PREVALENCE AND PATHOLOGY OF COLIBACILLOSIS IN DIFFERENT BROILER FARM AT DINAJPUR SADAR UPAZILA

A THESIS
BY

RAKIBA KHATUN

REGISTRATION NO. 1705442

SESSION: 2017-2018

SEMESTER: JULY-DECEMBER, 2018

MASTER OF SCIENCE
IN
PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
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Approved as to the style and content by

(Professor Dr. S.M. Harun-ur-Rashid)	**(Dr. Md. Golam Azam)**
Research Supervisor	Research Co-Supervisor

(Professor Dr. S.M. Harun-ur-Rashid)
Chairman, Examination committee
and
Chairman

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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ABSTRACT

This study was carried out to investigate the prevalence and pathological lesions of avian colibacillosis in different commercial broiler farm at Dinajpur sadar upazila with variation of age of birds from July to December, 2018. Colibacillosis in commercial broiler farms causes huge economic loss through a relatively high mortality and loss of production. In the present study, a total of 8800 birds from 6 farms were observed and diagnosed infected birds on the basis of clinical signs, post mortem lesions and histopathological lesions. Among them highest prevalence found in F1 farm (76.2%) and lowest prevalence found in F6 farm (9.16%) which was statistically highly significant ($p<0.05$). Highest mortality found in F1 farm (9.1%) and lowest in F6 farm (3.27%) which was statistically significant ($p<0.05$). Average prevalence and mortality of six farms of Dinajpur sadar upazila was 39.63% and 5.39%, respectively. On the basis of age, highest prevalence found in 0-2 weeks of age (69.3%) and lowest in 2-4 weeks of age (9.64%) which was statistically highly significant ($p<0.05$) for colibacillosis. The most frequent gross lesions of colibacillosis were air sacculitis omphalitis, pericarditis, perihepatitis, peritonitis and enteritis. The microscopic lesions of these conditions were haemorrhage and highly congestion in lung, destruction of intestinal wall and reactive cell infiltration, thickening of fibrous tissue in pericardium.

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LIST OF ABBREVIATION

APEC = Avian pathogenic *E. coli*

BBS = Bangladesh Bureau of Statistics

cAMP = Cyclic Adenosine Monophosphate

CDIL = Central Diseases Investigation Laboratory

CFU = Colony Forming Units

cGMP = Cyclic Guanosine Monophosphate

CRD = Chronic Respiratory Disease

DLS = Department of Livestock Services

E. coli = *Escherichia coli*

ELISA = Enzyme Linked Immunosorbent Assay

et al. = And others

FAO = Food and Agriculture Organization

HSTU = Hajee Mohammed Danesh Science and Technology University

IBD = Infectious Bursal Disease

IBV = Infectious Bronchitis Virus

IT = Intratracheal

ND = Newcastle disease

PCR = Polymerase Chain Reaction

RE = Reticulo Endothelial

SPSS = Statistical Package for Social Science

CHAPTER 1

INTRODUCTION

Bangladesh is an agro-based developing country in the world. Livestock is one of the most important sub-sectors in the agriculture which plays an important role to promote human health and national economy. There are about 47.48 million livestock and 206.98 million poultry (DLS, 2008-2009) in Bangladesh. Livestock not only help to upgrade the financial condition of the farmers of the Bangladesh but it has also made substantial contribution to human nutrition. Normal requirement of animal protein for a man is about 62.5 gm meat per day while people of our country get only 6.90 gm per day (Jabbar and Greeb, 1983). Poultry industries play an important role in poverty alleviation and economic development of Bangladesh. Poultry products are one of the most important protein sources for human beings throughout the world. Poultry meat contributes approximately 37% of total animal protein supplied in the country (Rahman *et al.,* 1998; FAO 1999), where broiler meat plays a vital role to meet the deficit.

According to the Bangladesh Bureau of Statistics (BBS) about 89% of the rural households rear poultry. Bangladesh population statistics also indicates poultry as the most important species of birds in this country. A total of 98.15% of poultry are kept in rural area and they are scavengers (BBS, 1987 Bangladesh livestock).

These birds scavenge in and around the farmers homesteads and meet a major part of their feed requirements in this way and require little additional feed (Fattah, 1999). For the last several years, poultry rearing specially broiler has been developed as an industry in Bangladesh. With great expansion of the poultry rearing and farming. Their production level is low as the breed quality is poor. However, this situation is continuously improving through introducing some pure and cross breeds from Department of Livestock Services (DLS) poultry farm. Moreover, these breeds introduce some new disease problems in the field level. Hence it is necessary to investigate the important diseases which are prevailing in the parent stock flocks of DLS poultry farm specially broiler, as various diseases are transmitted through the eggs, vertically or horizontally from infected parent stocks to the field level. Scientific breeding, feeding, management and disease control are the key points of success in poultry improvement programmed. Broiler farming in Bangladesh is now considered as a growing industry and plays an important role in the rural socio-economic system by contributing

significantly on economic growth and simultaneously creating numerous employment opportunities. More than 130 hatcheries produce 3.4 million day-old chicks per week and about 30,000 commercial broiler and layer farms supply 0.26 million tonnes of poultry meat and 5210 million eggs per year (Rahman, 2003a). About 78% of the country's eggs and 86% of poultry meat are produced by rural scavenging birds (Alam, 1995). The poultry population of Bangladesh has increased from around 71 million in 1986 to around 188 million in 2006, an increase of about 164 percent in 20 years (FAO 2008, BBS 2006).among poultry broiler rear very popular deu to short time and less investment. But one of the major constraints in the development of broiler industry in Bangladesh is the outbreak of diseases, which cause about 30% mortality of chickens (Ali, 1994).The advancement of broiler industry is facing a problem due to sudden outbreak of bacterial diseases, including colibacillosis, which posses a serious threat in Bangladesh.

Colibacillosis in chickens refers to any local or systemic infection caused entirely or partly by *E. coli* strains (Barnes *et al.*, 2003). *Escherichia coli*, strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC) Ewers *et al.* (2003).

Colibacillosis is one of the main causes of economic losses in the poultry industry worldwide (Yogaratnam, 1995; Ewers *et al.*, 2003). *Escherichia coli*, has been associated with a variety of diseases in poultry such as pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulites, colispticemia, coligranuloma and swollen-head syndrome (Saif *et al.*, 2003). However, fecal contamination of egg may result in the penetration of *E. coli* through the shell membrane and may spread to the chickens during hatching and is often associated with high mortality rates, or it may give rise to yolk sac infection. It has been noticed to be a major infectious disease in birds of all ages. Day-old chicks may become infected via the yolk sac, but in older chicks the infection is considered to be mainly airborne. Young broiler chickens up to three weeks of age are highly susceptible to the disease, but chickens of four weeks and older are considered quite resistant to primary colibacillosis (Goren, 1978). Various risk factors may increase the susceptibility of broilers to colibacillosis, e.g. respiratory viruses and environmental factors like dust and high concentrations of NH3 and CO_2. In addition, the *E. coli* concentration in the air of the broiler house is an

important factor. Airborne dust particles in broiler houses can contain 10 -106 *E. coli* bacteria/g and these bacteria may persist for long periods (Harry and Hemsley, 1965).

Strains of *E. coli* predominate among the aerobic commensal flora in the gut of humans and animals. The major species of *E. coli* are encountered in the lower intestine of warm-blooded animals and birds, where they cause gastroenteritis (Pelczar *et al.,* 1986). These bacteria are widespread and present wherever there is faecal contamination, causing strains, which may be purely commensal or possess combinations of pathogenic mechanisms that enable them to cause disease in man and other animals (Greenwood *et al.*, 2002).

Broilers suffering from colibacillosis are depressed, show respiratory distress and growth retardation. Mortality usually remains below 5%, but morbidity often reaches more than 50% (Wray *et al.,* 1996; Vandekerckhove *et al.,* 2004). But in severe cases, Mortality may rich up to 94% in colibacillosis. (Biswas *et al.,* 2006; Haider *et al.,* 2003; Roy *et al.,* 2006).

With expansion of poultry farming specially broiler, colibacillosis has become a widespread problem in Bangladesh (Islam *et al.,* 2003; Rahman, 2003b; Hossain *et al.,* 2004). It causes serious loss specially if there is bad management or stress in broilers such as complicating infections like chronic respiratory disease (CRD) or mycoplasma (Talha *et al.*, 2001). Heavy economic loss occurs in broilers and layers due to morbidity, mortality, reduced production and poor chick quality (Islam *et al.*, 2003; Rahman, 2003b; Rahman *et al.*, 2004; Hossain *et al.*, 2004). Investigations on colibacillosis in broiler are very scanty in Bangladesh. In Dinajpur there is no research about colibacillosis in broiler or layer.

Considering the above facts, the present study was designed with the following objectives:

- To study the prevalence of colibacillosis in different commercial broiler farm at Dinajpur sadar upazila
- To study the clinical signs of the colibacillosis affected flock
- To study the gross pathological lesions (different forms) of colibacillosis
- To study the microscopic lesions of colibacillosis affected dead bird.

CHAPTER 2

REVIEW OF LITERATURE

The main purpose of this chapter is to get up-to-date information regarding the research works addressed here. Literature related to the present study was presented below.

2.1 Etiology

Barmes *et al.* (2008) conducted study about Colibacillosis Diseases of Poultry that Escherichia genus consists of gram negative, non-acid-fast, uniform staining, non-spore forming bacillus, usually of 2-3 × 0.6 µm in size belonging to the family Enterobacteriacea. In most serologic typing schemes only the O and H antigens are determined.

La Ragione *et al.* (2002) conducted study about Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia that *E. coli* is considered as a member of normal microflora of the poultry intestine, but certain strains such as avian pathogenic *E. coli* (APEC), spread to various internal organs and cause colibacillosis characterised by systemic fatal disease.

Stenutz *et al.* (2006) conducted study about the structures of *Escherichia coli* O-polysaccharide antigens that Serotypes of *E. coli* are classified according to the Kauffmann scheme.

Kabir *et al.* (2010) conducted study about Avian colibacillosis and Salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns that, In domestic poultry, avian colibacillosis is frequently associated with *E. coli* strains of serotypes O78:K80, O1: K1 and O2: K1.

Dziva and Stevens (2008) examined Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian colibacillosis is caused by a group of pathogens designated avian pathogenic *Escherichia coli* (APEC). Despite being known for over a century, avian colibacillosis remains one of the major endemic diseases afflicting the poultry industry worldwide. Autologous bacterins provide limited serotype-specific protection, yet multiple serogroups are associated with disease, especially O1, O2 and O78 among many others.

Dhama *et al.* (2013) studied about *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance. Avian colibacillosis, caused by *Escherichia coli*, is one of the major bacterial diseases in the poultry industry worldwide, and along with salmonellosis, it is the most common avian disease communicable to humans. The organism is a normal inhabitant of the intestinal tract of birds and can survive in a wide range of temperature. Certain strains *viz.*, avian pathogenic *E. coli* (APEC), however, could spread to various internal organs and cause colibacillosis characterized by fatal systemic disease. Faeco-oral route is the main mode of infection, though vertical transmission is also possible.

