



AVIAN ESCHERICHIA COLI INFECTION IN MEAT-TYPE CHICKEN: PREVALENCE AND PATHOLOGY

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ABSTRACT

Avian colibacillosis is considered the major bacterial disease in the poultry industry worldwide. This study was conducted to determine prevalence and pathology by avian pathogenic *Escherichia coli* (APEC) in broiler chicken. Various organs of affected broilers were checked for the *E. coli* infection. One hundred and forty commercial poultry farms were divided into seven age groups (A, B, C, D, E, F, and G). Group A (1st week), B (2nd week), C (3rd week), D (4th week), E (5th week), F (6th week), G (7th to 9th week). Investigations were conducted from day 1 until the marketing of birds based on clinical findings. A total of 2491 (23.71% of sick birds) dead birds were observed positive for *E. coli* infection. Three forms of infection were observed i.e. omphalitis, colisepticaemia, and colibacillosis at the rate of 1.55, 17 and 56.65%, respectively. Omphalitis was confirmed by microbial culture and revealed in only groups A and B with 76.24 and 23.76 respectively. While, Colisepticaemia was observed at the rate of 3.11, 6.74, 20.18, 29.63, 19.16, and 22.18 in groups B, C, D, E, F, and G respectively. Whereas Colibacillosis was noticed as 0.075, 3.35, 5.99, 19.20, 26.70, 16.11 and 28.55% in groups A, B, C, D, E, F, and G respectively. Petechiation of heart, kidney, and gizzard besides hemorrhagic enteritis, hemorrhagic tracheitis, and necrotic foci on the liver and caseous exudates in air sacs were the prime gross lesions noticed in colibacillosis. Microscopically, no lesion was observed in serosa, muscularis externa, and submucosa. However, loss of epithelial tissue and breaches in the mucosal layer of the small intestine were observed. Furthermore, characteristic pink and grayish colonies were observed in *E. coli* cultures on McConkey's and Blood agar respectively.

Keywords: broiler, *Escherichia coli*, infection, pathology, prevalence

INTRODUCTION

The poultry industry, as a source of animal proteins for a balanced human diet, occupies an important place in the sphere of food production. The results of this study indicated that colibacillosis is highly prevalent in the study area; these observations raise serious concern and prompt attention and thus a huge economic could be reduced significantly and problematic aspects (i.e. under feeding and malnutrition) are directly related to the health of the nation. Pakistan commercial poultry is an important and significant contributor in livestock share in GDP that has added 1809 (000 tons) of meat, and 21, 285 millions eggs in FY 2021 as well as a major source of employment to almost 8 millions throughout the country (GoP, 2021). Producing more eggs and meat by growing more poultry

can combat the shortage of protein requirement by the cheapest available source of animal protein through filling the gap of protein availability as the poultry have a rapid growth rate and low generation gap as compared to other livestock.

Avian Pathogenic *Escherichia coli* (*E. coli*) cause severe economic losses in the animal and poultry industry by increasing morbidity and mortality rate as well as reducing the production of the animals. It causes a variety of infections through pan systemic involvement of various vital systems of the body. Amongst, colibacillosis is associated with respiratory and intestinal infections; it causes extra intestinal disease in humans and mammals. Infections progress to many other organs as salpingitis, peritonitis, cellulitis, perihepatitis, polyserositis, septicemia, and death (Dho-Moulin, 1999; Barnes 2003; Jeong *et al.*, 2012; Awad *et al.*, 2016).

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Various studies on Epidemiological and prevalence of *E. coli* infection described have been reported earlier in calves, pigs, ducks and turkeys. These findings revealed the prevalence of positive strains. These investigators found this infection as the most significant bacterial disease in poultry worldwide (Harel *et al.*, 1991; Dozois *et al.*, 1992, Dozois *et al.*, 1997; Stordeur *et al.*, 2002). *E. coli*, the predominant organism isolated from number of lesions in various avian studies showed reduced weight gain, poor feed conversion, down grading of carcasses and condemnation at slaughter, infection of soft tissues as well initiating cellulitis particularly in the broiler (Baracho *et al.*, 2006).

