Chapter I

Introduction

Bangladesh has recently stepped in the group of middle-income countries and the contribution of livestock in this achievement cannot be overlooked (World Bank, 2020). Livestock is not only a source of animal protein but also an inevitable component of the complex farming system of Bangladesh as well as a source of employment (Rahman et al., 2014). Share of livestock in Agricultural Gross Domestic Product is 13.5% and livestock contributes 1.5% of GDP in the national economy employing 20-50% people of Bangladesh (BBS, 2018-19). Within the livestock sector, poultry farming is playing important role. Total population of poultry in our country is approximately 347 million (DLS, 2018-19). There are 16 grandparent farms, 206 breeder farms and approximately 70000 commercial poultry farms employing 8 million people annually (OHPH, Prof Md. Ahasanul Hoque, CVASU, Personal Communication). Broiler chickens are broadly reared for meat purpose.The poultry meat alone contributes 35.3% of the total meat production in Bangladesh (Hamid et al., 2016). For this emerged poultry sector scientific breeding, feeding, management and disease control are utmost important. However, there are many challenges in driving the sectors to exploit its full potential among which disease is one of them (Hamid et al., 2016, Saleque, 2009). Among reported common poultry diseases, 29% are bacterial diseases (Salmonellosis, Collibacillosis, Necrotic enteritis, Infectious Coryza), 53.2% viral disease (IBD, Newcastle, Avian Influenza, Infectious Bronchitis, Egg Drop Syndrom), 7.1% mycoplasmal disease (Mycoplasma) and 6.5% protozoal disease (Coccidiosis) (Giasuddin et al., 2002; Uddin et al., 2010). Among the food borne zoonotic pathogens of poultry origin Campylobacter is a significant pathogen (Hsieh & Sulaiman, 2018) which is not well studied in Bangladesh.

Campylobacterios caused by *Campylobacter* spp (a gram-negative, non-spore forming, S-shaped or spiral bacteria). Currently there are 17 species with 6 subspecies, but most commonly reported species are *C jejuni, C. coli, C. lari, C. fetus and C. upsaliensis.*

Primary habitat of Campylobacter species is intestinal tract of warm blooded animals. *C jejuni* and *C. coli* are most commonly found in human. *C. lari* can cause recurrent diarrhoea in

children. *C. fetus* is found in cattle and sheep as well as opportunistic pathogen in human. *C. upsaliensis* is found in dog and cat (Stanley et al., 1992) *C jejuni* and *C. coli* are most common in case of poultry (Blaser et al., 2014).

Campylobacter has no detrimental effect on intestinal health of chicken. Hence, bird growth is not effected following natural exposure (Sakaridis et al., 2018). The major clinical sign caused by Campylobacter in human is acute diarrhea (Sotelo, 2011). Thrombophlebitis, endocarditis, neonatal sepsis and pneumonia are also reported (Igwaran & Okoh, 2019). Acute colitis and acute appendicitis are also found in some cases (Lagler et al., 2016). Guillain-Barre Syndrome (Scallan et al., 2015) and Miller-Fisher Syndrome (Skarp et al., 2016) are major post-infection complications.

Farm animals are the major cause of Campylobacteriosis since they are the major reservoir of *Campylobacter* species (de Vries et al., 2018). Most outbreaks of Campylobacteriosis are caused by consumption of poultry meats and poultry products (Taylor et al., 2013). Poultry meats and it products cause about 60–80% of the global Campylobacteriosis cases (EFSA, 2015).The risk of transmission is greater from broiler chickens because of high level of consumption. Campylobacter does not spread from broiler to human only via consumption of meat but also by handling of live birds (broiler and layer) and during the preparation of meat and meat products (Igwaran & Okoh, 2019).

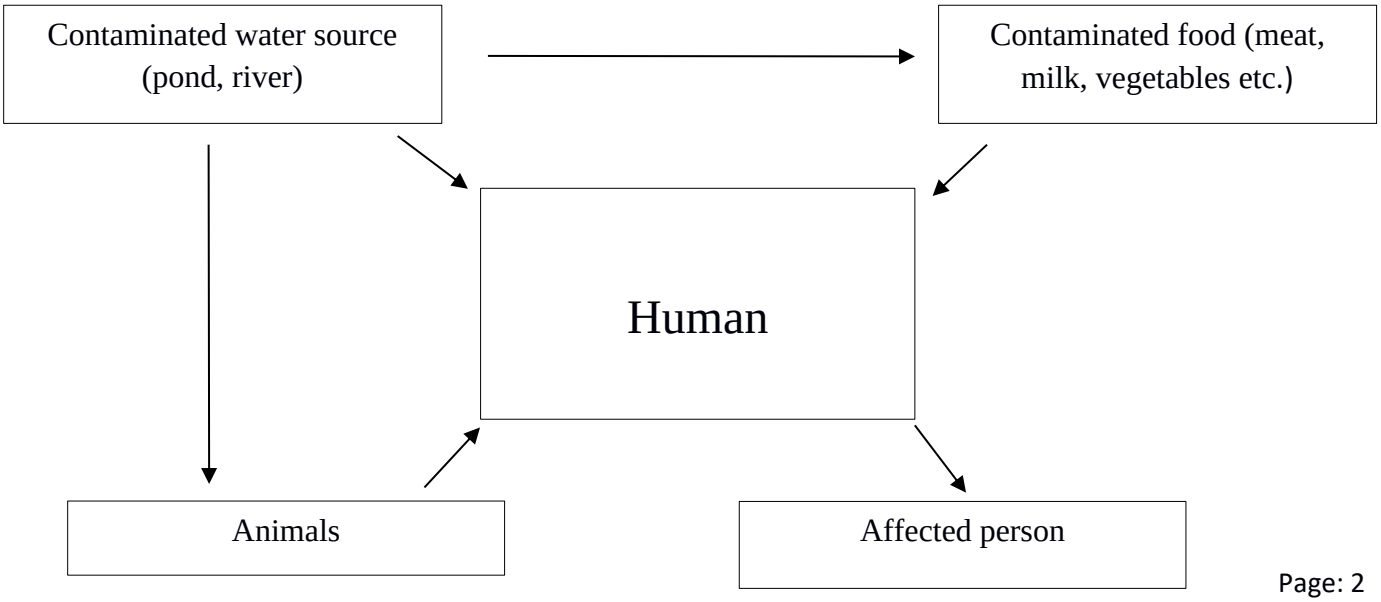

Figure 1: Transmission of Campylobacter through different routes (Kaakoush et al., 2015)

