

## CHARACTERIZATION AND ANTIBIOGRAM OF *ESCHERICHIA COLI* ASSOCIATED WITH MORTALITY IN BROILERS AND DUCKLINGS IN BANGLADESH

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### ABSTRACT

Mortality in broilers ( 6.56% ) and growing ducks ( 11.0% ) caused by *Escherichia coli* was recorded in the experimental flocks study during the period from May to August 2003. *E. coli* organisms isolated from broiler birds affected with characteristic lesions of omphalitis and yolk sac infection, fibrinous peri-carditis and peri-hepatitis, hemorrhagic enteritis, and accumulation of excessive pericardial and peritoneal fluid, whereas from ducks with lesions of hemorrhagic enteritis and extensive epicardial hemorrhages. Each of the 21 isolates collected from broilers and 11 isolates from ducks was characterized by cultural and biochemical studies, of which 8 isolates from broilers and 5 isolates from ducks were tested for antibiotic sensitivity with 9 different antibiotics. The antibiotic sensitivity pattern showed that the isolates were highly sensitive to ciprofloxacin but an increasing trend of resistance was recorded in broilers ( 7 / 9 ) than duck ( 4 / 9 ) isolates. It may be concluded from the results of this study that the high rate of *E. coli* infection in broilers and ducks along with the high resistance of isolates to antibiotics constitute a threat to the poultry industry in Bangladesh.

**Key words:** *Escherichia coli* infection, mortality, broilers, ducks, characterization, antibiogram

### INTRODUCTION

Avian colibacillosis, caused by *Escherichia coli* is an infectious disease of birds, which is regarded as one of the main causes of morbidity and mortality, associated with heavy economic losses to poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen. It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting death and in its subacute form by peri-carditis, airsacculitis and peri-hepatitis ( Calnek *et al.*, 1997 ). Colibacillosis was first described in chickens in 1894, and since then, there have been numerous reports on colibacillosis in poultry and considerable research on the disease has been made elsewhere but it has not so far been documented in poultry from Bangladesh ( Samad, 2000 ). Because of the lack of an effective commercial vaccine for the control of avian colibacillosis, the control of the disease mainly relies on the use of antimicrobial drugs. This leads to indiscriminate use of antimicrobial drugs in poultry industry without prior testing of the etiologic agent, might have resulted antibiotic resistance which is a serious problem because it limits the therapeutic possibilities in the treatment of bacterial diseases. Considering these facts, a study was undertaken to determine the present status of colibacillosis in broilers and ducks as well as its antibiotic sensitivity to the isolated organisms.

### MATERIALS AND METHODS

During the period May to August 2003, day-old broiler chicks were purchased from five hatcheries, of which 120 from Goalundo hatcheries, 50 from Kazi hatcheries, 50 from Aftab hatcheries, 50 from Paragon hatcheries and 50 from BRAC hatcheries. Similarly 4-week-old 50 Jinding breed of ducklings and 3-week-old 50 Khaki Cambel ducklings were purchased from the Government Poultry Farm, Kishoregonj and Government Duck Breeding Farm, Narayanganj respectively. All these birds were maintained in the newly constructed separate houses. The day-old broiler birds were purchased for trial of the available commercial Gumboro vaccines and ducks were purchased for trial of the available commercial Fowl cholera and Duck plague vaccines. These broiler birds were maintained with commercial feeds ( Quality Feeds Ltd., Dhaka ) in deep litter system and ducks were also maintained with adequate commercial feeds ( Quality Feeds Ltd., Dhaka ) and supply of water. In addition to general feed supply, vitamin-mineral premix ( Megavit<sup>®</sup> WS, Novartis, Bangladesh Ltd. ) was also supplied in the drinking water thrice in a week.

Sporadic mortality was recorded in all the groups of experimental broiler and duck flocks, and moribund and dead birds were subjected for necropsy examination. Swab samples from liver, heart, blood, lungs, pericardial and peritoneal fluids, intestines and yolk materials were collected from birds with the characteristic lesions for laboratory investigation. These swabs were streaked onto blood agar and nutrient agar plates and incubated at 37°C for 24 hours. Isolation studies were also carried out on MacConkey's lactose agar and Eosin Methylene blue ( EMB ) agar. The isolates were identified as *Escherichia coli* on the basis of cultural ( Fig. 12 ) and biochemical characters following the methods described by Cowan and Steel (1985).