2.2 Biological Characteristics

Dou *et al.* (2016) studied about Characterization of avian pathogenic Escherichia coli isolated in eastern China. In order to investigate the biological characteristics of avian pathogenic *Escherichia coli* (APEC) isolated in eastern China, a total of 243 isolates were isolated from diseased poultry on different farms during the period from 2007 to 2014. These isolates were characterized for serogroups (polymerase chain reaction and agglutination), the presence of virulence-associated genes (*fimC*, *iss*, *ompA*, *fyuA*, *stx2f*, *iroC*, *iucD*, *hlyE*, *tsh*, *cvaC*, *irp2*, and *papC*) and class I integrons (polymerase chain reaction), drug susceptibilities (disk diffusion method) and the biofilm-forming abilities (semi-quantitative method). The results showed that the most predominant serogroups were O78 (87 isolates, 35.8%) and O2 (35 isolates, 14.4%). Gene profiling found that *fimC* and *ompA* were frequently distributed among the isolates and that 77.4% of the isolates were positive for class 1 integrons. Overall, isolates displayed resistance to tetracycline (97.5%), nalidixic acid (82.3%), ampicillin (81.1%), sulphafurazole (80.7%), streptomycin (79.0%), trimethoprim (78.2%) and cotrimoxazole (78.2%). Multiple-drug resistance was exhibited in 80.3% of the isolates, and the presence of class 1 integrons is associated with multidrug resistance. Finally, 151 isolates had the ability to form biofilms in vitro, and drug resistance seemed relative to biofilm-forming abilities.

2.3 History

Barnes *et al.* (2008) studied about the colibacillosis disease of poultry.Deaths in birds caused by a bacteria consistent with *E. coli* was first reported in 1894. Experiential inoculations of different animals showed the bacteria's diverse number of hosts between 1894 and 1922. Colisepticemia was first described in 1907 as a virulent *E. coli* infection

that was killing chickens. In 1923, *E. coli* was isolated from birds with infectious enteritis and paralysis, which lead to more descriptions of colibacillosis. Between 1938 and 1965, *E. coli* was discovered as the cause of coligranuloma, plantar abscesses, omphalitis, and peritonitis, among other infections in chickens. The bacteria was also seen to possess resistance to vaccinations around this time. These discoveries provided the basis of colibacillosis knowledge, but there is still much to learn about the disease.

2.4 Epidemiology

Barnes *et al.* (1997) studied about colibacillosis disease of poultry that *E. coli* is a gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus that grows both aerobically and anaerobically and may be variable in size and shape. Many strains are motile and have peritrichous flagella. *E. coli* is considered as a member of the normal microflora of the poultry intestine, but certain strains, such as those designated as avian pathogenic *E. coli* (APEC), spread into various internal organs and cause colibacillosis characterized by systemic fatal disease. Rodents may be carriers of APEC and hence a source of contamination for the birds. Every damage to the respiratory system favours infection with APEC. Several pathogens, like NDV, IBV and MG, both wild type and vaccine strains, may play a part in this process. An unfavourable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract.

Rahman *et al.* (2004) examined bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in nature and experimental infections in chikens that In domestic poultry, avian colibacillosis is frequently associated with *E. coli* strains of serotypes O78:K80, O1:K1 and O2:K1 (2- Filali E). The avian colibacillosis was found widely prevalent in all age group of chickens (9.52 to 36.73%) with specially high prevalence rate in adult layer birds (36.73%).

Harry *et al.* (1965) studied about the relationship between environmental contamination with septicemia strains of *E. coli* that The most important reservoir of *E. coli* is the intestinal tract of animals, including poultry. In chickens, there are about 109 colony forming units (CFU) of bacteria per gram of feces and of these, 106 CFU are *E. coli*. *E. coli* has also been commonly isolated from the upper respiratory tract. In addition, it is present on the bird's skin and feathers. These strains always belong to both pathogenic and non-pathogenic types.

Harry *et al.* (1965) studied about the association between the presence of septicemia strain of *Escherichia coli* in the respiratory and intestinal tracts of chickens and the occurrence of coli septicemia. In the caecal flora of healthy chickens, 10 to 15% of the *E. coli* strains may belong to an O-serotype that can also be isolated from colibacillosis lesions.

Dho-moulin *et al.* (1999) studied about avian pathogenic *Escherichia coli* (APEC).the first hours after hatching, the birds start building up their *E. coli* flora. The bacteria drastically increase their numbers in the gut. In a single bird a large number of different *E. coli* types is present, obtained via horizontal contamination from the environment, more specifically from other birds, faeces, water and feed.

2.5 Prevalence

Ahmed *et al.* (2009) conducted a study to determine the occurrence of infectious diseases in broiler chickens at kapasia in Gazipur district during the period from 16th October to 16th December 2008. Detection was made on the basis of history, clinical findings and post-mortem lesions. A total of 199 broiler chickens were examined during the study where colibacillosis 104 (52.26%), mycoplasmosis 25 (12.56%), salmonellosis 02 (1.01%), omphalitis 23 (11.56%), coccidiosis 09 (4.52%), gumboro 22 (11.06%), mycotoxicosis 11 (5.53%) and mixed infection of gumboro and coccidiosis 03 (1.51%) were recorded. In the conclusion it has been remarked that collibacillosis is a major problem for broiler production and hence poultry farmers can not earn their profit perfectly due to adverse effect of those diseases.

Omer *et al.* (2010) studied on the epidemiological and economical effects of the disease to assist in disease control policies and planning research priorities in the region. The outbreak of colibacillosis was reported among broiler and layer chicks reared in closed and semi closed system In Kassala State, Eastern Sudan. Mortality rate of the disease was 6.8% in the broiler flocks and 1.9% in the layers ones. Diagnosis of the disease was made on the basis of the case history, clinical signs, postmortem findings and laboratory examinations. *Escherichia coli* (*E. coli*) isolates were obtained from infected organs of broiler and layer flocks. Isolation and identification of *E. coli* were achieved by using biochemical diagnostic test kits. The isolates were highly resistant to most tested antibiotics. The cost of losses in broilers and layer chicks due to the outbreak was

recorded. Factors which associated with the disease were discussed and some recommendations were outlined to avoid such outbreak.

Soon *et al.* (2008) examined 216 *E. coli* isolated from chickens and environmental specimens from hatcheries between 200S and 2006 in order to evaluate the epidemiological prevalence of avian pathogenic *E. coli* (APEC) in Korea tentatively by multiplex PCR. The multiplex PCR which was used as tentative criteria of APEC targets 8 virulence-associated genes; enteroaggregative toxin (astA), increased serum survival protein (iss), iron-repressible protein (irp2), P fimbriae (papC), aerobactin (iucD), temperature-sensitive hemagglutinin (tsh), vacuolating auto transporter toxin (vat), and colicin V plasmid operon (cva/cvi) genes. The number of detected genes could be used as a reliable index of their virulence. It was demonstrated that *E. coli* strains already typed as APEC always harbor 5 to 8 genes, but non-APEC strains harbor less than 4 genes. Assuming the criteria of APEC is a possession of more than 5 virulence-associated genes, we discriminated 24 APEC strains among the 216 *E. coli* strains. Contamination rates of APEC in the field were 31.3% in layers, 14.0% in broilers, 2.7% in broiler breeders, and 0.0% in environmental specimens from hatcheries. The combinational tendency of APEC examined is a fundamental possession of astA, iss and iucD genes and addition of cva/cvi, tsh, vat, and irp2 genes which have a critical importance for virulent traits of APEC. Compared with intravenous chicken challenge or embryo lethality assay, multiplex PCR method could be useful to discriminate APEC rapidly for convenient diagnosis.

Rahman *et al.* (2004) recorded 8.40% proportionate prevalence rate of colibacillosis in chickens, of which 67.35% recorded as single, 29.93% as two types and 2.72% as triple types of mixed infection with other diseases and also recorded widely prevalent of *E. coli* infection in all age groups of chickens (9.52 to 36.73%) with specially high prevalence rate in adult layer birds (36.73%). As the *E. coli* organism has been attributed to vertical transmission and to egg shell contamination followed by penetration, and high rate of *E. coli* infection in layer chickens again need keen attention towards its control to save the poultry industry in Bangladesh. Colibacillosis was recorded more or less uniformly in all the three seasons of the year with significantly higher rate during summer (40.82%) seasons.

Hossain *et al.* (2008) a study was conducted to isolate and identify *E. coli* from apparently healthy broilers and layers from different poultry farms adjacent to the Bangladesh Agricultural University, Mymensingh, Bangladesh, during the period of January to May 2006 and characterize their ability to produce enterotoxin and also the antibiogram of the isolates. A total of 110 fecal samples were collected from broiler (n=55) and layer (n=55) chickens. *E. coli* were isolated and identified by cultural, biochemical, motility test and the heat-stable toxins were determined by Infant Mouse Assay (IMA). In case of broilers, 35 (63.6%) samples were found positive while 31 (56.4%) from layers. The overall prevalence of E coli was 60%. Among the isolates of *E. coli*, 22.86% isolates from broiler and 38.71% isolates from layer were found positive for their ability to produce enterotoxin based on mice inoculation test. The antibiotic sensitivity pattern showed that the isolates were highly sensitive to chloramphenicol, ciprofloxacin, kenamycin and cephalexin and an increasing trend of resistance was recorded in both broiler and layer isolates. It may be concluded from the results of this study that the high resistance of *E. coli* to antibiotics constitutes a threat to poultry industry in Bangladesh.

Jeffrey *et al.* (2004) conducted a matched sampling of *E. coli* from broiler house litter and bird lesions of either cellulitis or colibacillosis was conducted to investigate the relationship of pathogenic *E. coli* to those found in the environment. Isolates were collected from six broiler flocks representing six geographically disparate ranches. Isolates were compared by flock for similarity in serotype and genotyped by pulsed- field gel electrophoresis. Serotyping revealed a considerable dissociation between the two groups of isolates. The prevalence of pathogenic *E. coli* that matched the environmental isolates from the same house was 0 to 3%. Statistical analysis of the serotype data showed a strong dependence of serotype on isolate source, indicating a high probability that a particular serotype would be found among lesions or litter but not in both groups. Genotyping of isolates on two farms supported the results of serotyping and provided differentiation of isolates that could not by typed by serology. These results suggested that the prevalence of pathogenic *E. coli* in the broiler house was independent of the prevalence of other commensal or environmental *E. coli*. Understanding the composition of *E. coli* populations in commercial poultry production may have bearing on the epidemiology and control of *E. coli* related diseases.

Shah *et al.* (2003) carried out to record the prevalence and pathology in different age groups of broiler. The clinical symptoms observed were dullness and depression with elevated body temperature, loss of appetite and diarrhea. Gross lesion indicated cloted blood beneath the heart, caseous exudates in the air sacs, fibrinous pericarditis and perihepatitis. The rate of infection varied with age as 3.11, 6.74, 20.18, 29.63, 18.16 and 22.18% in group B, C, D, E, F and G respectively. No lesion was observed in group A.