The prevalence of Campylobacter infection has raised more in developed and developing countries over last 10 years. The overall prevalence of Campylobacter colonization in broiler meat across Europe was 37.4% (EFSA and ECDC, 2018). In Asian context, prevalence of Campylobacter was reported to be 35.1% in Vietnam (Carrique-Mas et al., 2014) 67% in Sri Lanka (Kottawatta et al., 2017) and 38.6% in India (Khan et al., 2018). In Bangladesh, 62-75% prevalence was reported in case of broiler meat and 78% in cloacal swab sample (Kabir et al., 2014; Md. et al., 2018).

Risk factors for Campylobacter infection varies depending on farming practices, geographical location and climatic conditions (Hasan et al., 2020). However, there are some factors which are more or less considerable in every farm level Campylobacter colonization. Age of shed (Høg et al., 2016), disinfection of shed surroundings (Sommer et al., 2013), interval between introduction of new batch (Borck Høg et al., 2016), flock size (Newell et al., 2011), age of birds (Connerton et al., 2018), experience of farmers (Sibanda et al., 2018) and introduction of new birds in flock (Barrios et al., 2006) are some of the important risk factors related to Campylobacter colonization in broiler.

Major on farm strategies to prevent and reduce Campylobacter infection comprises **(i)** Biosecurity measures which is a must not only for Campylobacter but also for other diseases (Silva et al., 2011) **(ii)** Vaccination against Campylobacteriosis which was a partial success since an effective vaccine against Campylobacteriosis is still challenging (Janssen et al., 2008) **(iii)** Host genetic selection given significant difference in susceptibility for Campylobacter was found in different chicken lines (Li et al., 2008) and **(iv)** Antimicrobial alternatives such as bacteriophage and bacteriocin treatment to reduce or eliminate Campylobacter from colonized chicken (Kaakoush et al., 2015).

Considering public health significance of Campylobacter and limited scale of studies on Camphlobacter being conducted previously at local and national level in Bangladesh, the present study was there conducted with the following objectives:

(i) Estimate proportionate prevalence of Campylobacter colonization in Mirasarai upazilla

(ii) Identify potential risk factors associated with Campylobacter colonization in broiler farms

(iii) Observe mortality rate and causes of mortality in farms

(iv) Observe usage of antibiotic in farms and awareness of farmers regarding antibiotics.

Chapter II

Materials and method

2.1. Description of study areas

Mirasari is one of the largest and oldest upazilla (sub-district) of Chattogram. It is located in the South western part of Chattogram (22º39 and 22º59 N and 91º26 and 91º38 E) with an area of 482.88 sq km constituting 16 unions and 2 pourasava. Being a coastal area it is featured with sea, river, hills and low and high along with diverse ethnic groups. Most of people depend on agriculture for their livelihood (Anon, 2020).With 1.4 million poultry, there are around 600 poultry farms including 517 broiler farms, 12 layer farms, 2 breeder farms (Upazilla Livestock Office, Mirasharai, Chattogram 2019). Mirasharai Upazilla Livestock Hospital is the only veterinary establishment to offer veterinary and extension services to farmers.

2.2 Population and collection of sample and epidemiological data

Of 517 broiler farms, 20 farms, each consisting of at least 500 birds, were randomly selected for the present study. Necessary verbal permission was taken from individual farmer before sampling the birds and recording epidemiological information. Regardless of flock size 5 birds per farm were randomly sampled. Accordingly, 100 birds were brought under sampling. Cloacal swabs were obtained from birds by inserting swab sticks into the vent (until faecal contamination) and pooled 5 swab samples according to individual farm into a 15 ml falcon tube containing 7 ml buffer peptone water with unique identify number. Collected samples were then transferred through cooling box maintaining 4º C and stored at -20° C of the Clinical Pathology Laboratory, CVASU until conducting laboratory diagnosis.

A pre-tested structured questionnaire was used to record epidemiological data at farm level by face-to-face interview and physical observation. Data included number of houses, type of floor, water supply, litter materials, amount of litter materials used, number of flocks per year, number of employees, use of footwear and distinct cloth, foot bath facility, flock size, age of birds, number of dead birds per flock, all in all out system, disinfection of farm before restock, house

empty for >14 days before restock, information on vaccination and age of vaccination, usage of antibiotic and duration of usages along with farmers demographic information.

2.3: Sample evaluation

Samples were evaluated for *Campylobacter* spp by the methods described by (Lund et al., 2003).

2.3.1. Bacteriological evaluation

Sample was inoculated on Campylobacter agar by streaking method. The plate was then incubated at 42° in CO_2 atmosphere for 72 hours (Wedderkopp et al., 2000). Dew drop like colonies were found on the *Campylobacter* positive agar plate. *Campylobacter* isolates were stored in 300 µl glycerine and 700 µl Brain Heart Infusion Broth at -20°C until further testing.

