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# Prevalence and histopathological investigation of colibacillosis in Broiler chickens in the Rajshahi District, Bangladesh

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**ABSTRACT**

Avian colibacillosis is regarded as a serious bacterial disease in the poultry industry worldwide. Common bacteria known as *Escherichia coli* (*E. coli*) are naturally present in birds' digestive tracts and, consequently, in their surroundings. A strain of *E. coli* known as Avian Pathogenic Escherichia Coli (APEC) is capable of causing a clinical illness. Large financial losses result from colobacillosis in commercial broiler farms because of a comparatively high death rate and productivity loss. In the present study, a total of 300 broilers of 8 farms were examined and among them 170 (56.67%) were diagnosed as infected with any form of colibacillosis and 42 sick and dead birds (different organs) were collected from necropsy cases. The most frequent gross lesions of colibacillosis were cloud and thickened air sac, omphalitis, pericarditis, perihepatitis, peritonitis, colisepticemia, enteritis. The pericardium of the heart's thick fibrous layer, telangiectasis, blunting and sloughing off of the villus, and persistent passive congestion were the microscopic lesions of these illnesses, infiltration into the intestinal lamina propria. Average prevalence of colibacillosis, on the basis of age was higher (67.32%) in age group 0-2 weeks and lower in 4 weeks (0.2 %). The results obtained during the study period revealed that the higher prevalence of colibacillosis was in winter season (68.90%) and lower in summer season (41.91%). So, age and winter season was the risk factors of colibacillosis. Considering the factor, colibacillosis causes high mortality and winter season was the main risk factor and involved other factor in broiler at Rajshahi district.

## INTRODUCTION

In Bangladesh, livestock is an essential part of the complex farming system since they provide employment opportunities and a significant source of farm power services in addition to serving as a source of animal protein. According to data on livestock population in Bangladesh, poultry are the most significant bird species in the nation. In 2019-2020, Bangladesh produced 173.6 billion eggs and 7.674 million metric tons of meat from its 356.318 million broiler and layer chickens (DLS, 2020). The industry is expanding quickly and represents 14% of the value of livestock output overall (Raihan and Mahmud, 2003). It has been discovered that 37% of Bangladesh's total meat production comes from just poultry meat. About 22-27% of the nation's total animal protein supply comes from poultry (Prabakaran, 2003).

Over the past 20 years, Bangladesh's poultry farms have experienced rapid growth. Bangladesh's poultry industry includes both traditional village-based scavenging poultry production and commercial broiler and layer production. 89% of rural families raise chicken for commercial or subsistence purposes (Das et al., 2008). In Bangladesh's economy heavily depends on the production of chicken in its villages. This kind of manufacturing needs little labor, little acreage, and short generation intervals. Therefore, the government of Bangladesh and many NGOs are using a traditional village poultry production system as a tool to alleviate poverty and to improve the economic status of the rural people through self-employment. Because of their higher disease resistance and comparatively high productivity, sonali (a cross between RIR male and Fayoumi female) chickens are used in place of native local chickens in a model for semi-

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scavenging poultry rearing systems (Rahman et al., 1997). For the majority of people, poultry is a major source of animal protein. Since 1970, the production of poultry has increased significantly in comparison to the livestock industries. It makes up roughly 34% of the world's total meat production (MSadeq et al., 2015).

Provides a chance to feed the world's fastest-growing human population and poultry-growing regions. Furthermore, chicken is widely regarded as the primary source of both high-quality human food and less expensive animal protein in the modern world. The poultry's ability to convert plant protein into animal protein and the general public's acceptance of chicken meat and eggs are key factors in the industry's continued growth in many nations. Bangladesh's poultry industry is beset by several obstacles. The epidemic of diseases that kills roughly 30% of chickens annually is one of the main obstacles (Ali, 1994). In major poultry raising belts in and around Dhaka and Gazipur districts of Bangladesh, Saleque et al. (2003) conducted a survey on breeding flocks as well as commercial broiler and layer flocks from January 2000 to December 2001. We observed prevalence of 45%, 17%, 12.4%, 6.6%, 4.5%, 1.5%, and 1246 of bacterial, viral, mycoplasmal, protozoal, parasitic, fungal, and non-infectious diseases, respectively, in the birds examined.

