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**Research Paper**



# Pathomorphological assessment of naturally occurring colibacillosis in poultry raised in the Northern Himalayas

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ABSTRACT

**Avian Colibacillosis persists as a significant endemic disease in poultry, carrying substantial economic importance due to its ambiguous pathogenesis and potential implications for zoonosis and food safety. Therefore, it is important to understand the pathogenesis of the disease in any area to curtail its spread. In this context, a study was conducted to investigate the pathomorphological changes and pathogen-induced oxidative stress in naturally occurring cases of Colibacillosis in broiler chickens from commercial farms in the Ganderbal district of the Kashmir Valley. In the present study, 20 Colibacillosis outbreaks were recorded in different poultry farms. In infected chickens, elevated AST and ALT levels, decreased total protein, increased serum copper, and decreased zinc were noted. All isolates amplified the 500 bpecp gene fragment by PCR. Clinical signs ranged from asymptomatic to severe lethargy, with localized infections displaying milder symptoms than systemic ones. Colibacillosis in broiler chickens featured pronounced air sac swelling with deposition of fibrinous exudate around the heart and abdomen. Histologically, air sacs showed severe fibrinoheterophilic inflammation, while the pericardium and peritoneum exhibited fibrinous inflammation with heterophilic infiltration and necrotic foci in severe cases. The study underscored the ongoing economic threat posed by Colibacillosis in the poultry industry. It concluded that pathogenic E. coli can provoke systemic lesions and immunosuppression, with occurrence varying across different age groups.**

**Keywords:** Broiler, colibacillosis, diagnosis, hematology, histopathology, isolation

Agriculture involves cultivating plants and animals for human needs including farming, ranching, forestry, and fishing, vital for food security and economic growth. Livestock farming focuses on animals like cattle, pigs, and poultry for meat, milk, eggs, and other products, requiring proper nutrition, housing, healthcare, and breeding. Poultry farming, or aviculture, raises birds like chickens, turkeys, ducks, and geese for meat, eggs, and feathers, playing a key role in the food industry (Tahir *et al.*, 2021; Kromann and Jensen, 2022). Effective disease management in poultry farming is essential for bird health and productivity. Infectious agents and nutritional factors are key determinants of poultry diseases. Practices such as biosecurity protocols, vaccination strategies, sanitation, hygiene, regular monitoring, quarantine measures, feed quality control, and veterinary oversight are crucial (Kamil, 2022). Colibacillosis, or avian colibacillosis, is a bacterial infection, a common and economically impactful disease affecting young and adult birds, presenting in respiratory, septicemic, and localized forms. The pathogenic strains of *E. coli* possess virulence factors that enable them to cause disease in poultry (Adeyanju and Ishola, 2014). These strains originate from the environment, contaminated sources, or carriers. Stress factors like overcrowding and poor ventilation weaken birds' immunity, raising susceptibility to Colibacillosis. Inadequate hygiene, including sanitation, aids E. coli transmission. Clinical signs range from respiratory issues to septicemia symptoms and localized infections affecting various organs in hens. Keeping in view the paucity of information regarding Colibacilosis in broiler chickens in Kashmir, this study was undertaken to evaluate the pathological alterations on the structure and functionality of different organs of infected broiler chickens.

## MATERIALS AND METHODS

**Study area and study period:** The study, conducted at the Division of Veterinary Pathology, FVSc & AH, SKUAST-Kashmir, involved regular visits to organized and unorganized poultry farms to record mortality rates. One hundred deceased birds underwent postmortem examination at the division. Case confirmation was achieved through Polymerase Chain Reaction (PCR) analysis, as well as gross and histopathological examination of the organs. Besides, blood samples were randomly collected from suspected and normal birds (12 birds each) for hematobiochemical analysis.

**Hematological examinations:** Randomly collected blood samples from birds of both groups were analyzed for hematological parameters within 24 hours using Weiss and Wardrop methods. Biochemical parameters, including Total protein, SGOT/AST, SGPT/ALT, Albumin, Globulin, Creatinine, and BUN, were measured with an Olympus biochemistry analyzer and Aspen-compatible kits.

**Mineral estimation of zinc and copper:** Serum and plasma samples were analyzed for zinc and copper concentrations using an Atomic Absorption Spectrophotometer. For serum mineral estimation, 1 ml of serum was mixed with 5 ml of nitric acid in a 50 ml volumetric flask and left overnight. The next day, the mixture was heated to 0.5 ml and then diluted to 10 ml with distilled water for analysis.

**Relative organ weight ratio:** The spleen, thymus, caecal tonsils and Bursa of Fabricius were removed from deceased birds infected with colibacillosis. These organs were gently blotted and weighed, and their relative weights were calculated following the method outlined by Tahir *et al*. (2021).

