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RESEARCH ARTICLE

Prevalence and Pathological Investigation of Colibacillosis in Commercial Poultry Farms at Rajshahi District of Bangladesh

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Abstract

Avian colibacillosis is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry. The study was conducted for observation of prevalence and pathological investigation of colibacillosis in commercial poultry farm at city corporation area of Rajshahi, Bangladesh during the period from July 2023 to June 2024. *Escherichia coli* was isolated from 82.7% of cloacal swabs of apparently healthy birds and 85.0% of sick and dead birds. Average prevalence of colibacillosis, on the basis of age, was higher (65%) in age group 0–2 weeks and lower in >4 weeks (10%). The higher prevalence of colibacillosis was in winter (65%) and lower in summer season (35.71%). In colibacillosis, congested and consolidated lungs with cloudy and thickened air sacs, congested and thickened liver capsules, and enteritis were observed at necropsy. The microscopic lesions were hemorrhage in liver and huge infiltration of heterophils, highly congestion in lung, blunting of villus and reactive cell infiltration, and thickening of fibrous tissue in pericardium. In conclusion, a higher prevalence of colibacillosis (>80%) was observed in commercial poultry farms of city corporation area of Rajshahi, and age and season were the significant risk factors of colibacillosis in broiler chickens.

Keywords: Age, Colibacillosis, Prevalence, Pathology, Poultry Farms, Season.

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Introduction

Poultry industry can provide opportunities for the increase of Gross Domestic Product (GDP) growth rate through ensuring food security and self-employment and finally reducing poverty (Talukdar et al. 2017). Poultry farming plays a very important role for income generation and poverty alleviation particularly for the disregarded women and unemployed youths in Bangladesh by means of self-employment (Ahmed et al. 2021). The poultry population of Bangladesh is 396.038 million in 2023-2024 (BBS 2024). The advancement of poultry industry is facing a problem due to sudden outbreak of bacterial diseases, including colibacillosis, which creates a serious threat in economy of Bangladesh (Khaton et al. 2008). In chickens colibacillosis describes to any local or systemic infection caused entirely or partly by *Escherichia coli*, a major pathogen of commercially produced poultry all over the world (Barnes et al. 2003). *E. coli* strains causing systemic disease in poultry (avian colibacillosis) are called avian pathogenic *E. coli* (Nawaz et al. 2024). *E. coli* has been related to a variety of disease in poultry such as pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulitis, colisepticemia, coligranuloma and swollen-head syndrome (McMullin et al. 2020).

Avian pathogenic *E. coli* infection occurs in both broilers and layers. It is responsible for omphalitis in chicks and embryo mortality. Lesions seen are mainly polyserositis with fibrin in the air sacs, pericardium, and liver (Kabir 2010). It affects birds of all ages. Day old chicks get infected via the yolk sack, but in older chick become infected mainly airborne. Up to three weeks of ages young broiler chickens are highly sensitive to the disease, but chickens of four weeks and older are regarded quite resistant to primary colibacillosis (Kabir 2010). With expansion of poultry farming, colibacillosis has appeared as a widespread problem in Bangladesh (Islam et al. 2003, Hossain et al. 2004). It causes huge loss specially if there is poor management or stress in broilers such as complicating infections like chronic respiratory disease or mycoplasma (Talha et al. 2001) or under development of the immune system (Aktar et al. 2023 & 2024) causing immunosuppression. Considering these

factors, it needs to prevent and control that infectious disease like colibacillosis. Workable prevention and control measures cannot be undertaken unless the present status of colibacillosis is known. Investigations on colibacillosis in broiler are very scanty in Bangladesh. Therefore, the present study was aimed at determining the prevalence of *E. coli* in apparently healthy, sick and dead chickens on selected poultry farms at city corporation area of Rajshahi as well as studying their colony, staining and biochemical characteristics and also to study the changes in different organs found in chickens with colibacillosis.

