

## PATHO-MORPHOLOGICAL STUDIES ON SPONTANEOUSLY OCCURRING CASES OF COLIBACILLOSIS IN BROILER CHICKENS OF JAMMU REGION

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### SUMMARY

The present investigation was carried out to study the etiology, gross and histopathology of gastrointestinal tract lesions of broilers. To achieve the envisaged objectives, a survey of 200 different flocks of broiler chickens in and around Jammu was conducted during the period of July 2016 to June 2017 and bacteriological and patho-morphological studies were undertaken on naturally occurring cases of colibacillosis. *E. coli* isolates formed pink colonies when cultured on MLA and green metallic sheen on EMB agar. Isolates of *E. coli* belonged to sero-groups O1, O22, O37, O114, O118 and O149. Gross changes upon postmortem included fibrinous pericarditis, perihepatitis, air sacculitis and generalized serositis along with congestion and hemorrhages in different organs. Histopathologically, liver revealed multifocal areas of hepatic degeneration and necrosis with infiltration of heterophils and a thick layer of fibrin around liver capsule with moderate infiltration of heterophils. Pericardial layer was thickened due to congestion, fibrin deposition and heterophilic infiltration. Also, enteritis characterized by congestion, necrosis of villi and infiltration of heterophils in lamina propria or sub mucosa and thickening of serosal layer due to severe congestion and deposition of fibrinous exudate, was present.

**Keywords:** Broilers, Colibacillosis, Patho-Morphology, Pericarditis, Perihepatitis

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Colibacillosis caused by *Escherichia coli* is a leading cause of morbidity and mortality in broilers of all age groups, which inflicts heavy losses on poultry farming industry throughout the world including India (Bhalerao *et al.*, 2013; Shah *et al.*, 2019; Buragohain and Kalita, 2010; Tonu *et al.*, 2011). *E. coli* is a normal inhabitant of GIT but when host mucosal defenses are overwhelmed, invasion of various organs can occur to cause septicemic illness. In addition to compromised mucosal barriers, suppressed immune system due to prior infections with infectious bursal disease, mycoplasmosis or infectious bronchitis etc, can act as predisposing factors for occurrence of this disease.

Often, stressful environmental and poor managerial practices also cause the disease outbreaks to occur (Shankar *et al.*, 2010; Bhalerao *et al.*, 2013). Common manifestations of colibacillosis include cellulitis, coligranuloma, colisepticaemia, air sac disease, swollen head syndrome, peritonitis, salpingitis, yolk sac infection and enteritis. There are no previous reports on prevalent serotypes and the associated pathological lesions in *E. coli* infections in broilers in Jammu, hence, the present work was conducted to determine the presence of various serotypes of *E. coli* causing infection in broilers of Jammu and also to study the gross and histopathological lesions associated with this disease.

**Sample collection:** A total of 200 different poultry flocks in and around Jammu where disease outbreaks were reported to occur during the period from July 2016 to June 2017 were included in the study. The study period was divided into four seasons as per Meteorological Department of India, Pune, viz., monsoon (July to September), post monsoon (October to November), winter (December to February) and summer (March to June). Age wise and season wise disease occurrence and mortality pattern at the field level was determined. All affected age groups (0-6w) were included in the study. Occurrence of disease/pathological conditions and mortality pattern was determined as per the method described by Thrusfield (1995).

**Bacteriological studies:** Liver and heart blood swabs were collected from representative dead birds aseptically into sterilized petri-plates. Isolation and identification of *E. coli* was done as per the standard method. The samples were inoculated in nutrient broth and kept at 37 °C for 24 hours (h). The colonies from nutrient agar were then plated on MacConkey's lactose agar (MLA) and incubated for 24h at 37 °C. Organisms giving pink coloured colonies on MLA were also cultured on eosin methylene blue agar (EMB). Colonies giving metallic sheen were subjected to various biochemical and their IMViC pattern was recorded as per the procedure described by Quinn *et al.* (1994).

**Maintenance of cultures and serotyping of the *E. coli***

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**isolates:** After confirmation with biochemical tests, the purified cultures were inoculated on maintenance medium (nutrient agar slant) in duplicate and incubated at 37 °C for 24 h. These were sealed with paraffin wax and slants were stored at 4 °C in the refrigerator for further identification/conformation. Culture was revived each month for viability. After complete biochemical characterization, all the isolates of *E. coli* were sent for serotyping to the National Salmonella and Escherichia centre, CRI, Kasauli (HP), India.

