CHAPTER 1

INTRODUCTION

The poultry sub-sector is an important avenue in fostering agricultural growth and reduce malnutrition for the people in Bangladesh. It is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity including support services for about 6 million people. This sub-sector has proved as an attractive economic activity, thereby, indicating its importance for the entire economy. The sector accounts for 14% of the total value of livestock output and is growing rapidly. Poultry population in Bangladesh is estimated about 304.17 million where chicken population is about 255.31 million (Hamid et al., 2017). In developing countries, such as Bangladesh, poultry and poultry products are cheap and staple source of animal protein for all ethnic groups. Bangladesh produces an estimate of 4.52 million tons of poultry meat (Growth rate 24.86%) and estimate of 10168 million numbers of egg (Growth rate 33.48%)(Source: DLS, 2013-14). Farrell stated that, over the next 20 years significant expansion of most livestock industries, especially poultry eggs and meat is likely to occur mainly in developing countries. Bangladesh is one of the potential countries to catch this opportunity. The forecast of production eggs and meat within the year 2020 of about 47.0 million Mt of poultry and 54.0 million Mt of eggs (Source: Farrell).

Bangladesh started export of day old chicks and feed to other countries during 2002-2007 and it is temporarily stopped due to Avian Influenza. However if we control the AI, we will be in position to export not only the chicks and feed but also poultry meat.(Hamid et al.,2017).

This sector can create huge job opportunity. It offers full or part time employment of large number of peoples particularly women, children or elderly person on the farm operations. This clearly indicates that there is scope for expansion of this industry.(Rahman et al. 2017)

There are several constraints in poultry industries in Bangladesh. Some diseases of poultry specially in chicken act as a barrier in poultry industry which affects directly or indirectly. Among them Pullorum disease (caused by *Salmonella Pullorum*, recently used nomenclature) is one of the major constraints of poultry industries in Bangladesh (Hossain *et al.*, 2006). *Salmonella* infection is one of the most important bacterial diseases in poultry causing heavy economic loss through mortality and reduced production (Haider *et al.*, 2003).

Salmonellae are gram-negative bacteria, which belong to the genus *Salmonella* which is part of the family of Enterobacteriaceae (bacteria living in the intestine). Salmonellae are non-encapsulated bacteria that can grow under either aerobic or anaerobic conditions. The optimum temperature for growth is 35 to 43ºC while the optimum pH is 6.6 to 8.2. However, Salmonellae can continue to grow at pH values between 4.5 and 9.5 and at temperatures between 5 and 54 ºC. A water activity above 0.94 is also required (Hanes, 2003). Most of the salmonellae are motile bacteria that use flagella to move. Some serotypes like *S. Gallinarum* or *S. Pullorum* are non-motile. Among the different *Salmonella* serotypes, *S. Enteritidis* and *S.Typhimurium* are presented separately from others because, on the one hand, these bacteria are often specifically cited in zoonosis control legislation, and, secondly, because there are differences in the epidemiology as compared to other salmonellae. *S. Enteritidis* and *S. Typhimurium* are the predominant serotypes associated with human disease in most countries. Indeed, *Salmonella spp.* is one of the major causes of food poisoning in humans. According to the European Food Safety Authority (EFSA, 2009), a total of 154,099 cases of human salmonellosis were reported by the 25 EU Member States in 2004. The major sources of food borne salmonellosis are eggs and poultry meat (EFSA, 2005). More than 2300 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry.

Salmonella is also a globally widespread food-borne pathogen having major impact on public health. Salmonellosis is an infection with bacteria called *Salmonella*. Most persons infected with *Salmonella* develop diarrhoea, fever, and abdominal cramps 12 to 72 h after infection (Aditya, 2015). Poultry and poultry products are often implicated in sporadic cases and in outbreaks of human salmonellosis (Bryan and Doyle, 1995; Humphrey, 2000). Thus, it is inevitably of great importance for both animal and public health.