Islam *et al.* (2003) studied a pathological investigation on the occurrence of poultry diseases in Sylhet region of Bangladesh was conducted during the period from November 2001 to October 2002. A total of 1352 sample of either dead or sick birds was brought from different Upazillas of Sylhet region. Diagnosis of different disease conditions was made on the basis of the history, age of birds, clinical signs, gross and microscopic lesions. The diagnosed diseases included infectious bursal disease (IBD) (24.26%), Newcastle disease (ND) (6.73%), infectious bronchitis (0.29%), omphalitis (2.81%), fowl cholera (0.44%),salmonellosis (6.73%), colibacillosis (5.17%), necrotic enteritis (0.44%), aspergillosis (17.53%), infectious coryza (0.37%), chronic respiratory disease (CRD), /mycoplasmosis (5.32%), coccidiosis (9.46%) and deficiency disorders/stress condition (1.03%). In general, the highest number of cases were recorded in the age group of 8-21 days (42.60%), followed by 22-35 days age group (26.62%), 0-7 days age group (26.10%), 36-60 days age group (1.03%) and over 60 days age group (3.62%) of Poultry. Distribution and proportionate incidence of poultry disease of Bangladesh reveals that the poultry diseases occur mostly in rainy season (56.36%), followed by summer (28.11%) and the least in winter season (15.53%).

Talha *et al.* (2001) reported 5.51% proportionate prevalence rate of colibacillosis in chickens from Bangladesh and also reported higher proportionate prevalence rate of colibacillosis in growing chickens in comparison to adults whereas.

Giasuddin *et al.* (2002) postmortem and serological examination of poultry diseases were conducted in different farms of Bangladesh. A total of numbers of 1653 either dead or sick were examined. The incidence of IBD (11.80%), chronic respiratory disease (6.11%), Newcastle disease (7.60%), salmonellosis (5.56%) and colibacillosis (4.42%) were found.

Bhattachaijec *et al.* (1998) conducted a retrospective analysis of chicken diseases diagnosed at CDIL, Dhaka, among the bacterial diseases the incidence of avian colibacillosis was the highest in broiler chicks.

Bhattachaijee *et al.* (19%) also reported widely prevalent of colibacillosis in both the brooding (12.82%) and pre-peak-post production layer chickens (5.49 to 8.78%). It was found in all the seasons of the year in Bangladesh.

2.6 Pathogenesis

Barmes *et al.* (2008) conducted study about Colibacillosis Diseases of Poultry that Pathogenic strains of *E. coli* such as APEC and UPEC have the ability to cause disease and infections. These strains have acquired virulence factors through horizontal gene transfer and some are also opportunists that infect already immunocompromised mammals. The virulent factors they have acquired are genes that are iron-related, toxin-related, and adhesion-related. Iron-related genes encode for iron acquisition mechanisms which are significantly less common in commensal *E. coli*. The reason for this is not entirely understood, but it is believed that iron acquisition is an important part of pathogenesis in *E. coli*. Toxin-related genes include the gene stx1 which encodes for shiga toxins that inhibit protein synthesis and the gene cdtB which encodes for a toxin that blocks mitosis. Adhesion-related genes allow the pathogenic *E. coli* to attach itself to the host. These genes include the pap pilus and s fimbrial operons which both encode for pilus tip adhesion.

Matthijs (2008) reported that colibacillosis is caused by *E. coli* bacteria and occurs predominantly in broilers in the second half of the growing period. The disease is of economic importance worldwide due to growth retardation, increased feed conversion, mortality and high condemnation rate at slaughter. Moreover, colibacillosis results in impaired welfare. Infections of the respiratory tract, amongst others infection with infectious bronchitis virus (IBV), increase the susceptibility for colibacillosis of broilers significantly. From recently made field observations it was hypothesised that not only virulent IBV but also live IBV vaccine strains, which are commonly used in the field to prevent birds from clinical signs of infectious bronchitis (IB), are able to increase colibacillosis susceptibility in broilers substantially. This hypothesis was tested experimentally. The experiments showed that IBV vaccines HI20 and H52 increased colibacillosis susceptibility in four-weeks- old broilers to the same level as virulent IBV

strains (M41 and D387) did. It was also demonstrated that the IBV HI20 vaccine spread extensively between broilers. From the latter data it seems obvious that IBV H120 vaccine also might spread between flocks. In case not IB vaccinated flocks are infected with IB vaccine virus by that route, especially in the second half of the growing period, serious colibacillosis might occur. The significance of IBV vaccine strains in the occurrence of colibacillosis in broilers in the field has to be elucidated. The underlying mechanism of E coli super infections (*E. coli* infection after triggering by a previous viral infection) in IBV infected broilers was studied based on morphological and functional immunology. The results strongly suggest that preceding infection with vaccine or virulent IBV does not seem to impair the clearance of the E coli in the respiratory tract of broilers, but rather induces an exaggerated inflammatory response. It also seems that infection with virulent or vaccine strains of IBV altered the innate systemically immunity rather than the phagocytes cell function.

Petersen *et al.* (2006) investigated the epidemiology of an enrofloxacin resistant *Escherichia coli* clone during two separate outbreaks of colibacillosis in the Danish broiler production. In total five flocks were reported affected by the outbreaks. Recorded first week mortalities were in the range of 1.75%-12.7%. The clone was first isolated from dead broilers and subsequently demonstrated in samples from associated hatcheries and the parent flock with its embryonated eggs, suggesting a vertical transmission from the parents.

Landman and Comelissen (2006) isolated E coli bacteria isolated from localized and systemic disease processes in poultry and designated as Avian Pathogenic E coli (APEC). The disease inducing potential of these isolates has been explained by the occurrence of specific virulence factors. Despite the extensive literature on virulence factors for *E. coli*, unambiguous markers of virulence have not been identified yet The relationship between serotyping and virulence was not straightforward either and raises the question whether *E. coli* infections in poultry should mainly be considered as opportunistic. Investigations into the occurrence of certain (combinations of) virulence factors in APEC isolates as virulence markers should fulfill the molecular version of Koch's postulates if the former question is to be answered.

Loock *et al.* (2006) observed that *E. coli* infections were highly prevalent in Belgian turkeys and therefore they might contribute to the respiratory disease complex in turkeys.

They examined in their study the pathogenicity of an *E. coli* superinfection on *C. psittaci* predisposed turkeys. Turkeys were infected with *C. psittaci*, *E. coli* or with C. psittaci followed by *E. coli*. Simulating the impact of an *E. coli* infection during the acute phase or the latent phase of a *C. psittaci* infection, turkeys received E coli at 1 or 5 weeks post *C. psittaci* infection respectively. *E. coli* super infection during the acute phase of *C. psittaci* infection increased *C. psittaci* excretion and stimulated chlamydial replication in the respiratory tract resulting in exacerbated clinical disease. Interestingly, *E. coli* superinfection during the latent phase of *C. psittaci* infection induced chlamydial replication, leading to increased *C. psittaci*- specific antibody titles. In addition, chlamydial predisposition gave higher E coli excretion compared with turkeys that had only been infected with E coli.

Yoder *et al.* (1989) studied the relative pathogenicity of *E. coli* isolates from poultry by aerosol exposure of young chickens. Evidence of colisepticemia with airsacculitis and/or pericarditis and perihepatitis was evaluated. A system was devised that included the intratracheal (IT) inoculation of strain SE-17 infectious bronchitis virus (IBV) of chicks at 7 days of age followed by their aerosol exposure to *E. coli* culture suspensions 2 days later.. The *E. coli* isolate was consistently more pathogenic than the Congo Red-negative version of that isolate. Cultures of *E. coli* previously demonstrated to be pathogenic among the most pathogenic isolates evaluated in these experiments and were similar to the Congo Red-positive isolate.

Goims *et al.* (2001) stale that broilers may be affected by necrotic dermatitis, also known as cellulitis, characterized by a chronic inflammation of the subcutis on abdomen and thighs. The affected animals usually do not show any clinical signs, and the lesions are sometimes only detected at the slaughter bird but symptoms are probably only seen if a minimum infection pressure of APEC and possibly also other predisposing factors are present in the house.

2.7 Clinical Signs

Omer *et al.* (2010) studied about Outbreak of Colibacillosis among Broiler and Layer Flocks in Intensive and Semi intensive Poultry Farms in Kassala State, Eastern Sudan. The clinical signs of the affected birds were depression, respiratory distress, reduced food consumption, loss of weigh, weakness, ruffling of feathers, decrease in egg

production and yellowish diarrhea or sometimes vents pasted with faeces. Conjunctivitis was also observed as sporadic cases of layer chicks.

Awawdeh (2017) Studied on avian pathogenic *Escherichia coli* in commercial broiler Chicken in South East Queensland.Avian pathogenic *Escherichia coli* (APEC) is the causative agent of avian colibacillosis, a localised or systemic infection resulting in clinical diseases such as colisepticemia, chronic respiratory disease and swollen-head syndrome.

2.8 Transmission

WJ Landman *et al.* (2006) examined *Escherichia coli* salpingitis and peritonitis in layer chickens: an overview. Three routes of infection have been discussed in the literature: ascending faecal contamination from the cloaca, bacterial translocation from the respiratory tract (air sac and lungs) and bacterial translocation from the intestinal lumen. Only one study has reported the occurrence of ascending faecal contamination from the cloaca to the oviduct and subsequently to the peritoneum.

2.9 Pathology

2.9.1 Gross lesions

Dhama *et al.* (2013) studied about *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance. The disease occurs in various forms in poultry: colisepticemia and acute septicemia, air sac disease, pericarditis, perihepatitis, Mushy chick disease (yolk sac infection), peritonitis, panopthalmitis, synovitis, salpingitis, bumble foot, cellulitis, swollen head syndrome, infectious asthenia, and Hjarre's disease. Increased cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) due to *E. coli* infection affect the absorption of sodium as well as chloride and water balance ultimately producing watery diarrhea and death. APEC isolates also are of potential concern for public health professionals. Infected persons usually manifest diarrhea which may be complicated by other syndromes depending on the serotype.

Khaton *et al.* (2008) reported that the *E. coli* affected birds had cloudy thickened air sacs, pericarditis, and congestion in the liver, lung and spleen. On histopathological examination focal necrosis in liver and infiltration of heterophils, lymphocytes and

macrophages in liver and lung was found. Thickening of pericardium was found due to infiltration of reticulo endothelial (RE) cells. In duodenum, severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages was found in the sub mucosa.

Chowdhury *et al.* (2009) state that a cross sectional study was carried out to identify and observe pathological lesions of different forms of colibacillosis in commercial broiler and layer birds in Chittagong region. Colibacillosis in commercial broiler and layer farms causes huge economic loss through a relatively high mortality and loss of production. In this present study, a total of 4372 broiler and layer birds were examined through post-mortem to identify different forms of colibacillosis and among them 1893 (70.87%) broiler and 778 (29.13%) layer birds were diagnosed as affected with any form of colibacillosis. Broiler birds were found 1.17 (Odds ratio) times higher at risk than layer birds and it was evident from the Chi Square test that there is a significant ($p>0.01$) relationship between type of birds (broiler and layer) and occurrence of colibacillosis. The most frequent forms of colibacillosis were omphalitis, air sacculitis, pericarditis, perihepatitis, peritonitis, egg peritonitis and a large number (30.48%) of bird combination of different form of colibacillosis.