## Characterization and antibiogram of *Escherichia coli*

The *in vitro* antibiotic sensitivity test on *E. coli* isolates was performed using 10 different antibiotics ( Sanofi Diagnostics Pasteur 9242 Marnes-La-coquettes, France ) by standard disc diffusion technique as described by Bauer *et al.* (1966). Chi-square analysis was used to determine the differences of infection rate and level of antibiotic resistance and results were considered statistically significant when p values  $\leq 0.05$  were obtained ( Gupta, 1982 ).

### RESULTS AND DISCUSSION

A total of 320 broilers and 100 ducklings were purchased and maintained in intensive system of management, of which 21 ( 6.56% ) broilers and 11 ( 11.0% ) ducklings died during the study period ( Table 1 ). Necropsy changes and bacteriological investigation of all the specimens of dead broilers and ducks showed characteristic morphological and cultural properties of *Escherichia coli*. The mortality caused by *E. coli* started from two-day-old broilers, which maintained up to 12 days of age and then declined or completely absent during the 25 to 36 days of age and again the sporadic mortality caused by *E. coli* was observed from 38 days of age to 46 days of age ( Table 1 ). The mortality of ducklings caused by *E. coli* was recorded only during the period from 25 days old up to 36 days ( Table 1 ). These observations support the earlier reports of Jindal *et al.* (1899) who accounted 17.23% *E. coli* infections of total poultry diseases which caused 2.61% mortality due to colibacillosis. No published inland report on avian colibacillosis is available to compare the results but Sharma and Kaushik (1986) and Mahajan *et al.* (1994) reported *E. coli* infections as principal disease in broilers in Haryana State, India. The clinical signs of avian colibacillosis in broiler birds comprised inappetence, droopy head, respiratory distress, watery diarrhea which in some cases was blood tinged, pasting vent, ruffled feathers, occasionally lameness and stunted growth. The clinical findings of colibacillosis in broiler birds recorded in this study are in conformity with the earlier reports of Kaul *et al.* (1992) who reported an outbreak of colibacillosis with clinical signs in broiler chicks. Early chick mortality from two-day-old up to 12-day-old caused by *E. coli* infection might be transmitted through faecal contamination of egg shell, followed by penetration, and ovarian infection or salpingitis ( Calnek *et al.*, 1997; Mukhopadhyay *et al.*, 1999 ). Calnek *et al.* (1997) described the increased incidence of *E. coli* infection shortly after hatching which reduced after about six days.

Table 1. Mortality of broiler chickens and ducks obtained from different hatcheries caused by *E. coli*

Mortality age (days)	Source of broiler chickens ( Hatcheries )						Source of ducks ( Farms )		
	Goalundo (n = 120)	Kazi (n = 50)	Aftab (n = 50)	Paragon (n = 50)	BRAC (n = 50)	Total (n = 320)	KGDF* (n = 50)	NGDF* (n = 50)	Total (n = 100)
2	—	1	—	—	—	1	—	—	—
3	1	—	—	2	—	3	—	—	—
4	1	—	—	—	—	1	—	—	—
5	4	—	—	—	—	4	—	—	—
6	1	—	—	—	—	1	—	—	—
11	1	—	—	—	—	1	—	—	—
12	2	—	—	—	—	2	—	—	—
25	—	—	—	—	—	—	—	2	2
27	—	—	—	—	—	—	2	1	3
29	—	—	—	—	—	—	2	—	2
30	—	—	—	—	—	—	1	1	2
31	—	—	—	—	—	—	—	1	1
36	—	—	—	—	—	—	—	1	1
38	—	—	—	—	1	1	—	—	—
41	—	—	1	—	—	1	—	—	—
42	—	—	1	1	—	2	—	—	—
45	—	—	—	—	1	1	—	—	—
46	—	—	2	1	—	3	—	—	—
Total	10 ( 8.33 )	1 ( 2.0 )	4 ( 8.0 )	4 ( 8.0 )	2 ( 4.0 )	21 ( 6.56 )	5 ( 10.0 )	6 ( 12.0 )	11 ( 11.0 )

n = Total number of chickens / ducks, KGDF = Kishoregonj Govt. Duck Farm, NGDF = Narayangonj Govt. Duck Farm, \*Ducklings of 24-day-old age obtained.