Clinically *E. coli* infection is the most devastating disease that it consequences huge mortality, culling, treatment costs; infected birds show dissemination of infection. Several researchers have revealed purulent inflammation of avian lungs, presence of apoptotic cells. They also found dullness and depression with an elevation of temperature, in appetite, diarrhea, cyanosis, salpingitis and peritonitis in layers, infection of the reproductive tract, perihepatitis, pericarditis, damaged lymphatic organs and immune function. (Barnes, *et al.*, 2008; Horn *et al.*, 2012; Landman *et al.*, 2013; Pors *et al.*, 2014; Sun *et al.*, 2015; Jia *et al.*, 2017). Since, Avian *E. coli* infections are ubiquitous and widely spread in Pakistan and cause heavy economic losses to the poultry farmers. Thus, this study aimed to determine the prevalence and pathology produced by avian pathogenic *Escherichia coli* in different age groups of broiler in the surroundings of Karachi, Pakistan.

MATERIALS AND METHODS

Prevalence of infection in broiler chicken

The investigations were carried out to record the prevalence of *E. coli* infections in different age groups of broilers at different commercial poultry farms in surroundings of Karachi, Pakistan. To observe the pathogenesis in different age groups of chicks, 140 commercial broiler poultry farms were visited; the disease was investigated from day first up to the marketing. Total number of farms (140) was divided into seven groups (A, B, C, D, E, F and G), each group having twenty broiler poultry farms (comprising 1000-7000 chicks). Group-A represents the age group from day 1 to day 7 (1st week), from 08 to 14 days in group B (2nd week), from 15 to 21 days in group C (3rd week), 22 to 28 days in group D (4th week), 29 to 35 days in group E (5th week), 36 to

42 days in group F (6th week) and from day 43 up to marketing in group G, respectively.

Gross pathological observations

During the visits, a postmortem of dead birds was conducted, the lesions observed were recorded and positive samples were collected and preserved in 10% formal saline solution and brought to the Post Graduate Pathological Laboratory of the Department.

Histopathological observations

To determine the histopathological changes, collected samples were processed with ascending grades of ethyl alcohol (starting from 30% to absolute) for dehydration. Then the sample was cleared in xylene and embedded in paraffin wax. After embedding the tissues were cut in 4-5 μ section size. Ribbons were placed in the hot water bath to get rid of the wrinkle formation. These sections were mounted on slides and stained with Hemotoxyline and Eosin. These slides were then examined for histopathological changes.

Media prepared for isolation of *E. coli*

Commercially available McConkey's agar was prepared by mixing 05 grams of McConkey's agar with 100 ml-distilled water in conical flask. A homogenous mixture was obtained by gently mixing and heating on the hot plate. The mixture was cooled at the room temperature, pH was adjusted at 7.4, (by adding NaOH and NaHCO₃), autoclaved at 15 lbs. pressure at 121°C for 15 minutes, after autoclaving, the medium was cooled at 50°C and then 15ml was distributed into sterilized petri dishes.

Blood agar base

Commercially available Blood Agar media was used for the culture of *E. coli*. Media consisted Lab-Lemco Powder 10 gms, Peptone 10 gms, NaClO 5 gms, and Agar 15 gms. Forty grams of blood agar base medium was dehydrated in 1000 ml of distilled water and after mixing the pH of medium was adjusted at 7.3. Then the medium was autoclaved for 15mm. at 121°C under 15 lbs pressure. After cooling at 50°C about 10% fresh defibrinated sheep blood was added and subsequently distributed into petridishes. After solidification the plates were incubated for 24 hrs at 37°C for the sterility of the medium. The sterile blood agar plates were used for primary isolation of *E. coli*.

