RESEARCH ARTICLE

Pathomorphological Studies of Outbreaks of Avian Pathogenic *Escherichia coli* Infections in the Commercial Chicken

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Abstract

Colibacillosis, caused by *Escherichia coli*, affects domestic and wild birds. The economic losses in industrial and subsistence poultry farms were considerable due to high morbidity and mortality and higher costs of medical treatments. A total of 488 birds were examined, out of which pathogenic *Escherichia coli* was found in 308 (63.11%) birds based on the biochemical, cultural, morphological and staining characters and these were confirmed by the polymerase chain reaction (PCR). Clinically affected birds showed symptoms of dullness, depression, disinclination to move, chest and beak resting on the ground with closed eyelids, loss of weight, weakness, ruffled feathers, gasping and distended abdomen. The pathological gross lesions noticed were the congestion of all visceral organ, fibrinous pericarditis, perihepatitis, air-sacculitis, peritonitis and salpingitis. The histopathology of effected lungs, air-sacs, pericardium, heart, liver and its capsule and peritoneum showed fibrinous inflammation with infiltration of inflammatory cells. The study concluded that colibacillosis was the cause of death of birds with typical gross and histopathological lesions in various outbreaks.

Key words: Chicken, Colibacillosis, *Escherichia coli,* Gross lesion, Histopathology, PCR.

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Introduction

Colibacillosis still remains as one of the important disease of poultry in spite of advancements in the treatment and management in poultry industry. The disease is caused by *Escherichia coli*, a Gram-negative, motile, non-spore forming bacteria (Markey *et al*., 2013). The weak immune system, stressful environmental conditions like over-crowding, poor ventilation, excessive dust and other infections predispose the birds to the disease. It occurs as primary infection, secondary infection to respiratory diseases or concurrent infection with other etiological agents. These infections causing severe losses due to increased morbidity and high mortality, low weight gains, increased feed conversion ratios and decreased fertilization, hatching, laying and an increase discarding of carcasses and their parts due to pathological changes and ineffective treatment measures (Casagrande *et al*., 2017: Kim *et al*., 2020). It has been reported throughout the world including India (Abalaka *et al*., 2017: Prashant *et al*., 2023). The present study was aimed at confirmative diagnosis of colibacillosis in chicken by combining clinical, pathomorphological lesions, and isolation and identification of Avian Pathogenic *E. coli* in field outbreaks.

Materials and Methods

For this study birds were brought from various poultry farms of Andhra Pradesh. During the farm visit, history was taken from the farms and clinical symptoms of the birds were observed. From each poultry farm, representative dead birds were taken to the Department of Pathology of Veterinary College, Gannavaram (AP, India), and detailed necropsy was conducted. The heart blood, lung and trachea were collected aseptically and kept at 4°C for bacterial isolation and molecular studies. For histopathology, trachea, lung, liver, heart, air sacs, oviduct and spleen were collected in 10% formalin.

The collected tissue samples were triturated and incubated at 37°C for 24 h in MaConkey broth. A loopful of cultured bacteria were plated on MaConkey agar and again incubated at 37°C for 24 hr. These cultured plates were observed for the presence *E. coli* typical colonies based on morphological and cultural characteristics. Further on EMB

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agar also these pink colonies showed characteristic metallic sheen and confirmed their identity by biochemical assays.

For polymerase chain reaction assay tissue samples were collected and stored at -20°C. From the tissue homogenate, DNA was extracted using HiGenoMB® genomic DNA Purification Kit (Himedia) as per the manufacturer's protocol. The species specific 16s rRNA gene was detected by using the forward primer 5' ATCAACCGAGATTCCCCCAGT 3' and reverse primer 5' TCACTATCGGTCAGTCAGGAG 3' to amplify 230 bp fragment of *E. coli* (Dong-bo *et al.,* 2011). The PCR mixture used for amplification consisted of 10 µL PCR master mix (2x), 1 µL of each primer at a concentration of 10 pmol, 3 µL of DNA sample and 5 µL of nuclease free water. Thermal cycling conditions for PCR were 94°C for 5 min for initial denaturation and then 35 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 90 sec, and extension of 72°C for 30 sec and further final extension at 72°C for 7 min. In Geldoc the PCR products were visualized after agar gel electrophoresis.