Materials and Methods

A total of 52 cloacal samples from 52 apparently healthy chickens and a total of 40 swabs samples (10 from liver, 10 from lung, 5 from heart and 15 from duodenum of 40 sick and dead birds) and several lesion containing tissues (liver, lung, heart, duodenum) collected from necropsy cases at different poultry farms of Rajshahi city corporation area were used in the present study during the period from July 2023 to June 2024 at the Histopathology laboratory of the Department of Veterinary and Animal Sciences in University of Rajshahi, Rajshahi, Bangladesh. The first step was isolation and identification of *E. coli* on the basis of their colony morphology, staining property, motility and biochemical characteristics from the collected samples. The second step was gross and histopathological examinations of different organs of sick and dead chickens.

Identification of isolated organisms by microbiological examinations

Colony characteristics of the identified bacteria in different agar media

All cloacal and swabs samples were inserted into sterilized screw-capped test tubes containing nutrient broth and preserved at 4°C. During the time of examination, all samples were placed in nutrient agar plate and incubated overnight at 37° C for the growth of the organisms. After primary culture of the organisms, a small amount of inoculum from nutrient agar was sub-cultured in the nutrient agar and MacConkey agar to observe the colony morphology. Characteristic colony morphology of the organisms indicating the features of *E. coli* was selected for subculture on selective media such as Eosin Methylene Blue (EMB) agar. Morphological characteristics (shape, size, surface texture, edge and elevation, color, opacity etc.) of the suspected colonies on different agar media developed within 18 to 24 hours of incubation were carefully studied and recorded.

Study on morphological characteristics of the suspected organisms

Using Gram's staining technique; we examined the shape, size and density of the identified organisms under 100x of lighting compound microscope according to Tonu et al. (2011).

Motility test of the isolated organisms

The motility test of the isolated organisms was performed by hanging drop preparation to differentiate the motile bacteria from the non-motile one according to Tonu et al. (2011).

Identification of Isolated Organisms by Using Specific Biochemical Tests

Carbohydrate Fermentation Test

The carbohydrate fermentation test was performed by inoculating 5ml of nutrients in broth culture of the organisms into tubes containing different sugars and incubated for 72 hours at 37°C. Acid production was indicated by the colour change from red to yellow of the medium and gas production was noted by the appearance of gas bubbles in the inverted Durham's tube.

Methyl red test

After incubation 2-4 drops of methyl red solution were added to the test tube, which was incubated for 5 days. Positive test was indicated by the persistence of red colour, indicating acidity and negative by yellow colour.

Indole test

A sterilized test tube was taken containing 4 ml of tryptophan broth. The tube was inoculated aseptically by taking the growth from 18 to 24 hrs culture. The tube was incubated at 37°C for 24-28 hours. 0.5 ml of Kovac's reagent was to the broth culture. Then it was observed for the presence or absence of ring.

Pathological examination

Necropsy examination

The postmortem examination of all the cases was performed for the sick and dead birds. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathological studies.

Histopathological examination

Formalin-fixed tissues were dehydrated in alcohol, cleared in xylene and embedded in paraffin for histological examination. Then, sections were prepared with 5 µm thickness and, in accordance with Gofur et al. (2008), stained using the standard Mayer's Hematoxylin and Eosin. Stained tissue sections were examined thoroughly at magnifications of 10 and 40 using compound microscopes. A photographic microscope system (digital camera, model: C-B5, OPTIKA, Italy, attached with a microscope, Model B-293PLi, OPTIKA, Italy) was used to capture the photographs of the stained tissue sections.