2.3.2. Molecular evaluation

DNA extracts of all *Campylobacter* positive isolates were performed by using heat and boiling method (Pai et al., 1979). In brief, 5-6 single colonies from the freshly grown agar plate were taken and added to 200µl deionized water. After proper vortexing the contents were boiled in heat block in 99°C for 10 minutes. Then samples were kept in -20°C for 5 minutes and centrifuged at 12000 rpm for 5 minutes. Finally, 100µl supernatant was collected as extracted DNA. The DNA extracts were stored in -20°C freezer for conducting the conventional PCR. The DNA concentration obtained from each pooled sample was measured at 260nm using Qubit 4 fluorometer (ThermoFisher Scientific).

DNA extracts were then evaluated by a published multiplex PCR test (Klena et al., 2004) to determine lpx gene (Klena et al., 2004). The followings were the primer sequences: lpxAF9625 (5'- TGC GTC CTG GAG ATA GGC-3'), lpxAC.coli (5'-AGA CAA ATA AGA GAG AAT CAG -3') and lpxAC.jejuni (5'-ACA ACT TGG TGA CGA TGT TGT A-3') (Forward primers) and lpxARKK2m (5'-CAATCATGDGCDATATGASAATAHGCCAT-3') (Reverse primer).

The 20 µl reaction mixture constituted 10 µl New England BiolabsTaq 2X Master Mix (containing Taq DNA Polymerase, dNTPs, MgCl2, KCI and stabilizers), 0.5 µl each forward and reverse primer and 2µ of DNA extract template 6 µl Nuclease free water. The thermal cycling included 95°C for 5 min followed by 94 °C for 1 min (denaturation), 52 °C for 1 min (annealing), 72 °C for 1 min (extension) for 35 cycle and with final extension 72 °C 5 min. PCR products on the 1% agarose gel were visualized through ethidium bromide staining.100 bp DNA was used (New England BioLabs) as standard molecular ladder.

2.4. Data entry and statistical evaluation

All data obtained were entered into Microsoft office excel-2007,USA (MS excel 2007). Data were cleaned, sorted and coded in MS excel 2007 before exporting to STATA-14 *(StataCorp,4905,LakewayDrive,CollegeStation,Texas77845,USA)* for descriptive and univariable statistical analysis. The proportionate prevalance of Campylobacter colonization was calculated using the number of Campylobacter positive farm divided by total number of farms. Frequency distribution of *Campylobacter* spp was presented according to categories of each selected factors: number of houses, litter amount, slaughter age, vaccination, vaccination age, dead birds per flock, all in all out system, empty for >14 days between flocks, disinfection before restock, antibiotic use and duration of usage). Fisher'sexact test was performed to assess associations between the categorized response variable of Campylobacter colonization and the selected independent variables. The results were expressed in frequency number, percentage and P value.

