sq km and 361.5 sq km, respectively and the estimated humans population is 259,022 and 339,004, respectively (PHC, 2011). Bangladesh is a developing country, where 64% of the population lives in the rural area and nearly 71% rural households raise backward poultry (FAO, 2008). In our study area, the regions are widespread with household poultry production on the banks of Karnaphuli and Sangu rivers. Around 80% of households rear chickens or ducks or both under semi-scavenging production systems. The total household poultry population including ducks is 591043 in Anowara and 797191 in Rangunia (BBS, 2017; DLS, 2020). Besides, there is a good number of small- to large scale commercial poultry farms (Anowara: 252 broiler farms and 8 duck farms; Rangunia: 126 broiler farms and 3 layer farms) (BBS, 2017; DLS, 2020). According to the highest poultry population density, a total of six villages were selected randomly for the present study namely Roypur, Sarenga, Dudkumara and Gahira village from Anowara upazila, and Tinchadia, and Lalanagar village from Rangunia. The selected households under these six villages were previously used for an intervention study under the scope of Chattogram Veterinary and Animal Sciences University (CVASU), project supported by Department for International Development (DFID), the United Kingdom (UK) (2016-17) (MA Hoque, CVASU, Personal communication).

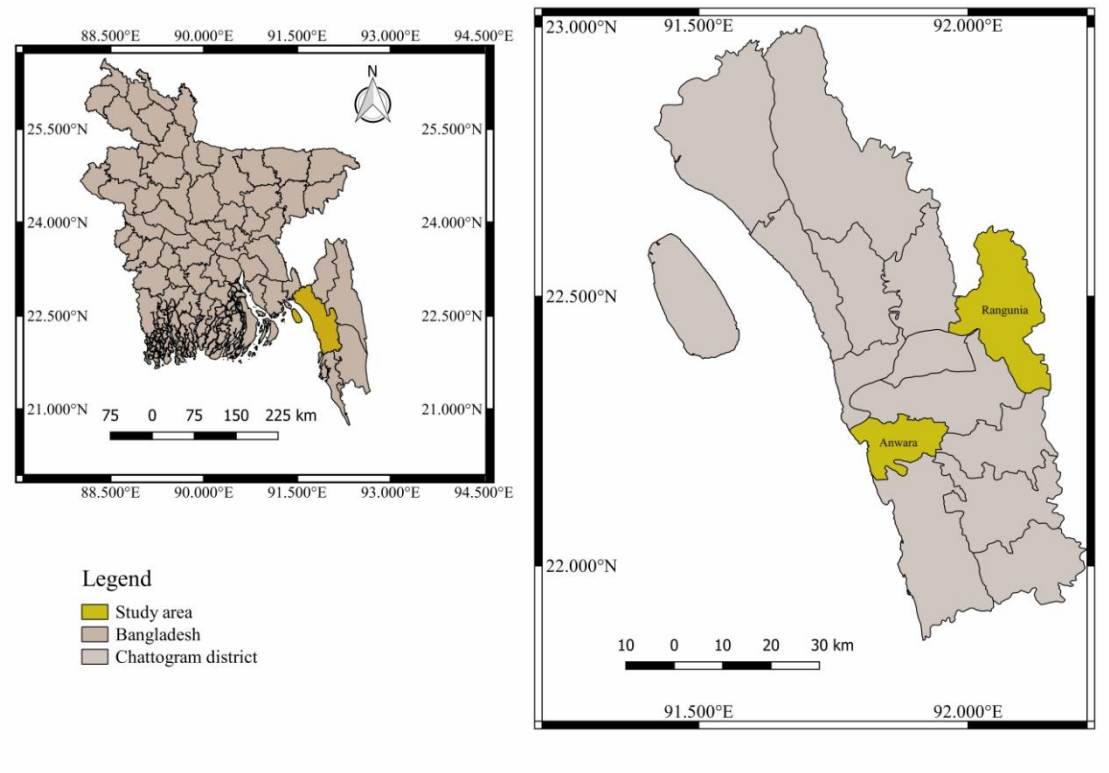

Figure: 3.1 Location of the Study areas in Chattogram district.

3.2 Study period

A cross-sectional study was designed on household chickens and ducks belonging to Anowara (repeated cross-sectional, covering both summer and winter seasons) and Rangunia (single cross-sectional, covering winter season only) upazila of Chattogram in between July 2017 and January 2018.

3.3 Target population

Households having poultry especially ducks and chickens of the study upazilas were selected as the target population

3.4 Study population and sampling frame

Households having chickens and ducks under the selected six villages of the two upazilas were considered as the source population for the investigation. A complete list of households of the villages, having at least 4 chickens and 4 ducks per household, was made and used as the sampling frame for selecting households for the study. The list consisted of 300 households, each village consisting of 45-55 households.

3.5 Sample size, calculation and sampling

A total of 292 households of duck rearing, 146 per season, including 272 household combine duck and chicken rearing, 136 per season were required for the study assuming 50% expected AI sero-prevalence ±10% precession, 95% confidence interval (CI), and 2% design effect (Rahman, 2019). However, we were able to collect samples from a total of 281 households of duck rearing and 251 from chicken rearing. A proportionate probability of random sampling was applied to recruit the required number of households for the study.

3.6 Sample collection, preservation and transportation

One chicken per household and one duck per household were selected randomly for sample collection. Swabs samples from cloacal were collected by wearing proper personal protective equipment. Swabs were taken from birds by inserting swab sticks (until fecal contamination) into the vent. Individual sample items were collected by using sterile cotton swab sticks (such as sterile cotton swab, model: PW005 by HiMedia Laboratories) and then were placed in 2 ml sterile cryo-vial containing 1-1.5 ml viral transport media (VTM). The VTM was prepared according to the recipe described by Healing and Organization (2006). Cryo-vials with samples were given unique identification numbers, placed within an insulated ice-box and transferred immediately (within 4 hours of collection) to the PRTC laboratory. Swab samples were stored at -80°C for testing.

3.7 Data collection

A structured was developed and pre-tested questionnaire was administered to the chicken and duck farmers to acquire the data. The information related to identity data (identity number), farmer's details (name, age, gender, educational status, time of duck rearing and income source of the farmer), farm demography (location, farm size, vaccination status and deworming status), farm management practices (scavenging, housing, feeding, cleaning, disposal of litter as well as dead birds and challenge) and risk factors (household level).

3.8 Laboratory assessment

3.8.1 RNA extraction and rRT-PCR

For conducting molecular assessment cloacal swab chicken samples were pooled and each pool consisted of 6 swab samples besides duck samples were pooled where each pool formed of 8 swab samples. Therefore, there were 42 chicken pool samples and 36 duck pool samples, which underwent AIV RNA extraction by using the MagMAXTM-96 Viral RNA Isolation Kit (QIAGEN, Hilden, Germany and its published protocol (AM1836 and AMB1836-5)). RNA extracts were then screened for AIV RNA using RT-PCR technology by using primers and probe specific for M gene of AIV in a Fast RT-PCR machine (ABI 7500) (Monne *et al.*, 2008; Heine *et al.*, 2015). The AgPath-ID One-Step RT-PCR kit (ThermoFisher Scientific, Waltham, MA, USA) (Catalogue no lot AM1005) was chosen for RT-PCR testing. A pool with a cycle threshold value (CT) <40 for the AIV Matrix gene was defined as an AIV RNA positive pool. Each swab of the positive pools was again extracted as explained previously and followed by screening for AIV RNA using the same rRT-PCR for M gene detection. Positive individual swabs (CT<40 for the AIV M gene) then were undergone for the H5 and H9 testing and considered to be rRT-PCR-positive if CT<38 and 40, respectively (Monne *et al.*, 2008; Heine *et al.*, 2015). Individual swab results for M/H5/H9 were defined as a chicken household as positive or negative. If any swab pool was regarded as negative in the initial rRT-PCR for M gene, then individual swab samples of these negative pools were defined as negative for M/H5/H9 and thus individual chicken households were treated as a negative household. The primers and probe sequences for M gene were as follows: 5´-AGATGAGTCTTCTAACCGAGGTCG-3´ (M+25), 5´-TGCAAAAACATCTTCAAGTCTCTG-3´ (M-124) and 5´-TCAGGCCCCCTCAAAGCCGA-TAMRA-3´ (M+64) (Hassan *et al.*, 2020c).