In many nations, including Bangladesh, colibacillosis is a major factor that causes large financial losses in poultry farms (Savageau, 1997). *Escherichia coli (E. coli)* is a Gram-negative member of the Enterobacteriacae family of bacteria that causes the disease syndrome known as avian colibacillosis. In mammals, colibacillosis is primarily an enteric disease; however, in poultry, it causes typical localized or systemic disease that primarily develops secondary to compromised host defenses. In its acute form, sepsis causes death; in its subacute form, reproductive tract infections such as salpingitis and/or peritonitis cause severe mortality; and in both cases, pericarditis, airsacculitis, and perihepatitis are the defining characteristics (Landman and Cornelissen, 2006; Ozaki and Murase, 2009).

Any local or systemic infection in chickens caused solely by strains of *E. coli* is referred to as colibacillosis (Barnes et al. 2003). The bacteria can spread throughout the surrounding environment through contaminated food chains and water supplies. It is typically found in the lower intestinal tracts of humans and other warm-blooded animals (Savageau, 1983) and can be disseminated in surrounding environment through contaminated food chain and water supply. They are the perfect indicator bacterium to test environmental samples for fecal contamination because of their capacity to multiply in water and soil, survive outside the body for extended periods of time, and survive (Byappanahalli and Fujioka, 1998; Byappanahalli et al., 2003; Byappanahalli et al., 2006; Ishii et al. 2006). It has been determined that avian colibacillosis is a serious infectious disease that affects birds of all ages. It can cause a wide range of symptoms in poultry, such as enteritis, cellulitis, swollen head syndrome, omphalitis, polyserositis, coligranuloma, yolk sac infection, and septicemia. Chicken colibacillosis is typified by pericarditis, airsacculitis, and peri-hepatitis in its subacute form and septicemia, which can lead to death in its acute form (Calnek et al., 1997).

As poultry farming and rearing have grown significantly, colibacillosis has spread throughout Bangladesh and the rest of the world (Talha et al., 2001; Islam et al., 2003 and Rahman et al., 2004). Both broiler and layer farms suffer significant financial losses as a result of morbidity, mortality, decreased output, and poor chick quality. In a severe colibacillosis outbreak, mortality could reach 94% (McPeake et al., 2005).The presence of one or more virulence factors, such as invasions, heat-labile and heat-stable enterotoxins, verotoxins, and colonization factors or adhesions, is what makes *E. coli* strains pathogenic (Smith and Haibs, 1967).

A variety of antibiotics may not work on *E. coli* but isolates from poultry are often resistant to one or more antibiotics; this is particularly true if the antibiotics, like tetracycline, have been used in the poultry industry for a long time (Castanon et al., 2007, Amara et al., 1995). Different authors conducted studies in Bangladesh on a variety of parameters, including the prevalence, isolation,

identification, and epidemiological analysis of all types of pathogenic and nonpathogenic *E. coli* (Chowdhury and Rahman, 1967).

Considering the economic significance of the poultry industry in Bangladesh and the devastating impact of colibacillosis on broiler production, it is crucial to understand the disease's prevalence and pathological characteristics. This study aimed to identify colibacillosis in broiler chickens through clinical and necropsy examinations and to characterize the disease based on gross and histopathological lesions. Furthermore, the study sought to determine the proportionate prevalence of colibacillosis in commercial poultry farms, analyzing risk factors such as age and seasonal variations. Based on these findings, preventive and therapeutic measures tailored to mitigate the disease's impact on poultry farms were proposed.

## MATERIALS AND METHODS


**Figure 1**: Samplings areas (Rajshahi District)

### Study design and area

This study was designed to investigate the prevalence and pathology of colibacillosis in broiler chickens. The study was conducted over a 12-month period from July 2021 to June 2022 in eight selected poultry farms within the Rajshahi district of Bangladesh (Figure 1). These farms were chosen to represent a diverse range of rearing practices and management systems in the region.

### Sample collection

A total of 300 broiler chickens from the eight farms were observed for clinical signs of colibacillosis. Among them, 42 sick and deceased birds showing clinical signs were selected for detailed pathological analysis. Samples were collected during necropsy, including tissues from the liver, heart, spleen, and intestine. These samples were preserved in 10% neutral buffered formalin and transported to the Department of Veterinary and Animal Sciences, University of Rajshahi, for histopathological processing and analysis.