**Bacterial isolation:** Liver samples from *Escherichia coli*-infected birds were collected in sterile petri plates for organism isolation. Samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. Inoculum from the broth was streaked onto MacConkey agar and incubated. Lactose-fermenting pink colonies were streaked onto Eosin Methylene Blue agar, where *Escherichia coli* showed a metallic sheen. These colonies were transferred to nutrient agar slants and stored at 4°C. Identification involved standard morphological and biochemical tests, including Gram staining and IMVIC tests.

**Molecular detection of isolated bacteria:** Bacterial colonies from agar plates were suspended in 150 µL sterile distilled water, lysed by boiling for 10 minutes, and cooled on ice for 20 minutes. The lysate was centrifuged at 10,000 × g for 5 minutes and the supernatant was used for PCR. *E. coli* isolates were screened for the ecp gene using PCR with specific primers (forward: 5’ TGGTAATTACCGACGAAAACGGC 3’; reverse: 5’ ACGCGTGGTTACAGTCTTGCG 3’) following Alqahtani *et al.* (2015). PCR was performed in a 25 µL reaction mixture containing 2.0 µL template DNA, 2.5 µL 10X buffer, 0.2 µL 100 mM dNTP mix, 1-unit Taq DNA polymerase, 2.5 mM MgCl2, and sterile distilled water. Amplification used a Gene Amp PCR System 2400 with initial denaturation at 95°C for 5 minutes, 30 cycles of 95°C for 45 seconds, 60°C for 45 seconds, 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. PCR products were visualized by electrophoresis on a 1.5% agarose gel.

**Assessment of oxidative parameters:** Live tissue homogenates were used to assess oxidative stress markers. MDA levels were quantified using a My Bio Source ELISA kit, validated against HPLC. ROS levels in supernatant homogenates were determined using DCFH-DA, which fluoresces upon oxidation; fluorescence was measured at 525 nm (emission) and 504 nm (excitation). SOD activity was quantified using a My Bio Source Chicken SOD ELISA Kit. GSH levels were measured in total homogenate using the OPT assay, with fluorescence measured as GSH. Catalase (CAT) and $H_2O_2$ levels were determined in *Escherichia coli*-infected birds. GPX activity was assessed by monitoring NADPH oxidation to NADP+, indicated by a decrease in absorbance at 340 nm.

**Gross and histological examination:** Post-mortem examinations were conducted and lesions were documented. Tissue samples including kidney, liver, heart, spleen, bursa, air sacs, thymus, and organs displaying gross typical lesions were collected for histopathological analysis. Samples were fixed in 10% formalin, processed routinely, and stained with Hematoxylin and Eosin.

**Statistical analysis:** All data are presented as mean (M) ± standard deviation (SD). T-test was applied to determine the significant differences among different groups.



## RESULTS AND DISCUSSION

**Hemato-biochemical analysis:** The study identified significant differences in haemoglobin, packed cell volume (PCV), total red blood cell (RBC) count, and total white blood cell (WBC) count in *Escherichia coli*-infected chickens compared to normal chickens. Infected chickens exhibited significantly higher mean AST and ALT values and lower mean total protein and albumin levels, with higher globulin levels than non-infected chickens. Detailed hemato-biochemical alterations are summarized in (Table 1). Affected broilers showed altered Hb, PCV, TEC, and TLC (Umar *et al.*, 2016). The increase in AST activity may be due to myocarditis (Tandale *et al.*, 2019), while the ALT increase likely indicates hepatic cellular damage and altered membrane permeability (Sharma *et al.*, 2015). Similar AST and ALT patterns were observed in previous studies (Adeke *et al.*, 2023; Abalaka *et al.*, 2017; Taunde *et al.*, 2021). Decreased total protein and albumin suggest severe hepatic damage, consistent with findings by Abalaka *et al.* (2017). Additionally, infected birds showed significant increases in blood urea nitrogen and creatinine levels, correlating with kidney damage as confirmed by gross and histopathological changes (Sharma *et al.*, 2015).

