

Colibacillosis in commercial chickens in Bangladesh

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Abstract

The prevalence of colibacillosis in layer chickens was studied from May to September 2007. Sixty five cloacal swabs from apparently healthy birds and 55 swabs of liver (n=15), lung (n=15) and intestine (n=25) from 30 dead birds were collected in sterile nutrient broth, with histopathological samples. Bacteria were isolated and identified. Tissue samples were studied under light microscope. *Escherichia coli* (*E. coli*) was isolated from 83% of cloacal swabs of apparently healthy chickens and 87% of samples from dead birds. Affected birds had cloudy thickened air sacs, pericarditis, 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. (*Bangl. vet.* 2007. Vol. 25, No. 1, 17-24)

Introduction

In Bangladesh 98% of poultry are kept in rural area as scavengers (BBS, 1987). 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 advancement of poultry industry is facing a problem due to sudden outbreak of bacterial diseases, including colibacillosis, which poses a serious threat in Bangladesh.

Colibacillosis, caused by *E. coli*, is a major disease of commercial poultry all over the world. 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). 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). With expansion of poultry farming, colibacillosis has become a widespread problem in Bangladesh (Islam *et al.*, 2003; Rahman, 2003b; Hossain *et al.*, 2004). 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). Mortality due to colibacillosis in Bangladesh

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may reach 94% in severe outbreaks (Biswas *et al.*, 2006; Haider *et al.*, 2003; Roy *et al.*, 2006).

Investigations on colibacillosis in poultry are still scanty in Bangladesh. The disease pattern is variable. This study was undertaken to know the status of colibacillosis in two commercial poultry farms with isolation and identification of causal agents.

Materials and Methods

Samples

The study was conducted from May to September 2007. Fifteen samples from layers of 68 weeks old chickens of SEVER 579 from the Bangladesh Agricultural University (BAU) Poultry Farm and 50 samples from layers of 75 weeks old chickens of ISA Brown from Krisibid Poultry Farm, Bhaluka, Mymensingh district were collected in nutrient broth. The samples [55 swabs from liver (n=15), lung (n=15), and intestine (n=25)] of 30 dead birds were collected in nutrient broth. The samples were carried in ice box for further study. Samples for histopathology were collected in plastic pot containing 10% neutral buffered formalin.

Sample collection

Samples were collected using sterile cotton-tipped swabs from the cloaca of healthy chickens. The swabs were inserted into sterilized screw-capped test tubes containing nutrient broth and preserved at 4°C. After dissecting the dead birds, the liver, lung and intestine were incised with sterile scissors and intestinal faeces were scraped with a sterile scalpel. Samples were collected from the liver, lung and intestine and swabs were handled carefully as above.

Identification of isolated E. coli

Cloacal and intestinal samples were placed on nutrient agar plate and incubated overnight at 37°C. After primary culture, a small amount of inoculum was subcultured in nutrient agar and MacConkey agar to observe colony morphology. Colonies with the features of *E. coli* were selected for subculture on selective media such as Eosin Methylene Blue (EMB) agar (Carter, 1986).

Morphological characterization by Gram-stain

Representative *Salmonella* colonies were characterized microscopically using Gram's stain as described by Freeman (1985).

Carbohydrate fermentation test

The carbohydrate fermentation test was performed by inoculating 5 ml of nutrient 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.

Voges-Proskauer (V-P) test

The VP test was performed by adding 0.6 ml of VP reagent-1 and 0.2 ml of VP reagent-2 for each ml of culture. The ingredients were mixed thoroughly and allowed to stand. A pink colour indicated a positive test.

Motility test

The motility test was performed as described by Carter (1986). A pure culture of the organism was grown in nutrient broth. The motile organisms were identified visually.

Maintenance of stock culture

Organisms from pure culture were inoculated into tubes containing EMB slant and incubated at 37°C for 24 hours. After the growth of organisms the tubes were sealed with sterile liquid paraffin (light) and kept at 4°C for further studies.

Gross pathology

At necropsy, gross tissue changes were recorded, and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathology (Stubbs, 1954).

Histopathology

The formalin-fixed tissues were processed using standard procedures (Luna, 1968).

Photomicrography

Photomicrography was taken using Olympus PM-C 35 camera on Olympus microscope (Olympus, Japan).

Results and Discussion

Prevalence of E. coli in cloaca of healthy chickens and dead chickens.

The prevalence of *E. coli* was 83% in apparently healthy chickens (Table 1). The prevalence of *E. coli* was 87% in 15 liver samples, 73% in 15 lung samples and 96% in

25 intestine samples from 30 dead chickens (Table 2). The prevalence was higher in intestine and liver than lung. However, these findings were lower than those of others (Derakhshantar and Ghanbarpour, 2002; El-Sukhon *et al.*, 2002; Haider *et al.*, 2003) possibly due to the age and breeds of the birds and the resistance of commercial chickens due to better management, vaccination and nutrition. *E. coli* is normally present in the digestive tract of poultry. Stress may enhance the virulence of *E. coli*, leading to disease (Talha *et al.*, 2001).

Table 1. Prevalence of *E. coli* from cloacal swabs of apparently healthy layer chickens

Samples/Swabs	No. of samples examined	Total number of +ve isolates	Overall prevalence (%)
Cloacal swabs	65	54	83

Table 2. Prevalence of *E. coli* from 30 layer dead chickens

Samples	No. of samples	No. +ve	Prevalence (%)	Total No. of +ve isolates	Overall prevalence (%)
Liver	15	13	87%	48	87%
Lung	15	11	73%		
Intestine	25	24	96%		

Colony characters

Pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar (Fig. 1) after overnight incubation were confirmed as *E. coli*: these colony characteristics correspond with the findings of others (Ali *et al.*, 1998; Sharada *et al.*, 1999).