### Pathological Examination

#### *Gross Pathology*

Postmortem examinations were performed on all collected birds to identify gross lesions in various organs. Lesions were documented systematically, and representative tissue samples showing abnormalities were preserved in formalin for further analysis.

#### *Histopathology*

Fixed tissue samples were processed using standard histological techniques (Luna, 1968). The processing involved fixation in 10% neutral buffered formalin, followed by dehydration through graded ethanol, clearing with xylene, and embedding in paraffin. Tissue sections of 5 µm thickness were prepared using a microtome, mounted on glass slides, and stained with Hematoxylin and Eosin (H&E). Hematoxylin stained nuclei blue, while eosin highlighted cytoplasmic structures in various shades of pink, enabling detailed microscopic evaluation of lesions.

#### *Data collection and photomicrography*

During the prevalence study, clinical symptoms and farm-related data were recorded, including flock size, age, breed, rearing practices, vaccination history, and disease outbreak details. Histological findings were captured using a photomicrographic setup (Kriis, Germany; MBL 2100), and the data were analyzed for a

comprehensive understanding of the pathological features of colibacillosis in broiler chickens.

## RESULTS

### Prevalence of Colibacillosis in Broilers and affected tissues

A total of 300 broiler chickens from eight commercial poultry farms in the Rajshahi district were evaluated for colibacillosis from July 2021 to June 2022. Out of these, 42 necropsy cases of sick and deceased birds were subjected to detailed pathological examination. The overall prevalence of colibacillosis among the 300 birds was 56.67%. Histopathological evaluations were conducted at the Department of Veterinary and Animal Sciences, Faculty of Veterinary Sciences, University of Rajshahi.

### Prevalence based on sample type

Colibacillosis was most frequently observed in the intestine (91.67%), followed by the liver (83.34%), heart (66.67%), and spleen (16.67%). Among the 42 necropsy cases, the intestine showed the highest infection rate, while the spleen showed the lowest. The distribution of positive cases by sample type is shown in Table 2.

**Table 2:** Sample-Based prevalence of colibacillosis

| Sample Type | Total Examined | Positive Cases | Prevalence (%) | P-Value |
|---|---|---|---|---|
| Liver | 12 | 10 | 83.34 | 0.031039 |
| Heart | 12 | 8 | 66.67 | - |
| Spleen | 6 | 2 | 16.67 | - |
| Intestine | 12 | 11 | 91.67 | - |

### Prevalence based on age group

The prevalence of colibacillosis varied significantly across different age groups. Birds aged 1–15 days showed the highest infection rate (67.32%), followed by 15–30 days (51.33%), while birds aged 30–40 days had the lowest prevalence (0.2%). The results are presented in Table 3.

**Table 3:** Age-based prevalence of colibacillosis

| Age Group (days) | Total Examined | Positive Cases | Prevalence (%) | P-Value |
|---|---|---|---|---|
| 1–15 | 101 | 68 | 67.32 | 0.082911 |
| 15–30 | 185 | 95 | 51.33 | - |
| 30–40 | 14 | 7 | 0.20 | - |

### Prevalence based on farm size

The size of the farm significantly influenced the prevalence of colibacillosis. Large farms (1,900–3,000 birds) exhibited the highest prevalence (82.67%), followed by medium-sized farms (900–1,800 birds) at 49.69%, and small farms (300–900 birds) at 43.33%.

**Table 4:** Prevalence of colibacillosis by farm size

| Farm Size | Total Examined | Positive Cases | Prevalence (%) | P-Value |
|---|---|---|---|---|
| Small (300–900) | 60 | 26 | 43.33 | 0.35178 |
| Medium (900–1800) | 165 | 82 | 49.69 | - |
| Large (1900–3000) | 75 | 62 | 82.67 | - |

Table 4 illustrates that larger farm sizes had a significantly higher prevalence, likely due to higher bird density and associated management challenges.

### Seasonal prevalence

The occurrence of colibacillosis was significantly higher during the winter season (68.90%) compared to the summer season (41.91%), as shown in Table 5. The season was identified as statistically significant factor ($P > 0.01$) influencing disease outbreaks.

**Table 5:** Prevalence of colibacillosis by season

| Season | Total Examined | Positive Cases | Prevalence (%) | P-Value |
|---|---|---|---|---|
| Winter | 164 | 113 | 68.90 | 0.214084 |
| Summer | 136 | 57 | 41.91 | - |

Table 5 shows the clear seasonal variation, with winter being the most favorable season for disease prevalence.