**Patho-morphological studies:** Representative carcasses were necropsied from the mortality in different flocks. Post-mortem examination of a total 632 birds was done. Systemic examination was carried out for the presence of any lesions in the GIT. Oesophagus, proventriculus, gizzard, pancreas, liver and intestine along with heart of dead birds were thoroughly examined and visible pathomorphological alterations were recorded. After thorough gross examination, representative pieces of less than 5mm thickness from respective visceral organs, viz., oesophagus, proventriculus, gizzard, liver, intestine and pancreas as well as heart were collected in 10% neutral buffered formalin solution and 4-5 µ sections were prepared as per standard procedure and stained by routine haematoxylin and eosin stain (Luna, 1968).

*E. coli* was isolated from 82 flocks, out of the 200 flocks examined with an occurrence of 24.16% and mortality of 8.33% as has been published previously by our group (Sanhga *et al.*, 2019). Colibacillosis affects all age groups (Tonu *et al.*, 2011). *E. coli* infection occurred among all age groups but it was the most common disease to strike birds between 0-2 weeks of age and *E. coli* affected birds of all age groups throughout the year in Jammu (Sanhga *et al.*, 2019). In contrast, Bhalerao *et al.* (2013) reported that the disease was most severe in birds of 3-4 weeks of age in Haryana. Since, *E. coli* is an opportunist and colibacillosis mostly occurs as a secondary bacterial infection when host defences are lowered or when poor managemental practices are followed, these factors may account for the differences in two studies. Mahajan *et al.* (1994) also reported that in Hisar, colibacillosis caused a mortality of 8.88% but Buragohain and Kalita (2010) observed a higher mortality rate in broilers affected by colibacillosis (19.23%) in Mizoram.

Morphologically, *E. coli* isolates formed pink colonies when cultured on MLA (Fig. 1), showed green metallic sheen on EMB agar (Fig. 2) and organisms were Gram-ve (Fig. 3). Biochemically, all the isolates were positive for indole, methyl red and negative for voges-proskauer and catalase test. Isolates of *E. coli* belonged to sero-groups O1, O22, O37, O114, O118 and O149. O1, O22 and O37

were the most common serotypes isolated which were isolated from 18, 15 and 9 cases, respectively.