3.9 Sequencing and phylogenetic analysis for M gene, HA gene, NA gene and PB1 gene:

RNA was extracted from all positive samples using Trizol method (Invitrogen, Carlsbas, CA, USA) and Direct-Zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) by the following of the manufacturers' protocol. cDNA was synthesized using Superscript III (Invitrogen, Carlsbas, CA, USA) as the manufacturers' indication

(Hassan *et al.*, 2020c). RT-PCR was performed for the amplification of M gene using primers FLUAW-M-U44 (GTCTTCTAACCGAGGTCGAAACG) and FLUAV-M-L287(GCATTTTGGACAAAGCGTCTACG) to produce 243-bp amplicon. Similarly primers were followed for the amplification of PB1 gene follow as:FLUAPB1-F(ATGATGATGGGNATGTTYAAYATG) and FLUAPB1-R(GCNGGNCCNAKDTCRYTRTTDATCAT)(Anthony *et al.*, 2016) and for HA gene primers were Bm-HA-1 (TATTCGTCTCAGGGAGCAAAAGCAGGGG) andBm-NS-890R (ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTTT)and for NA gene: Ba-NA-1 (TATTGGTCTCAGGGAGCAAAAGCAGGAGT) and Ba-NA-1413R (ATATGGTCTCGTATTAGTAGAAACAAGGAGTTTTTT) (Hoffmann *et al.*, 2001)

Sequences of M gene, HA gene, NA gene and PB1 gene were placed individually in the NCBI/GenBank database using Basic Local Alignment Tool (BLAST) search tool for searching similar sequences. Sequences of more than 95% were selected and aligned using the Clusta1W. Phylogenetic trees were constructed according to the maximum likelihood method using the Molecular Evolution Genetic Analysis (MEGA) version X (Kumar *et al.*, 2018).

3.10 Statistical analysis

Both field and laboratory data were recorded in Microsoft Access 2007. The data set was then cleaned, coded, recorded and checked for integrity in MS Access before exporting to STATA-13 (STATA Crop, 4905, Lake Way drive, College station, Texas 77845, USA) for conducting epidemiological data analysis.

3.10.1 Descriptive analysis

Descriptive analysis was done to calculate the household level RNA, M gene, H5 and H9 prevalence based on RT-PCR results. Distribution was revealed by study area, season and farm size. Descriptive analysis was also generated on some variables of chicken and duck farmers.

3.10.2 Risk factor analysis:

3.10.2.1 Univariable analysis

Univariable analysis was done for different factors related to farm demographic situation and farm management practices in the study area. Study area was categorized according to the 2 separate upazilas. Season of the same collection was classified into temporal pattern (winter and summer). Farm size was categorized based on the percentile. Cleaning of house was classified based on clean with broom or clean with disinfectant like ash, soap, detergents. Cleaning of feeder and waterer was categorized on the basis of cleaning with only water or with disinfectant. Cleaning frequency of house was divided into two separate groups cleaning daily and cleaning once or twice weekly or monthly. Cleaning frequency of waterer and feeder was categorized based on the cleaning every time after use and once in a day or week. Litter removal factor was categorized on cleaning of litter once in a day/ week and twice or more in a week. Location of litter disposal was grouped into 3 subcategories spread on fields/bushes, use for compost/bury/pit/yard and throw in water body. Disposal of dead bird factor was categorized into two different groups as bury in pit and feed to animal or throw into water body. 'Fisher Exact Test' was performed in STATA 13 to identify univariate association of factors with the prevalence of AI in backyard poultry.

Chapter-4: Result

4.1. Descriptive analysis:

4.1.1 Avian influenza viral prevalence at household level in backyard poultry in Chattogram:

The overall prevalence of AI viral RNA prevalence in backyard poultry was 6% (95% CI: 3.6 – 9.3; N=300) in Chattogram within the study period. In backyard poultry, the prevalence was 3.2% (95% CI: 1.4 – 6.2: N= 251) in chickens, whereas it was 3.6% (95% CI: 1.7 – 6.4; N=281) in duck. In Anowara the viral RNA prevalence was detected 7.1% (95% CI: 4.3 – 10.9; N= 254). In terms of temporal distribution, the AI viral RNA prevalence was 3.1% (95% CI: 0.8 – 7.7; N=129) and 8.2% (95% CI: 4.5 – 13.3, N=171) in the Summer and Winter season, respectively **(Table 4.1).** Within the AI viral RNA prevalence 2.7% (95% CI: 1.2 – 5.2; N=300) was recorded as viral prevalence of AIV H5 and 3.3% (95% CI: 1.6 – 6.1; N= 300) in H9 subtype, respectively.

Table No: 4.1 Distribution of AIV M gene and HA gene (H5 and H9 RNA subtypes) in household chickens and ducks (N= 300)

Variable	**Category (N)**	**AIV M gene, n (%); 95% CI**	**AIV H5 sub Type, n (%); 95% CI**	**AIV H9 sub Type, n (%); 95% CI**
Species	Household with Chickens (251)	8 (3.2); 1.4 – 6.2	4 (1.6); 0.4 – 4	4 (1.6); 0.4– 4
	Households with duck (281)	10 (3.6); 1.7 – 6.4	4 (1.4); 0.4 – 3.6	6 (2.1); 0.8 – 4.6
Area	Anowara (254)	18 (7.1); 4.3 – 10.9	8 (3.2); 1.4 – 6.1	10 (3.9); 1.9 – 7.1
	Rangunia (46)	0; 0 – 7.7	0; 0 – 7.7	0; 0 – 7.7
Season	Summer (129)	4 (3.1); 0.8 – 7.7	2 (1.6); 0.2 – 5.5	2 (1.6); 0.2 – 5.5
	Winter (171)	14 (8.2); 4.5 – 13.3	6 (3.5); 1.3 – 7.5	8 (4.7); 2 – 9

4.2. Risk factor analysis:

4.2.1 Univariable association between AI viral prevalence in backyard poultry species (chicken and duck) and the associated factors

The AI viral RNA prevalence was 3.9% in both poultry species in Anowara, whereas no positive cases found in Rangunia. In the temporal distribution, the viral RNA prevalence was recorded higher in winter for both species (6.6% in chicken and 3.5% in duck) compared to the summer season, recorded as 0% (0 – 2.8; N= 129) in chicken and 3.6% (1 – 7.5; N= 122) in duck. Among the scavenging system, the prevalence was recorded higher in duck (7.1%; 95% CI: 0.2 – 33.9; N= 14) than in chicken (3.2%; 95% CI: 1.4 – 6.2, N= 251) in house premises with rice paddy in chicken, whereas in duck, higher prevalence was recorded as 3.4% (1.6 – 6.3; N= 267) in house premises with water bodies sub category.

Based on the location of backyard poultry house, viral RNA prevalence was higher in duck (5.1%; 1.7 – 11.5; N=98) than chicken (1.1%; 0 - 6; N= 91) that were kept in yard, but this prevalence was recorded higher in chicken (4.4%; 1.8 – 8.8; N= 160) than in duck (2.7%; 0.9 – 6.3; N= 183) where poultry were rearing within the house. AI viral prevalence was detected higher in poultry house having no facility of free air movement in chicken (3.5%; 1.4 – 7.1; N= 199) and in duck (4.4%; 2.1 – 7.9; N= 229) than the poultry houses had open air movement facilities in both species. Viral RNA prevalence was found 3.3% (1.4 – 6.3; N= 245) and 3.7% (1.8 – 6.7; N= 268), respectively in chicken and duck where the feeders were cleaned with only water. Between the cleaning practice of waterers, AI viral RNA prevalence was recorded higher in duck (5.7%, 0.7 – 19.2; N= 35) compared to chicken (0%; 0 – 8.2; N= 43) that cleaned with only water whereas it was 3.8% (1.6 – 7.4; N= 208) in chicken and 3.3% (1.4 – 6.3; N= 246) in duck species that cleaned without water. The AI viral prevalence was calculated higher in chickens (7.7%; 2.9 – 16; N= 78) and ducks (4.5%; 1.2 –11.1; N= 89), where the poultry house was cleaned once daily than the prevalence in the poultry house that was cleaned weekly or monthly.