Results

Prevalence of *E. coli* in cloaca of apparently healthy chickens and different tissues of sick and dead chickens

The prevalence of *E. coli* in apparently healthy birds of different poultry farms and different tissues of sick and dead chickens are shown in Table 1. The prevalence of *E. coli* was 82.7% (layer 84.4% and broiler 80%) in cloacal swabs of apparently healthy chickens (Table 1), whereas the overall prevalence of *E. coli* was 85.0 % in sick and dead chickens, particularly 90% in liver, 60% in heart, 80% in lung and 93.33% in duodenum samples (Table 1). The prevalence of *E.* coli infection was higher in duodenum than liver, lung and heart samples of sick and dead chickens. The prevalence of *E. coli* considering various age groups of bird were 65%, 50% and 10% in 0-2 weeks, 2-4 weeks and >4 weeks, respectively. The higher prevalence of *E. coli* found was observed in age group 0- 2 weeks and the lower prevalence in >4 weeks of age. The season was very statistically significant factor for outbreak of colibacillosis in commercial broiler and layer chicken. The higher prevalence was found in winter season 65% followed by summer season 35.71%.

Table 1: Prevalence of *E. coli* in apparently healthy, sick and dead chickens.

Type of bird	No. of birds examined	No. of +Ve cases	Prevalence (%)	Overall prevalence (%)
Layer	32	27	84.4	82.7
Broiler	20	16	80.0	
Sick and dead birds				
Organs examined	No. of sample examined	No. of +Ve cases	Prevalence	Overall prevalence
Liver	10	09	90.0	85.0
Lung	10	08	80.0	
Heart	05	03	60.0	
Duodenum	15	15	93.3	

Colony characters, staining characters and motility test of the identified bacteria

Colony characters: Circular, moist, smooth, flat rose-pink colored colonies on MacConkey agar (Fig. 1) and greenish metallic sheen in reflected light, dark or even black centre in transmitted light on EMB agar produced by the organisms (Fig. 2) after overnight incubation were confirmed as *E. coli*.

Staining characters: Light microscopic examination of *E. coli* isolates after Gram's staining revealed Gram-negative, pink colored, short rod-shaped organisms arranged as single or in pair (Fig. 3).

Motility test: All the *E. coli* isolates were found to be motile with 'hanging drop' preparation under microscope.

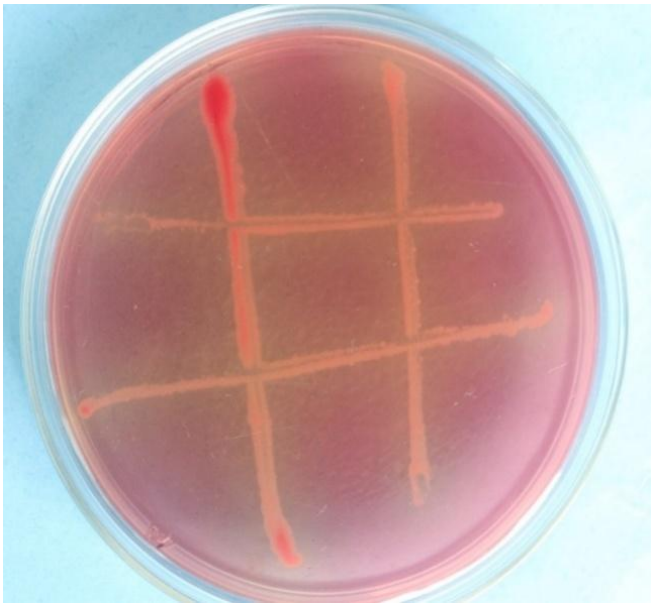

Fig. 1: *E. coli* isolates showing characteristics of rose-pink colored colonies on MacConkey agar.

Fig. 2: *E. coli* isolates showing characteristics greenish colored colonies with metallic sheen on eosin methylene blue agar (EMB).