Staining characters and motility test

Gram's stain

Microscopy revealed Gram-negative, pink, short rod-shaped organisms arranged singly or in pairs (Fig. 2).

Motility test

All the *E. coli* isolates were motile. The staining characteristics and motility test were similar to the findings of others (Buxton and Fraser, 1977; Thomas, 1988).

Biochemical tests of the identified bacteria

Sugar fermentation test

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 (Table 3). All the isolates

were methyl red positive and Voges-Proskauer test negative (Table 3). These findings support the findings of Buxton and Fraser (1977); Freeman (1985); Mishra *et al.* (2002).

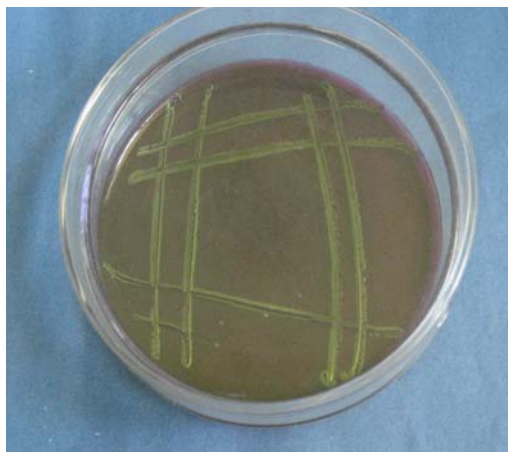


Fig. 1. *E. coli* colonies showing greenish colour with metallic sheen on Eosin Methylene Blue agar



Fig. 2. *E. coli* in Gram's stain showing Gram negative, pink, short rod-shaped organisms, singly or in pairs ($\times 830$)

Table 3. Biochemical characteristics of *E. coli*

Methyl red test	Voges-Proskauer test	Motility test	Sugar test				Bacteria isolated
			DX	L	ML	MN	
+	-	+	AG	AG	AG	AG	<i>E. coli</i>

Legends : DX= Dextrose, L = Lactose, ML = Maltose, MN = Mannitol, AG = Acid and gas, + = Positive, - = Negative

Pathological study

Gross lesions

Post-mortem findings revealed cloudy and thickened air sacs (air sacculitis), congested and thickened liver capsule (Fig. 3) and congested and consolidated lung (Fig. 4) in some chickens. The pericardium was thickened and the spleen enlarged with severe congestion. All these lesions indicated the septicaemic form of colibacillosis. The duodenum showed mucus, congestion and haemorrhage (enteritis) and there was haemorrhage in the caecal tonsil. *E. coli* can cause several disease conditions (Barens and Gross, 1997; Chauhan and Roy, 1996). In the present study not all conditions induced by *E. coli* were recorded.

Microscopic lesions

The livers showed coagulation type of focal necrosis, infiltration of heterophils, lymphocytes, and macrophages mainly in portal area (Fig. 5). Spleen showed

scattered pyknosis of lymphocytes and RE cell proliferation. Pericarditis was characterized by thickening of pericardium due to infiltration of RE cells. The lungs showed severe congestion, infiltration of heterophils, macrophages and lymphocytes in the wall of the bronchus as well as in the peribronchial alveoli (Fig. 6). In duodenum, there was severe infiltration of leukocytes, mainly heterophils, lymphocytes and macrophages in the submucosa. The colibacillosis in the present study could be categorized as entero-invasive. Similar lesions have been reported by Ghosh *et al.* (2006); Islam *et al.* (2003); Talha *et al.* (2001); Gagandeep *et al.* (2004); Zhou *et al.* (2002).



Fig. 3. Liver of *E. coli* infected chickens showing thickened capsule.



Fig. 4. Lungs of *E. coli* infected chickens showing congestion and consolidation

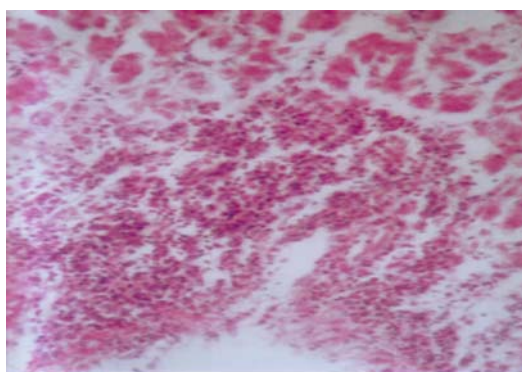


Fig. 5. Section of liver with *E. coli* infection showing infiltration of heterophils, lymphocytes and macrophages mainly in portal area (H&E, x 330)

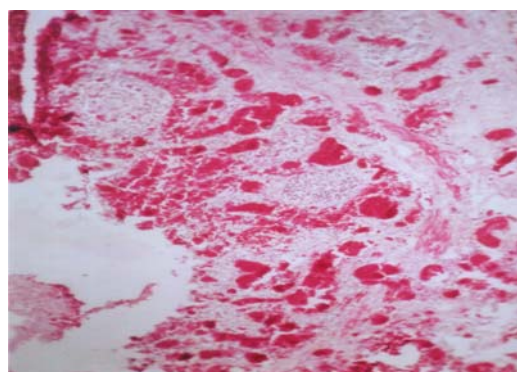


Fig. 6. Section of lung with *E. coli* infection showing severe congestion and infiltration of heterophils, macrophages and lymphocytes in the bronchial wall and peribronchial alveoli (H&E, x 830)

The present study was conducted to determine prevalence of *E. coli* in apparently healthy and dead chickens and the pathological lesions in dead birds. The prevalence of *E. coli* in apparently healthy chickens was 83% and in dead chickens

87%. Further investigation should be focused on typing of *E. coli* and antibiotic sensitivity test.

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