Suha *et al.* (2008) studied a total of 140 diseased or dead broiler chicks obtained from three different sources in Sulaimani city were necropsied; yolk sac samples for these chicks were cultured and the isolated bacteria were tested for their susceptibility to 8 systemic antimicrobial agents. Unabsorbed yolk sac was observed in 112 chicks associated with signs of septicemia in many cases. These unabsorbed yolk sac exhibit signs of moderate to mild inflammation indicating that the cause of death in YSI is the consequences of the infection rather than it's direct effects. One hundred and ninety one bacterial isolates were isolated and further identified using biochemical tests. E. coli was the most common bacteria followed by Enterobacter aerogenes, Staphylococcus aureus and Klebsiella pneumoniae. Other bacteria were also identified but in a lower proportion.

Ashraf *et al.* (2002) A study was conducted to observe the effect of intrayolk injection of gentamicin on experimentally induced omphalitis in broiler chicks. *E. coli* were injected into yolk sac to induce omphalitis and treatment was done by intrayolk injection of gentamicin. Results showed that mortality decreased and feed intake increased after

treatment. Intrayolk injection of antibiotics is suggested as an alternative to oral administration for the treatment of omphalitis in chicks.

Barnes (1997) observed that pathogenic serogroups of *E. coli* are common in the environments in which poultry are raised and may cause air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, cellulitis or yolk sac infection. Collectively, these diseases constitute a major economic loss. Colibacillosis refers to any localized or systemic infection caused entirely or partly by *E. coli*, including septicaemia, granuloma (Hjarre's disease), air sac disease, chronic respiratory disease, (CRD), avian cellulitis, swollen head syndrome, peritonitis, salpingitis, osteomyelitis/synovitis, panophthalmitis, and omphalitis/yolk sac infection.

Ganapathy *et al.* (2000) studied a flock of 3-week-old broiler chickens fed with antibiotic-free commercial feed developed cyanotic combs, depression, extended abdomens, reddened abdominal skin, faeces-stained vents, and diarrhoea. By the end of 6 week, mortality reached 19.3%, and important lesions seen were ascites, airsacculitis, swollen/congested kidneys, fibrinous perihepatitis, fibrinous pericarditis with or without hydro pericardium, haemorrhagic enteritis- typhilitis and ballooned caeca. Salmonella typhmurium var Copenhagen and *E. coli* were isolated from livers, hearts, intestines and caeca. *Histomonas* spp. were detected only in caeca. Thus, concurrent occurrence of salmonellosis, colibacillosis and histomoniasis was diagnosed.

Pourbakshsh *et al.* (1997) conducted study on gross pathology on visceral organs of broiler chicks infected with *E. coli* and observed airsaculitis, perihepatitis, pericarditis and splenic hypertrophy.

Barnes and Gross (1997) state that APEC causes yolk sac infections and embryo mortality. The chick can also be infected during or shortly after hatching. In these cases, retained infected yolk, omphalitis, septicaemia and mortality of the young chicks up to an age of three weeks is seen.

Gross (1994) carried out that coli form infection of the peritoneal cavity occurs in laying hens and is characterized by acute mortality, fibrin, and free yolk. Infection occurs when bacteria ascending through the oviduct grow rapidly in yolk material that has been deposited in the peritoneal cavity.

Bisgaard (1995) state that *E. coli* infects the left abdominal air sac, females may develop chronic salpingitis characterized by a large caseous mass in a dilated, thin/walled oviduct. The caseous mass contains necrotic heterophils and bacteria that persist for months. Size of the caseous mass may increase with time. Affected birds frequently die during the first 6 months. Post infection; those surviving rarely lay eggs. Salpingitis may also occur following entry of coli form bacteria from the cloaca in laying hens, ducks and geese.

2.9.2 Microscopic lesions

Nakamura *et al.* (2007) forty-eight of 134 chickens collected from a flock on a broiler farm were diagnosed pathologically and microbiologically to have colibacillosis. Both acute septicemia (seven birds, 1 to 36 days old) and subacute serositis (41 birds, 5 to 57 days old) were found. The former consisted of necrosis with fibrinous exudates in the ellipsoids and lymphoid follicles of the spleen, and fibrinous thrombi in sinusoids of the liver with occasional necrosis of hepatic cells. The latter had fibrinopurulent inflammation with granulomatous changes in the serosal tissues including the epicardium, pericardium, and hepatic peritoneal sac-accompanied by septicemic lesions in the spleen and liver. Respiratory lesions (airsacculitis, pneumonia, and tracheitis) were noted in most chickens affected with acute septicemia and subacute serositis. Degenerative changes also were observed in the bursa of fabricius.

Khaton *et al.* (2008) studied the prevalence of colibacillosis in layer chichens. On histopathological examination showed that focal necrosis in liver and infiltration of heterophils, lymphocytes and macrophages in liver and lung was found. Thickening of pericardium was found due to infiltration of reticulo endothelial (RE) cells. In duodenum, severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages was found in the sub-mucosa.

Piercya and Westa (2004) state that the pathogenicity in broiler chickens of three field isolates of *E. coli* was investigated, and the pathogenesis of the disease induced with log-phase cultures of one of these strains is described in detail. Mortality and/or gross pathological changes were only consistently induced after air sac or intraperitoneal injection. The pathogenesis of the experimental disease induced by air sac injection of diluted cultures of the strain was studied in two similar experiments. Bacteraemia was detected 3 hours, after injection, and was still present in 75 per cent, of birds at 96 hours.

The organism was recovered most frequently from the inoculated air sac, and then in descending order of frequency from pericardial fluid, liver, heart-blood, opposite air sac. Gross pathological changes were seen in the inoculated air sac from 6 hours, and histopathological changes from 3 hours, after injection. These changes progressed and spread across the thoracic viscera and into the peritoneal cavity by 72 hours. There appeared to be little correlation between the occurrence of bacteraemia and the observed progression of the disease and it is suggested that direct mechanical spread across areas of contact between air sac, pericardium, and liver capsule may be important.

2.10 Risk Factors

Foley *et al.* (2000) state those three to four weeks after introduction of the flock in the house, the maternal antibody titer becomes minimal and the bacterial infection pressure in the environment maximal. Viral diseases mostly occur around the age of three weeks. The biggest losses in broilers therefore are suffered in the age category of five to twelve weeks.

McGruder and Moore (1998) reported that the risk for colibacillosis increases with increasing infection pressure in the environment. Other principal risk factors are the duration of exposure, virulence of the strain, breed, and immune status of the bird.

Barnes and Gross (1997a) showed that stress promotes infection and induced bacteraemia after inoculation of APEC by fasting the animals for 36 - 48 hours or exposing them to high temperatures and damage to the respiratory system favors infection with APEC. Several pathogens, like NDV, IBV and MG, both wild type and vaccine strains, may play a part in this process.

Barnes and Gross (1997) also showed that an unfavorable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through debilitation of the upper respiratory tract.

2.11 Diagnosis

Omer *et al.* (2010) studied on the epidemiological and economical effects of the disease to assist in disease control policies and planning research priorities in the region. Diagnosis of the disease was made on the basis of the case history, clinical signs, postmortem findings and laboratory examinations. Escherichia coli (E. coli) isolates

were obtained from infected organs of broiler and layer flocks. Isolation and identification of E. coli were achieved by using biochemical diagnostic test kits. The isolates were highly resistant to most tested antibiotics. The cost of losses in broilers and layer chicks due to the outbreak was recorded. Factors which associated with the disease were discussed and some recommendations were outlined to avoid such outbreak.

Dhama *et al.* (2013) studied about *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance. Diagnosis is based on isolation and growth characteristics of the organism in wide variety of bacteriological media, biochemical tests, serological assays, enzyme linked immunosorbent assay (ELISA), molecular tools of polymerase chain reaction (PCR) and its various versions, phylogenetic analysis and other techniques. The disease must be differentiated from a wide variety of other bacterial diseases.

2.12 Prevention

Dhama *et al.* (2013) studied about *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance. Antibiotic sensitivity test is useful to select proper antibiotic but plasmid mediated resistance do occur for which vitamin as well as probiotic and bacteriophage therapy are gaining much attention nowadays. Live and inactivated mutant vaccines are available. Proper hygiene and sanitation along with good hatchery management are the prerequisites to prevent the occurrence of disease. Different disease manifestations caused by APEC, insights into this economically important avian pathogen, epidemiology, trends and advances in diagnosis, prevention and control, novel and emerging therapeutic regimens, and the associated public health concerns envisage the topic of discussion in the present review.

Huff *et al.* (2013) studied about Method of administration affects the ability of bacteriophage to prevent colibacillosis in 1-day-old broiler chickens. Bacteriophages are viruses that kill bacteria. They are plentiful in nature with no known activity in human or animal cells, making them an attractive alternative to antibiotics. The objective of this research was to determine if a coarse or a fine spray of bacteriophage would prevent colibacillosis induced by an intratracheal (IT) challenge with *Escherichia coli*. Two studies were conducted with 6 treatments: untreated control, birds treated with a spray administration of bacteriophage and not challenged, birds administered bacteriophage IT

and not challenged, birds not treated and challenged IT with *E. coli*, birds sprayed with bacteriophage and IT challenged with *E. coli*, and birds administered bacteriophage IT and challenged IT with *E. coli*. There were 3 replicate pens of 10 birds per pen, per treatment, and all treatments were administered at 1 d of age. Study 1 was concluded when the birds were 19 d of age, and study 2 was concluded when the birds were 21 d of age. In both studies, neither a coarse nor a fine spray protected the birds from an IT *E. coli* challenge; however, when bacteriophage was administered IT there was complete protection. This research demonstrates the necessity for the administration of bacteriophage therapeutics to deliver high bacteriophage titers to the site of a bacterial infection.