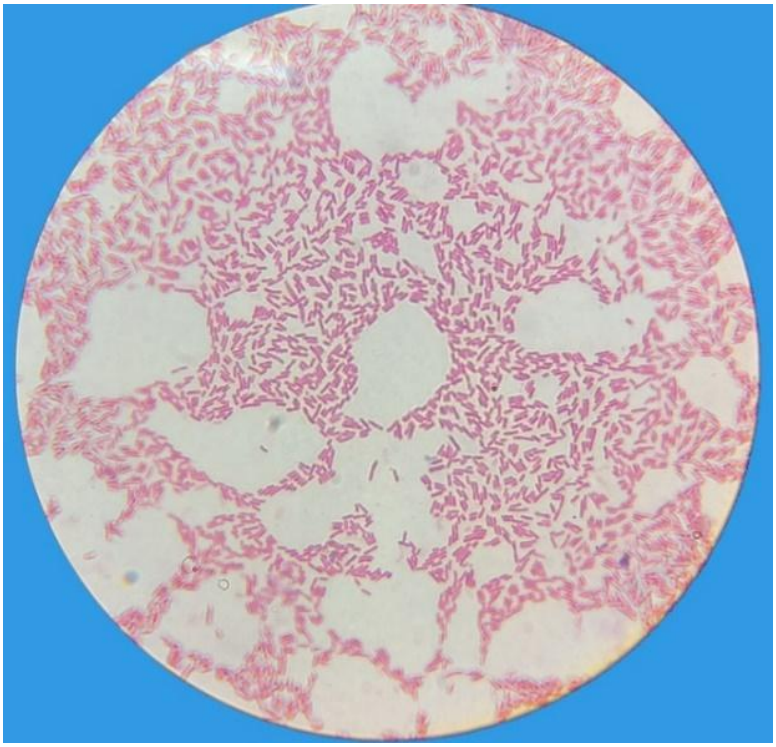

Fig. 3: Isolated *E. coli* in gram's straining (microscopic) showing gram negative, pink color, short rod shape organisms, arranged in single or paired.

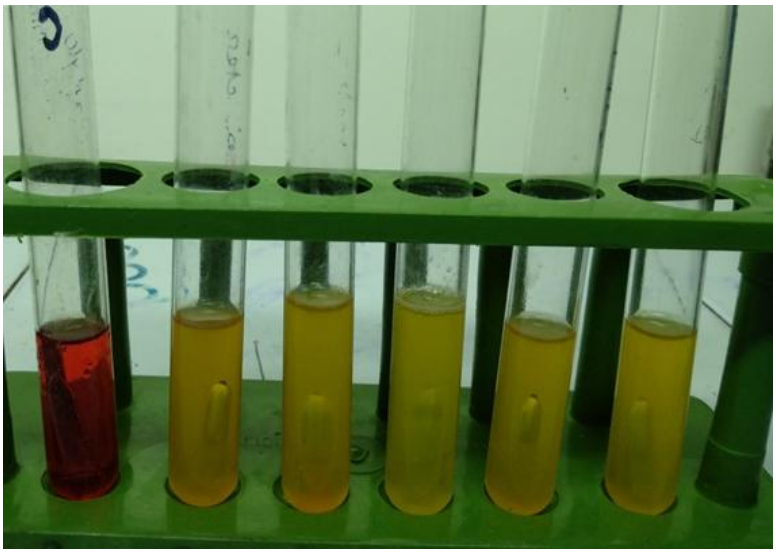

Fig. 4: Sugar fermentation test.

Biochemical test results of the identified bacteria

The results of biochemical tests of the identified bacteria were presented in Table 2. All the isolates fermented dextrose, lactose, maltose and mannitol with the production of acid and gas but did not ferment inositol. Acid production was indicated by the change from reddish to yellow and gas production by the accumulation of gas bubbles in the inverted Durham's tube (Fig. 4). All the isolates were methyl red test positive and presence of red colour ring in the tube, and indole test was also positive.

Table 2: Biochemical characteristics of *E. coli.*

Methyl red test	Indole test	Sugar test					Bacteria isolated
		DX	L	ML	MN	IN	
+	+	AG	AG	AG	AG	–	*E. coli*

Legends: DX, Dextrose; L, Lactose; ML, Maltose; MN, Mannitol; IN, Inositol; AG, Acid and gas; +, Positive; –, Negative.

Pathological study

Gross lesions

The observed postmortem findings were congested and consolidated lung with cloudy and thickened air sac (Fig. 5), congested and thickened capsule, haemorrhage, perihepatitis in liver, thickened pericardium due to fibrinous pericarditis and thickened pericardial sac with light yellow fibrinous exudates adhering to the heart (Fig. 6).