**Table 1: Hematological and biochemical parameters in different groups of broiler chickens**

| S. No | Group | Haemoglobin concentration (g/dl) | Packed cell volume (%) | otal erythrocy count ($M/mm^3$) | Total leukocyte count ($Th/mm^3$) | AST (U/L) | ALT (U/L) | Total Protein (g/dL) | Albumins (g/dL) | Creatinine (mg/dl) | BUN (mg/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Non infected group | 11.00 $\pm 0.57^{a}$ | 29.42 $\pm 1.64^{c}$ | 6.50 $\pm 0.13^{a}$ | 6.30$\pm$ $0.31^{c}$ | 55.03$\pm$ $1.28^{a}$ | 64.37 $\pm 1.25^{c}$ | 6.35 $\pm 0.06^{a}$ | 6.37 $\pm 0.08^{c}$ | 5.27 $\pm 0.24^{a}$ | 30.47 $\pm 1.82^{c}$ |
| 2 | Infected group | 9.00 $\pm 0.72^{b}$ | 24.27 $\pm 1.32^{d}$ | 14.72 $\pm 1.52^{b}$ | 19.52$\pm$ $1.43^{d}$ | 87.57$\pm$ $2.53^{b}$ | 85.72 $\pm 3.29^{d}$ | 5.37$\pm$ $0.05^{b}$ | 2.32$\pm$ $0.04^{d}$ | 9.73 $\pm 0.26^{b}$ | 36.05 $\pm 1.42^{d}$ |

Mean of various hematological parameters differ significantly

**Mineral estimation:** The study found increased serum copper levels and decreased zinc levels in *Escherichia coli*-infected birds (Table 2). Colibacillosis can cause fluctuations in copper and zinc levels, influenced by factors such as age, health, diet, and infection stage. A significant rise in serum copper was observed, consistent with Abalaka *et al.* (2017) and Jyotsna (2019), likely due to copper's antimicrobial properties and immune response. Conversely, serum zinc levels significantly decreased, aligning with Thakur *et al.* (2024), who noted zinc utilization in immune function and other critical processes during infection.

**Table 2: Copper and zinc levels in different groups of broiler chickens**

| S.No. | Group | Copper (ppm) | Zinc (ppm) |
|---|---|---|---|
| 1. | Non infected group | 0.59±0.05 [a] | 2.90±0.11 [c] |
| 2. | Infected group | 1.27±0.00 [b] | 1.76±0.43[d] |

The mean of various hematological parameters differs significantly

**Relative organ weight ratio:** The mean weights of spleen, bursa, thymus and caecal tonsils were comparable between infected and non-infected birds. However, a significant ($P \leq 0.05$) reduction in the relative weight of lymphoid organs was observed in infected birds compared to normal birds (Table 3). This weight loss is likely due to severe atrophy and lymphocyte depletion in the lymphoid follicles of the bursa, spleen, thymus, and caecal tonsils (Berthault *et al.*, 2018).

**Table 3: Relative weight of lymphoid organs of broiler chickens infected with Colibacillosis**

| S. No | Groups | Spleen (g/kg) | Bursa (g/kg) | Thymus (g/kg) | Ceacal tonsils (g/kg) |
|---|---|---|---|---|---|
| 1. | Non-infected group | 3.6[a] ± 0.15 | 3.50[c]± 0.37 | 3.7[a] ± 0.1 | 3.5[c]± 1.17 |
| 2. | Infected group | 1.20[b]± 0.45 | 1.40[d] ± 0.17 | 1.5 [b]± 0.27 | 1.5[d]± 0.52 |

Means in different columns bearing common superscript does not differ significantly

**Oxidative stress markers:** Infected birds showed a significant increase in oxidative markers, such as malondialdehyde (MDA) and reactive oxygen species (ROS), compared to normal birds. Antioxidant defenses (SOD, GSH, and catalase) were significantly lower in infected birds. Oxidative stress, induced by high levels of free radicals, is linked to various diseases, including bacterial infections. The host immune response and bacterial virulence factors generate oxidative stress, impacting poultry growth, production, and meat and egg quality (Israel *et al.,* 2023; Mishra and Rajesh, 2019).