Results and Discussion

In the present study out of 488 birds examined, pathogenic *Escherichia coli* were found in 308 (63.11%) cases. Among these, polyserositis was recorded in 158 (32.37%) cases, coli septicaemia in 65 (13.32%) cases, egg peritonitis in 44 (9.02%) cases, salpingitis in 32 (6.55%) cases, yolk-sac infection in 7 (1.43%) cases and pan ophthalmitis in 2 (0.40%) cases. Our results were in accordance with the results of Taunde *et al.* (2021), who studied colibacillosis in broiler chickens of Mozambique and observed air sacculitis and hepatomegaly in 100 % cases, splenomegaly in 75.5% cases, polyserositis in 75.5% cases and coli-septicaemia in 75% cases. Srinivasan *et al.* (2014) reported 27.45% salpingitis associated with *E. coli* infection. The variation in the incidence was due to difference in the age groups, rearing condition and managemental systems of different poultry flocks.

Clinical Signs

Birds affected with colibacillosis were dull, depressed, discharges from the eyes and nose, had ruffled feathers, revealed gasping, distended abdomen and diarrhoea. Some of the birds showed beak and chest resting on ground and difficulty in egg laying. The symptoms were in accordance with the previous studies (Abalaka *et al*., 2017: Jamoh *et al.,* 2018).

Gross Pathology

Grossly, the visceral organs heart, liver, spleen and kidneys were congested. The air sacs were cloudy, thickened and had fibrinous exudate in mild cases. They were thickened, opaque and contained fibrino-caseous exudate in moderate to severe cases. The tracheal mucosa showed severe degree of congestion and haemorrhages and lungs were severely congested and edematous. These all-respiratory lesions were in accordance with study of Veeraselvam *et al*. (2019), who isolated *E. coli* from the birds suffering with the respiratory distress. Birds infected with *E. coli* showed polyserositis involving pericardium, epicardium, hepatic capsule, pleura and air sacs with varying degree of severity. Fibrinous exudates were seen on pericardium, liver, peritoneum as threads to membranes based on severity (Fig. 1). These results were in agreement with the previous reports of Khaton *et al.* (2008) and Taunde *et al.* (2021) in colibacillosis of commercial layer chicken.

The pericardial sac was cloudy and filled with yellowish fibrinous exudates. The pericardium was thickened due to fibrinous exudate. Adhesions were noticed between pericardium and epicardium in severely affected birds. The lesions of air sacs and heart observed in this study were in line with colibacillosis lesions observed by Chaudhari *et al*. (2017) and Prashant *et al.* (2023).

Liver was soft, swollen, congested, mushy and contained haemorrhagic spots. The surface of the liver was covered by a layer of fibrinous material that caused adhesions between hepatic sac and the Glisson's capsule. The studies of Khaton *et al.* (2008) and Ashwin *et al.* (2013) also showed enlarged liver covered by white to yellow fibrinopurulent exudates.

The peritoneal cavity contained creamy yellowish peritoneal fluid and had fibrinous material adhering to the ovarian follicles, oviduct and intestines as noticed by Prashant *et al.* (2023). In some birds, the ovarian follicles were ruptured and the yolk material was observed in the peritoneal cavity, thus led to peritonitis. These findings were in accordance with the observations of previous authors (Srinivasan *et al*., 2014: Abalaka *et al*., 2017). Jamoh *et al*. (2018) found yolk sac infection. Similarly in this study, young chicks with age group of 1-2 weeks revealed unabsorbed yolk material (Fig. 2) in the abdomen with inflammation and necrosis along with congested lungs and liver.

In layers oviduct was impacted by inflammatory exudates and necrosed eggs. Watery yellowish fluid with fibrinous material adhering to the ovarian follicles, oviduct and intestines the peritoneal cavity was observed (Fig. 3). The ovarian follicles were ruptured in few birds showing the yolk material in the peritoneal cavity. Very few birds showed arthritis and panothalmitis. These results were similar to the earlier reports (Wafaa *et al.,* 2011: Srinivasan *et al*., 2014: Ozaki *et al*., 2018).

Histopathology

Microscopically, the tracheal mucosa revealed congestion, necrosis and desquamation of the epithelium along with mononuclear cell infiltration. The air sacs revealed congestion and deposition of fibrinous exudate and infiltration by heterophils and mononuclear cells. In few birds, the air sacs were severely thickened with fibrinopurulent to caseous exudates along with heterophils, mononuclear cells and plasma cells (Fig. 4). Congestion, presence of fibrinous strands and infiltration by heterophils and mononuclear cells were noticed on pleural surface of lungs. In the parenchyma

of lung, the blood vessels were severely congested along with infiltration of heterophils and mononuclear cells in the parabronchial area (Fig. 5). These findings are akin to those reported by Horn *et al.* (2012) and Veeraselvam *et al*. (2019) in *E. coli* infected birds. Veeraselvam *et al*. (2019) observed edema and heterophil infiltration and caseous exudates containing bacterial colonies in the air sacs.