In the cleaning frequency of feeders AI viral prevalence was calculated higher in duck (4.8%; 0.6 – 16.2; N= 42) than chicken (0%; 0 – 6.6, N= 54) in once in a day

or week whereas it was 4.1% (1.8 – 7.8; N= 197) in chicken and 3.4% (1.5 – 6.5; N= 239) in duck where feeders were cleaned every time after use. In the cleaning frequency of the waterer, AI viral prevalence was recorded higher in duck (8%; 1 – 26; N= 25) than chicken (0%; 0 – 12.8, N= 27) in every time after use whereas it was 3.6% (1.6 – 6.9; N= 224) in chicken and 3.1% (1.3 – 6.1; N= 256) in duck where waterer was cleaned once in a day or week. Between the removing frequency of litter, AI viral prevalence was calculated higher in both poultry species (4.6%; 2 – 8.8; N= 175 in chicken and 4.1%; 1.8 – 7.9; N= 195 in duck) once in a day or week than compared to the removing twice or more in a week (0% in chicken and 2.3% in duck). The prevalence was recorded higher in chicken as 5% (2.1 – 10.1; N= 139) than the duck (4.1%; 1.7 – 8.3; N= 169) where litter was disposed into water bodies as pond/ lake/ canal/ sea followed by buried in pit or used for compost (4.3%; 0.5 – 14.5; N= 47) in duck. Between the practice of dead bird disposal, this viral prevalence was higher in duck (4.2%; 2 – 7.5; N= 241) than the chicken (3.8%; 1.6 – 7.3; N= 212) in feeding to animals or throw into water bodies (pond, canal, sea etc.) **(Table 4.2).**

Table No: 4.2 Univariable associations between binary response of household-level AI in household chicken and ducks

Variable	**Category**	**Chicken (%); 95% CI**	**p (Fisher exact test)**	**Category**	**Duck (%); 95% CI**	**p (Fisher exact test)**
Area	Anowara (205)	8 (3.9); 1.7 – 7.5	0.173	Anowara (254)	10 (3.9); 1.9 – 7.1	0.294
	Rangunia (46)	0; 0 – 7.7		Rangunia (27)	0; 0 – 12.8	
Season	Summer (129)	0; 0 – 2.8	0.003	Summer (110)	4 (3.6); 1 – 9.1	0.955
	Winter (122)	8 (6.6); 2.8 – 12.5		Winter (165)	6 (3.5); 1.3 – 7.5	
Scavenging system	House premises with Rice paddy (251)	8 (3.2); 1.4 – 6.2	N/A	House premises with Rice paddy (14)	1 (7.1); 0.2 – 33.9	0.458

	House premises with water bodies (0)	0; 0 – 1.4		House premises with water bodies (267)	9 (3.4); 1.6 – 6.3	
Housing location	Yard (91)	1 (1.1); 0 – 6	0.155	Yard (98)	5 (5.1); 1.7 – 11.5	0.307
	Within house (160)	7 (4.4); 1.8 – 8.8		Within house (183)	5 (2.7); 0.9 – 6.3	
House ventilation	Wall opening/No Ventilation (199)	7 (3.5); 1.4 – 7.1	0.56	Wall opening/No Ventilation (229)	10 (4.4); 2.1 – 7.9	0.125
	Open Air (52)	1 (1.9); 0 – 10.2		Open Air (52)	0 (0); 0 – 6.8	
Cleaning of House	Clean With Boom (239)	7 (2.9); 1.2 – 5.9	0.298	Clean With Boom (272)	10 (3.7); 1.8 – 6.6	0.558
	Clean with Disinfectant (12)	1 (8.3); 0.2 – 38.5		Clean with Disinfectant (9)	0; 0 – 33.6	
Cleaning feeder	Clean with water (245)	8 (3.3); 1.4 – 6.3	0.653	Clean with water (268)	10 (3.7); 1.8 – 6.7	0.478
	Cleaning with disinfectant (6)	0; 0 – 45.9		Cleaning with disinfectant (13)	0; 0 – 24.7	
Cleaning waterer	Clean with water (43)	0; 0 – 8.2	0.191	Clean with water (35)	2 (5.7); 0.7 – 19.2	0.462
	Clean without water (208)	8 (3.8); 1.6 – 7.4		Clean without water (246)	8 (3.3); 1.4 – 6.3	
Cleaning frequency	Once daily (78)	6 (7.7); 2.9 – 16	0.006	Once daily (89)	4 (4.5); 1.2 – 11.1	0.564

of poultry house	Once or twice weekly or monthly (173)	2 (1.2); 0.1 – 4.1		Once or twice weekly or monthly (192)	6 (3.1); 1.2 – 6.7	
Cleaning frequency of feeder	Every time after use (197)	8 (4.1); 1.8 – 7.8	0.132	Every time after use (239)	8 (3.4); 1.5 – 6.5	0.648
	Once in a day or week (54)	0; 0 – 6.6		Once in a day or week (42)	2 (4.8); 0.6 – 16.2	
Cleaning frequency of waterer	Every time after use (27)	0; 0 – 12.8	0.318	Every time after use (25)	2 (8); 1 – 26	0.209
	Once in a day or week (224)	8 (3.6); 1.6 – 6.9		Once in a day or week (256)	8 (3.1); 1.3 – 6.1	
Litter removed	Once in a day/Once in a week (175)	8 (4.6); 2 – 8.8	0.058	Once in a day/Once in a week (195)	8 (4.1); 1.8 – 7.9	0.459
	Twice or more in a week (76)	0; 0 – 4.7		Twice or more in a week (86)	2 (2.3); 0.3 – 8.1	
Litter disposed	Spread on fields/ throw in bushes (62)	1 (1.6); 0 – 8.7	0.159	Spread on fields/ throw in bushes (65)	1 (1.5); 0 – 8.3	0.604
	Compost/ bury/ throw in pit/ left in yard (50)	0; 0 – 7.1		Compost/ bury/ throw in pit/ left in yard (47)	2 (4.3); 0.5– 14.5	
	Throw in pond/ lake/ canal/ sea (139)	7 (5); 2.1 – 10.1		Throw in pond/ lake/ canal/ sea (169)	7 (4.1); 1.7 – 8.3	

Dead bird disposal	Bury and throw in pit (39)	0; 0 – 9.1	0.218	Bury and throw in pit (40)	0; 0 – 8.8	0.190
	Feed to other animals /throw in pond/ lake/ canal/ sea/ throw in bushes/ road side (212)	8 (3.8); 1.6 – 7.3		Feed to other animals /throw in pond/ lake/ canal/ sea/ throw in bushes/ road side (241)	10 (4.2); 2 – 7.5	

4.2.2 Univariable association between AI viral RNA subtype (H5 and H9) - prevalence in backyard poultry and the associated factors

Based on the scavenging system of backyard poultry, the viral prevalence was recorded higher in H9 subtype as 3.6% (1.7 – 6.4; N= 282) than H5 subtype (2.8%; 1.2 – 5.5; N= 282) where poultry species were scavenging in house premises with water bodies. Between the location of backyard poultry house, AI viral prevalence was resulted higher in H9 (3.6%; 1.4 – 7.3; N= 195) than in H5 (2.6%; 0.8 – 5.9; N= 195) in within house whereas it was 2.9% (0.6 – 8.1; N= 105) in yard in both viral subtypes. On the basis of air ventilation facilities, the viral prevalence was recorded higher in poultry house having no ventilation in both viral subtypes as 3.3% (1.4 – 6.4; N= 243) in H5 and 3.7% (1.7 – 6.9; N= 243) in H9 than the house having free air movement facilities as 0% (0 – 6.3; N= 57) and 1.7% (0 – 9.4; N= 57) in respective viral subtype.

Between the cleaning process of poultry house, the viral prevalence was recorded higher in H5 (8.3%; 0.1 – 38.5; N= 12) than H9 (0%, 0 – 26.5; N= 12) in cleaning with disinfectant whereas it was higher in H9 (3.5%; 1.7 – 6.3; N= 288) than in H5 (2.4%; 1 – 4.9; N= 288) in cleaning with only boom. The RNA prevalence was calculated higher in cleaning of feeders with only water as 3.4% (1.6 – 6.2; N= 293) in H9 and 2.4% (1 – 4.9; N= 293) in H5 subtype than in cleaning with disinfectants in

both viral subtypes. For the cleaning process of waterer, the AI viral prevalence was recorded higher in H9 (3.5%; 1.6 – 6.5; N= 257) than in H5 (2.7%; 1.1 – 5.5; N= 257) in cleaning without water whereas in cleaning with water, it was 2.3% (0 – 12.3; N= 43) in both H5 and H9 subtypes.

The prevalence was recorded higher in H5 subtype as 7.1% (2.9 – 14; N= 99) than H9 subtype (3%; 0.6 – 8.6; N= 99) where poultry house was cleaned once daily whereas it was higher in H9 (3.5%; 1.4 – 7; N= 201) than in H5 (0.5%; 0 – 2.7; N= 201) in cleaning once or more in week or month. Between the cleaning frequency of the feeder, the AI viral prevalence was calculated higher in H9 subtype (3.7%; 1.7 – 7; N= 241) than H5 subtype (2.9%; 1.2 – 5.9; N= 241) in cleaning of feeder every time after use whereas it was 1.7% (0 – 9.1; N= 59) in cleaning every time after use in both viral subtypes. Based on the cleaning frequency of waterer the viral prevalence was observed higher in H9 (3.3%; 1.5 – 6.2; N= 273) than in H5 (2.6%, 1.1 – 5.2; N= 273) in cleaning of waterer once in a day or week, whereas it was 3.7% (0.1 – 19; N= 27) in cleaning every time after use in both viral subtypes.