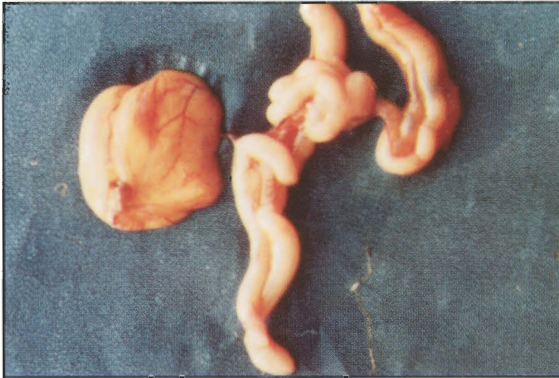


Fig. 1. Omphalitis and yolk sac infection with *Escherichia coli* in a 4-day-old broiler chick showing inflamed unabsorbed yolk sac with abnormal colour and consistency.

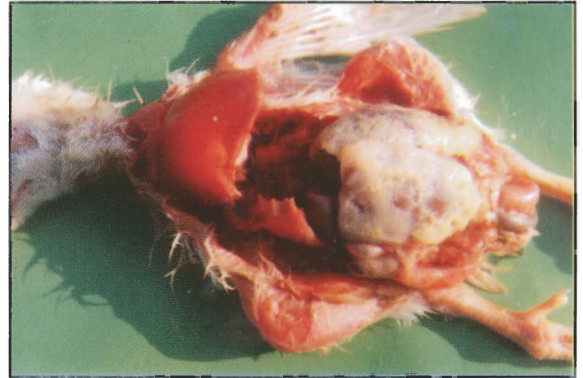


Fig. 2. A 20-day-old broiler chick died of *E. coli* showing deposition of light-yellow fibrinous material which covered the entire liver surface.



Fig. 3. A 20-day-old broiler died of *E. coli* showing fibrinous pericarditis and thickened pericardial sac with light-yellow fibrinous exudate adhering to the surface of the heart.

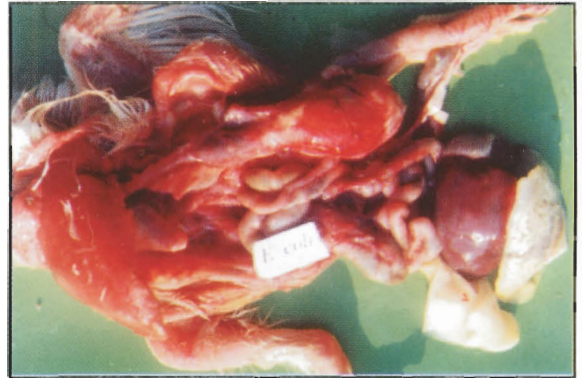


Fig. 4. A 20-day-old broiler chick died of *E. coli* showing dark and congested liver after removal of the deposited fibrinous layer.



Fig. 5. *E. coli* infection in the intestine of a 12-day-old broiler showing pin-point haemorrhagic spot in the intestine.

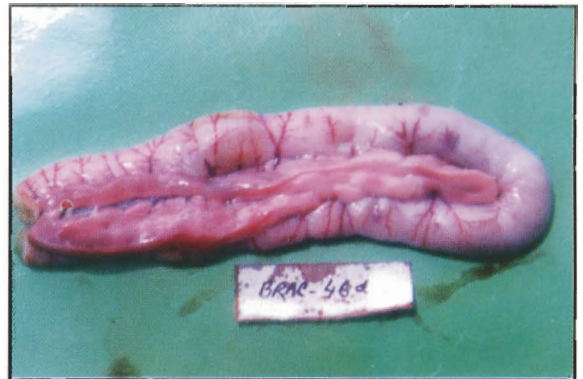


Fig. 6. *E. coli* infection in the intestine of a 46-day-old broiler chicken showing congestion and haemorrhage of the intestine.

