Asian Australas. J. Biosci. Biotechnol. 2017, 2 (2), 173-180

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Prevalence and pathological investigations of avian colibacillosis in commercial broiler at Chittagong district in Bangladesh

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Received: 07 August 2017/Accepted: 27 August 2017/ Published: 31 August 2017

Abstract: This study was carried out to identify and observe prevalence and pathological lesions of avian colibacillosis in commercial broiler in variation of litter type, age, farm size, source of water and season of year in Chittagong region. 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 275 broilers of 104 farms were examined through post-mortem and among them 150 (54.55%) broiler was diagnosed as affected with any lesion of colibacillosis. The most frequent gross lesions of colibacillosis were air sacculitis (20.67%), omphalitis (12.00%), pericarditis (13.33%), perihepatitis (13.33%), peritonitis (1.33%), colisepticemia (8.00%), enteritis (8.00%) and a large number 35 (23.33%) of broiler combination of different form of colibacillosis. The microscopic lesions of these conditions were chronic passive congestion, fibrosis of liver capsule, telangiectasis, thick fibrous layer in the pericardium of heart, blunting and sloughing off villus and infiltration of lamina propria of intestine. The results obtained during the study period revealed that winter season of the year was significant ($p>0.05$) high risk factors of colibacillosis in broiler but litter, age, farm size, source of water were not significantly risk factor. Considering the factor it could be concluded based on pathology that colibacillosis causes high mortality and winter season was the main risk factor in broiler at Chittagong District.

Keywords: broiler; colibacillosis; prevalence; pathology

1. Introduction

Avian colibacillosis is an infectious disease of birds caused by *Escherichia coli* which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen (Kabir, 2010; Kabir *et al*., 2017). It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis. colibacillosis of poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by peri-carditis, airsacculitis and peri-hepatitis (Calnek *et al*., 1997). 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). 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). Considering the above facts the present study was designed to investigate the prevalence, risk factors and of pathological lesions colibacillosis in commercial broiler farm at different region of Chittagong district.

2. Materials and Methods

2.1. Experimental design

Data was collected from broiler farms through public service rendered by the Department of Pathology and Parasitology and the Department of Physiology and Biochemistry, Pharmacology of Chittagong Veterinary and Animal Sciences University. Sometimes farmers came to the department to take the service to diagnose the cause of mortality in their birds. Then data was recorded into a register note book regarding the location of farm, type of litter used, source of drinking water, and number of birds, age and diagnosis with forms of colibacillosis. After that samples (liver, heart and intestine) were collected from live and dead birds for histopathological and bacteriological examination respectively that was positive by postmortem examination.

2.2. Study Period and place

This study was carried out in the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Chittagong from July 2009 to June 2010.

2.3. 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.

2.4. Post mortem examination and sample collection

Post mortem of the birds and diagnosis of the disease with forms of colibacillosis were done by expert technical hands by following standard procedures. All samples (liver, heart, lungs and intestine) were collected from post mortem birds in separate zipper lock bag and then it was kept in deep freeze for bacteriological examination to identify the *Escherichia coli* colony.

2.5. Bacteriological examination

Among birds came for post mortem in the campus, *E.coli* suspected samples were randomly selected and were sent to the Department of Microbiology for the identification of colony of *E. coli*. Neutrien broth, MacConkey agar and Eosin methyline blue (EMB) agar were used to identification of the colony of *E. coli*.

2.6. Inoculation of the sample on agar plate

The tissues were collected in sterile petridish. At first the tissue surface was touched with hot spatula or knife and then was cut with sterile sharp knife. Then the freshly cut surface was touched with solid media on plate that was further separated by bacteriological loop. All inoculation work was done within laminar flow to avoid chance of contamination. Then the petridish were incubated overnight at 37^0C temperatures. The petridishes were placed in inverted position to avoid dissolving by condensed vapors.