The *Salmonella* spreading with the presence of antimicrobial resistance genes have some global public health impacts, because of their transmission to other countries, by travelers or by trade are impossible to prevent (Collard et al., 2007) but differences in hygiene practices and handling between slaughterhouses, could significantly eliminate the risk of *Salmonella* contamination of broiler meat (Heyndrickx et al., 2002). In developed countries it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have gained their resistance in an animal host before being moved to humans through the food chain (Molbak et al., 2002; Threlfall et al., 2002). This resistance occurs due to the indiscriminate and improper use of sub-therapeutic doses of antibiotics. Present study has designed to determine prevalence

and antibiogram analysis by collecting fecal sample of 30 broiler farms from Patiya,Chittagong.

Antimicrobial uses are not well regulated in most of the developing countries including Bangladesh that encourage poultry farmers to abuse antimicrobials on their own without concern of the veterinarians. This abuse of antibiotics might lead to the development of antimicrobial resistance in zoonotic pathogens such as Salmonella. Through the trades of poultry and poultry products, and human movements this antimicrobial-resistant Salmonella can spread over the borders. Thus, local emergence of a multidrug-resistant Salmonella strain in poultry has an international importance.

Reducing *Salmonella* contamination on poultry carcasses requires a complete approach that includes the entire integrated broiler operation, the breeding flocks, hatchery, broiler growers, feed mills and transporters. Therefore, in order to control *Salmonella*, one should consider the critical points of the farm. In order to efficiently control *Salmonella spp*. at the farm level, a set of biosecurity measures need to be applied. Therefore, a combination of different interventions will allow achieving significant reductions in the frequency of *Salmonella*-contaminated broilers sent to slaughter.

Objectives:

1. To estimate prevalence of *Salmonella* in broiler farm at Patiya, Chittagong.
2. To isolate *Salmonella* from pooled fecal samples of broiler farm.
3. To investigate antibiotic resistance pattern of *Salmonella* isolated from broiler farm at Patiya, Chittagong.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples and Sampling/Study area and animals

A cross-sectional survey was undertaken by collecting pooled fecal sample from 30 broiler farms in different ages of Patiya, Chittagong. The sample was collected during Upazilla Veterinary Hospital internship placement schedule for two months. The duration of placement was about 01 March 2017 to 06 April 2017 and 05 July 2017 to 03 August 2017.Pooled sample (200 gms) was taken from the broiler farm.

2.2 Epidemiological survey

A questionnaire was designed and applied to shop owners during an interview at the time of sampling.

2.3 Sample collection and processing

Fecal samples were firstly collected in separate clean plastic zipper bag immediately after voiding. Then fecal samples were transferred scientifically in a clean sterile screw capped falcon tube containing buffer peptone water. Soon after collection, the samples were kept into a cool box with ice for ceasing the growth and activity microorganism. Each sample was labeled with date of sampling, type of sample and the place from where the sample was collected. The samples were transported to the Department of Microbiology and Veterinary Public Health or to the Poultry Science and Research Centre, Chittagong Veterinary and Animal Sciences University (PRTC-CVASU) as early as possible and stored at 4°C until bacteriological investigation.

2.4 Study Design

Broiler farm of Patiya, Chittagong

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Collection of pooled fecal samples

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Put the samples into plastic zipper bags after voiding

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Peptone broth

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Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar (Selective enrichment)

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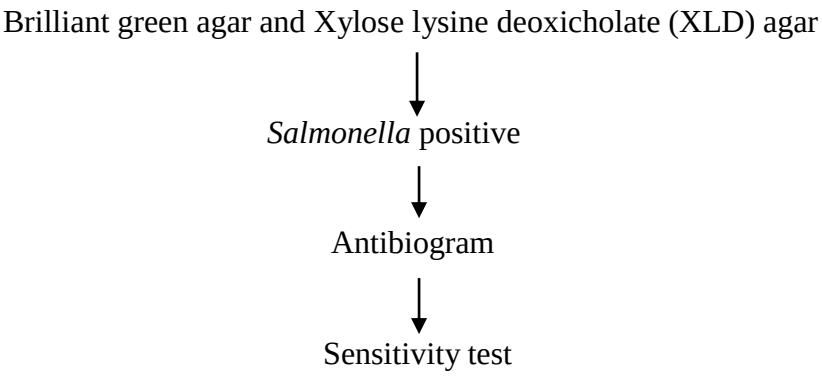