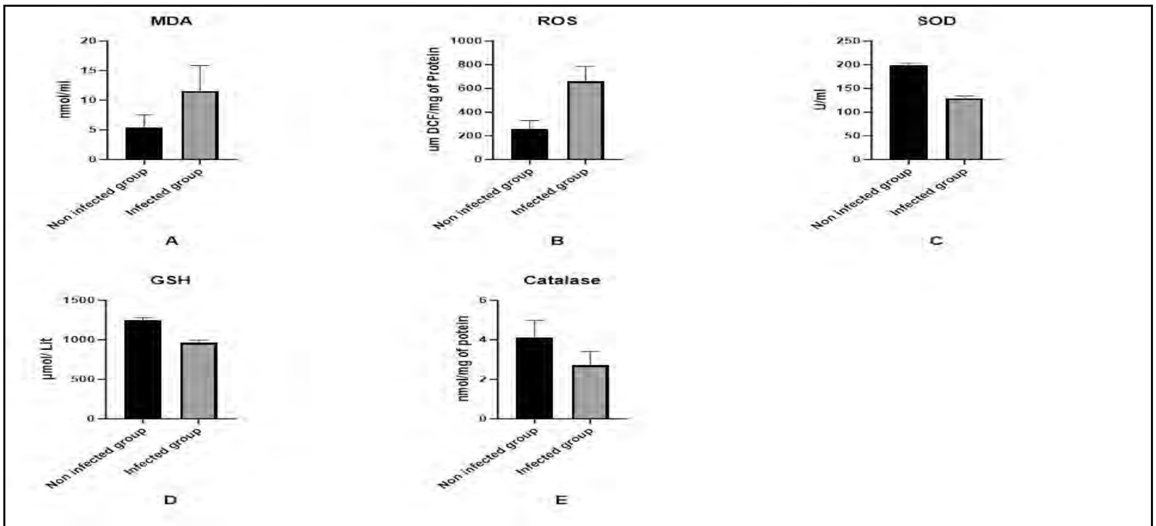


**Fig 1: Graphs depicting the levels of oxidative and anti-oxidative markers in infected birds and non-infected birds in liver homogenates. Data is expressed as Mean ± S.D {P<0.05}**

In this study, infected poultry exhibited altered levels of MDA, ROS, SOD, GSH, and catalase. Elevated MDA indicated tissue and cellular membrane damage (Fig. 1). Increased ROS levels signified oxidative damage related to disease (Israel *et al.,* 2023). Reduced superoxide dismutase (SOD), an enzyme defending against oxidative stress, was observed (Alkazemi *et al.,* 2021). Lower levels of reduced glutathione (GSH), a key ROS scavenger, suggested heightened proinflammatory cytokines and lipid peroxidation. Catalase, important for $H_2O_2$ breakdown in stressed cells, was also reduced (Mahomoodally and Mohamad *et al.,* 2022). Further research is needed on these markers in bacterial infections.

**Isolation and identification of *Escherichia coli:*** *Escherichia coli* was identified through isolation and biochemical characterization. E. coli was isolated using MacConkey and Eosin Methylene Blue (EMB) agars, with E. coli colonies showing greenish metallic sheen on EMB and bright pink lactose-fermenting colonies on MacConkey agar. Microscopic examination revealed gram-negative, pink, short rods, arranged singly or in pairs (Fig. 1). Biochemical tests confirmed E. coli: lactose fermentation on MacConkey agar and metallic sheen on EMB, indicating lactose fermentation and amide linkage formation. Standard tests (Gram staining, IMViC, and TSI Agar) confirmed gram-negative rods and typical *E. coli* reactions: positive for indole and methyl red, negative for Voges-Proskauer and citrate. These findings align with previous research (Gupta *et al.,* 2023; Dadheech and Rastogi, 2016).

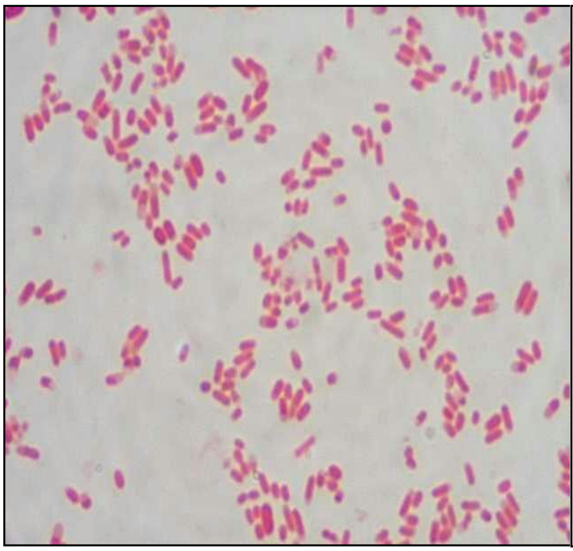

**Fig. 1: Gram's stained smear of *E. coli* showing pink coccobacillary bacteria**

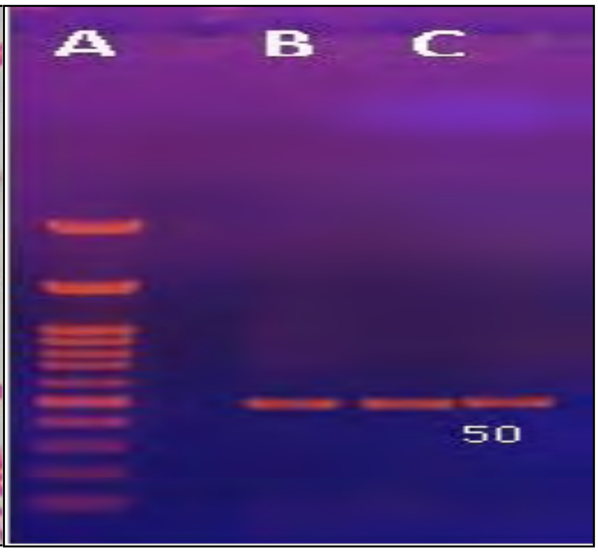


**Fig 2: Detection of ecp gene by polymerase chain reaction lane A: 100bp ladder, lane B: Negative control, lane C-E: Positive sample for ecp gene**



**Molecular detection by polymerase chain reaction:** All the isolated colonies showed amplification of 500 bp of the ecp gene by polymerase chain reaction (Fig. 2). These findings are in accordance with Kravik *et al*. (2023) and Adeyanju and Ishola (2014), who detected *E. coli* from chickens by amplification of 500 bp of the ecp gene.