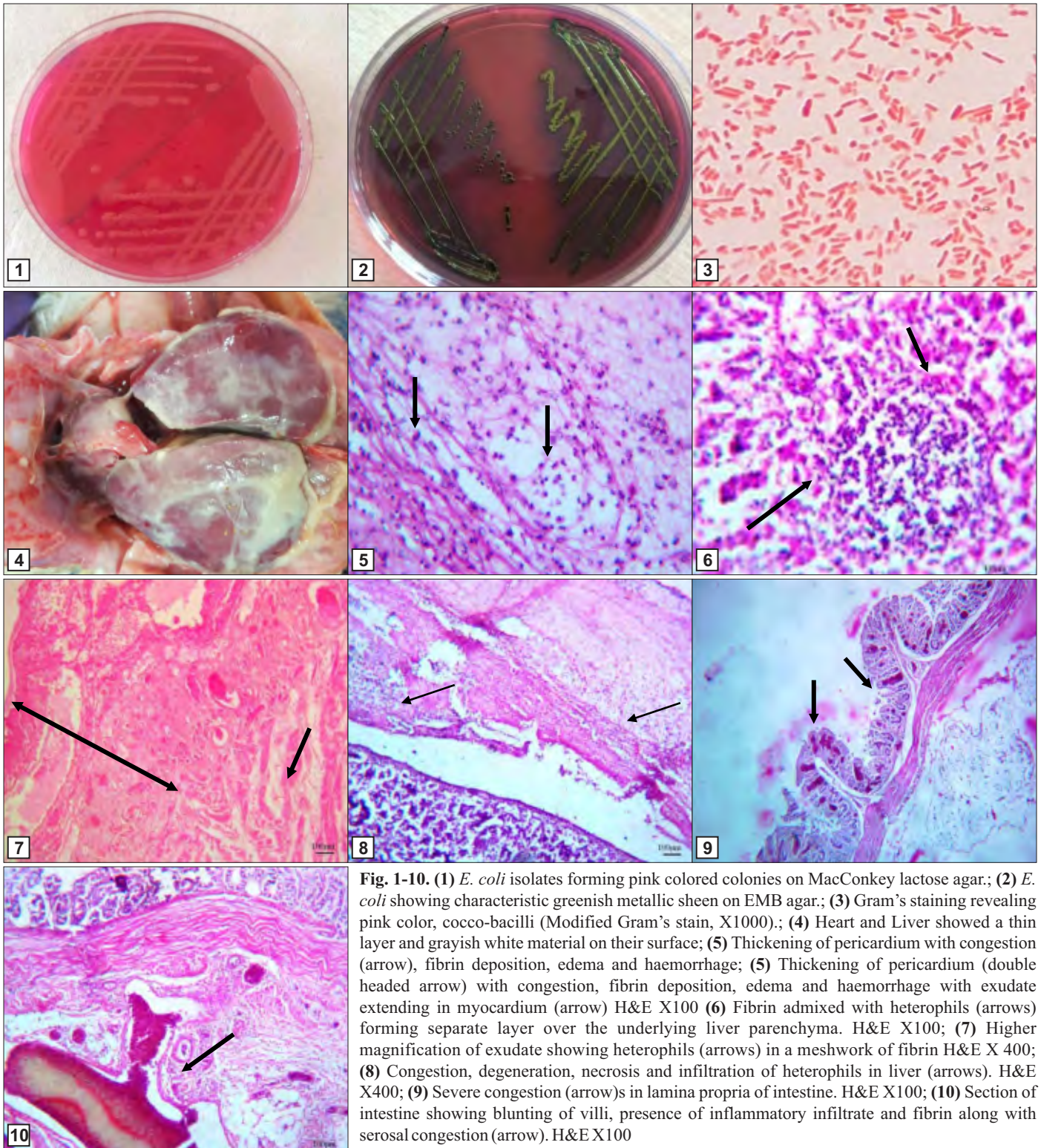
Different serotypes previously have been isolated by different workers in different geographical regions. Shankar *et al.* (2010) recorded presence of O78, O75, O2, O6 and O111 in Hisar, whereas Sahoo *et al.* (2012) recorded O1, O9, O23, O33, O103 and O119 serotypes in different farms of Odisha. Sarker *et al.* (2013) revealed O2, O8, O9, O19, O37, O86, O101, O133, O151 and O173 in West Bengal.

The gross lesions characteristic of *E. coli* in our study were fibrinous pericarditis, perihepatitis and air sacculitis (Fig. 4). The pericardial sac was opaque, whitish and adherent to the epicardium. The liver had a yellowish fibrin layer covering its serosal surface. The air sacs were opaque and thickened.

In our study, birds from 82 flocks tested positive for *E. coli* and the organism could be isolated among 152 out of the total 632 birds necropsied. Intestine and liver were the most commonly affected organs in GIT. As far as intestines were concerned, lesions were seen in 85% (129/152) cases. Grossly, the lesions consisted of congestion, hemorrhages, necrosis and enteritis which were seen in 78.3% (101/129), 20.9% (27/129), 12.4% (16/129) and 71.3% (92/129) cases, respectively. Liver grossly was affected in 92.05% (139/152) birds and lesions, viz., congestion, hemorrhages, enlargement, necrosis, fatty changes and perihepatitis were seen in 84.8% (118/139), 23.7% (33/139), 76.2% (106/139), 31.6% (44/139), 20.8% (41/139) and 73% (101/139) cases, respectively. Examination of esophagus and gizzard didn't show any appreciable changes except occasional fibrinous covering on serosa of gizzard. Rarely, proventriculus also showed presence of mucoid exudate adhering to the underlying mucosa in addition to presence of serosal fibrinous layering. Pancreas only revealed mild congestion in some birds.

Microscopically, pericardium was thickened due to presence of fibrin, necrotic tissue, edema, congested blood vessels and heterophilic infiltration (Fig. 5).

Fibrinous pericarditis is a characteristic lesion of colibacillosis and in our study also thick fibrin layer enveloped heart grossly and microscopically pericardium was expanded by presence of deeply eosinophilic fibrin fibrils, congested blood vessels and heterophilic infiltration, the underlying myocardium was also necrotic staining deeply eosinophilic with loss of cross striations and pyknotic nucleus (Fig. 5). These findings are in concurrence with the results of Bhalerao *et al.* (2013) and Renu *et al.*, (2012).