The prevalence was calculated higher in H9 subtype (2.3%; 0.3 – 7.9; N= 89) than H5 subtype (0%; 0 – 4.1; N= 89) in removing of litter twice or more in a week a week whereas it was 3.8% (1.6 – 7.3; N= 211) in both subtypes where litter was removed once in a week. Among the process of litter disposal, the viral prevalence was calculated higher in throwing into water bodies as 4.7% (2.1 – 9.1; N= 169) in H9 and 3.6%; 1.3 – 7.6; N= 169) in H5 than using as compost or in pit (1.9%; 0 – 9.9; N= 54) and spreading on fields or throw in bushes (1.3%; 0 – 7; N= 77) in both species correspondingly. Between the dead bird disposal practice, the viral prevalence was recorded higher in H9 subtype (3.9%; 1.9 – 7.1; N= 256) than H5 subtype (3.1%; 1.4 – 6.1; N= 256) in feeding to animals or throw into water bodies whereas no positive observation was found in burying or throwing in pit **(Table 4.3).**

Table No: 4.3 Univariable associations between binary response of household-level AI virus RNA subtype (H5 and H9) prevalence in backyard poultry and the associated factors

Variable	**Category**	**H5 (%); 95% CI**	**H9 (%); 95% CI**	**p (Fisher exact test)**
Area	Anowara (254)	8 (3.2); 1.4 – 6.1	10 (3.9); 1.9 – 7.1	0.268
	Rangunia (46)	0; 0 – 7.7	0; 0 – 7.7	
Season	Summer (129)	2 (1.6); 0.2 – 5.5	2 (1.6); 0.2 – 5.5	0.232
	Winter (171)	6 (3.5); 1.3 – 7.5	8 (4.7); 2 – 9	
Scavenging system	House premises with Rice paddy (282)	8 (2.8); 1.2 – 5.5	10 (3.6); 1.7 – 6.4	1
	House premises with water bodies (18)	0;0 – 18.5	0;0 – 18.5	
Housing location	Yard (105)	3 (2.9); 0.6 – 8.1	3 (2.9); 0.6 – 8.1	1
	Within house (195)	5 (2.6); 0.8 – 5.9	7 (3.6); 1.4 –7.3	
House ventilation	Wall opening/No Ventilation (243)	8 (3.3); 1.4 – 6.4	9 (3.7); 1.7 – 6.9	0.444
	Open Air (57)	0; 0 – 6.3	1 (1.7); 0 – 9.4	
Cleaning of House	Clean With Boom (288)	7 (2.4); 1–4.9	10 (3.5); 1.7 – 6.3	0.323
	Clean with Disinfectant (12)	1 (8.3); 0.2 – 38.5	0; 0 – 26.5	
Cleaning feeder	Clean with water (293)	7 (2.4); 1–4.9	10 (3.4); 1.6 – 6.2	0.191
	Cleaning with disinfectant (7)	1 (14.3); 3.6 – 57.9	0; 0 – 40.9	
Cleaning waterer	Clean with water (43)	1 (2.3); 0 – 12.3	1 (2.3); 0 – 12.3	1
	Clean without water (257)	7 (2.7); 1.1 – 5.5	9 (3.5); 1.6 – 6.5	
	Once daily (99)	7 (7.1); 2.9 – 14	3 (3); 0.6 – 8.6	0.004

Cleaning frequency of poultry house	Once or twice weekly or monthly (201)	1 (0.5); 0 – 2.7	7 (3.5); 1.4 – 7	
Cleaning frequency of feeder	Every time after use (241)	7 (2.9); 1.2–5.9	9 (3.7); 1.7 – 7	0.895
	Once in a day or week (59)	1 (1.7); 0 – 9.1	1 (1.7); 0 – 9.1	
Cleaning frequency of waterer	Every time after use (27)	1 (3.7); 0.1 – 19	1 (3.7); 0.1 – 19	0.643
	Once in a day or week (273)	7 (2.6); 1.1 – 5.2	9 (3.3); 1.5 – 6.2	
Litter removed	Once in a week (211)	8 (3.8); 1.6 – 7.3	8 (3.8); 1.6 – 7.3	0.169
	Twice or more in a week (89)	0; 0 – 4.1	2 (2.3); 0.3 – 7.9	
Litter disposed	Spread on fields/ throw in bushes (77)	1 (1.3); 0 – 7	1 (1.3); 0 – 7	0.609
	Compost/ bury/ throw in pit/ left in yard (54)	1 (1.9); 0 – 9.9	1 (1.9); 0 – 9.9	
	Throw in pond/ lake/ canal/ sea (169)	6 (3.6); 1.3 – 7.6	8 (4.7); 2.1 – 9.1	
Dead bird disposal	Bury and throw in pit (44)	0; 0 – 8	0; 0 – 8	0.352
	Feed to other animals /Throw in pond/ lake/ canal/ sea/ throw in bushes/ road side (256)	8 (3.1); 1.4 – 6.1	10 (3.9); 1.9 – 7.1	

4.2.3 Univariable association between AI viral RNA subtype (H5 and H9) - prevalence in backyard poultry species (chicken and duck) and the associated factors

The AI viral prevalence in chicken was 2% (95% CI: 0.5 – 4.9; N= 205) in both the H5 and H9 viral RNA subtype but in duck this prevalence was higher in H9 (2.4%; 0.9 – 5.1; N= 254) than in H5 (1.6%; 0.4 – 4; N= 254) and in Anowara whereas no positive observation was calculated in both Rangunia. On the temporal distribution, the RNA prevalence was recorded higher in H9 (2.3%; 0.6 – 5.9; N= 171) than in H5 (1.2%, 0.1 – 4.1; N= 171) in duck whereas it was 3.3% (0.9 – 8.2; N= 122) in both H5 and H9 subtypes of chicken in winter season but in summer the AI viral prevalence was higher in duck as 1.8% (0.2 – 6.4; N= 110) in both viral subtypes than in chicken (0%, 0 – 2.8; N= 129) in both subtypes. The Al viral prevalence in H9 viral subtype in duck was calculated higher in both scavenging systems as 7.1% (0.2 – 33.9; N= 14) in house premises with rice paddy and 1.9% (0.6 –4.3; N= 267) in house premises with water bodies than in H5 as 0% and 1.5% in respective scavenging categories in duck whereas in chicken it was observed as 1.6% (0.4 – 4; N= 251) in house premises with rice paddy in both viral subtypes.

On the location of the poultry house, the AI viral prevalence in duck was recorded higher in H9 as 3.1% (0.6 – 8.7; N= 98) in yard and 1.6% (0.3 – 4.7; N= 183) in within house than in H5 as 2% (0.2 – 7.2; N= 98) and 1.1% (0.1 – 3.9; N= 183) in respective category whereas in chicken it was calculated higher in H5 (1.1%; 0.3 – 6; N= 91) than in H9 (0%; 0 – 4; N= 91) in yard but in within house it was higher in H5 (2.5%; 0.7 – 6.3; N= 160) than in H9 (1.9%; 0.4 – 5.4; N= 160). The AI viral prevalence in poultry house having no ventilation facilities were recorded higher in H5 (2%; 0.6 – 5.1; N= 199) than in H9 (1.5%; 0.3 – 4.3; N= 199) in chicken whereas in duck this prevalence was calculated higher in H9 (2.6%; 1 – 5.6; N= 229) than in H5 (1.7%; 0.5 – 4.4; N= 229).

Based on the cleaning practice of poultry house, the AI viral RNA prevalence in cleaning with boom was calculated higher in H9 of both poultry species as 2.2% (0.8 – 4.7; N= 272) in duck and 1.7% (0.5 – 4.2; N= 239) in chicken than in H5 in both species (1.5% in duck and 1.3% in chicken). Between the cleaning processes of feeder, the viral prevalence in cleaning with only water was resulted higher in H9 (2.2%; 0.8 –

4.8; N= 268) than in H5 (1.5%; 0.4 – 3.8; N= 268) in duck whereas in chicken it was 1.6% (0.4 – 4.1; N= 245) in both RNA subtypes. On the cleaning facilities of waterer, the viral prevalence in cleaning without water was higher in H9 (2%; 0.7 – 4.7; N= 246) than H5 (1.2%; 0.2 – 3.5; N= 246) in duck whereas in chicken it was 1.9% (0.5 – 4.8; N= 208) in both RNA subtypes.

According to the cleaning frequency of poultry house, the viral prevalence in cleaning of house once daily was resulted higher in H5 (5.1%; 1.4 – 12.6; N= 78) than in H9 (2.6%; 0.3 – 8.9; N= 78) in chicken but in duck it was observed higher in H9 (3.4%; 0.7 – 9.5; N= 89) than in H5 (1.1%; 0 – 6.1; N= 89), whereas in cleaning house once or twice weekly or monthly the viral prevalence was calculated higher in H9 in both species as 2.6% (0.8 – 6; N= 192) in duck and 1.2% (0.1 – 4.1; N= 173) in chicken than in H5 as 0.5% (0 – 2.9; N= 192) and 0% (0 – 2.1; N= 173) in respective species.