Culture of sample

The samples from infected yolk and liver with *E. coli* were collected from different chicks and

cultured by sterilized platinum loop into the McConkey's nutrient agar and blood agar and incubated for 24 hours at 37°C for characteristic and morphological observations. The study was conducted at Central Veterinary Research and Diagnostic Laboratory, Tandojam.

RESULTS

The systematic investigations were carried out to record the prevalence of *E. coli* infections in the boiler at commercial poultry farms in the surroundings of Karachi. The period of studies lasted five months. During the period, 140 commercial poultry broiler farms were visited to confirm the disease.

Prevalence of *E. coli* infections

During the study period, the prevalence of *E. coli* infections was recorded at various broiler farms. The highest rate of prevalence was found in group E (33.2%), followed by group G (27.7%), whereas, the prevalence of *E. coli* infection was observed as 14.6%, 13.6%, 17.7%, 27.5%, and 26.9% in groups A, B, C, D and F respectively (Table1).

Table 1. Prevalence of *E. coli* in different groups

Groups	Total No. of birds reared	No. of Sick Birds (%)	No. of Positive Birds (%)
A (1 st Week)	12500	996 (7.69 %)	146 (14.65)
B (2 nd Week)	15000	1499 (9.99%)	204 (13.60)
C (3 rd Week)	15700	1587 (10.10%)	282 (17.76)
D (4 th Week)	14000	1620 (11.57%)	447 (27.59)
E (5 th Week)	12000	1675 (13.95%)	557 (33.23)
F (7 th Week)	14600	1490 (10.20%)	401 (26.91)
G (8 th Week)	17000	1637 (9.62%)	454 (27.73)
Grand Total	100800	10504 (10.42%)	2491 (23.71)

Gross pathology

Gross pathological lesions varied at different broiler farms comprising the birds of various age groups. The lesions observed in different organs

indicated higher infarction of *E. coli* in birds of the 5th week and subsequently decreased in older birds as indicated in (Table 2).

Omphalitis

The maximum rate of infection was observed in group A with 76.24% and minimum in group B with 23.76% whereas, omphalitis was not seen in other groups (Table 3). The gross lesions observed were panophthalmitis, unabsorbed yolk, congestion of the small intestine, and necrotic foci in the liver (Figure 3A and 3B).

Colisepticaemia

The maximum rate of infection 29.63% was observed in group E whereas, minimum of 3.11% in group B while no infection was observed in group A. However, the rate of infection in other groups was as 22.18, 20.18, 18.63 and 6.74% were seen in group G,D,F and C respectively (Table 3).

The gross pathological lesions about colisepticaemia were observed in certain visceral organs such as the liver, heart, and air sacs. The most common lesions were fibrinous perihepatitis, hepatomegaly with pale-colored necrotic foci, fibrinous pericarditis along clotted blood present in the heart, the air sacs were found inflamed due to presence of caseous exudates.

Colibacillosis

The maximum rate of infection was observed in group G as 28.55% whereas, the minimum was 0.075% in group A. However, the rate of infection was 26.70, 19.20, 16.11, 5.99, and 3.35 in group E, D, F, and B respectively as shown in (Table 3).

Table 2. Specific lesions were observed in various age groups infected with *E. coli*