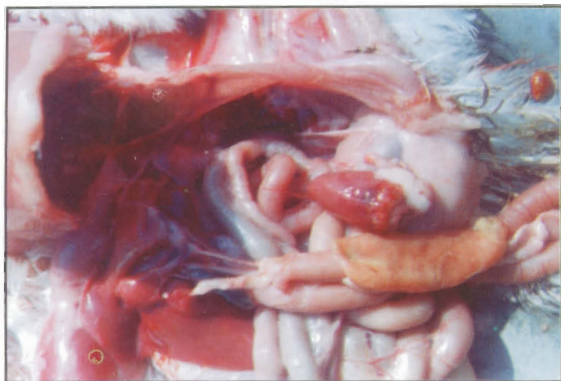


Fig. 7. *E. coli* infection in 18-day-old broiler showing enteritis with inflammatory exudate in the mucosa and perihepatitis.

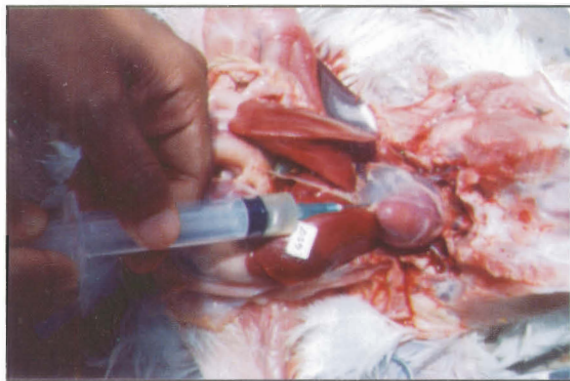


Fig. 8. A 40-day-old broiler died of *E. coli* infection showing excessive accumulation of fluid in the pericardial sac.

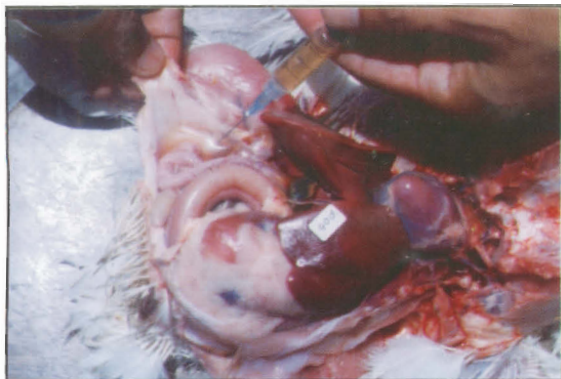


Fig. 9. Collection of peritoneal fluid from a 40-day-old broiler chicken died of colibacillosis.



Fig. 10. A cut portion of intestine of 27-day-old Jinding breed of duck naturally affected with *E. coli* showing enteritis with extensive haemorrhages.



Fig. 11. A photograph of a heart of 27-day-old duck died of *E. coli* showing extensive epicardial haemorrhage.



Fig. 12. A pure culture colonies of *E. coli* on EMB agar showing characteristic dark with a metallic sheen.

Colibacillosis in ducks was recorded only in aged between 25 to 36 days whereas it recorded in broilers aged between 2 to 12 days as well as 38 to 46 days of age ( Table 1 ). These observations indicate both horizontal and egg transmission of *E. coli* in broilers but only horizontal transmission in ducklings.

### Necropsy findings

The pathological lesions of avian colibacillosis are varied and wide and, have been reported to be included omphalitis and yolk sac infection, pericarditis, salpingitis, peritonitis, synositis, panophthalmitis, coligranuloma, swollen head syndrome, avian cellulitis, and enteritis. In this study omphalitis and yolk sac infection ( Fig. 1 ), fibrinous pericarditis and perihepatitis ( Fig. 2 & 3 ), dark and congested liver ( Fig. 4 ), pin-point haemorrhagic spot in the intestine ( Fig. 5 ), congestion and haemorrhage of the intestine ( Fig. 6 ), enteritis with inflammatory exudates in the mucosa and perihepatitis ( Fig. 7 ), accumulation of excessive fluid in the pericardial sac ( Fig. 8 ) and peritoneum ( Fig. 9 ), were recorded in broiler birds. Enteritis with extensive haemorrhage ( Fig. 10 ) and extensive epicardial haemorrhage ( Fig. 11 ) were recorded in ducks died of colibacillosis. These lesions observed in this study are in conformity with the earlier reports of Kaul *et al.* (1992) and Calnek *et al.* (1997).

### Antibiotic sensitivity test

The results of antibiotic sensitivity of different isolates of *E. coli* isolated from broiler birds and ducks are presented in Table 2. It appears from this Table 2 that ciprofloxacin was found highly effective ( 100% ) against both the broiler and duck isolates. This high sensitivity might be due to the fact that it has been recently introduced, have broad spectrum of action and limited used so far by the poultry farmers.