2.7. Histopathological Examination

All samples (liver, heart and intestine) were collected from live birds after postmortem that were brought to the Department of Pathology and Parasitology and the Department of Biochemistry, Pharmacology and physiology

and then it was preserved in 10% buffered formalin/Bouin's solution for histopathological examination. Formalin fixed tissue samples were processed and stained as per standard method **(**Luna, 1968).

2.8. Statistical analysis

Data were entered into a database (spreadsheet of Microsoft Excel) and was analyzed with P value by using Graph pad software.

3. Results

3.1. Prevalence of colibacillosis in commercial broiler

Prevalence of colibacillosis on the basis of type of litter was 58.82% and 53.14% in rice-husk and wood-shaving respectively (Table 1), where the highest outbreak in rice-husk as compare to wood shaving. The outbreak of colibacillosis considering various age groups of bird was 67.36%, 52.76% and 0.00% in 0 - ≤ 2 weeks and >2 - ≤4 weeks and >4 weeks respectively (Table 2). The highest significance (P=0.4468) outbreak in age group 0 - ≤ 2 weeks and the lowest in group >4 weeks. Occurrence of colibacillosis on the basis of farm size was 42.85%, 46.20% and 82.35% in small, medium and large size farm respectively (Table 3). The highest (82.35%) outbreak was in large farm followed by medium (46.20%) and small (42.85%) farm.

Table 1. Prevalence of colibacillosis on the basis of type of litter used.

Type of litter	No. of PM Birds	No. of *E. coli* affected Birds	Percentage	P-value
Wood Shaving	207	110	53.14	0.3718
Rice Husk	68	40	58.82	

Table 2. Prevalence of colibacillosis on the basis of age group.

Age group (week)	No. of PM birds	No. of *E. coli* affected bird	Percentage	P-value
0 - ≤ 2	95	64	67.36	0.4468
>2 - ≤4	163	86	52.76	
>4	17	0	0.00	

Table 3. Prevalence of colibacillosis on the basis of farm size.

Farm size	No. of PM birds	No. of *E. coli* affected bird	Percentage	P-value
Small (300-800)	49	21	42.85	0.3224
Medium (900-1900)	158	73	46.20	
Large (2000-3000)	68	56	82.35	

Table 4. Prevalence of colibacillosis on the basis of source of water.

Source of water	No. of PM Birds	No. of *E. coli* affected Bird	Percentage	P-value
Tubewell	252	133	52.78	0.2026
WASA	23	17	73.91	

Table 5. Prevalence of colibacillosis on the basis of season.

Season	No. of PM birds	No. of *E. coli* affected bird	Percentage	P-value
Winter	151	102	67.54	0.0056**
Summer	124	48	38.71	

**=Means p<0.01

The *Escherichia coli* infection was higher (73.91%) in farm with supplied WASA water than tube well water (52.78%) (Table 4). Tube well and washa supplied water was not statistically significant (P=0.2026) factor for outbreak of colibacillosis. The season was very statistically significant (p<0.01) factor for outbreak of colibacillosis in commercial broiler chicken. The highest occurrence in winter season (67.54%) followed by summer season (38.71%) (Table 5).

3.2. Pathology of colibacillosis

3.2.1. Gross lesions of colibacillosis

During post mortem, different forms of colibacillosis were observed, those are, airsacculitis (Figure 7), omphalitis (Figure 4 & 6), pericarditis (Figure 3), perihepatitis (Figure 5), peritonitis (Figure 6), colisepticemia (Figure 7, petechial haemorrhages in the spleen, heart and liver, enteritis (Figure 2), haemorrhage and mucus in intestine and complex occurrence of colibacillosis with different forms and different diseases at a time (Figure 1). Among different forms of disease, airsacculits was the highest percent (20.67%) of occurrence and lowest was peritonitis (1.33%) (Figure 1). There after, pericarditis and perihepatitis were (13.33%). On the other hand coliseptecemia and enteritis were (8.00%). 23.33% birds was a complex form of colibacillosis. Among complex forms, there were observed colibacillosis complexes with mycoplasmosis, necrotic enteritis, coccidiosis, newcastle, gumboro, brooder pneumonia and chronic respiratory disease. Different forms of colibacillosis were also observed occur together.