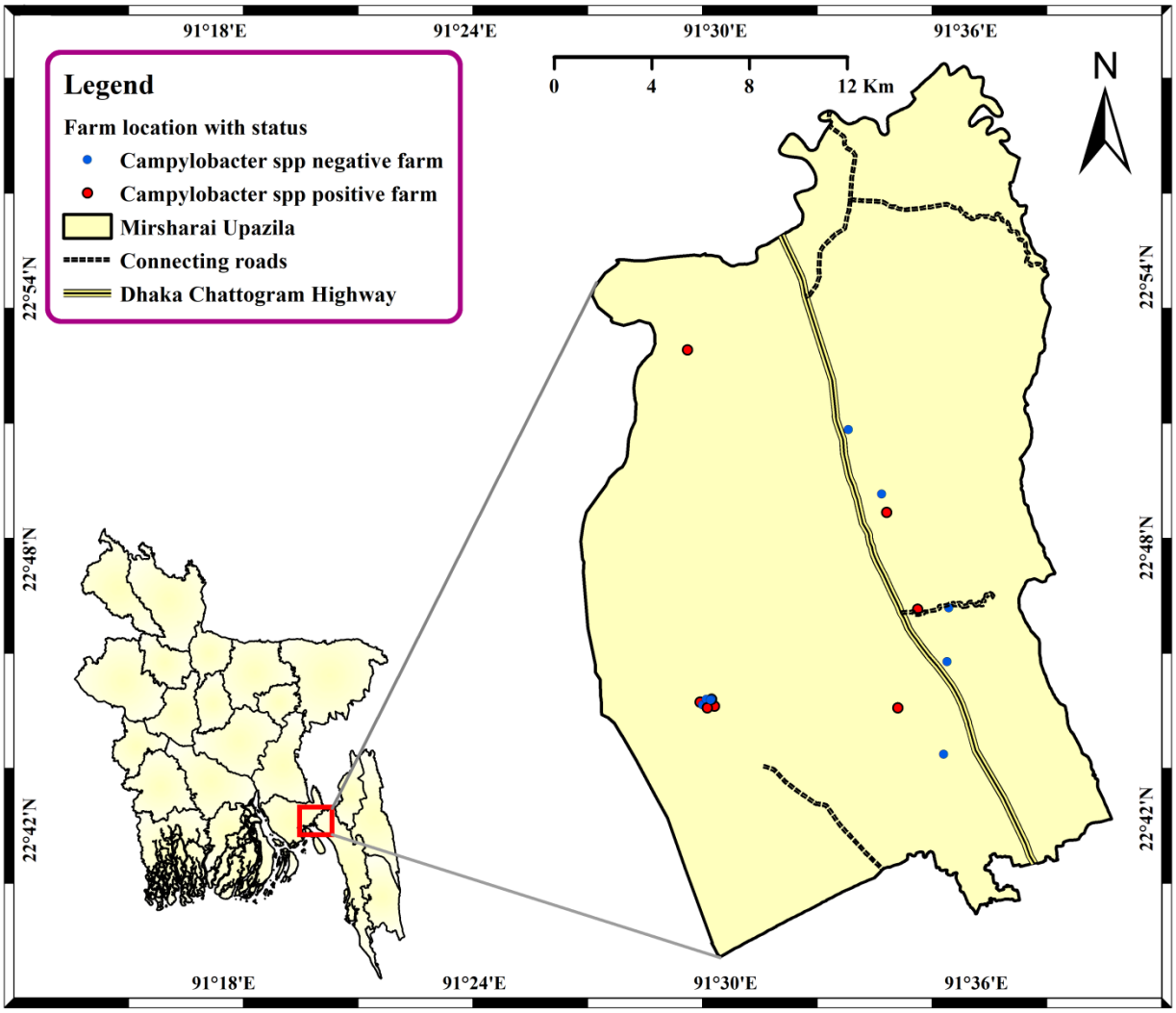

Fig: Spatial distribution of Campylobacter positive farms in Mirasarai

Chapter III

Results

3.1. Farm characteristics and farmers' demography

According to the findings of the present study, educational status of most of the farmers had below SSC (60%). Only10% of the farmers had graduation. 70% farms had the flock size of 500 to 2000. Most of the farmers (70%) had experience of farming for less than 4 years and 30% farmers had experience of farming for more than 5 years. Poultry sheds were made of mud floor (80%) and tin-made ceiling (70%). 80% farms used saw dust as litter. Most of the farmers responded they don't have distinct cloth for entering into the farm, however, 25% farmers use separate footwear. 30% farms had foot bath facility having potassium per manganete. Almost all farmers (90%) reported that they follow "all-in-all-out" system and keep house empty for 14 days before restocking (Table 3.1).

Table 3.1: Characteristics of farm and farmers' demography

Factors	Category	Frequency	Percentage
Number of houses per farm	1	7	35
	2	6	30
	3	7	35
Flock size	500-900	7	35
	901-2000	7	35
	2001-4000	6	30
Education of farmers	Graduated/BBA	2	10
	HSC	4	20
	SSC	1	5
	Below SSC	12	60
	Illiterate	1	5
Establishment of farm	2007-2011	3	15

	2012-2015	3	15
	2016-2019	14	70
Experience on poultry farming	Less than 4	14	70
	5-8	3	15
	More than 8	3	15
Water source	Deep tube well	6	30
	Tube well	14	70
Type of floor	Brick	4	20
	Mud	16	80
Type of ceiling	Bamboo	6	30
	Tin	14	70
Type of litter	Rice husk	3	15
	Saw dust	16	80
Use of distinct cloth	Yes		1
Use of separate footwear	Yes	5	25
Footbath facility	Yes (using $KMnO_4$)	6	30
"All In All Out" system	Yes	18	90
House kept empty before restocking of new flock	Yes	18	90

3.2. Farm prevalence of Campylobacter and its distribution

The proportionate prevalence of Campylobacter was greater in farms containing multiple sheds (61.5%; 95% CI: 36-88.9%), small (500) to medium flock (1000) size (70%), water supplied with deep tube wells (66.7%), floor made of mud (68.8%), saw dust litter (56.3%) and no use of distinct cloth (57.9%) or separate footwear (60%) while entering the farm than that of the counterpart of each variable (Table 3.3)

Table 3.2: Association between prevalence of *Campylobacter spp* and selected factors through Fisher's exact test

Factors	**Categories**	**Campylobacter spp**		***P***
		Yes	**No**	
No. of sheds	Single shed	3 (42.9)	4	0.370
	Multiple sheds (2-3)	8 (61.5)	5	
Flock size	500-1000	7 (70)	3	0.185
	1001-4000	4 (40)	6	
Establishment of farm	2008-2014	4 (44.4)	5	0.342
	2015-2019	7 (63.6)	4	
Water supply	Deep tube well	4 (66.7)	2	0.426
	Tube well	7 (50)	7	
Type of floor	Brick	0 (0)	4	0.026
	Mud	11 (68.8)	5	
Litter type	Rice husk	2 (50)	2	0.625
	Saw dust	9 (56.3)	7	
Distinct cloth	No	11 (57.9)	8	0.450
	Yes	0	1	
Separate footwear	No	9 (60.0)	6	0.396
	Yes	2 (40.0)	3	

3.3. Mortality status within each farm

According to the responses of farmers average maximum mortality per flock over a year was recorded as up to 0-2.5% mortality in 15% farms, 2.5-5% mortality in 60% farms and >5% mortality in 25% farms. Reported causes of mortality were Newcastle disease (30%), necrotic enteritis (40%), coccidiosis (40%), infectious bursal disease (1.45%), and avian influenza (1.45%) (Table 3.3).

Table 3.3: Mortality status within each broiler farm and the associated diseases (July 2019-2020) (According to farmers' response)

Farm ID	Flock size	Morbidity per flock (min-max)	Mortality per flock (%, min-max)	Disease causes
M1	500-1000	0-50	0-5	Newcastle Disease (ND) and Necrotic Enteritis (NE)
M2	500-1000	0-50	0-5	Coccidiosis
M3	500-1000	0-50	0-5	Coccidiosis and NE
M4	500-1000	0-50	0-5	ND
M5	500-1000	0-50	0-5	ND
M6	500-1000	60-70	6-7	ND and IBD
M7	500-1000	60-70	6-7	ND and NE
M8	1001-2000	50-100	2.5-5	NE
M9	1001-2000	50-100	2.5-5	Coccidiosis
M10	1001-2000	50-100	2.5-5	AI
M11	500-1000	50-100	5-10	NE and Coccidiosis
M12	500-1000	0-50	0-5	Coccidiosis
M13	500-1000	0-50	0-5	Coccidiosis
M14	2000-4000	50-100	1.25-2.5	NE
M15	2000-4000	50-100	1.25-2.5	ND
M16	2000-4000	0-50	0-1.25	Coccidiosis and NE

M17	2000-4000	200<	5<	Avian influenza (AI) and Infectious Bursal Disease (IBD)
M18	2000-4000	200<	5<	AI and IBD
M19	2000-4000	100-200	2.5-5	Coccidiosis
M20	2000-4000	100-200	2.5-5	NE

3.4. Pattern of antimicrobial usage

According to the responses of farmers on antimicrobial usage over six months (July 2019-December 2020) multiple antimicrobials were used in the farmers for different purposes. Among antimicrobial usage amoxicillin, doxycycline and sulfur drugs (sulfaclonazin, sulfadimidine and sulfadimerthoxine) were frequently used (15% each) followed by combination of Amoxycillin with Enrofloxacin or Ciprofloxacin (10%) and Ciprofloxacin, Cloxacillin and Oxytetracycline (5% each) (Table 3.4).