2.5 Bacterial isolation

Conventional bacteriological procedures be followed for the isolation and identification of *Salmonella*. Briefly, naturally pooled fecal samples will be mixed with buffered peptone water (BPW) and 25 gm of this mixture will be transferred to 200 ml of BPW, and incubated at 37°C for 16-18 hours, followed by selective enrichment in Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar for 24 hours at 42°C. Any opaque growth observed on the MSRV agar plates will be suspected for *Salmonella* and streaked on to brilliant-green and xylose lysine deoxicholate (XLD) agar by using an inoculating loop. Following incubation at 37°C for 24 hours, suspected *Salmonella* colonies will be transferred on to 5% blood agar plate with incubation for 16 to 18 hours at 37°C.

Table 1: Methods on culture test

SN	Agar/Test	Incubation time & Temparature	Observations
1	MSRV Agar	Incubated at 42^0C for 24 hours	Grey white turbid zone visible.
2	Brilliant Green Agar	Incubated at 37^0C for 24 hours	Red to pink white colonies surrounded by a red zone.
3	XLD Agar	Incubated at 37^0C for 24 hours	Pink color colonies with black center.
4	Blood Agar	Incubated at 37^0C for 16-18 hours	Produce non-hemolytic smooth white colonies.

2.6 Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing of *Salmonella* isolates will be performed by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2008) standards with a panel of 5 antimicrobials including Amoxicillin(10µg), Ampicillin (10µg),Ciprofloxacin (5µg), Colistin sulphate (10µg), Salphamethazole (25µg) isolate the zone of inhibition around each disk will be measured and interpreted as Susceptible (S), Intermediate (I) or Resistant (R) according to CLSI documents for veterinary pathogens (CLSI, 2008).

Table 2: Standard measurement of diameter of zone of inhibition.

SL	Name of Antimicrobial agents	Diameter of zone of inhibition (millimeter)		
		Resistant	**Intermediate**	**Sensitive**
1	Ampicillin	≤13	14-16	≥17
2	Amoxicillin	≤13	14-17	≥18
3	Ciprofloxacin	≤15	16-20	≥21
4	Colistin sulphate	≤10	11-13	≥14
5	Salphamethazole	≤10	11-15	≥16

Source: CLSI, 2007; Seol et al., 2005; LO-Ten-Foe et al., 2007

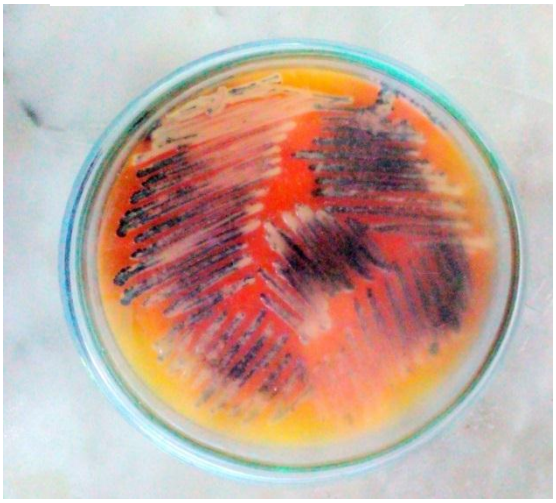
‘ R’=Resistance, ‘I’=Intermediate, ‘S’=Sensitive

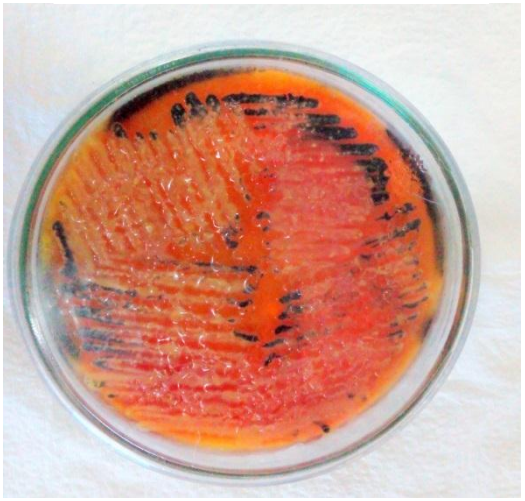
2.7 Data analysis

Data was stored in MS excel (Microsoft Word 2007) and descriptive analysis was done in this study.