Form of Infection	Specific Lesions observed	Groups							Total
		A	B	C	D	E	F	G	
1. Omphalitis and its forms 1.1 Mushy	Unabsorbed yolk, congested blood vessels, and panophthalmitis	125	15	-	-	-	-	-	140
1.2 Naval ill	Emaciation	13	28	-	-	-	-	-	41
2. Colisepticaemia	2.1 Fibrinous perihepatitis	-	-	38	112	291	139	168	748
	2.2 Fibrinous Pericarditis	-	-	33	72	68	74	88	335
	2.3 Air Sacculitis	-	62	63	217	230	148	185	905
3. Colibacillosis	3.1 Enteritis	5	33	59	225	323	202	294	1141
	3.2 Necrotic foci on liver	-	39	71	228	192	173	231	934
	3.3 Hemorrhages on heart	-	20	23	139	179	43	83	487
	3.4 Tracheitis	-	50	73	207	297	171	327	1125
	3.5 Nephritis	-	50	74	160	280	185	331	1080
	3.6 Osteodystrophy	-	-	35	161	233	155	332	916
	3.7 Petechiation of Gizzard	-	30	62	152	265	138	293	940
4. <i>E. coli</i> and other infections	4.1 H.P.S	-	51	3	55	223	202	162	696
	4.2 N.D.	-	-	60	80	-	49	-	189
	4.3 I.B.D.	-	-	-	106	120	20	80	326
	4.4 Coccidiosis	-	-	105	50	32	35	100	322
	4.5 Water belly (Ascites)	-	-	13	-	-	27	72	112
	4.6 C.R.D	3	70	92	276	323	194	296	1254
Grant Total		146	448	804	2240	3056	1955	3042	11691

Group A represents 1st week, B 2nd Week, C 3rd Week, D 4th Week, E 5th Week, F 6th Week, and G 7th up to marketing.

Table 3. Rate of infections observed in various forms of *E. coli* in different age groups

Form of Infection (Omphalitis and its form)	Specific Lesions observed	Groups with Percentage														No. of birds examined
		A	%	B	%	C	%	D	%	E	%	F	%	G	%	
Types Mushy	Unabsorbed yolk, congested blood vessels and panophthalmitis	125	76.24	15	23.76	-	-	-	-	-	-	-	-	-	-	181
Naval ill infection	Emaciation	13		28		-		-		-		-		-		
Colisepticaemia	2.1 Fibrinous Perihepatitis	-	-	-	3.11	38	6.74	112	20.18	291	29.63	139	18.63	168	22.18	1988
	2.2 Fibrinous Pericarditis	-		-		33		72		68		74		88		
	2.3 Air Sacculitis	-		62		63		217		230		148		185		
Colibacillosis	3.1 Enteritis	5	0.075	33	3.35	59	5.99	225	19.20	323	26.70	202	16.11	294	28.55	6623
	3.2 Necrotic foci on liver	-		39		71		228		192		173		231		
	3.3 Hemorrhages on heart	-		20		23		139		179		43		83		
	3.4 Tracheitis	-		50		73		207		297		171		327		
	3.5 Nephritis	-		50		74		160		280		185		331		
	3.6 Osteodystrophy	-		-		35		161		233		155		332		
	3.7 Petechiation of Gizzard	-		30		62		152		265		138		293		

Group A represents 1st week, B 2nd Week, C 3rd Week, D 4th Week, E 5th Week, F 6th Week, and G7th up to marketing.