**Pathological findings**

***Gross lesions***

Postmortem examination revealed characteristic gross lesions in affected birds. The liver exhibited a whitish fibrinous layer and necrosis. The heart was completely enclosed by a sero-fibrinous covering, while the spleen was hemorrhagic. The intestine was distended and congested with prominent lesions (Figure 2).

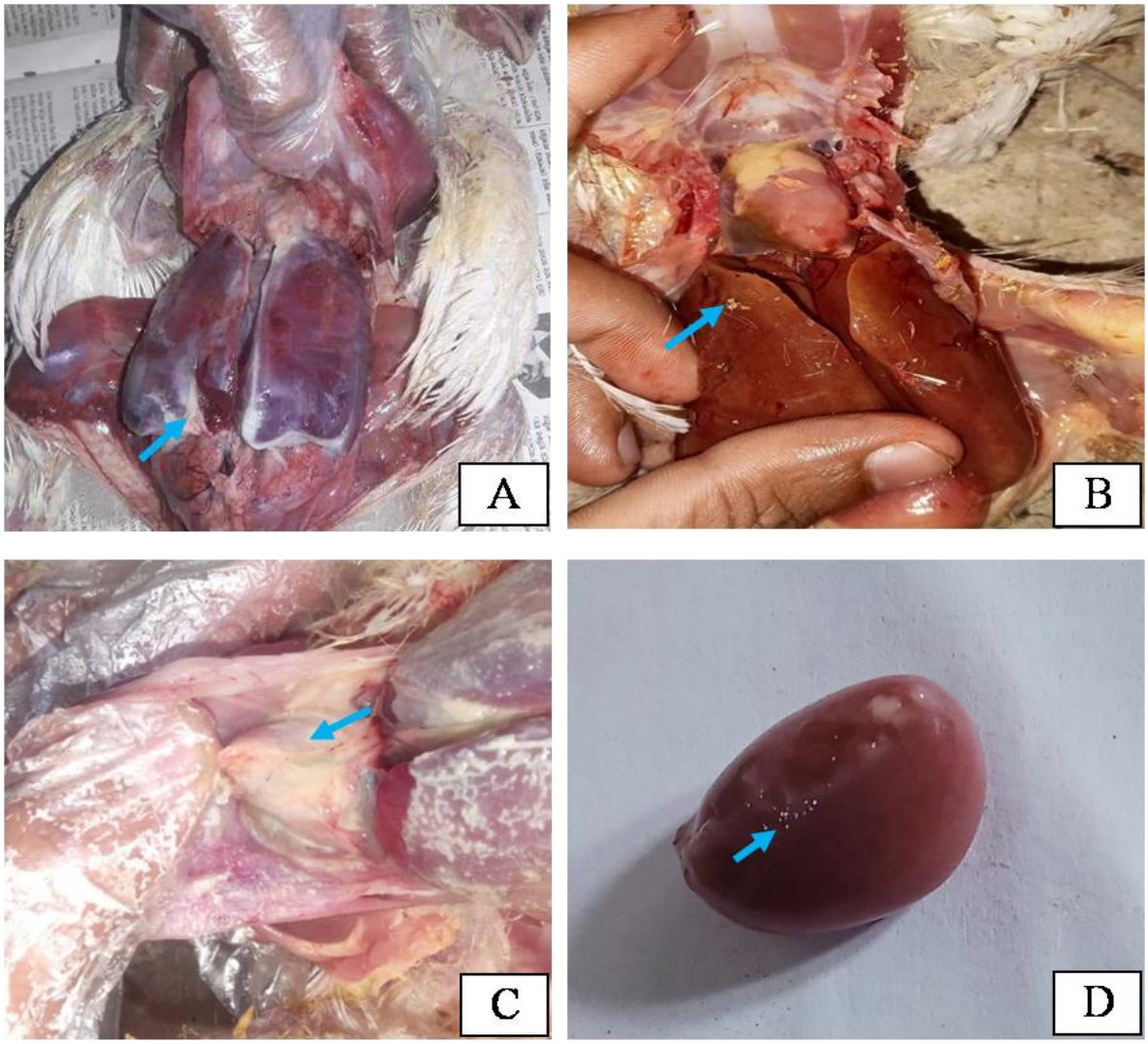


**Figure 2:** Gross lesions, including the whitish fibrinous liver (A) and necrotic areas (B), completely enclosed heart by sero-fibrinous covering (C) and Hemorrhagic of spleen (D)

**Microscopic lesions**

Histopathological analysis showed coagulative necrosis of the liver and infiltration of inflammatory cells (Figures 3a and 3b). The heart exhibited thickened pericardium and cellular infiltration (Figure 3c). The spleen revealed multiple necrotic areas (Figure 3d). Intestinal sections showed blunted and sloughed villi with inflammatory infiltration in the duodenum (Figure 3e).