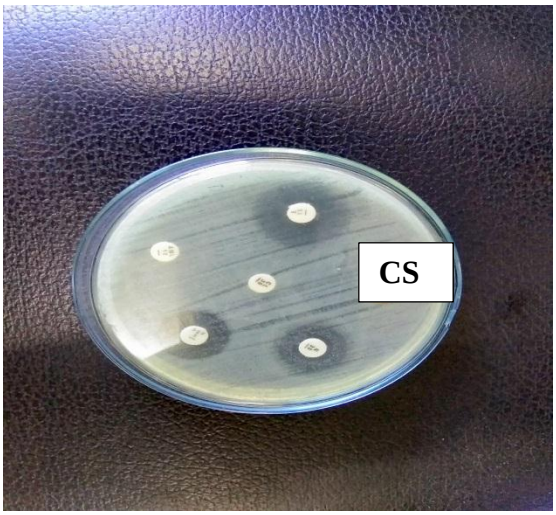
Broiler Farm

Collecting fecal sample





***Salmonella* on XLD agar**



Culture Sensitivity test

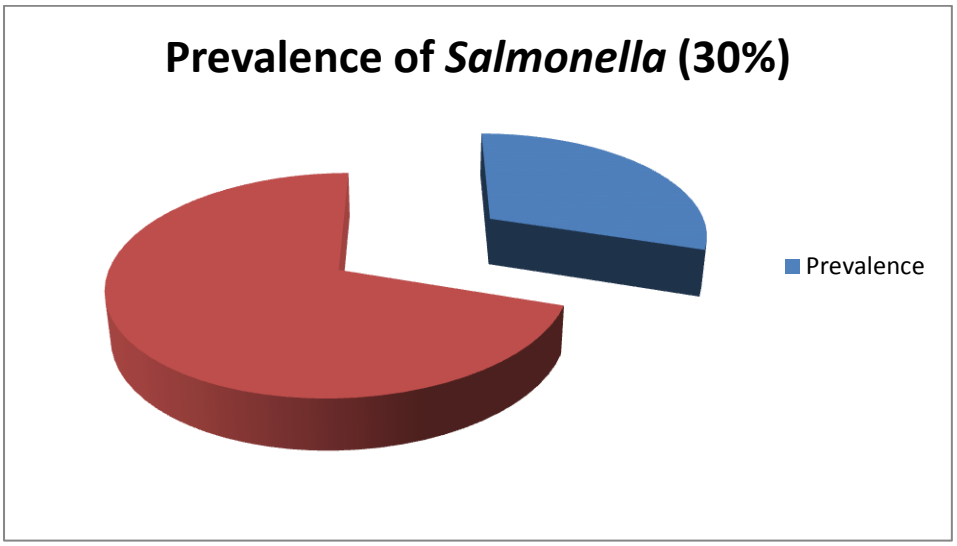
Observing zone of inhibition

CHAPTER 3

RESULTS

Among 30 samples of feces of broiler farms 9 (30%) pooled fecal samples were positive and individual colonies of *Salmonella* were isolated through different tests sequentially.

Graph 1: Graphical representation on prevalence of *Salmonella*



Results of culture examination

Salmonella was cultured on Brilliant-green agar (BGA) & Xylose lysine deoxicholate (XLD) medium for morphological characterization. After completing sequential processing, 30% samples were found positive and formed two types of colonies were isolated under microscopic examination. All the isolated colonies were Red to pink-white colonies surrounded by red zone on BGA, while Pink color colony with black center on XLD agar. In the microscopic examination of Gram's staining, all the positive samples are found as Gram-negative, pale pink colored, short rod shaped transparent bacteria which are arranged in single or in pairs.