According to WHO classification (WHO, 2019) reserve group of antimicrobials was used in 2 farms, whereas watch group of antimicrobials was used in 18 farms (Table 3.4).

Table 3.4: Commonly used antimicrobials in broiler poultry farms in Mirarsarai, Chattogram

Name of antibiotics	**Frequency**	**Percentage (%)**	**Antimicrobial class as per WHO**
Amoxicillin	3	15	Access
Amoxicillin and Colistin sulfate	2	10	Access and reserve
Ciprofloxacin	1	5	Watch
Ciprofloxacin andOxytetracycline	1	5	Watch
Cloxacillin	1	5	Access

Doxycycline	3	15	Watch
Enrofloxacin and Amoxicillin	2	10	Watch
Fluoroquinolone and Ciprofloxacin	1	5	Watch
Neomycin	2	10	Access
Oxytetracycline	1	5	Watch
Sulfer drug	3	15	Access

Chapter IV

Discussion

Poultry intestines constitute a favorable environment for Campylobacter colonization; therefore, it increases the chance of human Campylobacteriosis caused by the consumption of its contaminated meat, which is of great concern for human health (Kaakoush et al., 2015). Campylobacter is an extremely important zoonotic, food borne pathogen which worldwide infects millions of people each year. Human can get infection in various ways, but studies indicate that broiler is the most important source of infection since poultry intestine constitutes a favorable environment for Campylobacter colonization (Mirzaie et al., 2011). There are not many reported studies on Campylobacter in poultry Bangladesh. Therefore, this study added fresh scientific information to the literature. The present study was aimed to estimate proportionate farm level prevalence of *Campylobacter* in broiler poultry at Mirarsarai in Chattogram, to know the distribution *of Camphylobacter* by different factors, describe overall mortality and antimicrobial usage pattern. This section of report has discussed significant findings of the present study along with limitations, conclusion and recommendations.

The overall farm level prevalence of *Campylobacter* in Mirasarai was 45% which corresponds to the findings of (Hasan et al., 2020) (40.5%). However, variable Campylobacter farm prevalence was reported in 4.9% and 100% ((Hasan et al., 2020). Reasons of prevalence vary due to rearing system, farm management and biosecurity and hygiene (Cardinale et al., 2004; Guerin et al., 2007; Medicine et al., 1999; Näther et al., 2009).

Like the present study an increased risk of *Campylobacter* was associated with increasing number of sheds in a farm (Arsenault et al., 2012; Cardinale et al., 2004; Guerin et al., 2007; Medicine et al., 1999; Näther et al., 2009; Refrégier-Petton et al., 2001). Several houses in the same premise may lead to an increased prevalence of Campylobacter colonization through introduction of bacteria into the sheds possible because of increased movement of personnel (Hasan et al., 2020). Many studies reported higher Campylobacter prevalence with increasing number of flock size which does not support the finding of the present study (Barrios et al., 2006a; Lin, 2009). Some earlier studies however found no link between flock size and

Campylobacter occurrence (Cardinale et al., 2004; Humphrey et al., 2007). Larger flock might give more chance of *Campylobacter* infection because of large volume of water, food, litter as well as larger movement of personnel. The effect of small flock size on Campylobacter status in the present study might be due to specific production system and management of farm (Kaakoush et al., 2015)

In previously reported studies, source of water supply had no influence on the Campylobacter colonization. (Näther et al., 2009) though the present study found some influence of source of water supply on *Campylobacter* occurrence. Farms where water is supplied from tube well tend to have more *Campylobacter* prevalence than those where water is supplied from deep well, but this explanation has not been tested in the present study. A previous study identified the use of groundwater as a risk factor if it is not sanitized, which is consistent with the present finding (Sasaki et al., 2011). Depth of underground water level might have effect on this factor. This possibility is still not much explored and needs further study.

Use of rice husk as litter material was previously reported to increase the level of prevalence of *Campylobacter* (Hasan et al., 2020), but the present study did not identify such connection with Campylobacter occurrence. Using saw dust as litter material can cause respiratory problems resulting decrease in body immunity of birds and thus saw dust might play a role in *Campylobacter* infection.

Unlike urban farmers, the social-economic status of farmers of country side areas is generally poor (Alam et al., 2016). They start farming with low investment. Hence, the sheds are not well built. Most of the houses were mud-made floor. In the present study floor type was found with a significant influence on *Campylobacter* occurrence.

Farming experience is an important factor for the occurrence of *Campylobacter*. Better farm hygiene and biosecurity along with personnel training can reduce *Campylobacter* occurrence (Sibanda et al., 2018). And experienced farmers tend to be more compliant in these matters (Racicot et al., 2012). Similarly the present study found less *Campylobacter* occurrence in the farms which were established before 2014 (more than 5 years' experience). Although a few

earlier studies found no real effect of biosecurity measures such as use of separate footwear or distinct cloth on *Campylobacter* occurrence (Bouwknegt et al., 2004; Näther et al., 2009). Many other studies determined the significant effect of such bio-security measures on *Campylobacter* occurrence (Cardinale et al., 2004; Evans & Sayers, 2000). The present study found an influence of using separate footwear and cloth on *Campylobacter* occurrence where usage of separate footwear and cloth reduced *Campylobacter* occurrence. That might be because changing shoes and cloth before entering farm prevents environmental contamination from outside farm.