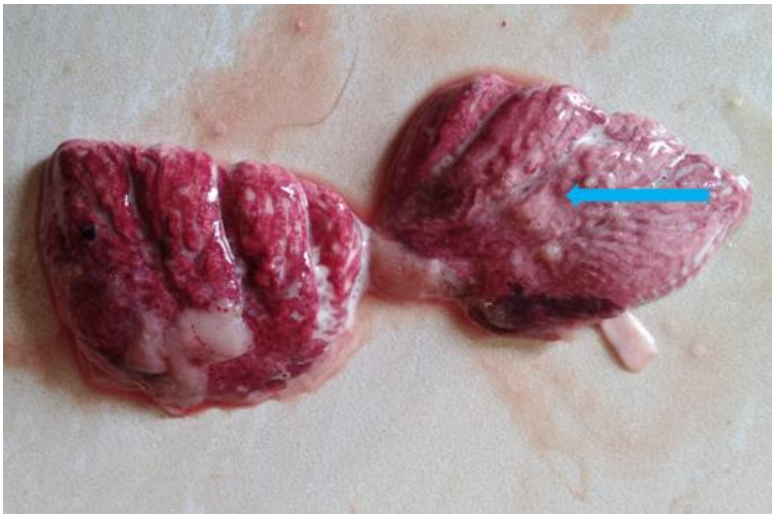

Fig. 5: Colibacillosis affected birds showing congested and consolidation lungs.

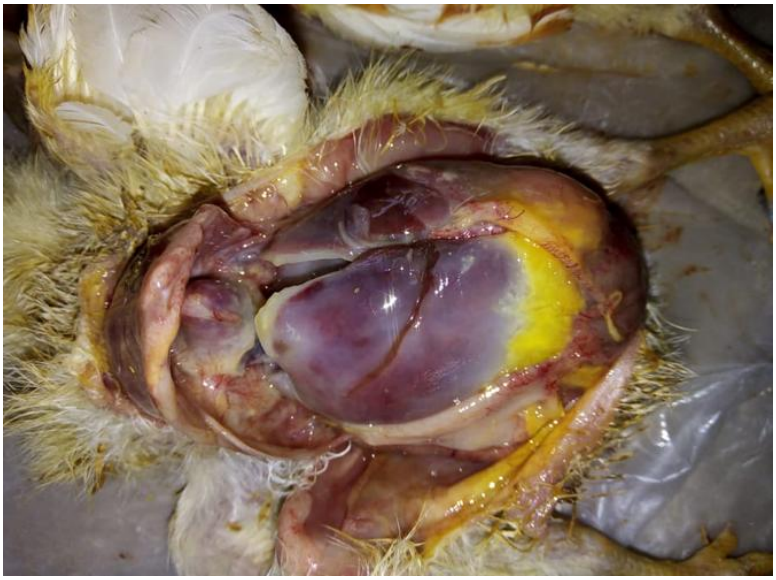

Fig. 6: Liver of *E. coli* infected chicken showing thickened liver capsule.

Microscopic lesions

The microscopic section of liver showed coagulation type of focal necrosis (Fig. 7), infiltration of heterophils, lymphocytes and macrophages mainly in portal area. In heart, pericarditis, characterized by

thickening of pericardium due to infiltration of RE cells, was observed (Fig. 8). The section of lungs showed congestion, hemorrhage, infiltration of heterophils, macrophages and lymphocytes in the wall of bronchus as well as in the peribranchial alveoli (Fig. 9). Blunting and sloughing off villus, infiltration of inflammatory cells were observed in duodenum (Fig. 10).