3.2.2. Microscopic lesions of colibacillosis in different organs

In liver, there are found chronic passive congestion, infiltration of heterophils, lymphocytes and macrophages surrounding the portal veins (Figure 10). Fibrosis of liver capsule due to perihepatitis. Blood vessels become dilated (telangiectasis) (Figure 11). In heart, observed thick fibrous layer in the pericardium due to infiltration of reticulo endothelial (RE) cells (Figure 10 & 11). In intestine Blunting and sloughing off villus (Figure 9) are observed. Infiltration of lamina propria due to severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages was found in the sub-mucosa (Figure 8).

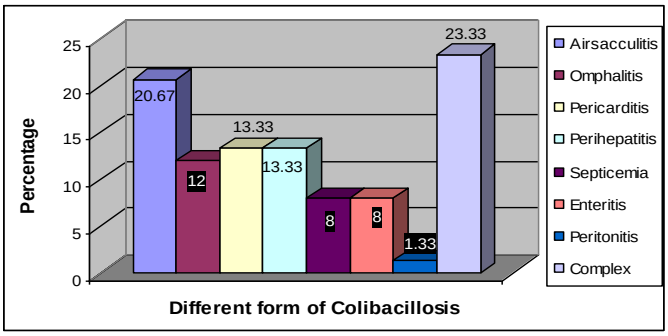

Figure 1. Occurrence of different forms of colibacillosis in commercial broiler farm.

Figure 2. *Escherichia coli* infection in 18-day-old broiler showing enteritis with inflammatory exudates in mucosa

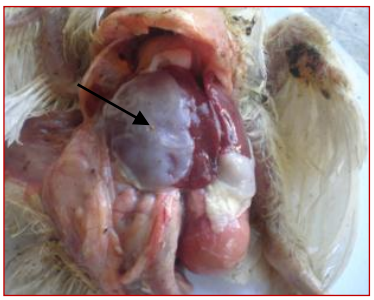

Figure 3. A 23-day-old broiler died of *Escherichia coli* showing fibrinous pericarditis and thickened pericardial sac with light yellow fibrinous exudates adhering to the heart

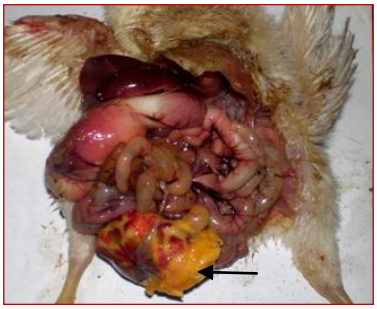

Figure 4. *Escherichia coli* infection in 4-day-old broiler showing unabsorbed yalk

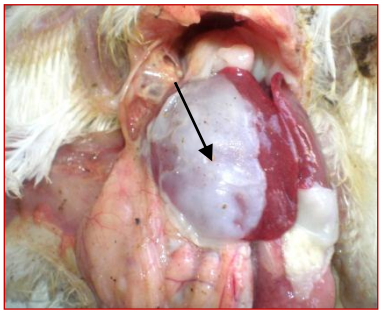

Figure 5. A 17-day-old broiler died of *Escherichia coli* showing perihepatitis and thickened light yellow fibrinous exudates adhering to the liver

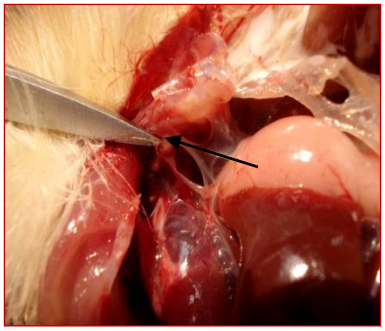

Figure 6. A 24 days old broiler died of *Escherichia coli* infection showing Omphalitis & Peritonitis