El-Ghany and Madian (2011) studied about control of experimental colisepticaemia in broiler chickens using sarafloxacin. Conducted to detect the effect of using sarafloxacin (5 mg/kg body weight) in the drinking water of broiler chickens to control experimental colisepticaemia in broiler chickens. One hundred and seventy, day old broiler chicks were used in the study. Twenty chicks at the day of arrival were sacrificed and cultured to ensure absence of *E. coli* infection. One hundred and fifty chicks were divided into three equal groups, each consists of 50 birds. Group (1) was challenged with *E. coli* and not treated with sarafloxacin (control positive), group (2) was challenged with *E. coli* and treated with sarafloxacin, while group (3) was neither challenged with *E. coli* nor sarafloxacin treated (blank control). Challenge was done intramuscularly (I/M) at 2 weeks of age in groups (1 and 2) as each bird received 0.5 ml of the nutrient broth culture containing 108 colony forming unit (CFU) *E. coli* O78 / ml. One appearance of signs, sarafloxacin was added to the drinking water for 3 successive days. All the birds were kept under complete observation for 6 weeks for estimating the bird's performance (body weight and feed conversion rate) and recording signs, mortalities, gross lesions, re-isolation of the organism and microscopical examination of the organs. The obtained results indicated significant ($P<0.05$) improvement in chickens performance in chickens challenged with *E. coli* and treated with sarafloxacin than those challenged and not treated. On the other hand, significant ($P<0.05$) decrease in morbidity and mortality rates, gross organs lesion score and re-isolation of *E. coli* O78 from the internal organs of chickens treated with sarafloxacin when compared with *E. coli* challenged non treated birds. Also, improvement of the microcscopical lesion scores was also detected in sarafloxacin treated group. It could be concluded from the above results that sarafloxacin

used in a dose of 5 mg/kg body weight in the drinking water for 3 consecutive days is very effective in controlling of colisepticaemia in broiler chickens.

2.13 Vaccination

Rawiwet and Chansiripornchai (2009) studied about the efficacy of *Escherichia coli* aroA-live vaccine in broilers against avian E. coli serotype O78 infection. The efficacy of an *Escherichia coli* (*E. coli*) aroA-live vaccine in the prevention of colibacillosis in chickens following intratracheal challenge with a virulent strain of E. coli O78 was investigated. Thirty-six, one day old broiler chickens were divided into 3 groups of 12 each. Chickens in each group were randomly divided into 2 replicates. The chickens in group 1 that were not vaccinated and challenged served as a negative control. The chickens in group 2 that were not vaccinated but received E. coli serotype O78 served as a positive control. The chickens in group 3 were vaccinated by the oral route at 5 days of age with E. coli aroA-vaccine and challenged with E. coli serotype O78. All the chickens in groups 2 and 3 were challenged intratracheally at 4 weeks of age with 0.5 ml (1.2×109 cfu/ml) per dose of E. coli O78. The chickens were monitored for 7 days after infection for feed conversion ratio (FCR), and the post-mortem pathology was assessed. The results revealed that the vaccine tends to prevent E. coli infection. The chickens in group 3 tended to show lower pathological findings including airsacculitis, pericarditis, perihepatitis, peritonitis and arthritis than the chickens in group 2 but the FCR was not different in each group ($p>0.05$).

CHAPTER 3

MATERIALS AND METHODS

The present study was conducted in different farm of Dinajpur sadar upazila. Histopathological study was conducted in the pathology laboratory, Department of Pathology and Parasitology, Faculty of Veterinary and animal Science, Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh for a period of 6 month from July to December, 2018. The details outline of materials and methods are given below:

3.1 Study area: Study was undertaken in six different farm in Dinajpur.

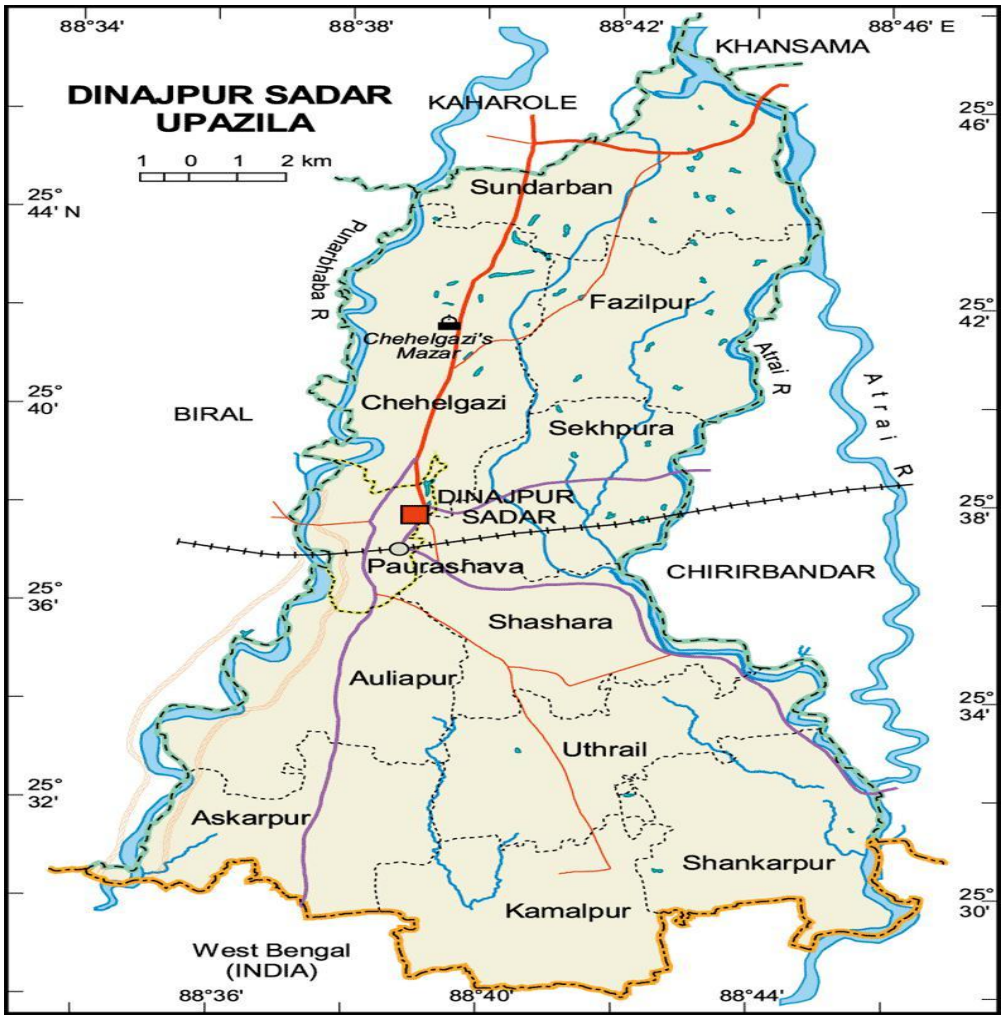

Fig. 3.1: Map of Dinajpur sadar upazila

3.2 Study period

Study was conducted from July to December 2018 in different broiler farm of Dinajpur sadar upazila.

3.3 Experimental birds

A total of 8800 birds from different broiler farms of Dinajpur sadar upazila were observed and some sick, dead birds were collected on the basis of clinical signs for post mortem examination.

3.4 Case definition

Cases were defined on the basis of clinical signs and postmortem findings and different forms of colibacillosis were identified according to a predefined case definition. Case definitions of different forms of colibacillosis were as follows:

- Acute septicemia: Green liver and congested pectoral muscles. Sometimes, small white foci were found in liver. There is a tendency toward pericarditis and peritonitis.
- Omphalitis or Yolk sac infection: Unabsorbed yolk sac with viscid, yellow-green to watery, yellow-brown or caseous material.
- Pericarditis: Cloudy pericardial sac and edematous epicardium and covered with a light-colored exudate. Light yellow and fibrinous exudates in the pericardial sac.
- Peritonitis: In laying hen, fibrin and free yolk infection in the peritoneal cavity.
- Perihepatitis: A yellowish white covering on liver
- Air saculitis: Airsac contain a caseous exudates and the airsac membranes become thicker and cloudy in appearance.

3.5 Experimental Layout

Six broiler farm of Dinajpur Sadar upazila were visited and observed the clinical signs of colibacillosis affected flock. The farms were divided into three groups on the basis of age respectively

- 0-2 weeks
- 2-4 weeks and
- >4 weeks.

Some sick and dead birds were collected from different broiler farm of Dinajpur Sadar Upazilla on the basis clinical signs according to bird age for necropsy. After necropsy suspected sample (lung, intestine, heart) were collected for histopathological study and preserved in 10% formalin. Histopathological research works were done at research laboratory of Department of Pathology and Parasitology at Hajee Mohammad Danesh Science and Technology University, Dinajpur.

Experimental flow chart are given below

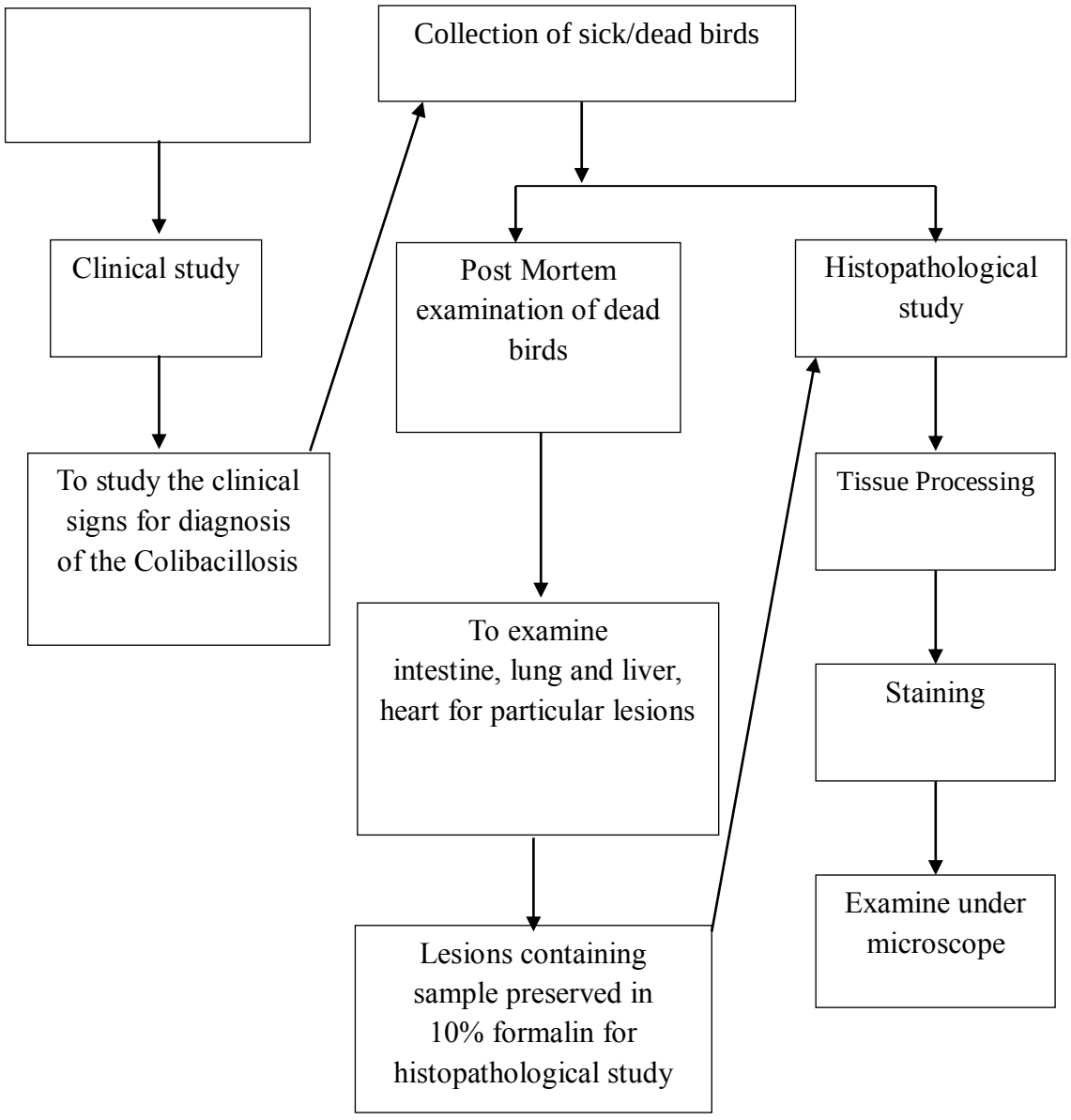

Fig. 3.2: Experimental layout

3.6 Study Item

During necropsy suspected liver, lung, heart sample were collected, preserved in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin according to standard method (Luna, 1968). Details of tissue processing, sectioning and staining are given below.