Table 3: The examination result at a glance done for isolation of *Salmonella*

SL	Name of the media / Test	Total no. of sample	No. of positive sample	Percentage (%)
1.	Brilliant-green agar	9	9	30%
2.	XLD agar	9	9	30%
3.	Gram's staining	9	9	30%

Result of antimicrobial sensitivity test:

All the 9 positive isolates were subjected to do antibiotic sensitivity test to 5 different antimicrobial agents. From the isolates 77.78% samples were sensitive & 11.11% samples were intermediate to Colistin Sulphate which was the highest in sensitivity. 55.56% samples showed sensitive to Ciprofloxacin. From the isolates 88.89%, 88.89% and 66.67% samples showed resistance to Amoxicillin, Ampicillin and Salfamethazole respectively.

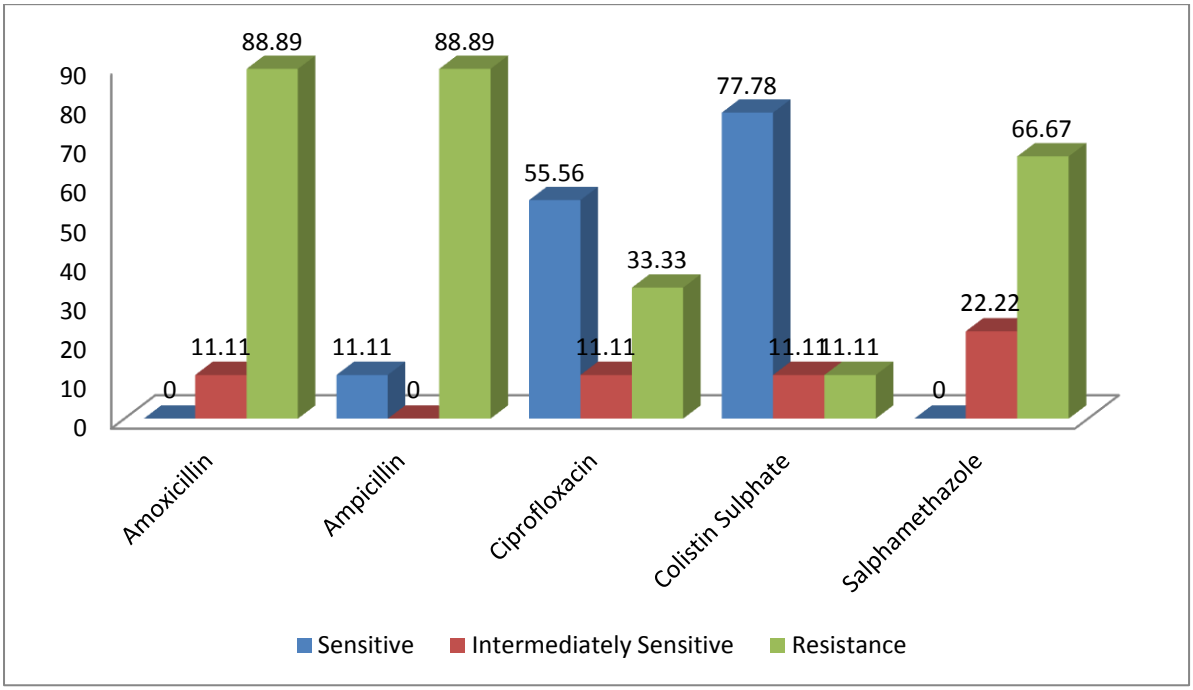
Table 4: Antimicrobial resistance pattern against *Salmonella* isolates

Sample NO	Amoxicillin (AMX)	Ampicillin (AMP)	Ciprofloxacin (CIP)	Colistin Sulphate (CT)	Salphamethazole (SXT)
Sample 1	R	R	S	S	R
Sample 2	R	S	S	S	R
Sample 3	R	R	R	S	I
Sample 4	R	R	S	I	R
Sample 5	R	R	R	S	I
Sample 6	R	R	S	R	S
Sample 7	R	R	I	S	R
Sample 8	I	R	S	S	R
Sample 9	R	R	R	S	R

Table 5: Prevalence of antimicrobial resistance pattern against *Salmonella* isolates

Antimicribial agents	No. of Positive isolates	Resistance No (%)	Intermediately Sensitive (%)	Sensitive (%)
Amoxicillin	09	8(88.89)	11.11	0.00
Ampicillin	09	8(88.89)	0.00	11.11
Ciprofloxacin	09	3(33.33)	11.11	55.56
Colistin sulphate	09	1(11.11)	11.11	77.78
Salfamethazole	09	6(66.67)	22.22	0.00