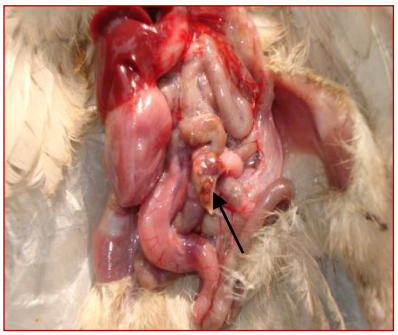

Figure 7. Air sacculitis and septecemia showing in 27 days *Escherichia coli* infected broiler birds

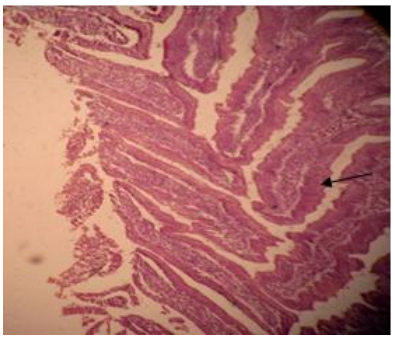

Figure 8. Infiltration of lamina propria due to inflammatory cells in intestine of broiler chicks

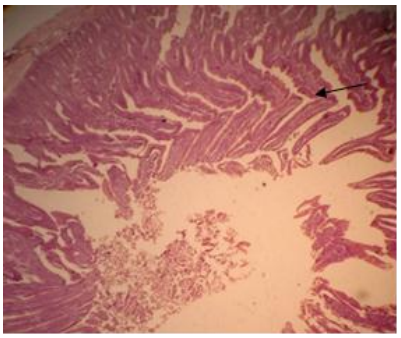

Figure 9. Blunting and sloughing off villus of broiler chicks

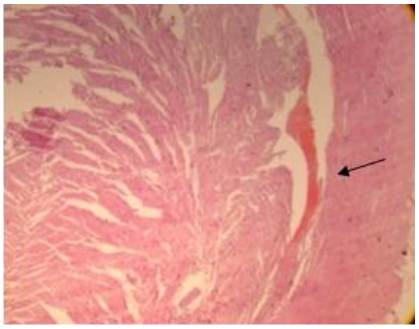

Figure 10. Thick fibrous layer in the pericardium due to pericarditis in heart of broiler chicks

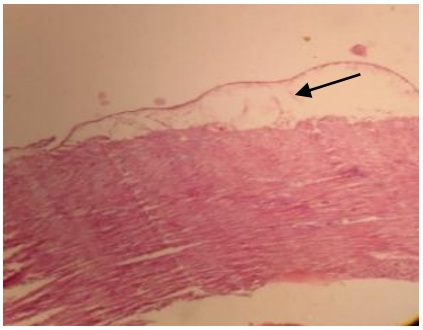

Figure 11. Thick fibrous layer in the pericardium due to pericarditis in heart of broiler chicks

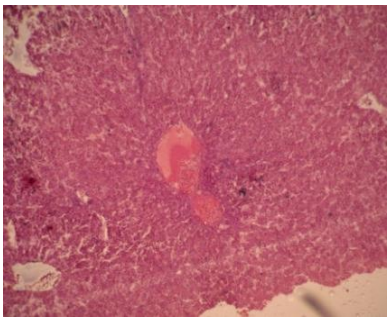

Figure 12. Chronic passive congestion due to infiltration of inflammatory cells surrounding the portal vein of liver of broiler chicks

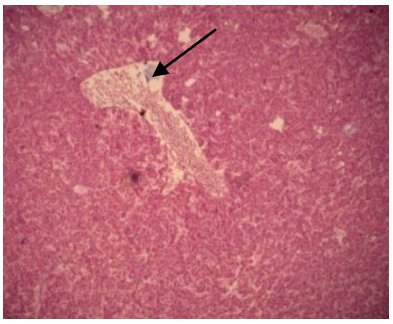

Figure 13. Blood vessels become dilated resulting talengioactasis in liver of broiler chicks