Microscopically, lesions were noticed in the pericardium, epicardium and myocardium. There was marked congestion of blood vessels in all the layers. Pericardial sac revealed edema, marked fibrinous exudation and infiltration by heterophils and mononuclear cells along with fibrinous exudates (Fig. 6). Pericardium and epicardium showed marked fibrinous exudation and infiltration by heterophils and mononuclear cells and the myocardium revealed degenerative changes and interstitial myocarditis with diffuse infiltration of mononuclear cells. The microscopic lesions were similar to the reports of Prasant *et al*. (2023) and Chaudhari *et al*. (2018). Wafaa *et al*. (2011) and Srinivasan *et al*. (2014) noticed degenerative changes in heart muscles.

Hepatic capsule/Glisson's capsules showed fibrinous to caseous exudation with infiltration of inflammatory cells (Fig. 7). In few cases in liver, the fibrinous exudation was more extending into the hepatic peritoneal cavity along with infiltration of heterophils and mononuclear cells. In addition, congestion of blood vessels and sinusoids, degeneration and necrosis of hepatocytes, focal accumulation of mononuclear cells and mild fibrinous exudate on the surface were seen as noticed by Ashwin *et al*. (2013) in *E. coli* infections.

Spleen showed congestion, haemorrhages and mild lymphoid depletion. In the kidney, congestion of blood vessels, intertubular haemorrhages and degenerative changes of tubular epithelial cells were found. Intestines revealed degeneration and desquamation of intestinal epithelial cells and infiltration of heterophils and mononuclear cells in the mucosa and submucosa. These changes were in conformity with the earlier findings of Tonu *et al.* (2011) and Ashwin *et al.* (2013) in birds affected with colibacillosis.

Microscopically, the infected yolk sac wall was edematous along with infiltration of inflammatory cells. The peritoneum and ovarian tissue also showed congestion, serofibrinous exudation, cellular infiltration by heterophils and mononuclear cells. These results were similar to earlier reports (Wafaa *et al.,* 2011: Ozaki *et al*., 2018).

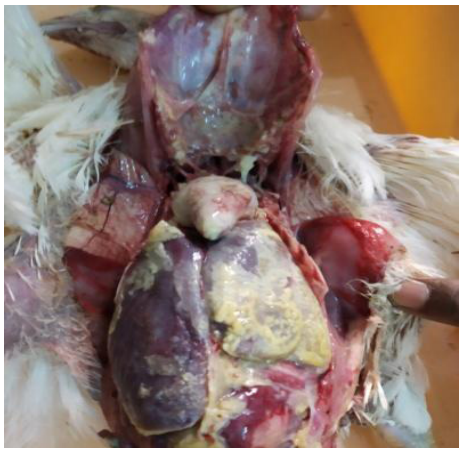

Fig.1: Bird showing fibrinous exudates on heart, liver, peritoneum

Fig. 2: Chick showing unabsorbed yolk material in the abdomen

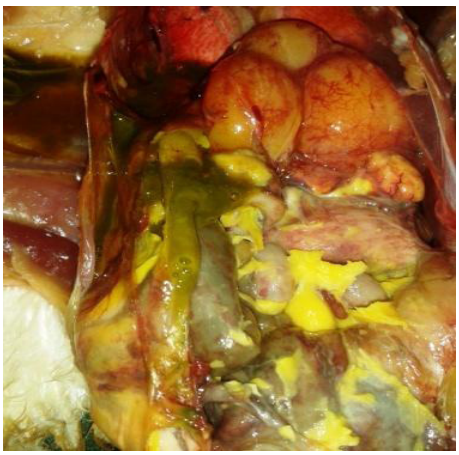

Fig. 3: Bird showing fibrinous material adhering to the ovarian follicles, oviduct and intestine.

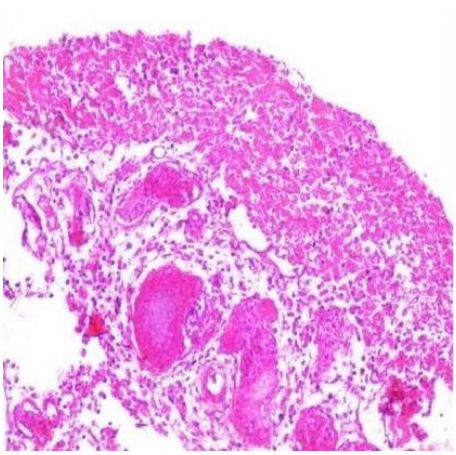

Fig. 4: Air sacs thickened with caseous exudates and infiltration of heterophils, mononuclear cells and plasma cells.