These pathological findings provide detailed insights into the progression and tissue-specific impacts of colibacillosis in broilers.

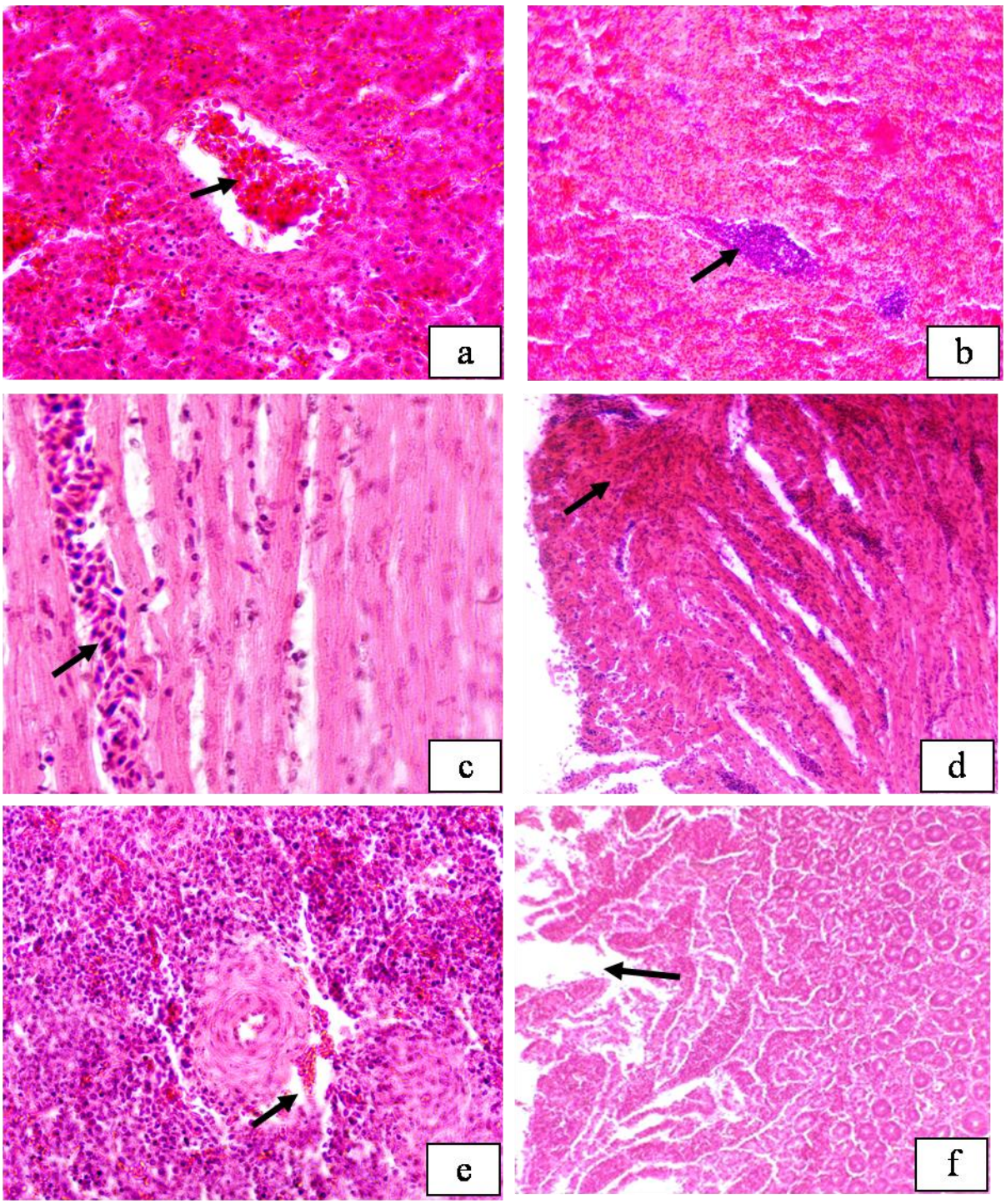


Figure 3: (a) Coagulative necrosis of liver (H & E × 40), (b) Infiltration of inflammatory cells of liver (H&E ×10), (c) Cellular infiltration of heart muscle (H & E ×40), (d) Thickening of pericardium due to infiltration of RE cells, (e) Multifical necrosis along with depletion of lymploid cells in spleen, (f) In duodenum, blunting and sloughing off villus, infiltration of inflammatory cells.