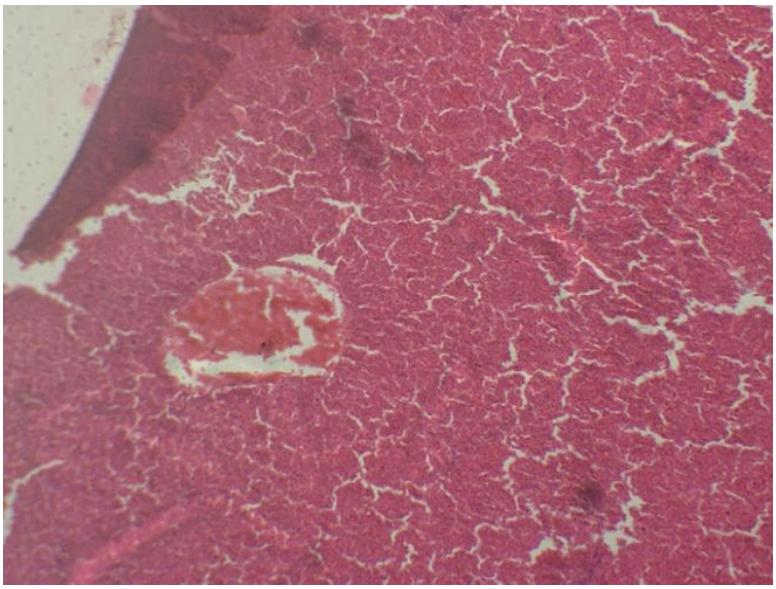

Fig. 7: The section of the liver of *E. coli* affected birds at 33 weeks of age showing coagulation type of necrosis.

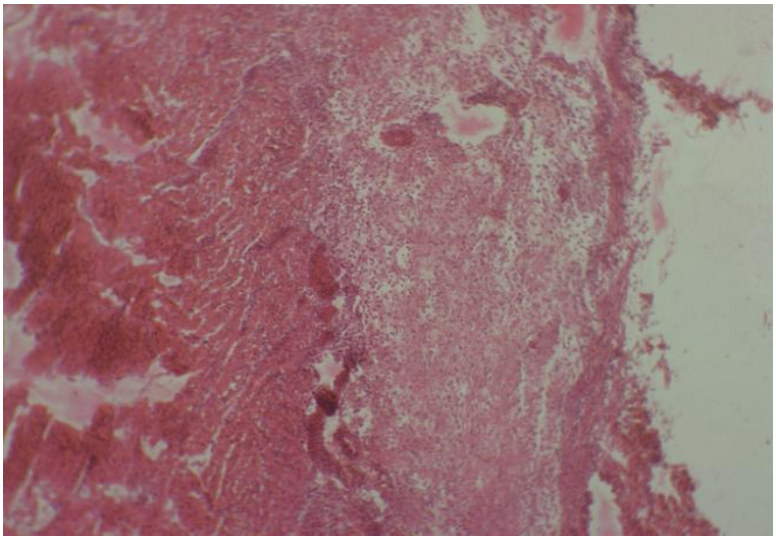

Fig. 8: The section of heart in *E. coli* infected chickens exhibiting pericarditis characterized by thickening of pericardium due to infiltration of RE cells.