Farmers under the present investigation maintained 14 days interval between batches and practiced "All in all out system" which are indicatives of practices. However, other factors might have attributed to the occurrence *Campylobater.*

According to farmers' response, most of the farms (60%) had 2.5-5% mortality rate in a production cycle which is considered as expectable (Akbay & Azeez, 2016) . Most frequent causes of mortality were Coccidiosis and Necrotic Enteritis (40%) which is generally occurred because of their endemicity. Though vaccine against ND and IBD is commonly practiced in broiler poultry farms available, death occurred due to ND and IBD which might be due to the failure of the vaccine. Farmers collect vaccines from Upazilla Veterinary Hospital coming from variable distance. They often do not maintain cool chain. Also they rarely consider health condition of bird before vaccination. These may contribute to cause the infection from vaccine itself. Sometimes farmers don't follow booster doses of the vaccines.

A wide range of antibiotics was used in the studied farms. Reserve group of antimicrobials was reported to be used in a couple of farmers though most of the farms used watch group of antimicrobials. It was discovered in 1950 that adding antibiotics to the diet of animals at the sub-therapeutic level may increase the rate of growth of the animal (Kaakoush et al., 2015). Since growth rate is the most important for broiler production in the present study farmers used antibiotics as growth promoter. Farmers are not educated about the proper use of antibiotics and also not aware of antimicrobial resistance. Hence this widespread use of antiobiotics has led to an increase in antibiotic resistance. Moreover, it was found that farmers used Reserve group antibiotics. Reserve antibiotics should be applied as a last resort to treat multi- or extensively-

drug resistant bacteria. They are a valuable and non-renewable resource. So, farmers should be trained about antibiotics with the risk and danger of their misuse. Government officials as well as various non-government organizations should come forward in this regard.

Chapter V

Limitations

Proper sample size using statistical assumption was not followed. So, the study was not able to estimate the true prevalence of *Campylobacter* at farm level. Although a face-to-face interview was conducted for the present study, farmers do not maintain any written farm record and the responses were relied on farmers' memories mostly. Therefore, information bias might have introduced in this study.

As sample size was small and hence it was not possible to apply multivariate logistic regression. Therefore, it was not able to identify the adjusted effect of the factors on the occurrence of *Campylobacter*.

Chapter VI

Conclusion

The overall farm level *Campylobacter* prevalence was quite high. Common occurrence of *Campylobacter* in farms having multiple sheds, small to medium flock size (500-1000), water supplied with deep tube wells, mud floor, use saw dust litter. A wide range of antibiotics was used in the studied farms. Reserve group of antimicrobials was used in 2 farms which should be banned. However, most of the farms used watch group of antimicrobials. Mortality due to Necrotic Enteritis, Coccidiosis and NewCastle Disease were observed.

As the prevalence is high, so improved farm hygiene and bio-security measures should be practiced. Farms should be built with more caution with cemented floor and with pure water supply. Wider usage of antibiotics with reserve and watch group should be prevented. Use of reserve group antibiotics should be stopped. For the aforementioned aspects farmers' education and awareness would be utmost important.

Chapter VII

Reference

. M. G., . B. K. S., . J. A., . I. K., . M. R. I., & . M. M. R. (2002). Prevalence of Poultry Diseases in Bangladesh. *Journal of Biological Sciences*, *2*(4), 212–213.

Akbay, C., & Azeez, J. A. (2016). Factors affecting on mortality rate in the broiler chicken production farms in Erbil , Iraq. *Pakistan Journal of Food Sciences*, *26*(3), 119–128.

Alam, M., Sultana, S., Hassan, M., Hasanuzzaman, M., & Faruk, M. (2016). Socio-economic status of the farmers and economic analysis of poultry farming at Gazipur district in Bangladesh. *International Journal of Natural Sciences*, *4*(2), 8–12.

Arsenault, J., Berke, O., Michel, P., Ravel, A., & Gosselin, P. (2012). Environmental and demographic risk factors for campylobacteriosis: Do various geographical scales tell the same story? *BMC Infectious Diseases*, *12*(1), 318.

Barrios, P. R., Reiersen, J., Lowman, R., Bisaillon, J. R., Michel, P., Fridriksdóttir, V., Gunnarsson, E., Stern, N., Berke, O., McEwen, S., & Martin, W. (2006a). Risk factors for Campylobacter spp. colonization in broiler flocks in Iceland. *Preventive Veterinary Medicine*, *74*(4), 264–278.

Barrios, P. R., Reiersen, J., Lowman, R., Bisaillon, J. R., Michel, P., Fridriksdóttir, V., Gunnarsson, E., Stern, N., Berke, O., McEwen, S., & Martin, W. (2006b). Risk factors for Campylobacter spp. colonization in broiler flocks in Iceland. *Preventive Veterinary Medicine*, *74*(4), 264–278.

Blaser, M., Campylobacter, J. E.-, Edition, T., & 2008, U. (2014). Clinical Aspects of Campylobacter jejuni and Campylobacter coli Infections. In *Campylobacter*, *Third Edition* (pp. 99–121).

Borck Høg, B., Sommer, H. M., Larsen, L. S., Sørensen, A. I. V., David, B., Hofshagen, M., &

Rosenquist, H. (2016). Farm specific risk factors for Campylobacter colonisation in Danish and Norwegian broilers. *Preventive Veterinary Medicine*, *130*, 137–145.