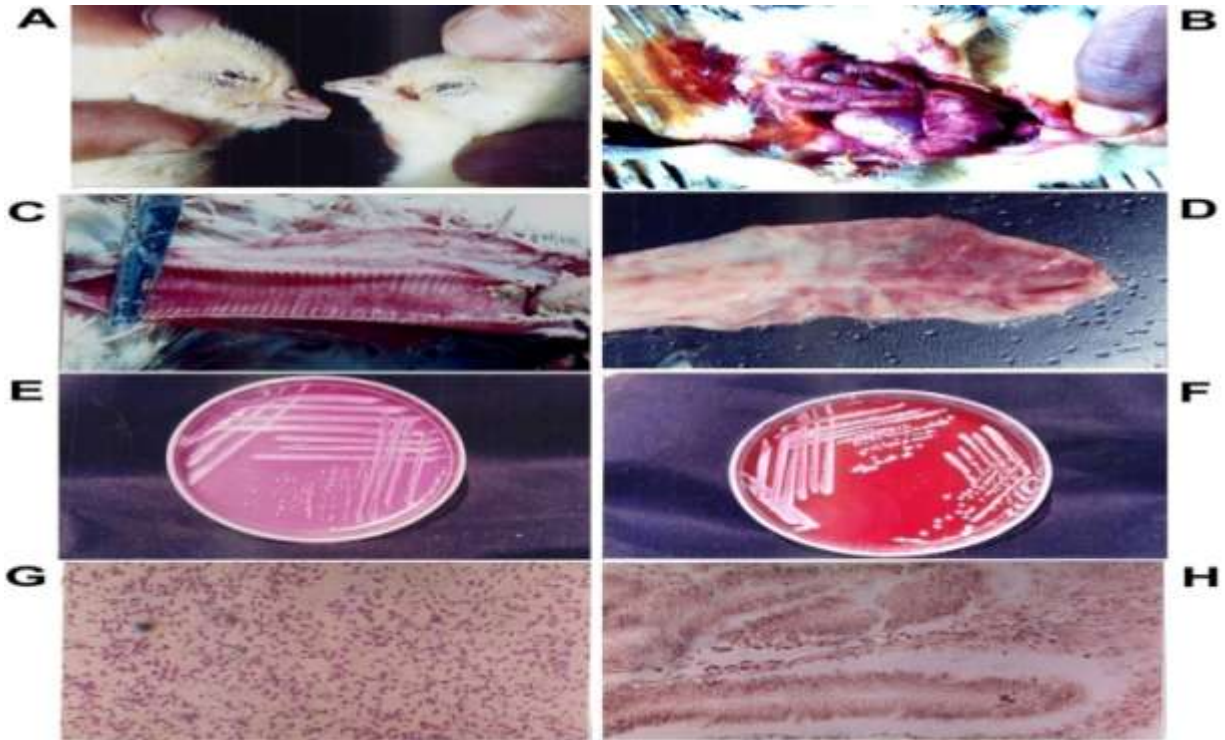


Figure 3. Various pathological conditions caused by *E. coli* in broiler chicken. Figure 3A shows panophthalmitis in day-old chick. Figure 3B shows classical postmortem signs for omphalitis due to *E. coli*. Figure 3C and D, Shows hemorrhagic tracheitis and enteritis respectively, severe hemorrhages can be observed. Figure 3E and F, represent cultural characteristics of *E. coli* in the broiler on blood agar and McConkey's agar. Figure 3G represents the morphology of *Escherichia coli* after successful culture and Figure 3H shows disruption of villus due to *E. coli* in a histological study (H and E, magnification 40X).

The gross pathological lesions varied in different visceral organs in the case of colibacillosis which included necrotic foci and hemorrhages in the liver, hemorrhagic patches in both the heart and gizzard. The kidney was also inflamed and in some cases showed necrotic lesions in the peripheral region. Gall bladder was enlarged and distended in some of the cases, whereas in some cases, the lesions of C.R.D were associated (Table 3) with hemorrhagic tracheitis (Figure 3C), hemorrhagic enteritis (Figure 3D) along with the lesions of mixed infections (Table 3). In general, colibacillosis with 56.65% was noticed as maximum, whereas, colisepticaemia with 17% was observed as a minimum. While other associated diseases with 24.80% and omphalitis with 1.55% were 2nd and 3rd week respectively (Table 4).

Table 4. Rate of infection in various types of *E. coli* along with mixed infection

Disease	No. of Lesions observed	Percentage
Omphalitis	181	1.55
Colisepticaemia	1,988	17.00
Colibacillosis	6,623	56.65
<i>Escherichia coli</i> and other infections	2,899	24.80
Grand Total	11,691	100

Histopathological observations

There was no any histopathological lesion observed in serosa, muscularis externa and sub-mucosa but the lesions in the mucosa revealed loss of epithelial tissue along with desquamation (Figure 3H), leukocytic infiltration of necrotic foci were found in liver, heart, and kidney.

Cultural and morphological characteristics of *Escherichia coli*

The morphological study was conducted by culturing the specimens on different media revealed either singly or paired colonies with pinkish, small, circular, smooth, translucent, and convex colonies on McConkey's agar and grey-white (Figure 3E), glistening circular with convex colonies on blood agar (Figure 3F).