Based on the cleaning frequency of feeder, the AI viral prevalence in every time after use was recorded higher in H9 (2.1%; 0.7 – 4.8; N= 239) than H5 (1.3%; 0.3 – 3.6; N= 239) whereas it was 2.4% (0.1 – 12.6; N= 42) in both subtypes in duck. But in chicken this prevalence was recorded higher in every time after use as 2% (0.6 – 5.1; N= 197) than once in a day or week (0%, 0 – 6.6; N= 54) in both subtypes. The AL viral prevalence in cleaning of waterer once in a day or week was reported higher in H9 (1.9%; 0.6 – 4.5; N= 256) than in H5 (1.2%; 0.2 – 3.4; N= 256) in duck whereas in chicken it was recorded 1.8% (0.5 – 4.5; N= 224) in both subtypes. This prevalence in cleaning of waterer every time after use was recorded higher in duck as 4% (0.1 – 20.3; N= 25) than in chicken (0%; 0 – 12.8; N= 27) in both subtypes.

On the frequency of litter removing, the viral prevalence was showed 3.1% (1.1 – 6.6; N= 195) in both RNA subtype duck and in chicken it was 2.3% (0.6 – 5.7; N= 175) in both RNA subtypes where litter was removing once in a day or week. According to the disposal place of litter, the viral prevalence in throwing of litter into water bodies was recorded higher in H9 RNA subtype in both species as 2.4% (0.6 – 4.9; N= 169) in duck and 2.9% (0.8 – 7.2; N= 139) in chicken than in H5 as 1.8% (0.4 – 5.1; N= 169) in duck and 2.2% (0.4 – 6.1; N= 139) in chicken. Based on the site of the dead bird disposal, the viral prevalence in feeding to animals or throw into water bodies was reported higher in H9 RNA subtype (2.5%; 1.4 – 6.4; N= 241) than H5 subtype (1.7%;

0.1 – 3; N= 241) in duck while it was similar in both RNA subtype in chicken (1.9%; 0.5 – 4.8; N= 212). **(Table 4.4)**

Table No: 4.4 Univariable associations between binary response of household-level AI RNA subtypes in household chicken and ducks in Chattogram and the selected factors

		Chicken				Duck			
Variable	**Category**	**N**	**H5 (%); 95% CI**	**H9(%); 95% CI**	**p**	**N**	**H5 (%); 95% CI**	**H9(%); 95% CI**	**p**
Study area	Anowara	205	4 (2); 0.5 – 4.9	4(2); 0.5 – 4.9	1	254	4 (1.6); 0.4 – 4	6 (2.4); 0.9 – 5.1	1
	Rangunia	46	0; 0 – 7.7	0; 0 – 7.7		27	0; 0 – 12.8	0; 0 – 12.8	
Season	Summer	129	0; 0 – 2.8	0; 0 – 2.8	0.003	110	2 (1.8); 0.2 – 6.4	2 (1.8); 0.2 – 6.4	0.888
	Winter	122	4 (3.3); 0.9 – 8.2	4 (3.3); 0.9 – 8.2		171	2 (1.2); 0.1 – 4.1	4 (2.3); 0.6 – 5.9	
Scavenging areas	House premises with Rice paddy	251	4 (1.6); 0.4 – 4	4 (1.6); 0.4 – 4	-	14	0; 0 – 23.2	1 (7.1); 0.2 – 33.9	0.405
	House premises with water bodies	0	0	0		267	4 (1.5); 0.4 – 3.8	5 (1.9); 0.6 – 4.3	
Poultry house location	Yard	91	1 (1.1); 0.3 - 6	0; 0 - 4	0.510	98	2 (2); 0.2 – 7.2	3 (3.1) ;0.6 – 8.7	0.505
	Within house	160	3 (1.9); 0.4 – 5.4	4 (2.5); 0.7 – 6.3		183	2 (1.1); 0.1 – 3.9	3 (1.6); 0.3 – 4.7	
Poultry house ventilation facility	Wall opening/No Ventilation	199	4 (2); 0.6 – 5.1	3 (1.5); 0.3 – 4.3	0.826	229	4 (1.7); 0.5 – 4.4	6 (2.6); 1 – 5.6	0.661
	Open Air	52	0; 0 – 6.8	1 (1.9); 0 – 10.2		52	0; 0 – 6.8	0; 0 – 6.8	

Cleaning of house	Clean With Boom	239	3 (1.3); 0.3 – 3.6	4 (1.7); 0.5 – 4.2	0.328	272	4 (1.5); 0.4 – 3.7	6 (2.2); 0.8 – 4.7	1
	Clean with Disinfectant	12	1 (8.3); 0.2 – 38.5	0; 0 – 26.5		9	0; 0 – 33.6	0; 0 – 33.6	
Cleaning feeder	Clean with water	245	4 (1.6); 0.4 – 4.1	4 (1.6); 0.4 – 4.1	1	268	4 (1.5); 0.4 – 3.8	6 (2.2); 0.8 – 4.8	1
	Cleaning with disinfectant	6	0; 0 – 45.9	0; 0 – 45.9		13	0; 0 – 24.7	0; 0 – 24.7	
Cleaning waterer	Clean with water	43	0; 0 – 8.2	0; 0 – 8.2	1	35	1 (2.9);0.7 -14.9	1 (2.9);0.7 -14.9	0360
	Clean without water	208	4 (1.9);0.5 – 4.8	4 (1.9); 0.5 – 4.8		246	3 (1.2); 0.2 – 3.5	5 (2); 0.7 – 4.7	
Cleaning frequency of house	Once daily	78	4 (5.1);1.4 – 12.6	2 (2.6); 0.3 – 8.9	0.01	89	3 (3.4); 0.7 – 9.5	1 (1.1); 0 – 6.1	0.134
	Once or twice weekly or monthly	173	0; 0 – 2.1	2 (1.2); 0.1 – 4.1		192	1 (0.5); 0 – 2.9	5 (2.6); 0.8 - 6	
Cleaning frequency of feeder	Every time after use	197	4 (2); 0.6 – 5.1	4 (2); 0.6 – 5.1	0.507	239	3 (1.3); 0.3 – 3.6	5 (2.1); 0.7 – 4.8	0.597
	Once in a day or week	54	0; 0 – 6.6	0; 0 – 6.6		42	1 (2.4); 0.1 – 12.6	1 (2.4); 0 – 12.6	
Cleaning frequency of waterer	Every time after use	27	0; 0 – 12.8	0; 0 – 12.8	1	25	1 (4); 0.1 – 20.3	1 (4); 0.1 – 20.3	0.220
	Once in a day or week	224	4 (1.8); 0.5 – 4.5	4 (1.8); 0.5 – 4.5		256	3 (1.2); 0.2 – 3.4	5 (1.9); 0.6 – 4.5	
Litter removed	Once in a day/Once in a week	175	4 (2.3); 0.6 – 5.7	4 (2.3); 0.6 – 5.7	0.215	195	4 (2.1); 0.6 – 5.2	4 (2.1); 0.6 – 5.2	0.493

	Twice or more in a week	76	0; 0 – 4.7	0; 0 – 4.7		86	0; 0 – 4.2	2 (2.3); 0.3 – 8.1	
Litter disposed	Spread on fields/ throw in bushes	62	1 (1.6); 0 – 8.7	0; 0 – 5.8	0.618	65	0; 0 - 5.5	1 (1.5); 0 – 8.3	0.918
	Compost/ bury/ throw in pit/ left in yard	50	0; 0 – 7.1	0; 0 – 7.1		47	1 (2.1); 0 – 11.3	1 (2.1); 0 – 11.3	
	Throw in pond/ lake/ canal/ sea	139	3 (2.2); 0.4 – 6.1	4(2.9); 0.8 –7.2		169	3 (1.8); 0.4 – 5.1	4 (2.4); 0.6 – 4.9	
Dead bird disposal	Bury and throw in pit	39	0; 0 – 9	0; 0 – 0.9	1	40	0; 0 - 8.8	0; 0 - 8.8	0.783
	Feed to other animals /Throw in pond/ lake/ canal/ sea/ throw in bushes/ road side	212	4 (1.9); 0.5 – 4.8	4 (1.9); 0.5 – 4.8		241	4 (1.7); 0.4 – 4.2	6 (2.5); 0.9 – 5.3	

4.3 Sequencing and phylogenetic analysis for AIV M gene, PB1 gene, HA gene, NA gene:

4.3.1 M gene:

The phylogenetic analysis of M gene of 6 H5 and 2 H9 RNA positive samples suggests most of the M gene sequences were similar to the previous isolated H9 and H5 sequences reported in different poultry species and sectors. From the 8 M gene sequences 6 H5 (2 of household duck and 4 of household chicken) were almost identical

and placed in one cluster and 2 H9 M gene (household chicken) were in another cluster. They were also closely related the sequences of neighboring countries like India, Vietnam, Bhutan and Nepal. However, 1 H5 (household chicken) M gene was phylogenetically distinct and separate from others **(Figure -4.1).**

4.3.2 PB1 gene:

The phylogenetic analysis of PB1 gene of 4 H5 positive samples shows the PB1 gene sequences were similar to the previously isolated H5 PB1 gene sequences that recorded in different poultry species in different years in Bangladesh. 4 PB1 gene sequences were almost similar and placed in a cluster **(Figure -4.2).**

4.3.3 HA gene:

The phylogenetic analysis of HA gene of 4 H5 positive samples reveals that the HA gene sequences were identical to the previously isolated H5 gene sequences that were reported in Bangladesh in different poultry species over the different years. They were also showed similarity to the sequences of India, Bhutan. 3 HA gene were almost similar from the 4 HA gene sequences where one (household chicken) was distinct from the others **(Figure -4.3)**.