3.7 Laboratory preparation

All the instruments were placed in their appropriate place to conduct laboratory operation correctly and accurately personnel's who works in the laboratory must were apron and hand gloves before laboratory work all the surgical instruments were kept clean and also disinfect to prevent any kinds of contamination after finishing laboratory work all personnel put off their apron, hand gloves and wash their hand before leave the laboratory the dissecting table and laboratory room kept clean after each postmortem operation.

3.8 Necropsy findings of suspected Chickens

The necropsy was done on the selected chicken taken from different farms, Dinajpur. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection. The samples were also collected in 10% neutral buffered formalin for the histopathological study.

Equipment and appliances for necropsy

1. Birds
2. Scissors
3. Forceps
4. Gloves
5. Musk
6. Bone cutting saw
7. Scalpel
8. Chisel
9. 10% neutral buffered formalin

Procedure

1. At first the chicken was wet in a detergent solution thoroughly to lessen the chances of feathers floating around the area while the examination.

2. The bird was laid on a pad of newspaper on post mortem table. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.

3. The bird was positioned in such way so that the legs and feet were facing the examiner. Then an incision was given on skin in between the thighs towards the back and through skinning was done to observe paleness condition of carcass for detection of anaemia. Body cavity of bird was opened.

4. Segments of the intestines, lung, liver, heart were observed carefully for importants post mortem lesions. Then the parts opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal contents.

3.9 Gross lesion

Gross morbid lesions of different organs were observed after necropsy examination of the birds.

Fig. 3.3: Different organs examination of the birds during necropsy

3.10 Histopathological Examination

During necropsy, various organs having gross lesions were collected, preserved at 10% formalin, processed. Formalin-fixed samples of the lung, Intestine from the dead birds of broiler were processed for paraffin embedding, sectioned and stained with haematoxylin and eosin according to standard method (Luna, 1968) for histopathological study. Details of tissue processing, sectioning and staining are given below.

Fig. 3.4: Chemical solution Used for Histopathological Examination

Fig. 3.5: Microtome Machine

Fig. 3.6: Histopathological Lesions Examination under Microscope

3.11 Equipment and appliances

1. Sample (lung, Intestine)
2. Formalin
3. Chloroform
4. Paraffin
5. Alcohol
6. Tape Water
7. Xylene
8. Hematoxylin and Eosin Stain
9. Distilled water
10. Microtome
11. Clean Slides
12. Cover slips
13. Mounting media (dpx)
14. Microscope

3.12 Processing of tissues and sectioning

1. The tissues were properly trimmed to obtain a good cross section of the tissue.

2. The tissues were washed under running tape water for overnight to remove the fixative.

3. The tissues were dehydrated in ascending grades of alcohol using 50%, 70%, 80%, 90% alcohol, and three changes in absolute alcohol, for 1hr in each.

4. The tissues were cleared in two changes in chloroform, 1.5hr in each.

5. The tissues were embedded in molten paraffin wax at 560C for two changes, 1.5hr in each.

6. Paraffin blocks containing tissue pieces were made using templates and molten paraffin.

7. The tissues were sectioned with a microtome at 5 micrometer thickness, which were allowed to spread on warm water bath (42°C) containing small amount of gelatin and taken on oil and grease -free glass slides. The slides were air dried and kept in cool place until staining.

3.13 Hematoxylin and Eosin Staining Procedure

Preparation of Harris' hematoxylin solution

Hematoxylin crystals 5.0g

Alcohol (100%) 50.0 ml

Ammonium or potassium alum 100 g

Distilled water 1000.0 ml

Mercuric oxide (red) 2.5 g

Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately

plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

3.14 Preparation of eosin solution

1. 1% stock alcoholic eosin
2. Eosin Y, water soluble 1 g
3. Distilled water 20 ml
4. 95% alcohol 80 ml
5. Eosin was dissolved in water and then 80 ml of 95% alcohol was added.
6. Working eosin solution
7. Eosin stock solution 1part
8. Alcohol, 80% 3 parts
9. 0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

3.15 Staining protocol

1. Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 mins in each),
2. Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 mins in each; 95% alcohol for 2 mins; 80% alcohol for 2 mins; 70% alcohol for 2 mins) and distilled water for 5 mins,
3. The tissues were stained with Harris‟ hematoxylin for 10 mins,
4. The sections were washed in running tap water for 10 mins,
5. Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol), 2-4 dips,
6. The tissue sections were then washed in tap water for 5 mins and dipped in ammonia water (2-4 times) until sections became bright blue,
7. The sections were stained with eosin for 1 min and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 mins in each),
8. The stained sections were then cleaned by 3 changes in xylene, 5 mins in each and finally the sections were mounted with cover slip using DPX,

9. The slide were dried at room temperature and examined under a low (10X) and high (40X) power objects.

3.16 Some formula for calculation:

3.16.1 Prevalence rate (%)

Prevalence were calculated as number of infected birds of disease divided by total number of birds and multiple by 100

$$\text{Prevalence rate (\%)} = \frac{\text{No. of infected birds}}{\text{Total No. of birds}} \times 100$$

3.16.2 Mortality rate (%)

Mortality rate were calculated as number of dead birds of disease divided by number of infected birds and multiple by 100

$$\text{Mortality rate (\%)} = \frac{\text{No. of dead birds}}{\text{No. of infected birds}} \times 100$$

3.17 Statistical Analysis

The data were analyzed statistically by using software 'SPSS' (version 22). Chi-Square Test were performed and the results were expressed in percentage with P-value and level of significance was determined significant ($P<0.05$).

CHAPTER 4
RESULTS

Pathological investigation of colibacillosis diseases encountered in different commercial broiler farms in Dinajpur sadar upazila were studied and different clinical, necropsy and microscopic conditions were recorded during the study period.

4.1 Clinical Examination

The clinical signs of the birds affected with colibacillosis were

Respiratory signs, coughing, sneezing, feather, weakness, ruffling of reduced appetite, poor growth, omphalitis, depression, loss of weight, dullness, lethargic**,** soiling of cloaca with semi-solid cheesy material, fecal material is often green with containing white-yellow**,** dehydrated bird have dark dry skin which is more noticeable on shank and feet, younger birds with omphalitis (navel/yolk sac infection), distended abdomen of chicks, aggregation near to the source of heat, low performance in older birds (due to tumors), high mortality in younger birds (due to yolk-sac infections), high embryonic mortality in breeder flocks.

4.2 Prevalence of colibacillosis in different broiler farms at Dinajpur sadar upazila:

Six different broiler farm of Dinajpur sadar upazila were visited on the basis of clinical signs. A number of infected birds and dead birds were recorded. Determined the prevalence percentage of colibacillosis and mortality percentage of colibacillosis. In which highest prevalence of colibacillosis found in F1 which was 76.2% and lowest prevalence of colibacillosis found in F6 which was 9.16%. The highest mortality rate of colibacillosis found in F1 which was 9.1% and lowest mortality rate of colibacillosis found in F6 which was 3.27%. Average prevalence of colibacillosis was 39.63%. Average Mortality of colibacillosis was 5.39%.

Table 4.1: Prevalence of colibacillosis in different broiler farms at Dinajpur sadar upazila

Name of farm	Total no. of birds	No. of infected birds	Prevalence of colibacillosis (%)	P Value	No. of dead birds	Mortality rate (%)	P Value
F1	500	381	76.2	0.00***	35	9.1	0.047**
F2	2000	207	10.35		07	3.38	
F3	1000	449	44.9		24	5.34	
F4	800	520	65		41	7.88	
F5	1500	483	32.2		16	3.31	
F6	3000	275	9.16		09	3.27	
Total	8800	2315	39.63		188	5.39	

Prevalence of Colibacillosis on the basis of age group in broiler farms at Dinajpur Sadar Upazila:

Colibacillosis infected birds in broiler of Dinajpur sadar upazila were divided into three groups respectively 0-2 weeks, 2-4 weeks, >4 weeks. In which highest prevalence of colibacillosis found in 0-2 weeks which was 69.3% and lowest prevalence of colibacillosis found in >4 weeks which was 9.64%.

Table 4.2: Prevalence of Colibacillosis on the basis of age group in broiler farms at Dinajpur Sadar Upazila

Age group	Total no. of birds	No. of infected birds	Prevalence (%)	P Value
0-2 weeks	1300	901	69.3	0.00***
2-4 weeks	2500	932	37.28	
>4 weeks	5000	482	9.64	

4.3 Pathology of Colibacillosis

4.3.1 Gross lesions of colibacillosis during necropsy:

During post mortem, different forms of colibacillosis were observed, those are, Airsacculitis, omphalitis, perihepatitis, peritonitis, pericarditis, colisepticemia, enteritis, haemorrhage and mucus in intestine. Complex occurrence of colibacillosis with different forms and different diseases at a time.