DISCUSSION

One hundred forty commercial broiler farms were surveyed to record the prevalence of *E. coli* infections in the surroundings of Karachi Pakistan. The studies were carried out to record the infection of *E. coli* in different age groups of birds from day one up to the marketing. The

infection rate was recorded as 14.6%, 13.6%, 17.7%, 27.5%, 33.2%, 26.9% and 27.7% in group A,B,C,D,E,F and G, respectively.

The gross pathological lesions varied in different organs of various age groups. Omphalitis was observed as 76.2% in group A and 23.7% in group. Colisepticaemia as 3.1%, 6.7%, 21.1%, 18.1%, and 22.1% in birds of group B, C, D, E, F, and G, while in group A, Colisepticaemia was not observed. Whereas colibacillosis was found in all groups as 0.07% in A, 3.3% in B, 5.9% in C, 19.2%, in D, 26.7% in E, 16.1% in F, and 28.5% in G groups of birds. The specific macroscopic lesions observed in the case of omphalitis included unabsorbed yolk material (sac) congested intestinal mucosa along with panophthalmitis whereas in the case of colisepticaemia there were fibrinous pericarditis, perihepatitis, and airsacculitis. While, in the case of colibacillosis, gross lesions observed were petechiation in the heart, kidney and gizzard besides enteritis tracheitis and necrotic foci in liver and caseous exudate in air sacs. Histopathological lesions revealed congestion, degenerative changes in the intestine, and leukocytic infiltration in liver, heart and kidneys with sometimes necrotic foci especially in the heart. Based on the observed lesions, the form of colibacillosis in the current study could be a hepatic form of colibacillosis. Histopathological lesions in the intestine, lungs and liver, were also identified by previous studies (Gosh *et al.*, 2006; Talha *et al.*, 2001; Riaz *et al.*, 2016).

Colibacillosis is a contagious disease of chicken caused by *E. coli* (Barnes *et al.*, 2008), it is classified as one of the major causes of an increase in morbidity and mortality rate in commercial farms, consequently leading to large economic losses to the poultry farming through its association with other diversified diseases, as a primary or secondary pathogen (McPeake *et al.*, 2005; Tonu *et al.*, 2012; Mbanga and Nyararai 2015).

Avian colibacillosis noticed to be a major infectious disease in all age birds, and has various local and systemic symptoms. The current study agrees with previous findings, in our finding various lesions were found in the liver, intestine and lungs. Died chicks showed postmortem signs for omphalitis intestinal hemorrhagic with tracheitis and enteritis respectively with severe hemorrhages observed. The poor flock performance and managements practices correlated with increased early

mortality rates in broiler forms, Histopathological observations in the present study had similarities to observations earlier (Barnes *et al.*, 2008; Riaz *et al.*, 2016).

In conclusion, the results of this study confirm the prevalence of avian pathogenic *E. coli* in the field, based on morphological and histological lesions and cultural findings. The economic and poultry health burden of this disease emphasizes the effective surveillance and control measures to reduce economic losses inflicted to the farmer due to this hazard.

Novelty statement

We report that no such data have been published earlier and this is first ever report on natural infection of Avian *Escherichia coli* in Pakistan. This data will prove to be complete data on Avian *Escherichia coli* in meat-type chicken from day 1, upto marketing.

AUTHOR'S CONTRIBUTION

Q. A. Shah: Executed the experiment, data collection, analysis and overall manuscript write-up

N. M. Soomro: Helped in the development of experiment and generation of the data

J. M. Shah: Provided technical support in data and sample collection from the study area

M. A. Hussain: Helped in data collection

S. F. Sayed: Helped in data collection and culture of *E. coli* (Prevalence)

CONFLICT OF INTEREST

There is conflict of interest among authors.

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(Received: September 29, 2021; Accepted: October 28, 2021)