Bouwknegt, M., Van De Giessen, A. W., Dam-Deisz, W. D. C., Havelaar, A. H., Nagelkerke, N. J. D., & Henken, A. M. (2004). Risk factors for the presence of Campylobacter spp. in Dutch broiler flocks. *Preventive Veterinary Medicine*, *62*(1), 35–49.

Cardinale, E., Tall, F., Guèye, E. F., Cisse, M., & Salvat, G. (2004). Risk factors for Campylobacter spp. infection in Senegalese broiler-chicken flocks. *Preventive Veterinary Medicine*, *64*(1), 15–25.

Carrique-Mas, J. J., Bryant, J. E., Cuong, N. V., Hoang, N. V. M., Campbell, J., Hoang, N. V., Dung, T. T. N., Duy, D. T., Hoa, N. T., Thompson, C., Hien, V. V., Phat, V. V., Farrar, J., & Baker, S. (2014). An epidemiological investigation of Campylobacter in pig and poultry farms in the Mekong delta of Vietnam. *Epidemiology and Infection*, *142*(7), 1425–1436.

Connerton, P. L., Richards, P. J., Lafontaine, G. M., O'Kane, P. M., Ghaffar, N., Cummings, N. J., Smith, D. L., Fish, N. M., & Connerton, I. F. (2018). The effect of the timing of exposure to Campylobacter jejuni on the gut microbiome and inflammatory responses of broiler chickens. *Microbiome*, *6*(1), 88.

de Vries, S. P. W., Vurayai, M., Holmes, M., Gupta, S., Bateman, M., Goldfarb, D., Maskell, D. J., Matsheka, M. I., & Grant, A. J. (2018). Phylogenetic analyses and antimicrobial resistance profiles of Campylobacter spp. from diarrhoeal patients and chickens in Botswana. *PLOS ONE*, *13*(3), e0194481.

Evans, S. J., & Sayers, A. R. (2000). A longitudinal study of campylobacter infection of broiler flocks in Great Britain. *Preventive Veterinary Medicine*, *46*(3), 209–223.

Guerin, M. T., Martin, W., Reiersen, J., Berke, O., McEwen, S. A., Bisaillon, J. R., & Lowman, R. (2007). A farm-level study of risk factors associated with the colonization of broiler flocks with Campylobacter spp. in Iceland, 2001 - 2004. *Acta Veterinaria Scandinavica*, *49*(1), 1–12.

Hasan, M. M., Talukder, S., Mandal, A. K., Tasmim, S. T., Parvin, M. S., Ali, M. Y., Sikder, M. H., & Islam, M. T. (2020). Prevalence and risk factors of Campylobacter infection in broiler and cockerel flocks in Mymensingh and Gazipur districts of Bangladesh. *Preventive Veterinary Medicine*, *180*(April), 105034.

Hsieh, Y. H., & Sulaiman, I. M. (2018). Campylobacteriosis: An Emerging Infectious Foodborne Disease. In *Foodborne Diseases* (Vol. 15, pp. 119–155).

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*(3), 237–257.

Igwaran, A., & Okoh, A. I. (2019). Human campylobacteriosis: A public health concern of global importance. In *Heliyon* (Vol. 5, Issue 11, p. e02814). Elsevier Ltd.

Janssen, R., Krogfelt, K. A., Cawthraw, S. A., Van Pelt, W., Wagenaar, J. A., & Owen, R. J. (2008). Host-pathogen interactions in Campylobacter infections: The host perspective. In *Clinical Microbiology Reviews* (Vol. 21, Issue 3, pp. 505–518). American Society for Microbiology Journals.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of campylobacter infection. *Clinical Microbiology Reviews*, *28*(3), 687–720.

Kabir, M. M., Jonayat, A. S. M., Patel, S., & Toussaint, K. C. (2014). Graphics processing unit-based quantitative second-harmonic generation imaging. *Journal of Biomedical Optics*, *19*(09), 1.

Klena, J. D., Parker, C. T., Knibb, K., Claire Ibbitt, J., Devane, P. M. L., Horn, S. T., Miller, W. G., & Konkel, M. E. (2004). Differentiation of Campylobacter coli, Campylobacter jejuni, Campylobacter lari, and Campylobacter upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid A gene lpxA. *Journal of Clinical Microbiology*, *42*(12), 5549–5557.

Kottawatta, K., Van Bergen, M., Abeynayake, P., Wagenaar, J., Veldman, K., & Kalupahana, R.

(2017). Campylobacter in Broiler Chicken and Broiler Meat in Sri Lanka: Influence of Semi-Automated vs. Wet Market Processing on Campylobacter Contamination of Broiler Neck Skin Samples. *Foods*, *6*(12), 105.

Lagler, H., Kiesewetter, B., & Raderer, M. (2016). Infection with multidrug-resistant Campylobacter coli mimicking recurrence of carcinoid syndrome: A case report of a neuroendocrine tumor patient with repeated diarrhea. *BMC Infectious Diseases*, *16*(1), 409.

Li, X., Swaggerty, C. L., Kogut, M. H., Chiang, H., Wang, Y., Genovese, K. J., He, H., Stern, N. J., Pevzner, I. Y., & Zhou, H. (2008). The paternal effect of Campylobacter jejuni colonization in ceca in broilers. *Poultry Science*, *87*(9), 1742–1747.

Lin, J. (2009). Novel approaches for campylobacter control in poultry. *Foodborne Pathogens and Disease*, *6*(7), 755–765.

Md., K. I., S., M. L. K., A., K. M. Z. H., Y., A. S., & M., H. S. (2018). Molecular detection and characterization of Escherichia coli, Salmonella spp. and Campylobacter spp. isolated from broiler meat in Jamalpur, Tangail, Netrokona and Kishoreganj districts of Bangladesh. *African Journal of Microbiology Research*, *12*(32), 761–770.