4.3.4 NA gene:

The phylogenetic analysis of the four NA gene sequences study indicates that the NA gene sequences were related to the previously circulated H5N1 gene sequences published in different poultry species in different years in Bangladesh. All NA gene sequences were in a cluster and were closely related in phylogenetic context **(Figure -4.4).**

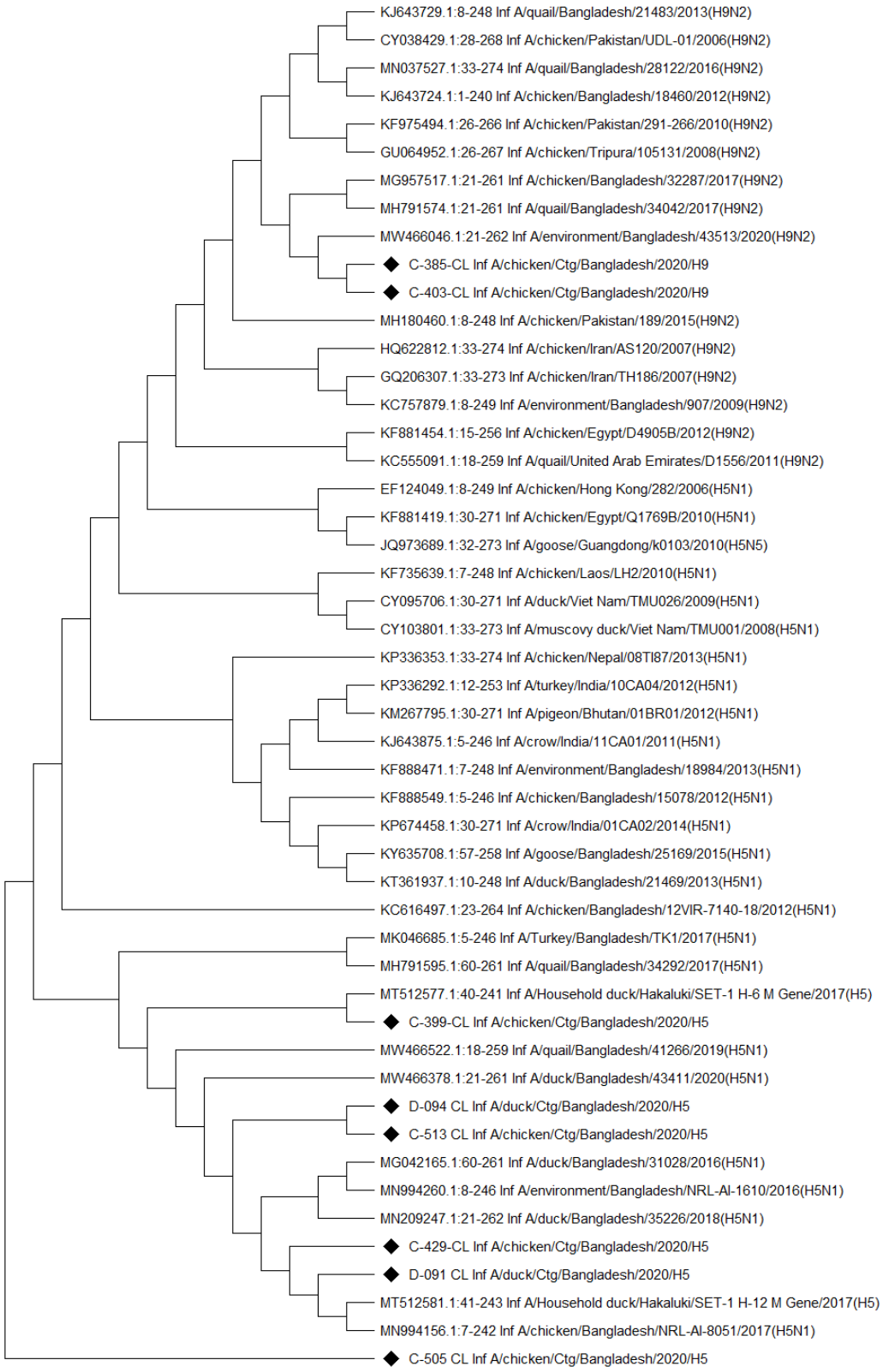

Figure 4.1 Phylogenetic relationships of the M gene sequences detected in chickens and ducks in the Chattogram, generated by maximum likelihood method in MEGA X. Black diamond (◆) with the taxon name in the tree indicated the M-gene sequences identified in this study.

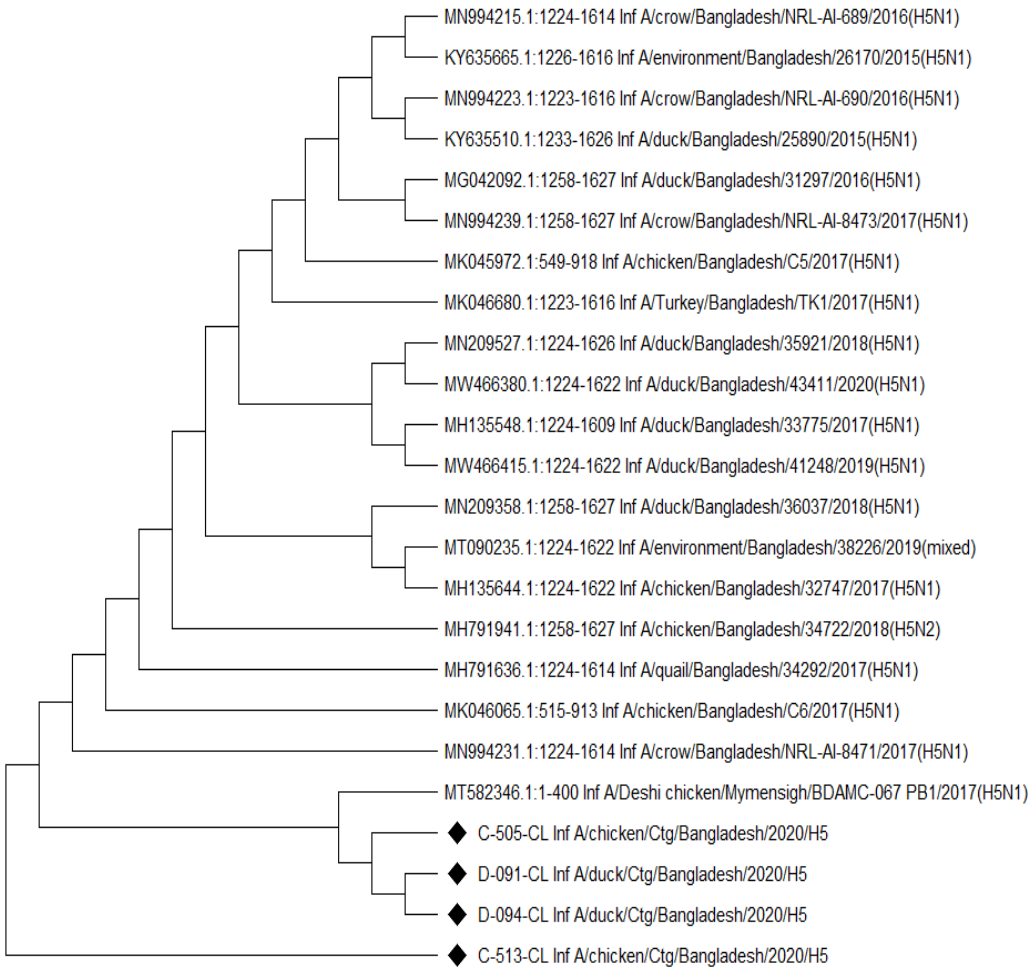

Figure 4.2 Phylogenetic relationship of PB1 gene sequences detected in backyard poultry in the Chattogram, generated by maximum likelihood method in MEGA X. Black diamond (◆) with the taxon name in the tree indicated the PB1-gene sequences identified in this study