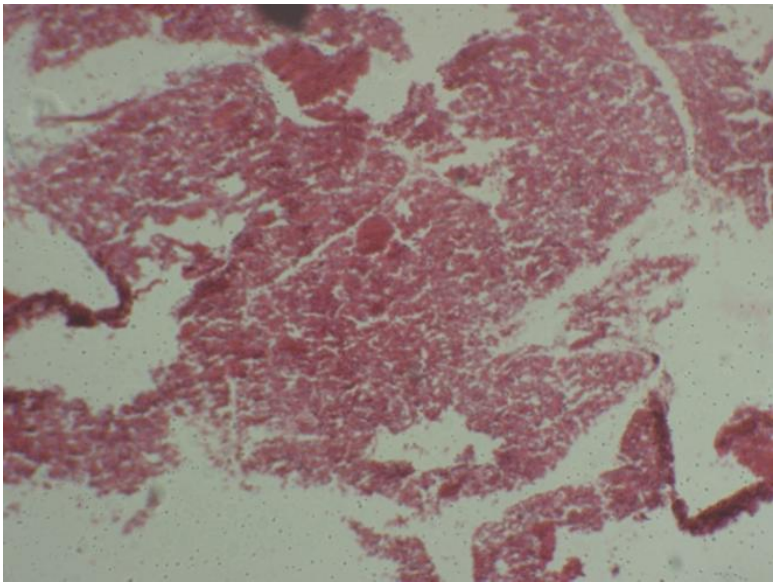

Fig. 9: Lungs of *E. coli* infected birds showed congestion, infiltration of heterophils in the wall of bronchus.

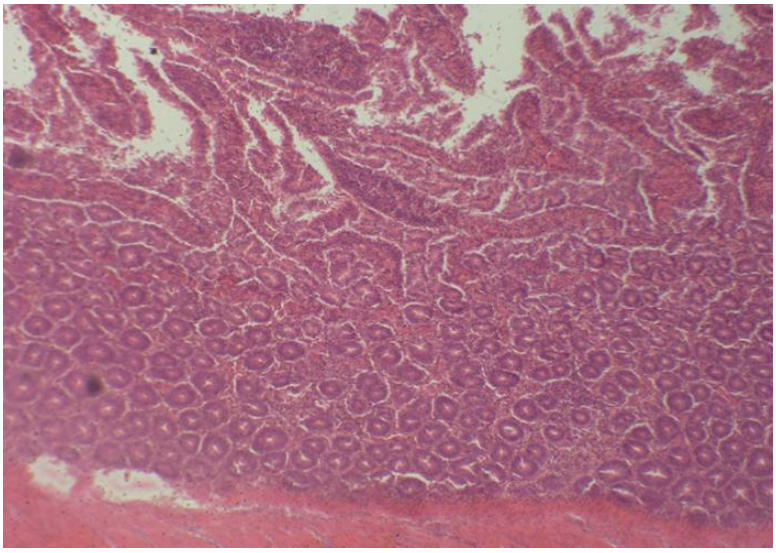

Fig. 10: Duodenum of *E. coli* infected birds showed blunting and sloughing off villus, infiltration of inflammatory cells.

Discussion

The prevalence of *E. coli* was 82.7% in cloacal samples and 85.00% in liver, lung, heart and duodenum samples in the present research work: these findings were in support with the findings of (Khaton et al. 2008) where they showed the prevalence is 83.07% and 87.27% respectively. However, the present findings were lowered than those of others (El-Sushon et al. 2002, Haider et al. 2003). On the other hand, Ahmed et al. (2009) reported 52.26% prevalence of *E. coli* in broiler chicken, which was much lower than the present study. Hashem et al. (2017) also found the average occurrence of colibacillosis was 54.55% in commercial broiler in Chittagong district in Bangladesh. The difference in *E. coli* prevalence might be due to the geographic location of farm, age and age and breeds of the birds and also for the resistance power of the commercial chickens due to proper management, vaccination and nutrition. Normally *E. coli* is present in the digestive tract of poultry. The stress factors of any types may enhance the virulence of *E. coli* with the production of disease (Talha et al. 2001). In the present study, the prevalence of *E. coli* isolation in healthy, sick and dead chickens was almost similar. The reasons of similar prevalence in the present work might be due to stress factors in the healthy chickens. The study revealed rose pink colored colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar

produced by the organisms after overnight incubation were confirmed as *E. coli*; these colony characteristics corresponded with the findings of previous studies (Sharada et al. 1999, Khaton et al. 2008, Tonu et al. 2011). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative, pink color, short rod to coccoid shape and in the hanging drop technique, all the isolates revealed motile. These findings supported the findings of earlier studies (Khaton et al. 2008, Tonu et al. 2011). In the present study, the entire isolated *E. coli* organisms revealed a complete fermentation of several sugars like dextrose, lactose, maltose, mannitol; and the organisms were motile but did not ferment inositol. These findings also supported the findings of Beutin et al. (1997). The results of methyl red test of all isolates of *E. coli* were positive, like the findings of Mishra et al. (2002) and indole test positive, similar to the findings of Wang et al. (2001). Susceptibility of *E. coli* varies with the age of birds (Awad et al. 2020). The highest prevalence of colibacillosis was 65% in age group 0–2 weeks and the lowest in >4 weeks of age which was 10%. 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 (5.49-8.78%), and this study also recorded widely prevalent of *E coli* infection in all age groups of chickens (9.52-13.73%). The occurrence of *E. coli* was higher in winter season (65%) followed by summer season (35.71%). Hashem et al. (2017) also reported higher *E. coli* infection in winter season (67.54%) in Bangladesh. Lambie et al. (2000) reported higher *E coli* infection during rainy season (67.2%). We found an association of risk factors like age, and season of the year with the occurrence of colibacillosis which is supported by the earlier report of Rahman et al. (2004).

To characterize the colibacillosis, pathological study was done by necropsy and histopathological examination. The present study showed different forms (gross lesions) of colibacillosis by necropsy. The observed postmortem findings were congested and thickened capsule, hemorrhage, perihepatitis in liver; congested and consolidated lung with cloudy and thickened air sac, thickened pericardium due to fibrinous pericarditis and thickened pericardial sac with light yellow fibrinous exudates adhering to the heart, mucus, congestion and hemorrhage in the duodenum. The severity of gross lesions was variable in chickens in the present study. These necropsy findings of colibacillosis were also observed by different authors (Islam et al. 2023, Rahman et al. 2004, Ghosh et al. 2006, Chowdhury et al. 2009, Dhama et al. 2013). The histopathological lesions of colibacillosis were coagulation type of focal necrosis in liver, hemorrhage and highly congestion in lung, destruction of intestinal wall and reactive cell infiltration, thickening of fibrous tissue in pericardium. Similar histopathological lesions have been reported by (Talha et al. 2001, Islam et al. 2003, Ghosh et al. 2006, Khaton et al. 2008, Tonu et al. 2011) in colibacillosis infected broiler chickens. *E. coli* singly does not produce typical gross lesions. The lesions are most prominent when simultaneously infected with other organisms such as other *bacteria,* mycoplasma etc. (Tonu et al. 2011, McMullin 2020). In this study identification of other *bacteria or* mycoplasma was not carried out. *E. coli* can produce diseases becoming attached with mucosal epithelia and another form by invasion to the mucosal epithelia. The recorded lesions of intestine were in the form of degeneration, necrosis and desquamation of mucosal epithelia associated with severe inflammation. On the basis of the recorded lesions, the form of colibacillosis in the present study could be categorized into entero-invasive form of colibacillosis. These types of histopathological lesions in recorded forms of colibacillosis were supported by different authors (Talha et al. 2001, Islam et al. 2003, Ghosh et al. 2006).

Conclusion

The occurrence of colibacillosis was different in apparently healthy, sick and dead birds, and also varied with the age of broilers and in different seasons. The higher prevalence was in age group 0–2 weeks and in winter season. The most frequent gross lesions were congested and thickened liver capsule; congested and consolidated lungs; cloudy and thickened air sac; mucus, congestion and hemorrhage in duodenum. The major microscopic lesions were hemorrhage and highly congestion in lungs, destruction of intestinal wall and reactive cell infiltration, and thickening of fibrous tissue in pericardium. In summary, a higher prevalence of colibacillosis (>80%) was in commercial poultry farms of city corporation area of Rajshahi district, and age and season were the significant risk factor of colibacillosis in broiler chickens.

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Conflict of interest: The authors declare no conflict of interest regarding the publication of this article.

Author's contribution: AK collected samples and data, conducted experiments. RK designed the experiment, supervised the study, and corrected the manuscript. All authors have read and approved the final manuscript.

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Data availability: All data generated in the study are mentioned in the article and unprocessed data will be available to the corresponding author upon request.

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