**Clinical signs:** Infected birds with E. coli in this study showed symptoms including depression, lethargy, watery diarrhea, dehydration, ruffled feathers, trembling, gasping, and vent picking. Dehydration, evidenced by sunken eyes and increased thirst, resulted from diarrhea. Respiratory issues such as coughing, sneezing, and labored breathing were prevalent, consistent with Rosa *et al.,* (2019) and Shah *et al.,* (2019). Neurological signs included tremors, convulsions, and uncoordinated movements, with affected birds often displaying drooping wings. Respiratory failure generally preceded death, following signs of distress and sudden collapse.

## Pathomorphology

***Gross pathology:*** Throughout the study, a range of gross lesions were observed in *E. coli*-infected birds. Liver samples exhibited varying degrees of fibrin coverage, from thin films to thick layers presenting a "bread and butter" appearance (Fig. 3 A&B), often with discoloration and necrotic areas. The gross pathological lesions were severe in the liver and comprised of congestion, necrotic foci, deposition of fibrinous exudate on the surface of the liver along with adhesions and rounding of edges, besides swelling of the gall bladder and the presence of fluid in the abdomen (Sharma *et al.,* 2015 and Kromann and Jensen, 2022).

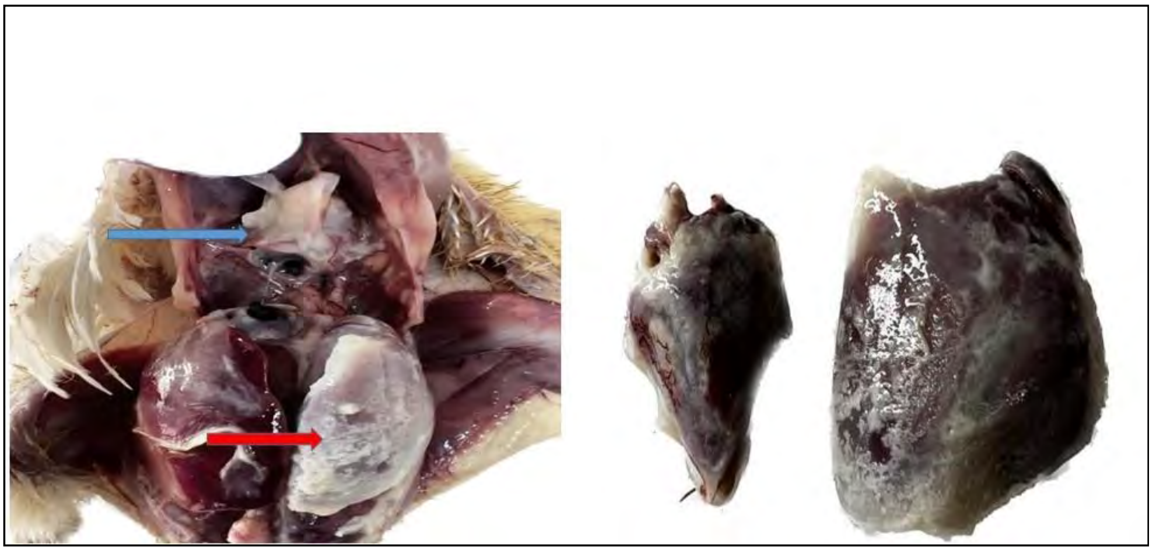

**Fig. 3: A&B: Broiler chicken affected with severe colibacillosis showing perihepatitis (Red arrow) and pericarditis (Blue arrow) as evident with thick fibrin layer attached to liver and heart**