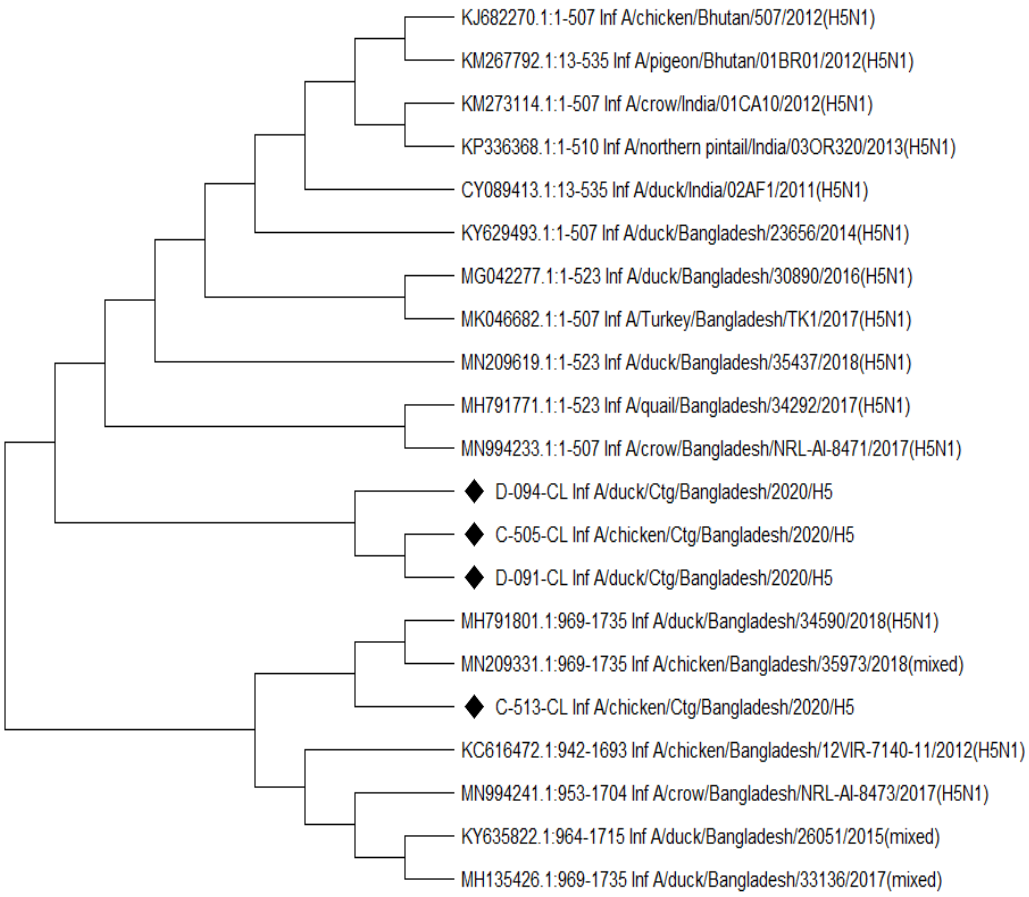

Figure 4.3 Phylogenetic relationships of the hemagglutinin (HA) gene sequences detected in backyard poultry in the Chattogram, generated by maximum likelihood method in MEGA X. Black diamond (◆) with the taxon name in the tree indicated the HA-gene sequenced in this study.

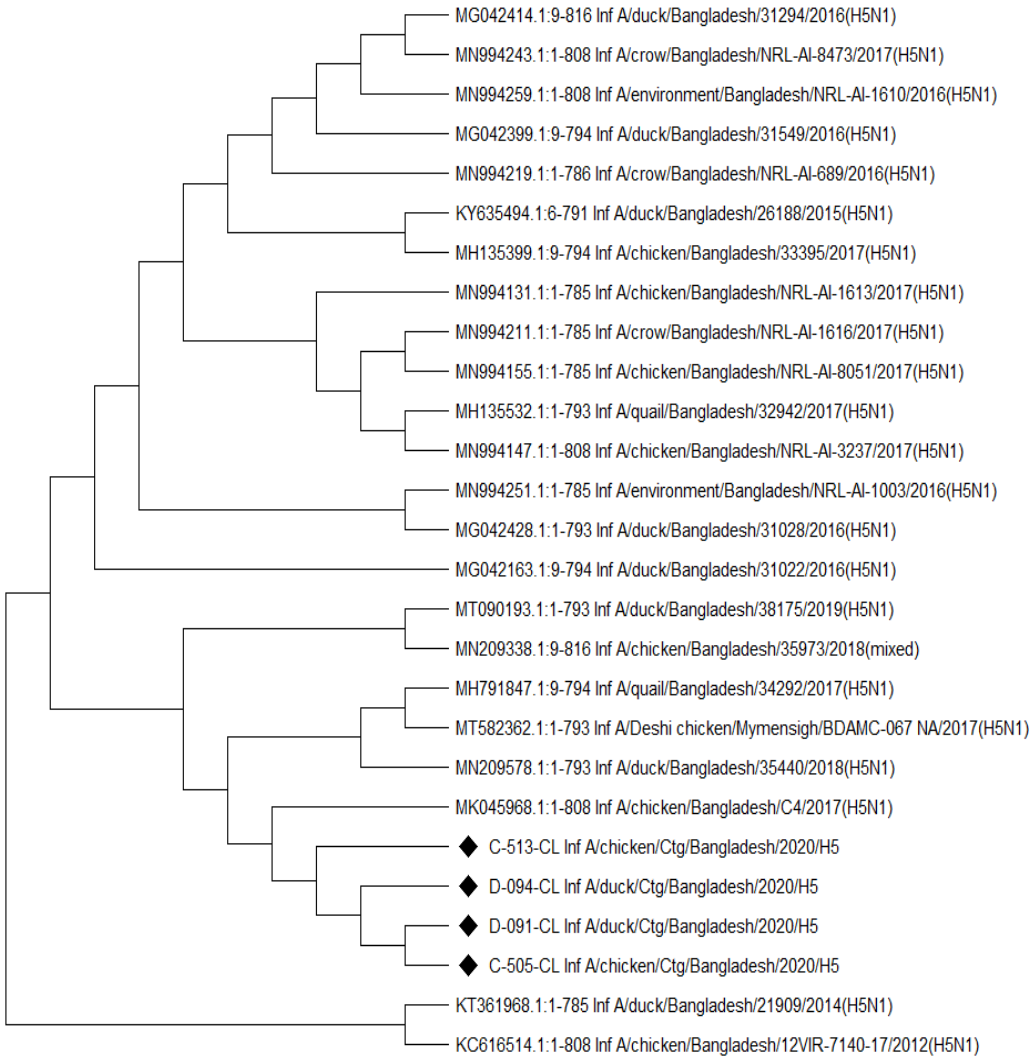

Figure 4.4 Phylogenetic relationships of the NA gene sequences detected in backyard poultry in the Chattogram, generated by maximum likelihood method in MEGA X. Black diamond (◆) with the taxon name in the tree indicated the NA gene sequences amplified in this study.

Chapter-5: Discussion

AI virus persistently prevailed in economic impact on poultry sectors of Bangladesh by causing infection and continued as potential threats for human's health (Biswas *et al.*, 2008; Parvin *et al.*, 2018; Rimi *et al.*, 2019). In Bangladesh epidemiological surveillance and studies of avian influenza (AI) have been mainly focused on commercial chicken farming systems and live bird markets (Biswas *et al.*, 2009; Sayeed *et al.*, 2017). There are a few studies of AI available on backyard poultry rearing systems. In this segment of the thesis important findings of the present study, their implications, limitations, conclusion and recommendation have been discussed.

The estimated AIV RNA prevalence of present study at household level was almost similar to the finding of Kayali et al. (2016) where the viral prevalence was detected 3.6% in backyard poultry and slightly higher than the findings of (Turner *et al.*, 2017) where the RNA prevalence was recorded 2.2% in backyard poultry.

This viral RNA prevalence for AIV in chickens of current study was almost similar with the findings of other studies conducted on backyard chicken in different countries such as 4% in China (Offeddu *et al.*, 2016), 4.7% in Egypt (Kayali *et al.*, 2016) and slightly higher than the study in Bangladesh was recorded as 2.8% (MS Abdullah, CVASU, Personal communication, 2019).