Gross pathological changes in different samples with figure are given below

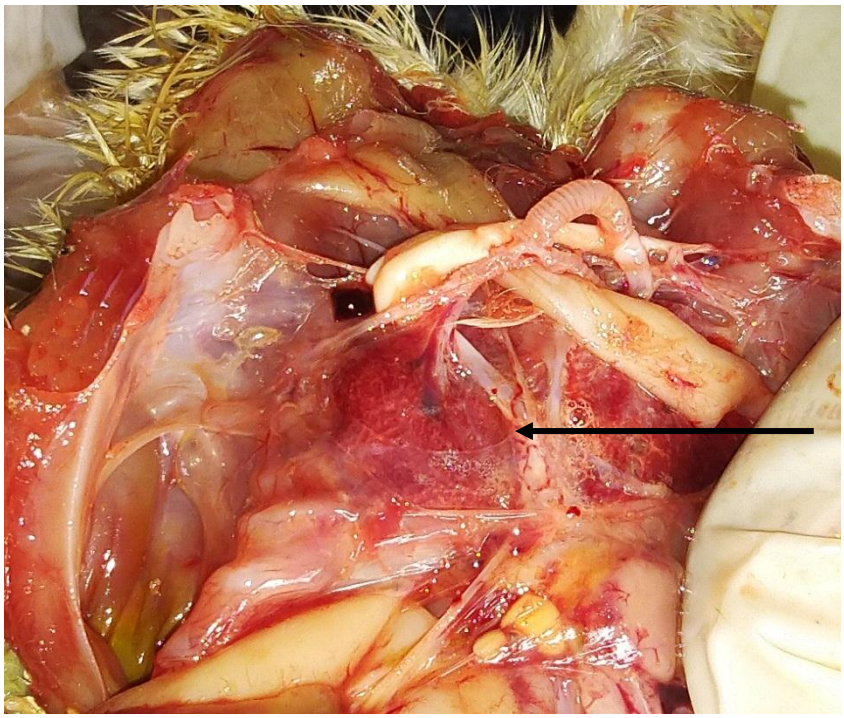

Fig. 4.1: Air Saculitis

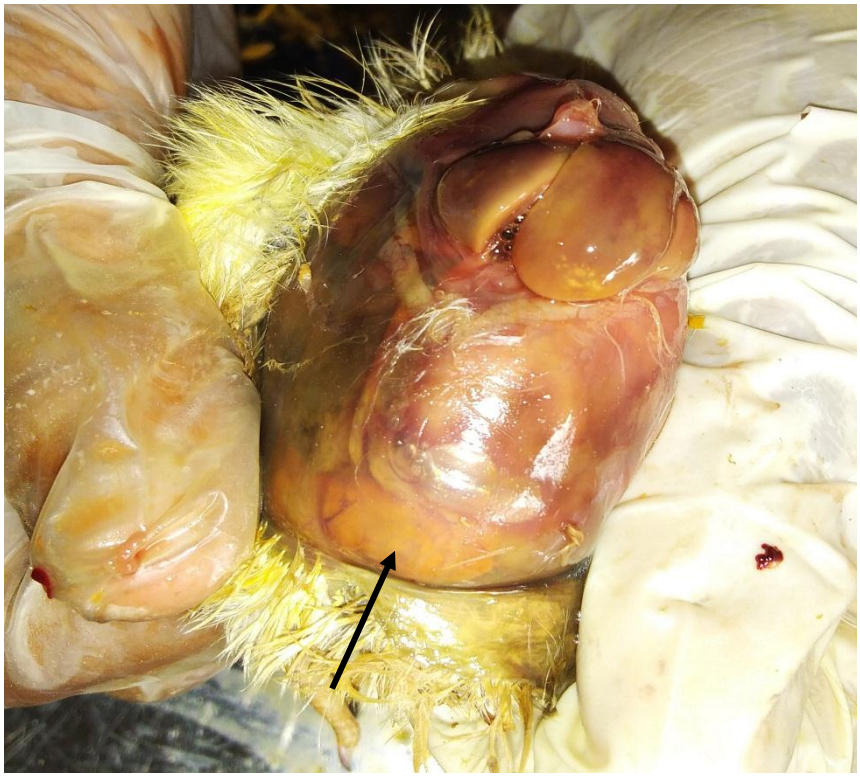

Fig. 4.2: Omphalitis

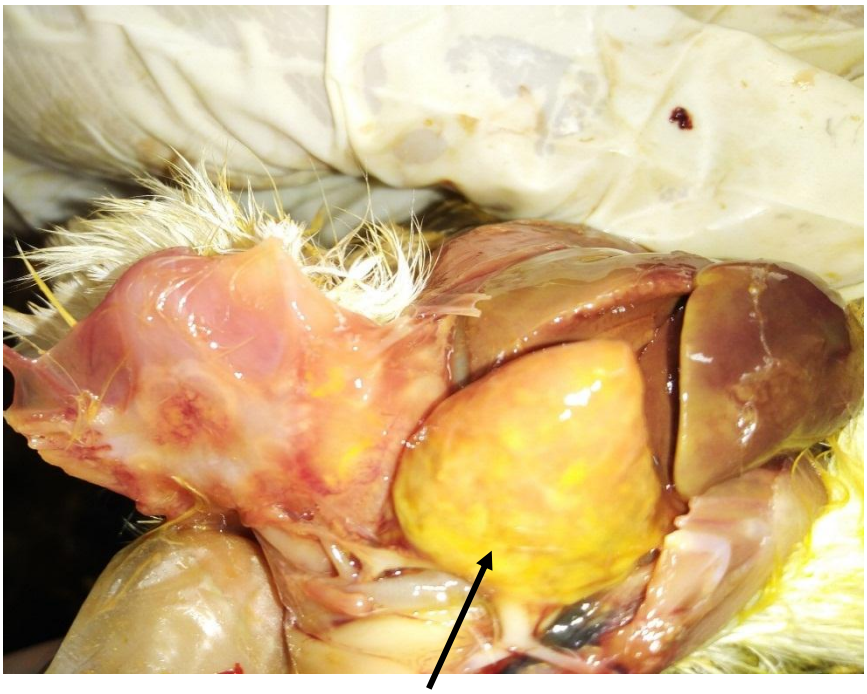

Fig. 4.3: Colibacillosis infected broiler showing fibrinous pericarditis and thickened pericardial sac with light yellow fibrinous exudates adhering to the heart

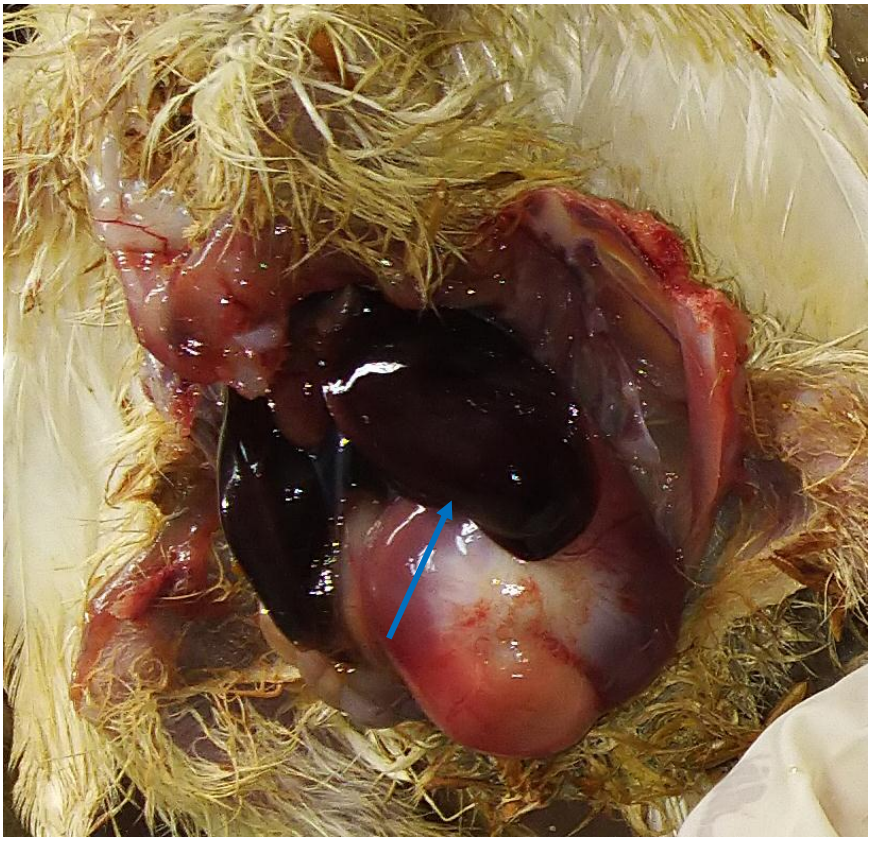

Fig. 4.4: Perihepatitis

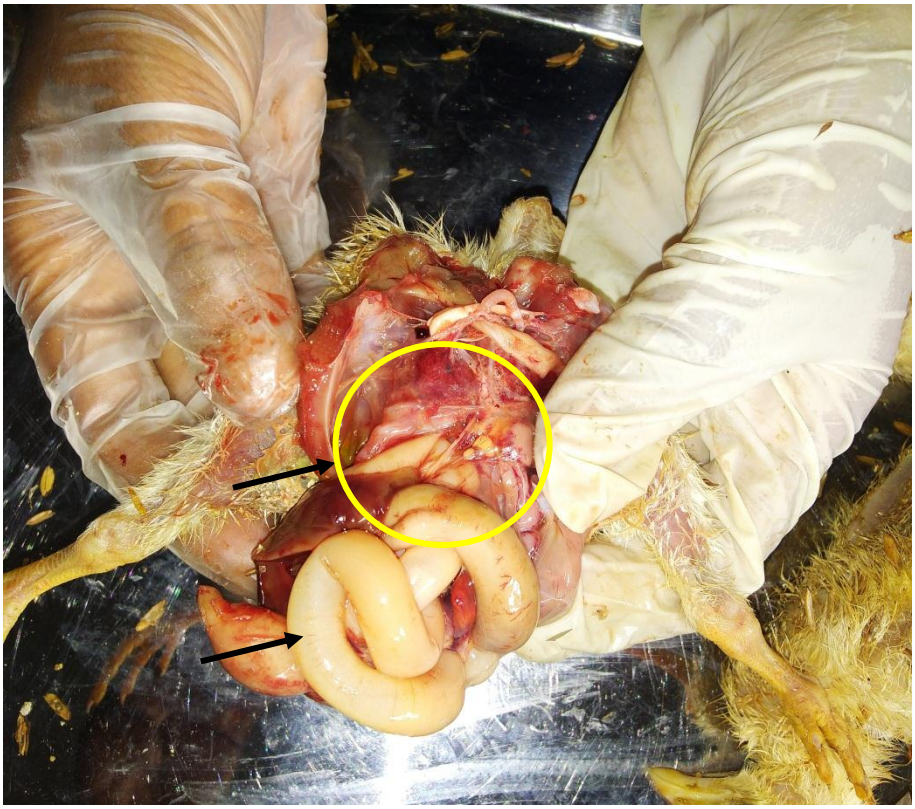

Fig. 4.5: Deposition of exudation of fluid in intestine.