Medicine, V., Sciences, V., Health, A., & Development, L. (1999). *Prevalence of Campylobacter jejuni in broilers and the adhesion and invasion abilities*.

Näther, G., Alter, T., Martin, A., & Ellerbroek, L. (2009). Analysis of risk factors for Campylobacter species infection in broiler flocks. *Poultry Science*, *88*(6), 1299–1305.

Newell, D. G., Elvers, K. T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N. J., Davies, R., Connerton, I., Pearson, D., Salvat, G. S., & Allen, V. M. (2011). Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. *Applied and Environmental Microbiology*, *77*(24), 8605–8614.

Pai, C. H., Sorger, S., Lackman, L., Sinai, R. E., & Marks, M. I. (1979). Campylobacter gastroenteritis in children. *The Journal of Pediatrics*, *94*(4), 589–591.

Racicot, M., Venne, D., Durivage, A., & Vaillancourt, J. P. (2012). Evaluation of the relationship between personality traits, experience, education and biosecurity compliance on poultry farms in Québec, Canada. *Preventive Veterinary Medicine*, *103*(2–3), 201–207.

Rahman, S., Begum, I. A., & Alam, M. J. (2014). Livestock in Bangladesh: Distribution, growth, performance and potential. *Livestock Research for Rural Development*, *26*(10).

Refrégier-Petton, J., Rose, N., Denis, M., & Salvat, G. (2001). Risk factors for Campylobacter spp. contamination in French broiler-chicken flocks at the end of the rearing period. *Preventive Veterinary Medicine*, *50*(1–2), 89–100.

Sakaridis, I., Ellis, R. J., Cawthraw, S. A., van Vliet, A. H. M., Stekel, D. J., Penell, J., Chambers, M., La Ragione, R. M., & Cook, A. J. (2018). Investigating the Association Between the Caecal Microbiomes of Broilers and Campylobacter Burden. *Frontiers in Microbiology*, *9*(MAY), 927.

Scallan, E., Hoekstra, R. M., Mahon, B. E., Jones, T. F., & Griffin, P. M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology and Infection*, *143*(13), 2795–2804.

Sibanda, N., McKenna, A., Richmond, A., Ricke, S. C., Callaway, T., Stratakos, A. C., Gundogdu, O., & Corcionivoschi, N. (2018). A Review of the Effect of Management Practices on Campylobacter Prevalence in Poultry Farms. *Frontiers in Microbiology*, *9*(AUG), 2002.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). Campylobacter spp. As a foodborne pathogen: A review. In *Frontiers in Microbiology* (Vol. 2, Issue SEP). Frontiers Research Foundation.

Skarp, C. P. A., Hänninen, M. L., & Rautelin, H. I. K. (2016). Campylobacteriosis: The role of poultry meat. In *Clinical Microbiology and Infection* (Vol. 22, Issue 2, pp. 103–109). Elsevier B.V.

Sotelo, J. (2011). Clinical manifestations, diagnosis, and treatment of neurocysticercosis. *Current Neurology and Neuroscience Reports*, *11*(6), 529–535.

Stanley, J., Burn Ens, " A P, Lint, ^ D, On, S. L. W., Costas', ^ M, & Ow En^, R. J. (1992). Campylobacter heheticus sp. nov., a new thermophilic species from domestic animals: characterization, and cloning of a species-specific DNA probe. In *Journal of General Microbiology* (Vol. 138).

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2013). Common source outbreaks of Campylobacter infection in the USA, 1997-2008. *Epidemiology and Infection*, *141*(5), 987–996.

Uddin, M. B., Syed, A., Uddin, S., Hassan, M. M., & Al Mamun, M. (2010). *Prevalence of poultry diseases at Narsingdi, Ban gladesh Epidemiological investigation of highly pathogenic avian influenza at birds (wild and domestic)-human interface in Bangladesh View project clinical cases View project*.

Wedderkopp, A., Rattenborg, E., & Madsen, M. (2000). National surveillance of Campylobacter in broilers at slaughter in Denmark in 1998. *Avian Diseases*, *44*(4), 993–999.

Acknowledgements

I would like to express my gratitude to Dr. Shemol Chandra Poddar, Upazilla Livestock Officer, Mirasarai; Dr. Jayita Basu, Veterinary Surgeon, Mirasarai and all the staffs of Upazilla Veterinary Hospital, Mirasari for their cordial co-operations and time during the research period.

I am grateful to Prof. Abdul Ahad, Dean, Faculty of Veterinary Medicine and Prof. Dr. AKM Saifuddin, Director, External Affairs of CVASU for the provision of this unique internship programme and research exposure.

I would like to pay my immense graciousness to my internship supervisor Md.Ahasanul Hoque, Professor, Department of Medicine and Surgery of CVASU for his intellectual supervision.

Special thanks to Dr. Sharmin Chowdhury, Professor, Dept. of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University for her kindful facilitation during work in lab.

I would also like to thank Dr. Abu Sayeed, Lecturar, Jhenaidah Veterinary College for guiding in map construction.

Thanks to DR. Md. Sirazul Islam, MS fellow, Dept. of Pathology and Parasitology, who helped me in collecting data for this study and lab work.

I would also like to acknowledge my family members, my batchmates and laboratory staffs for being supportive in this wonderful journey.

Biography

I am Tahia Ahmed Logno, daughter of Md. Shamsul Alam and Gulshan Ara Parveen. I passed Secondary School Certificate examination in 2010 (G.P.A-5.00) followed by Higher Secondary Certificate examination in 2012 (G.PA-5.00). Now I am an intern veterinarian under the Faculty of Veterinary Medicine in Chattogram Veterinary and Animal Sciences University, Bangladesh. In the future I would like to work as a veterinary practitioner and do research on clinical animal diseases in Bangladesh.