4. Discussion

In this study, the average occurrence of colibacillosis was 54.55% 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. Bhattacharjee *et al.* (1996) reported 40.82% and Ahmed *et al.* (2009) reported52.26% 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 ($p>0.01$) influence of age group of birds was found to be related with the increase susceptibility of colibacillosis. The highest significant ($P>0.01$) outbreak was in age group 0 - ≤ 2 weeks and the lowest in age group of >4 weeks. 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 Bhattacharjee *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 it was found that the highest (82.35%) outbreak of colibacillosis in large size of farm followed by medium (46.20%) and small (42.85%) size farm. The farm size was not significant for outbreak of colibacillosis in broiler. There was no published data on that context. The *E. coli* infection was higher (73.91%) in farm with supplied WASA water than tube well water (52.78%). Tube well and WASA supplied water was not statistically significant ($P=0.2026$) factor for outbreak of colibacillosis. There are little or no published data on that context.

Colibacillosis was recorded more or less uniformly in all the three seasons of the year. The season was very statistically significant ($p<0.01$) factor for outbreak of colibacillosis in commercial broiler chicks. In this study, the highest occurrence in winter season (67.54%) followed by summer season (38.71%). Bhattacharjee *et al.* (1996) also reported avian colibacillosis in all the seasons of the year in Bangladesh. Pandey *et al.* (1998) reported outbreaks of *E. coli* infection during November to March, and Lambie *et al.* (2000) reported higher *E coli* infection during rainy season. Finally in this study the associated risk factors of colibacillosis were age, farm size and season of the year which is supported the earlier report of Rahman *et al.* (2004).

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 Cornelissen 2006; Rahman *et al*. 2004; Shah e*t al*. 2003). Among different forms of colibacillosiis, airsacculits was the highest percent (20.67%) of occurrence and the lowest was peritonitis (1.33%). There after, pericarditis and perihepatitis were (13.33%). On the other hand coliseptecemia and enteritis were (8.00%). Jordan *et al.* (1995) has found 46% omphalitis in his study and then 15% peritonitis in broiler. The microscopic lesions in liver showed chronic passive congestion, infiltration of heterophils, lymphocytes and macrophages surrounding the portal veins, fibrosis of capsule, dilated blood vessels. pericarditis was characterized by thickening of pericardium due to infiltration of RE cells. The intestine showed blunting and sloughing off villus, Infiltration of lamina propria due to severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages was found in the sub-mucosa. 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) and Talha *et al*. (2001).

In this study a large number of birds (23.33%) showed a complex forms of *E. coli* infection which includes the occurrence of different forms of colibacillosis at a time as well as the occurrence of colibacillosis with different diseases including mycoplasmosis, necrotic enteritis, coccidiosis, sometimes with newcastle, gumboro,brooder pneumonia and CRD etc. It may be due to damages in respiratory tract of birds caused by these viruses and bacteria and this damaged respiratory tract becomes extremely susceptible to invasion by *E. coli* (Gross, 1961). But Landman and Cornelissen (2006) said that, unlike colibacillosis in broilers, recent research has failed to demonstrate an association between several pathogens of the respiratory tract and the occurrence of *E. coli* pathology in layer chickens. Though Piercy and Westa (2004) had shown in their study that several respiratory pathogens (mycoplasma, Newcastle disease) had an unknown relationship with the occurrence of colibacillosis in both layer and broiler.

5. Conclusions

The results obtained during the study period revealed that litter, source of water, age of birds and farm size were not significant for colibacillosi but season of the year was statistically significant ($p<0.01$) risk factor of colibacillosis in broiler.

Acknowledgements

The authors are thankful to the authorities of research and extension, Chittagong Veterinary and Animal Sciences University for providing financial supports for this study.

Conflict of interest

None to declare.

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