## DISCUSSION

The purpose of the research was to determine the prevalence and pathological lesions of avian colibacillosis in commercial broiler chickens and to generate some hypotheses about the risk of transmission and infection with *E. coli* in poultry to humans from industrial broilers.

In this study, the average occurrence of colibacillosis was 56.67% in commercial broiler. These results support the earlier reports of Shah 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 Khaton et al. (2008) who reported 60.00% colibacillosis in commercial broiler. The current study was prevalence of E. coli considering different age groups of bird to be related with increase susceptibility of E. coli. The highest prevalence 67.32% in age group 0-2 weeks and the lowest prevalence of colibacillosis found in 4 weeks of age which was 0.2%. 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 E. coli in growing chickens in comparison to adults whereas Bhattacharjee et al. (1996) reported widely prevalent of E. coli 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 present study, the prevalence of E. coli is higher occurrence in winter season 68.90% followed by summer season 41.91%. Hashem et al. (2017) also noted that Bangladesh had a higher rate of *E. coli* infection (67.54%) during the winter. According to Lambie et al. (2000), there is a 67.2% increase in *E. coli* infection during the rainy season. The present study has confirmed the previous report by Rahman et al. (2004) by identifying age, farm size, and season as associated risk factors for colibacillosis.

Necropsy and histological examination of hens that seemed sick or dead were used to characterize the diseases in the pathological investigation. Gross pathological ledion in the current study, perihepatitis, hazy and thickened air sac, haemorrhage, and congested and thickened capsule. The heart's pericardium thickened as a result of fibrinous pericarditis, and the pericardial sac thickened as well, with light yellow fibrinous exudates sticking to the heart. Mucus, congestion, and septicemic bleeding (enteritis) were discovered in the duodenum. Under a microscope, the liver segment revealed focal necrosis of the coagulation type along with heterophil, lymphocyte, and macrophage infiltration, primarily in the portal area. The thickening of the pericardium as a result of RE cell infiltration is a characteristic of pericarditis in the heart. Inflammatory cell infiltration, blunting and sloughing off of the villus in the duodenum. Colisepticemia, coligranuloma (Hjarre's disease), avian cellulites (inflammatory process), enteritis, pericarditis, peritonitis, salpingitis, osteomyelitis/ synovitis, panophthalmitis, and omphalitis/yolk sac infection are among the severe disease conditions caused by *E. coli* (Chauhan, 1996; Barens and Gross, 1997). There may be a significant danger of disease transmission from commercial broilers to humans due to *E. coli's* fecal-oral pathway as well as food- and water-borne germs, though this could rely on other circumstances such as the need for aseptic procedures while processing bird goods, the potential for live microorganisms in cooked meat, etc.

## CONCLUSION

The present research work was conducted in order to observe the prevalence and pathological lesions of avian colibacillosis in commercial broiler farm in Rajshahi district. A total of 300 broiler of 8 farms were examined through post-mortem and among them 170 (56.67%) broiler were diagnosed as affected with any lesion of colibacillosis. The most frequent gross lesions of colibacillosis were air sacculitis, omphalitis, pericarditis, perihepatitis, peritonitis, colisepticemia, enteritis and a large numberof birds with combination of different form of colibacillosis and in combination with different diseases, mainly newcastle disease, gumboro, necrotic enteritis, coccidiosis, chronic respiretory disease, infectious bronchitis etc. The microscopic lesions of this disease was chronic passive

congestion, infiltration of liver, thick fibrous layer in the pericartium of heart, blunting and sloughing off billus and infiltration of lamina propria of intestine. The results obtained during the study period revealed that age of birds and farm size were not significant for colibacillosis but season of the year was statistically significant ($p>0.01$) risk factor of colibacillosis in broiler.

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