Hearts showed congestion and fibrinous pericarditis, sometimes leading to cardiomegaly and dilated blood vessels. Severe cases displayed mottled spleens, swollen livers with rounded borders, and severe haemorrhages on the liver surface (Fig. 3 C). Kidneys were consistently engorged, enlarged, and edematous, occasionally displaying haemorrhages and necrotic foci. The lesions were in concurrence with the findings of other scientists (Taunde *et al.,* 2021), who also reported congestion and oedema in kidneys in experimental colibacillosis (Rosa *et al.,* 2019 and Sharma *et al.*, 2015). Tracheal inflammation ranged from hyperemia to fibrin deposits obstructing the lumen. Air sacs exhibited fibrinous coatings and in severe cases, caseous exudates and necrotic spots. Lungs showed edema, congestion, and consolidation, often unilateral or bilateral. Sharma *et al.* (2015) reported the deposition of fibrinous mass on air sacs in natural cases of colibacillosis particularly in birds more than 3 weeks of age. The fibrinous deposition on air-sacs could be attributed to the severity of the outbreak and an important pathological lesion in colibacillosis in chickens (Kromann and Jensen, 2022). The Bursa of Fabricius appeared edematous, hemorrhagic and inflamed with gelatinous transudate and fibrin deposits. The thymus exhibited atrophy and congestion, indicative of lymphoid depletion. Intestinal lesions included increased vascularity, hemorrhages, thickened walls, ulcers, excessive mucus, and fibrinous plaques. Proventriculus displayed congestion, hemorrhages, necrosis, and edema with serous exudates in the lumen.

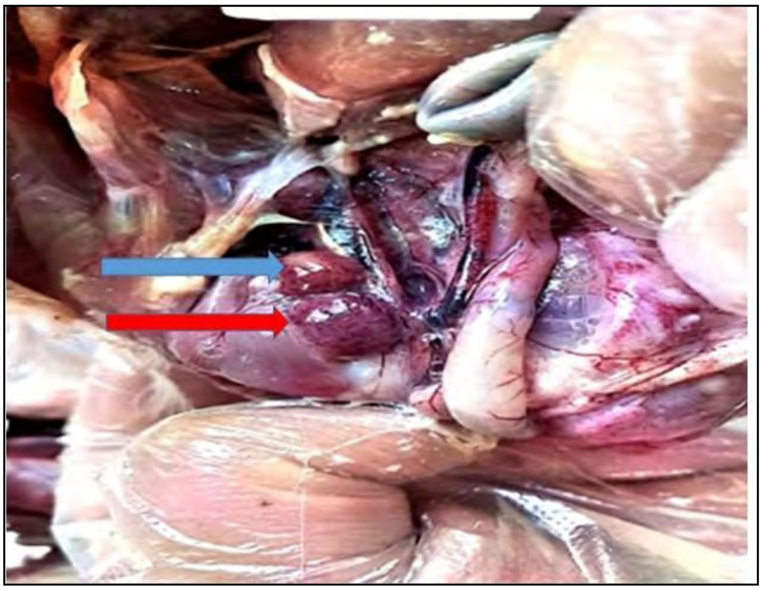

**Fig. 3C: Broiler chicken affected with severe colibacillosis showing mottled spleen with enlarged kidneys**

***Histopathology:*** In cases of moderate to severe colibacillosis, various histopathological findings were observed across different organs. The liver exhibited hepatocellular degeneration with enlarged cells, distorted hepatic cords, and congested central veins. Severe cases showed thickened capsules, heterophilic infiltration and fibrin deposition. Hepatitis was consistently observed across all age groups of affected birds (Fig. 4 A&B). Similar types of lesions have been described by researchers in a natural outbreak of colibacillosis in chicks (Sharma *et al.*, 2016; Rosa *et al.*, 2019; Kromann and Jensen, 2022) and Sharma *et al*., 2015).