Graph 2: Graphical representation of antimicrobial susceptibility profiling test



CHAPTER 4

DISCUSSION

Overall prevalence of *Salmonella* infection

The study was conducted to the aim of isolation and identification of *Salmonella* present in pooled fecal sample of broiler farms in Patiya, Chittagong. Antibiogram was also done to know the sensitivity and resistance pattern against different antibiotics. The isolates were confirmed as *Salmonella* by cultural staining. Finally, antibiotic sensitivity and resistance patterns of the isolates of broiler chickens identified in this study. Among 30 pooled samples of 30 broiler farms, 9 isolates were positive for *Salmonella.* Here our study observed that 30% prevalence were found .The overall prevalence of Salmonellosis was recorded as 43.4% (Islam *et al.*, 2006). Yang *et al.,* (1996) reported as (39.02%) which are higher than that of the present study. It might be due to geographical location, age, managemental variation. Naurin et al, 2012 reported that the prevalence of *Salmonella* in Mymensingh region was 52% which is higher than our observation (30%) might be also the reason of geographical variation, seasonal variation, sample size etc. The highest prevalence of *Salmonella* in broiler chickens recorded in the current study might be due to overcrowding and improper sanitary measures of the farms. The results of this study indicated that broilers could be an important reservoir of *Salmonella* spp.

Antimicrobial Susceptibility Profiling of *Salmonella sp*

Six drugs were used for antibiogram study. These were Amoxicillin, Ampicilin, Ciprofloxacin, Colistin Sulphate, Salfamethazole. My observed result was Amoxicillin(88.89%), Ampicillin(88.89%), Ciprofloxacin(33.33%), Colistin Sulphate(11.11%), Salfamethazole(66.67%) resistant to *Salmonella.* Aditya, 2015 reported that Colistin sulphate, 8(50%) isolates were resistant while the remaining 8 (50%) were intermediate but my result was 77.78% sensitive and 11.11% intermediate which is fully different from our observation.It might be the reason of variation in antimicrobial drug trial by veterinarians/farm owners.Nath et al., 2017 state that 100% resistance grow in case of Amoxicillin which is similar (88.89%) to our result. Similar type of finding was observed by Hemen *et al.*, (2012). It is indicating that these antibiotics were used very frequently in treating large animal. The global situation concerning antibiotic resistance worldwide is at least alarming. In the present time, the recognition of the importance of antibiotic resistance is almost catholic. Therefore, certain measures have been implied by the states so as to mitigate this problem.

CHAPTER 5

LIMITATIONS

Although all the pooled samples were handled very scientifically from the beginning to the end of the study, there might have some error or mistake unwillingly. Moreover the sample size was too small to get precise result. The agar medium which was used for culture might have some manufacturing problem or contamination on Agar plate. The period of sampling time was also limited.

CHAPTER 6

CONCLUSION

The result of the study is directly indicated that isolates from broilers are being multi-drug resistant which may be due to indiscriminate and continuous use of sub-therapeutic doses of antibiotics during rearing in commercial production system. These findings would certainly help the veterinarians to select the specific antibiotics against *Salmonella* infections. This study will create public awareness about *Salmonella* in healthy poultry and would be helpful for controlling *Salmonella* associated food-borne infections originating from chickens. Moreover, the disease caused by *Salmonella* has a great public health importance. Therefore, broiler sector should be provided with immediate attention by the government to maintain strict hygienic measurement in farm all over the country.

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BIOGRAPHY

Myself Sushyam Biswas, son of Mr. Shyamal Mitra Biswas and Mrs. Suchitra Biswas. I passed my Secondary School Certificate examination in 2009 from Chittagong Collegiate School, Chittagong and passed my Higher Secondary School Certificate examination in 2011 from Govt. City College, Chittagong. Now I am an intern doctor under the Faculty of Veterinary Medicine in Chittagong Veterinary and Animal Sciences University. In future, as a veterinarian I want to furnish and develop my veterinary profession. I have immense interest on medical research, public health and small animal medicine.