Fig. 4.6: Blackish deposition of fluid in intestine

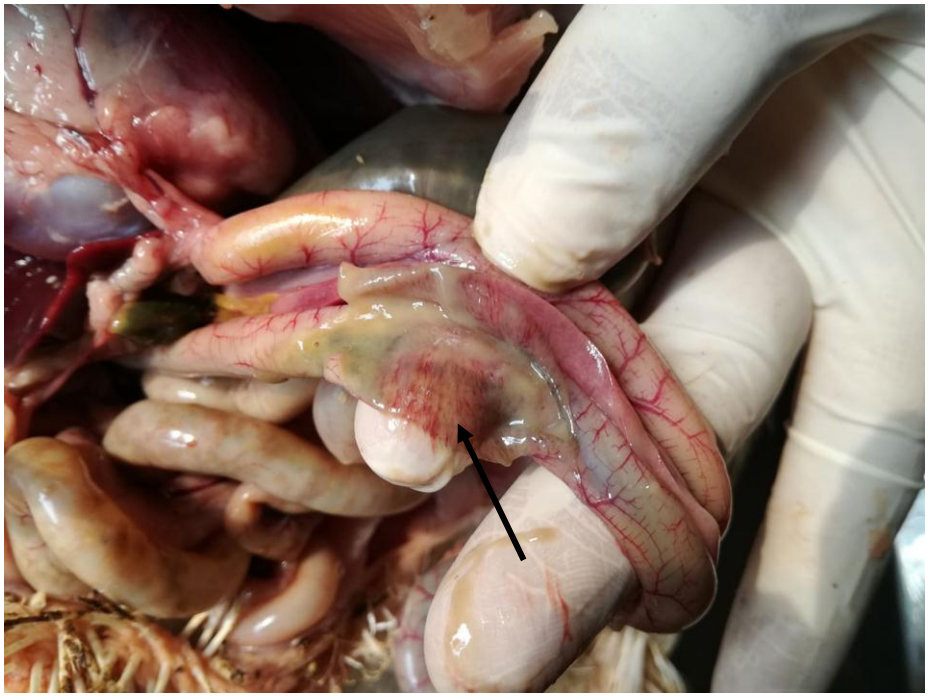

Fig. 4.7: Haemorrhage in Intestine Catarrhal exudation in intestinal lumen

4.3.2 Microscopic lesions of colibacillosis in different organs

In lung

- **Haemorrhage and highly congestion in lung**

In intestine

- destruction of intestinal wall
- Infiltration of reactive cells.

In heart

- Thickening of fibrous tissue in pericardium

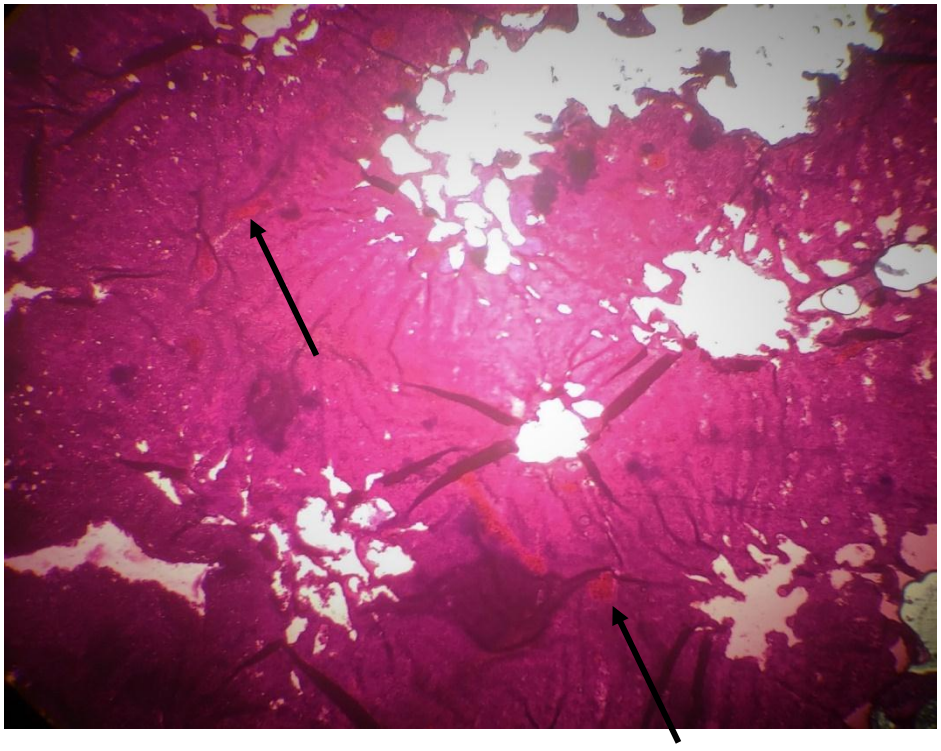

Fig. 4.8: Haemorrhage and congestion in lung of *E. coli* affected bird

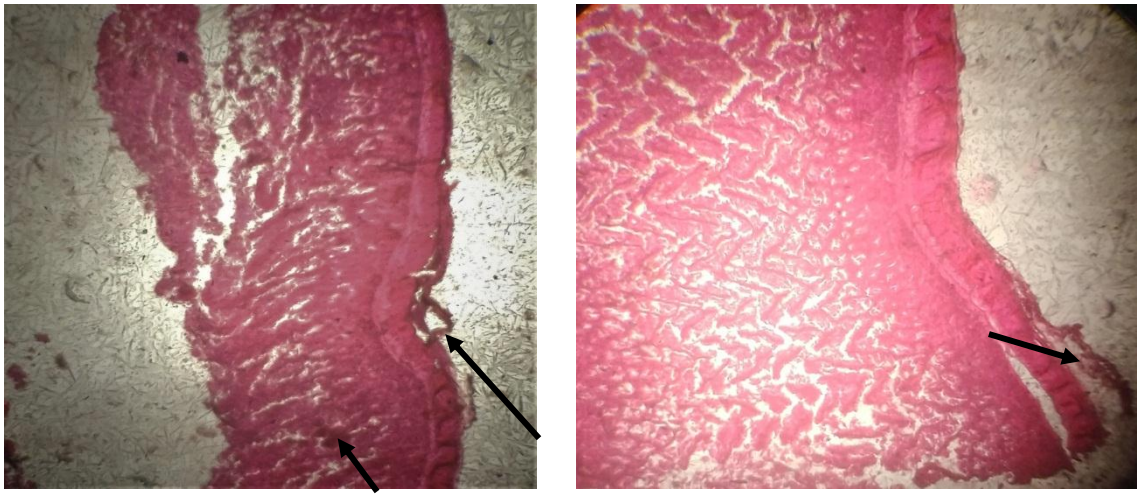

Fig. 4.9: Destruction of Intestinal wall and reactive cell infiltration

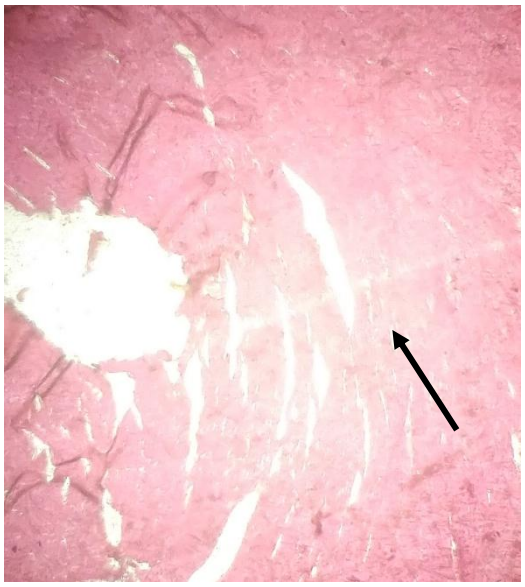

Fig. 4.10: Thickening of fibrous tissue in pericardium due to pericarditis

CHAPTER 5

DISCUSSION

The research work was conducted to know the prevalence and pathological lesions of avian colibacillosis in commercial broiler chickens at Dinajpur sadar upazila.

In this study, the clinical signs of affected flock were respiratory signs, coughing, sneezing, diarrhoea, reduced appetite, poor growth, omphalitis, dull, lethargic**,** soiling of cloaca with semi-solid cheesy material, fecal material is often green with containing white-yellow, dehydrated bird have dark dry skin which is more noticeable on shank and feet, younger birds with omphalitis (navel/yolk sac infection), distended abdomen of chicks, aggregation near to the source of heat, low performance in older birds (due to tumors), high mortality in younger birds (due to yolk-sac infections), high embryonic mortality in breeder flocks. These clinical signs of colibacillosis was already mentioned by different authors (Omer *et al.* 2010, Awawdeh 2017, Ganapathy *et al.* 2000)

In this study, the prevalence percentage of colibacillosis in F1, F2, F3, F4, F5 and F6 farm was respectively 76.2, 10.35, 44.9, 65, 32.2 and 9.16. The average occurrence of colibacillosis was 39.63% in commercial broiler. These results support the earlier reports of Suha *et al.* (2008) who reported 43.50% and reports of Rahman *et al.* (2004) who reported 67.73% colibacillosis in commercial broiler and layer. These results also support the earlier reports of Hossain *et al.* (2008) who reported 60.00% colibacillosis in commercial broiler and layer birds. Bhattachaijee *et al.* (1996) reported 40.82% and Ahmed *et al.* (2009) reported 52.26% prevalence of E. coli in chicken from Bangladesh but Nazir (2004) stated the over all prevalence was 62.5% from chicken, which is closed to the present findings.

A significant ($p>0.05$) influence of age group of birds was found to be related with the increase susceptibility of colibacillosis. The highest significant ($P>0.05$) outbreak was in age group 0 - < 2 weeks (69.3) and the lowest in age group of >4 weeks (9.64). It revealed that the age group was considered to be statistically significant for outbreak of colibacillosis in broiler. Talha *et al.* (2001) reported higher proportionate prevalence rate of colibacillosis in growing chickens in comparison to adults whereas Bhattachaijee *et al.* (1996) reported widely prevalent of colibacillosis in both the brooding (12.82%) and pre-

peak-post production layer chickens (5.49 to 8.78%), and this study also recorded widely prevalent of *E. coli* infection in all age groups of chickens (9.52 to 36.73%).

In this study efforts had been made to identify different forms (gross lesions) of colibacillosis by postmortem. Those forms were air sacculitis, omphalitis, pericarditis, perihepatitis, septicaemia, enteritis, peritonitis and a combination of different forms at a time. These forms of colibacillosis was already mentioned by different authors (Chowdhury *et al.* 2009, Someya *et al.* 2007, Nakamura *et al.* 2007, Landman and Comelissen 2006, Rahman *et al.* 2004 and Shah *et al.* 2003)

In this study the histopathological lesion was haemorrhage and highly congestion in lung, destruction of intestinal wall and reactive cell infiltration, thickening of fibrous tissue in pericardium. Similar lesions have been reported by Khaton *et al.* (2008), Nakamura *et al.* (2007), Ghosh *et al.* (2006), Gagandeep *et al.* (2004), Islam *et al.* (2003), Zhou *et al.* (2002). Talha *et al.* (2001).

CHAPTER 6

CONCLUSION

The present research work was conducted in order to observe the prevalence and pathological lesions of avian colibacillosis in different commercial broiler farm at Dinajpur sadar upazila. A total of 8800 broiler were observed from 6 farm in which 2315 birds were infected and number of dead birds were 188. Sick and dead birds were examined on the basis of clinical signs, post-mortem and histopathological examination. among them 39.63% broiler were infected with any lesion of colibacillosis and 5.39% birds were dead. The most frequent gross lesions of colibacillosis were air sacculitis, omphalitis, pericarditis, perihepatitis, peritonitis, enteritis. The microscopic lesions of this disease was haemorrhage and highly congestion in lung, destruction of intestinal wall and reactive cell infiltration, thickening of fibrous tissue in pericardium. The results obtained during the study period revealed that the prevalence percentage among different farm, age of birds were statistically highly significant ($p<0.05$) for colibacillosis and mortality rate in different farm was statistically significant ($p<0.05$).

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