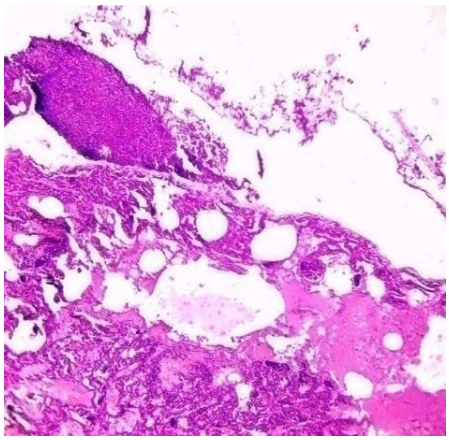

Fig. 5: Pleura and lungs showing oedema, marked fibrinous exudation and infiltration heterophils and mononuclear cells.

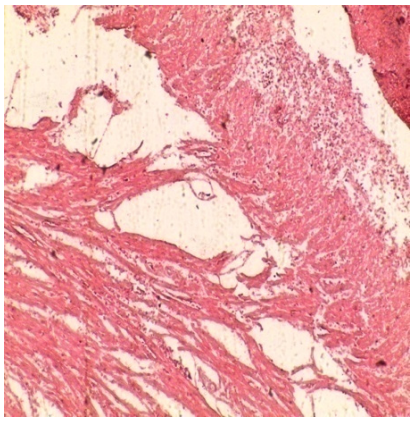

Fig. 6: Pericardial sac revealed oedema, marked fibrinous exudation and infiltration heterophils and mononuclear cells.

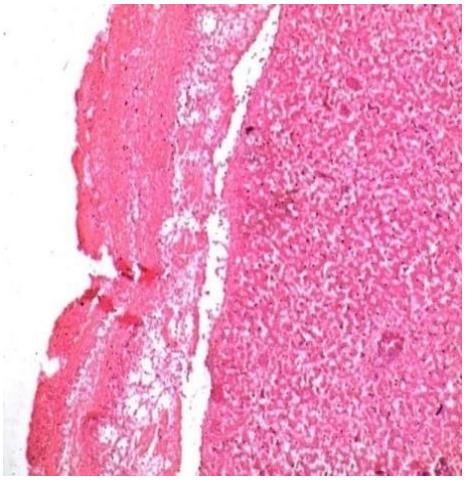

Fig. 7: Hepatic capsule and liver showing showed fibrinous to caseous exudation with infiltration of inflammatory cells

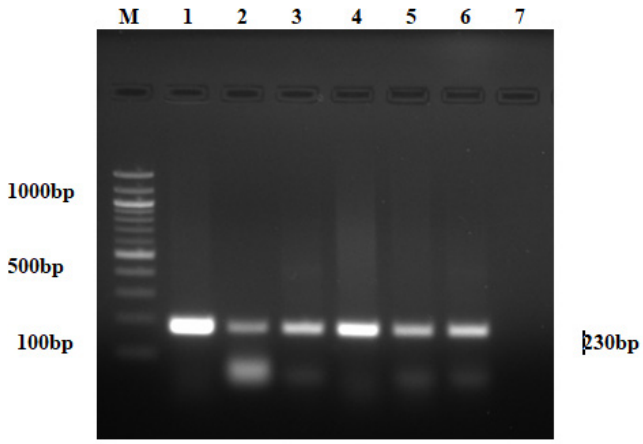

Fig. 8: Gel electrophoresis showing PCR products of 16S rRNA in *E. Coli* (230 bp), M- Ladder 100bp, 1- Positive control, 2 to 6- DNA samples, L7- Negative control

Molecular Detection

In this study, *E. coli* was confirmed by using specific primers of 16S rRNA that produced an expected amplicon size of 231 bp in PCR assay (Fig. 8), and these results are in agreement with the findings of Tonu *et al.* (2011), Islam *et al.* (2014) and Matin *et al.* (2017), who used same primers for molecular confirmation of *E. coli* in chicken.

Conclusion

The study concluded colibacillosis as the cause of death of all birds in the present study and it was confirmed by polymerase chain reaction, and it severely affected the health status and performance of commercial chickens in the field.

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