**Fig. 1-10.** (1) *E. coli* isolates forming pink colored colonies on MacConkey lactose agar; (2) *E. coli* showing characteristic greenish metallic sheen on EMB agar; (3) Gram's staining revealing pink color, cocco-bacilli (Modified Gram's stain, X1000); (4) Heart and Liver showed a thin layer and grayish white material on their surface; (5) Thickening of pericardium with congestion (arrow), fibrin deposition, edema and haemorrhage; (5) Thickening of pericardium (double headed arrow) with congestion, fibrin deposition, edema and haemorrhage with exudate extending in myocardium (arrow) H&E X100 (6) Fibrin admixed with heterophils (arrows) forming separate layer over the underlying liver parenchyma. H&E X100; (7) Higher magnification of exudate showing heterophils (arrows) in a meshwork of fibrin H&E X 400; (8) Congestion, degeneration, necrosis and infiltration of heterophils in liver (arrows). H&E X400; (9) Severe congestion (arrow)s in lamina propria of intestine. H&E X100; (10) Section of intestine showing blunting of villi, presence of inflammatory infiltrate and fibrin along with serosal congestion (arrow). H&E X100

Microscopically, lesions in liver consisted of congestion, hemorrhage, degeneration or necrosis, hepatitis, and perihepatitis which were seen in, 92% (128/139), 51.7% (72/139), 48.2% (67/139), 33.8% 47/139, 43.1% 60/139 cases, respectively. Histopathologically, perihepatitis (Fig. 6) was characterized by presence of fibrin strands admixed with heterophils (Fig. 7) forming a

separate layer over the underlying liver parenchyma which showed varying degrees of degeneration and necrosis (Fig. 6). Multifocal areas of hepatic degeneration, necrosis, with infiltration of heterophils were also commonly seen (Fig. 8). Other workers have also observed hepatocyte degeneration, congestion along with sinusoidal dilatation and distorted hepatic cords in livers of poultry birds

affected with colibacillosis (Hooda *et al.*, 2011; Bhalerao *et al.*, 2013, Gangane *et al.*, 2006). Perihepatitis with deposition of thick fibrin layer over liver surface was a characteristic finding in our study which histopathologically was seen as an overlay of pink fibrillar material admixed with large number of heterophils. Underlying hepatocytes were invariably either degenerated or necrotic. Similar changes were also recorded by other investigators (Hooda *et al.*, 2011; Bhalerao *et al.*, 2013; Shah *et al.*, 2019).

Proventriculus revealed necrosis of epithelium of mucosal folds, edema, congestion and severe infiltration of inflammatory cells mainly heterophils in lamina propria and underlying sub mucosa. In some cases, tunica muscularis and serosal layers of proventriculus were also severely congested. Serosal layer showed large amounts of fibrinous exudate comprising of fibrin strands and heterophilic infiltration. Microscopic pathological lesions in the intestines comprised mainly of congestion, hemorrhage, edema, degeneration and necrosis, enteritis were observed in 82.1% (106/129), 31.7% (41/129, 48.8% (63/129), 86% (111/129), 75.1% (97/129) cases, respectively. Enteritis was characterized by congestion, necrosis of villi and infiltration of heterophils in lamina propria along with sub mucosa. Severe congestion in lamina propria was often observed (Fig. 9). Occasionally intestinal serosal layer was thickened due to severe congestion and deposition of fibrinous exudate (Fig. 10). Similar lesions in intestines due to *E. coli* infection were also described by Tonu *et al.* (2011), who noticed hemorrhage, mucus and hemorrhages in intestines.

In the present study, *E. coli* was found to be widely prevalent in broiler flocks of Jammu, causing significant morbidity and mortality in affected farms. Since *E. coli* is an opportunistic microbe, colibacillosis mostly occurs as a secondary bacterial infection when host defences are lowered due to environmental stress or presence of other infections. This problem can be controlled by improving sanitary conditions and adopting effective biosecurity measures at the farm. Implementations of better managerial practices are therefore recommended to minimize occurrence of colibacillosis and the consequent economic losses. The present study furnished useful data on the range of pathological lesions associated with *E. coli* in broilers of Jammu.

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