The viral RNA prevalence of ducks of our study was quite similar with the findings that detected 2.5% in Egypt (Kayali *et al.*, 2016); 4.4% (Khan *et al.*, 2018a) and 2.4% (MA Rahman, CVASU, Personal communication, 2019), respectively in Bangladesh; lower than our finding as 0.05% in Mohanganj sub-district of Netrokona District (Sarkar *et al.*, 2017) where some studies have higher prevalence, reported as 22% in Dhaka and Shylet (Khatun *et al.*, 2013); 24.6% in Dhaka (Turner *et al.*, 2017) and 89% in Netrokona (Haider *et al.*, 2017).

In the present study, H9 viral RNA prevalence was higher than the H5 viral RNA prevalence at household level in backyard poultry. This finding supported the decision of other studies (Negovetich *et al.*, 2011; Turner *et al.*, 2017). However, overall results suppose that both H5 and H9 RNA subtype are sustaining and co circulating in backyard poultry in the study areas reflecting the ordinary pattern of AI

subtypes in other poultry sector across the country (Biswas *et al.*, 2008; Biswas *et al.*, 2009; Ahmed *et al.*, 2012a) and in other countries (Naguib *et al.*, 2015).

In our study we found higher prevalence of H9 viral RNA prevalence in ducks than in chicken in backyard poultry. Peng et al. (2013) notify that low pathogenic AIV as H9 viral prevalence is higher in duck than in chicken. The viral RNA prevalence of H5 was almost similar in backyard chicken and duck in the study area. However, in Bangladesh both H5 and H9 subtype of AIV are considered as endemic subtypes in different poultry sectors (Loth *et al.*, 2010; Turner *et al.*, 2017) and also neighboring countries (Abbas, 2012; Naguib *et al.*, 2015).

In current study, we found a higher prevalence of AIV in winter than the summer season. Winter season is considered a favorable period for AI occurrence as many outbreaks have been reported in the past in Bangladesh and other countries (Potter, 2001; Gilbert *et al.*, 2008; Liu *et al.*, 2018). It might be due to low temperature and less humidity where AIV remains highly activated and persists for longer duration in infected materials (Kraidi *et al.*, 2017). In our study we found higher prevalence of H9 viral RNA subtypes in winter than the summer season in the study area which is usually known as H9N2, considering endemic in different poultry sectors in Bangladesh (Turner *et al.*, 2017; Kim *et al.*, 2018). The viral RNA prevalence of H5 was almost similar in household chicken and duck in summer. H5 subtype of AIV as HPAI, may remain largely confined to domestic poultry, has been spreading worldwide successfully in a very wide range of climatic conditions (Gilbert *et al.*, 2008). Bangladesh remained as a HPAI endemic country where H5N1 were isolated all over the year, previously it was reported mainly during the cold months before 2013 (Marinova-Petkova *et al.*, 2016). In Bangladesh, H5N1 subtype of AIV is more often found as a co-circulating with H9N2 subtype supposes that reassortment between two subtypes should be anticipated (Marinova-Petkova *et al.*, 2016).

Backyard poultry rearing system where domestic chicken and household ducks are reared together in free scavenging system for food during day in the study areas, rich with the wetland having direct contact with wild and migratory birds during winter season may consider one of the potential sources of AIV transmission (Chen *et al.*, 2005; Kwon *et al.*, 2011). Backyard chickens are more vulnerable to HPAI virus infection and can get the infection from the domestic ducks where the virus can sustain

as a reservoir (Biswas *et al.*, 2009; Hassan *et al.*, 2020a). As the domestic duck considered a 'Trojan horse' for the HPAI virus (Hulse-Post *et al.*, 2005; Sturm-Ramirez *et al.*, 2005; Songserm *et al.*, 2006) that might contaminate water bodies and the surrounding areas with HPAI virus by infected duck where backyard chickens might be exposed during scavenging sharing the same places (Biswas *et al.*, 2009).

The presences of H5 and H9 in the backyard poultry species will be a potential threat to nearby small-scale commercial poultry where less bio-security is maintained (Biswas *et al.*, 2008; Biswas *et al.*, 2009). Backyard poultry infected with H5 and with subsequent spread of H5 in other avian species can potentially affect humans because of their closeness and direct contact and exposure with infected poultry flocks (Claas *et al.*, 1998; Peiris *et al.*, 2007; Gilbert and Pfeiffer, 2012; Parvin *et al.*, 2014b). In rural areas of Bangladesh, backyard poultry are kept inside the living room at night (Sultana *et al.*, 2012a; Siddiky, 2017).

The close living arrangement of the rural people with backyard poultry put them at a raised risk of zoonotic transmission. Moreover lack of knowledge and lack of awareness of AIV and less practicing of bio-security among the backyard poultry raiser are often observed (Sultana *et al.*, 2012b; Shanta *et al.*, 2017) and also contributed to continuing HPAI and LPAI in Bangladesh (Parvin *et al.*, 2018). Backyard poultry raisers should be educated on household hygiene practice and efforts have focused on awareness about AIV to prevent zoonotic transmission (Rimi *et al.*, 2019).

The phylogenetic analysis reveals that most of the M genes of isolated positive samples are closely related to the previous identified M gene sequences of different poultry sector of Bangladesh. The phylogenetic result of M gene reflects the isolated H9 gene sequences are closely related to the sequences of H9N2 in different poultry species of neighboring country (Pakistan, India, Iran, UAE, Egypt) (Negovetich *et al.*, 2011; Monne *et al.*, 2013a; Nang *et al.*, 2013; Parvin *et al.*, 2014b; Turner *et al.*, 2017; Kim *et al.*, 2018). This isolates may be introduced to household chicken in Bangladesh by crossing border and illegal trading of poultry species from neighboring countries (Shanmuganatham *et al.*, 2013). On the other hand phylogenetic result shows most of the M gene sequences of the isolated H5 positive samples are closely identical to the M gene sequences of previously identified H5N1 in different poultry species of India, Bhutan, Nepal, Laos, China, Egypt, and Hong Kong which reveals that H5N1 is

introduced in household poultry in Bangladesh from those countries. However, phylogenetic analysis shows M gene sequences of H5 and H9 are lined and found as a confecting in the study area which is supported by the findings of (Marinova-Petkova *et al.*, 2016)

Phylogenetic analysis of PB1 gene of H5 shows that, the sequenced PB1 genes of H5 positive samples are in same cluster and similar to the different poultry species and sector of Bangladesh that previously identified in different years which indicates that the PB genes are circulating in Bangladesh (Barman *et al.*, 2017).

Phylogenetic exploration of HA gene sequences of H5 reveals that the isolated HA gene are in a cluster and similar to the HA gene sequence of different poultry species of Bangladesh and surrounding country (India, Bhutan) and spreading around the countries (Hoque *et al.*, 2013; Barman *et al.*, 2017). Phylogeny of HA gene suggested that a Muscovy duck in Vietnam are considered as the closest ancestor of clade 2.3.2 viruses in Nepal and Bangladesh which was the descend of the viruses from Guizhou, China that isolated from environment in 2009 (Hoque *et al.*, 2013).

Phylogenetic analysis of NA gene sequences of H5 reveals that NA gene sequences are closely similar to NA gene sequences of previously isolated H5N1 in different poultry species of Bangladesh which is supported the finding of (Hoque *et al.*, 2013) and circulating through the Bangladesh.

5.1 Limitations of the Study:

1. We had to interview many backyard poultry raisers within a short period of time, which could have created information bias in some cases.
2. Due to time and financial constraints, the present study was not able to use the larger sample size covering the extended areas and seasons.
3. Most of the ducks were sampled from Anowara upazila as the Rangunia upazila having the lower density of duck that's why fewer number of duck household number of ducks and sampled from Rangunia upazila.
4. Due to resource constraints, we sequenced partially 04 segments of AIV only.

Chapter-6: Conclusion, Recommendation

6.1 Conclusion:

AI RNA prevalence was found higher in household duck than the household chicken in backyard poultry rearing systems. The prevalence was almost concentrated in coastal areas of Chattogram. H5 and H9 subtypes were detected in the study areas where the prevalence of H9 subtype was higher than the H5 subtype of AIV. Winter season was a more favorable period of AIV transmission in this study where H9 subtype was found more detected in winter season than summer season and H5 subtype. The findings of phylogenetic analysis of M gene, PB gene, HA gene and NA gene revealed that identified gene were closely similar with the previous gene sequences of AIV that isolated and circulating within Bangladesh and neighboring countries. These findings of the present study recommended to implementation of regular surveillance and control of the AIV and strength the awareness among the backyard poultry raisers.

6.2 Recommendation:

1. Separate housing should be practiced for duck and chicken for prevent AIV transmission
2. Farmers should be educated on household bio-security and hygiene practice to reduce AIV transmission.
3. Regular surveillance and early detection of AIV should be done and it can be helpful to quick response against the disease and limit the transmission.

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Brief biography

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