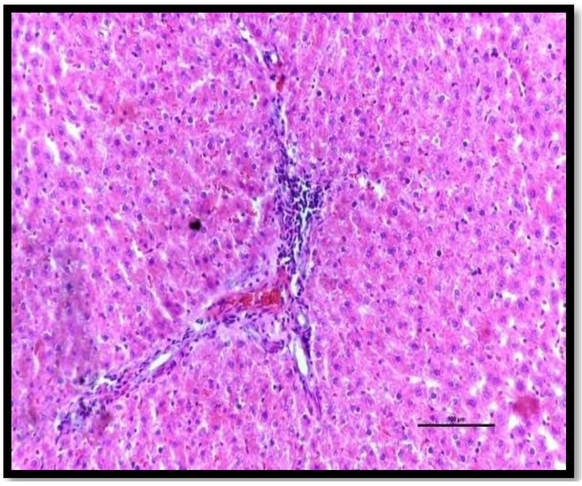

**Fig. 4A: Photomicrograph of liver revealing hepatitis as evidenced with heterophilic infiltration H&E,10X)**

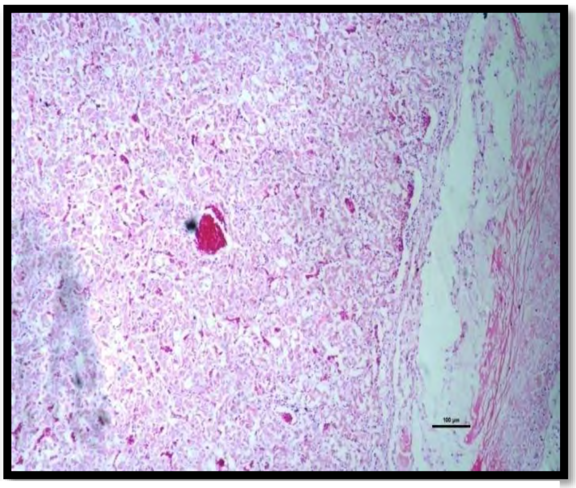

**Fig. 4B: Photomicrograph of liver revealing deposition of fibrin layer with hepatocellular degeneration (H&E, 10X)**



Hearts exhibited pericarditis with fibrinous exudate, myocardial degeneration, and necrosis (Fig. 5 A& B). Kidneys displayed interstitial congestion, glomerular nephritis and tubular necrosis with leukocytic infiltration (Fig.6 A&B). Spleens revealed severe loss of lymphoid tissue from the splenic follicles of the affected birds with several foci of necrosis in the affected tissue section.

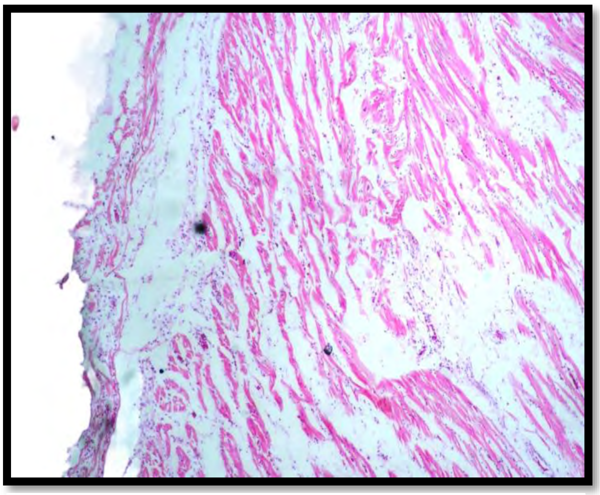

**Fig. 5A: Photomicrograph of heart revealing Fibrinous Pericarditis (H&E,10X)**

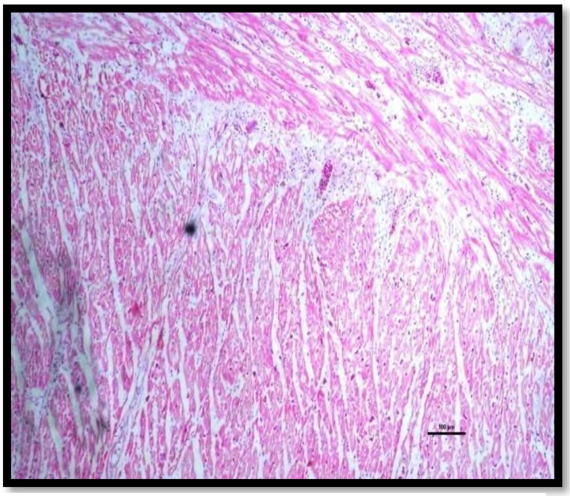

**Fig. 5B: Photomicrograph of heart revealing muscle fiber degeneration with cellular infiltration (H&E,10X)**

(Fig.7). The observations were in concurrence with the findings of various researchers (Sharma *et al.,* 2016; Sharma *et al*., 2015). Tracheal mucosa showed hyperplasia, inflammatory cell infiltration, and fibrin deposits. Air sacs had enlarged membranes with leucocytic infiltration and fibrinous exudate. The results are in concurrence with those of various previous reports where severe air sacculitis from clinical cases of different field outbreaks are noticed during postmortem examination (Aranda-Rivera *et al*., 2022; Rosa *et al*., 2019). Lungs displayed congestion, pneumonia and bronchial inflammation with parabronchial haemorrhages (Fig: 8). Similar type of lesions has been observed in various colibacillosis cases in broiler chickens which include congestion, oedema and pneumonic foci (Rosa et al., 2019; Sharma et al., 2015). Bursa of Fabricius showed lymphoid depletion, tissue degeneration and atrophy.