As the *E. coli* is a gram negative organism, it was 100% ineffective against penicillin in both the broiler and duck isolates ( Table 2 ). Although other antibiotics were found less to moderate sensitive against isolated *E. coli* but resistant *E. coli* isolates were also recorded with ampicillin ( broiler isolates 50% & duck isolates 100% ), tetracycline ( broiler isolates 25% and duck isolates 20% ), doxycycline ( only broiler isolates 25% ), cephradine ( only broiler isolates 37.5%), erythromycin ( broiler isolates 62.5% and duck isolates 20% ) and chloramphenicol ( Only broiler isolates 12.5% ). It also appears that higher resistance of isolates was recorded for broilers ( 7 / 9 ) than duck ( 4 / 9 ) isolates ( Table 2 and Fig. 1 ). This higher level of resistance recorded in broiler isolates than ducks isolates suggest the possibility of indiscriminate use of these antibiotics for the treatment of general bacterial infection more in broilers than ducks. A high level of resistance to antimicrobial drugs has been reported among pathogenic isolates ( Kaul *et al.*, 1992; Ngeleka *et al.*, 1996; Lambie *et al.*, 2000 ). The high level of resistance of isolates to antimicrobial drugs, as observed in this study, may reflect an extensive use of these drugs in local poultry farming. Such practices, especially without prior antibiotic sensitivity testing of bacterial isolates, may lead to the development of a pool antibiotic resistant genes and thus to the selection of increasing numbers of *E. coli* resistant clones, many with potential negative impact on the broiler industry. Overall, on the basis of our data, the antibiotic of choice for *E. coli* infection treatment in broilers and ducks appeared to be ciprofloxacin.

Table 2. Antibiotic sensitivity of isolated *E. coli* from broiler chickens and ducks

Experimental birds	No. of isolated tested	Sensitivity grade	No. ( % ) of sensitive isolates to different antibiotics									
			P-G (10 unit)	AP (10 µg)	T (30 µg)	DXT (30 µg)	GM (10 µg)	CRD (30 µg)	E (15 µg)	CIP (5 µg)	C (30 µg)	
Broilers	8	3+	0 (00.0)	0 (0.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	8 (100)	2 (25.0)
		2+	0 (00.0)	0 (0.0)	4 (50.0)	0 (00.0)	7 (87.5)	1 (12.5)	0 (00.0)	0 (0.0)	5 (62.5)	
		1+	0 (00.0)	4 (50)	2 (25.0)	6 (75.0)	1 (12.5)	4 (50.0)	3 (37.5)	0 (0.0)	0 (00.0)	
		R	8 (100)	4 (50)	2 (25.0)	2 (25.0)	0 (00.0)	3 (37.5)	5 (62.5)	0 (0.0)	1 (12.5)	
Ducks	5	3+	0 (00.0)	0 (0.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	5 (100)	1 (20.0)
		2+	0 (00.0)	0 (0.0)	1 (20.0)	0 (00.0)	3 (60.0)	2 (40.0)	2 (40.0)	0 (0.0)	2 (40.0)	
		1+	0 (00.0)	0 (0.0)	3 (60.0)	5 (100)	2 (40.0)	3 (60.0)	2 (40.0)	0 (0.0)	2 (40.0)	
		R	5 (100)	5 (100)	1 (20.0)	0 (00.0)	0 (00.0)	0 (00.0)	1 (20.0)	0 (0.0)	0 (00.0)	

P-G = Penicillin-G, AP = Ampicillin, T = Tetracycline, DXT = Doxycycline, GM = Gentamicin, CRD = Cephradine, E = Erythromycin, CIP = Ciprofloxacin, C = Chloramphenicol.

Sensitivity grade : 3+ = High, 2+ = Moderate, 1+ = Low / less, R = Resistant.

## Characterization and antibiogram of *Escherichia coli*

Considering the higher mortality rates in broilers and ducks caused by *E. coli* infection and that of the isolates were found resistant to most of the selected antimicrobial drugs used in local poultry farming, colibacillosis may be considered as a threat to the broiler and duck industry in Bangladesh. Therefore, this disease problem can be checked by adopting sound management, good sanitation and judicious selection of suitable antibiotic based on antibiogram studies.

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