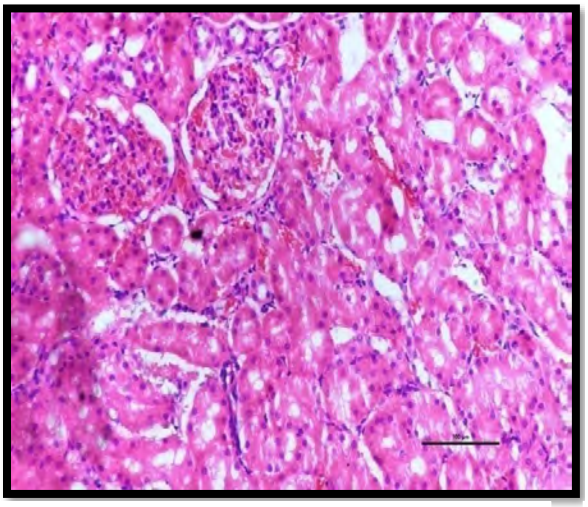

**Fig. 6A: Photomicrograph of kidney revealing glomerular Nephritis (H&E,10X)**

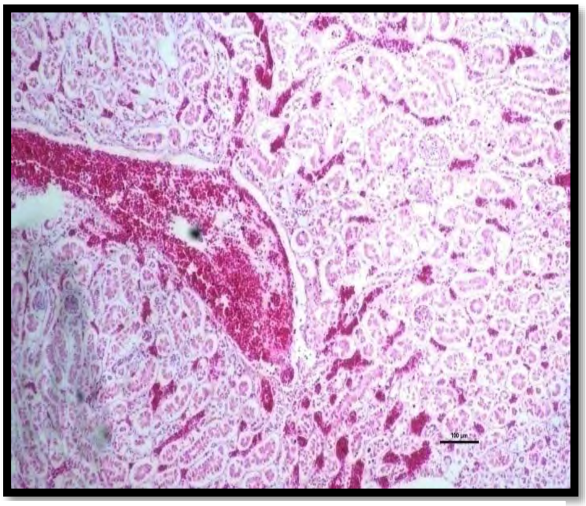

**Fig. 6 B: Photomicrograph of kidney revealing renal tubular degeneration with vascular congestion (H & E, 10X)**

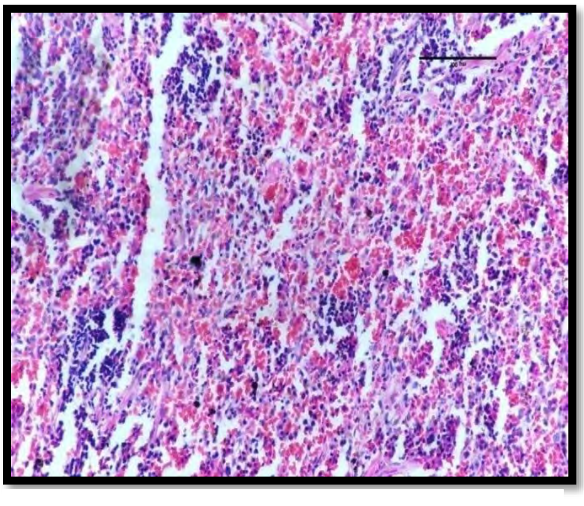

**Fig. 7: Photomicrograph of spleen revealing lymphoid depletion (H&E,10X)**

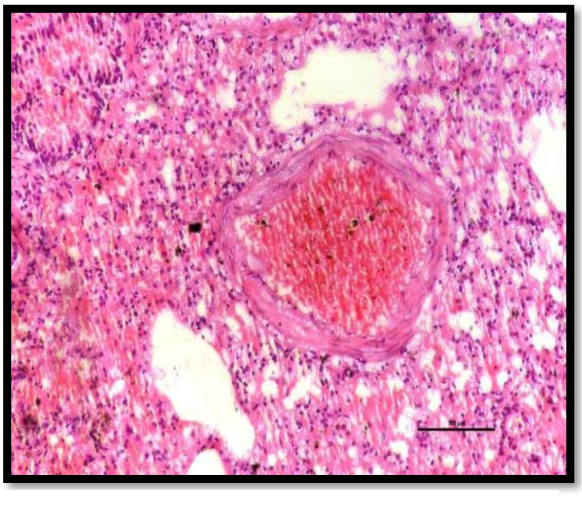

**Fig. 8: Photomicrograph of lungs revealing Vasiculitis (H&E,10X)**

The thymus exhibited lymphocyte depletion, inflammatory cell infiltration, and necrosis. Intestinal tissues showed villus blunting, mucosal necrosis, glandular atrophy, and inflammatory cell infiltration, often with haemorrhage in severe cases. Microscopically, lymphoid depletion was observed in all the lymphoid organs like the spleen, bursa, caecal tonsils and thymus of the affected birds (Shah *et al.,* 2019).

## CONCLUSION

This study demonstrated significant pathomorphological, hematological, and oxidative changes caused by Colibacillosis in broiler chickens. Elevated AST and ALT levels, altered serum minerals, and ecp gene amplification were noted. Symptoms ranged from mild to severe, with systemic infections showing pronounced damage and immunosuppressive effects. These findings underscore the economic threat of Colibacillosis, highlighting the need for effective management strategies to mitigate *E. coli*-induced lesions and mortality in broiler chickens.

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## CONFLIT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

## AUTHOR CONTRIBUTION

All the authors contributed equally in the present study.

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