RESEARCH ARTICLE

Clinico-Pathological and Bacteriological Insights into Colibacillosis in Broiler Chickens

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Abstract

Colibacillosis caused by *Escherichia coli* infection is a major bacterial disease of poultry leading to significant economic losses and potential zoonotic risk. This study investigated clinico-pathological and bacteriological aspects of the disease in broiler chickens around Patna, Bihar (India), over six months. A total of 250 suspected cases were examined through necropsy, with tissues collected for gross and histopathology. Additionally, 62 swabs (34 cloacal, 28 tracheal) and 188 organ samples were processed for bacterial isolation. Gross lesions commonly observed included fibrinous perihepatitis (57.5%) and pericarditis (52.5%), along with air sacculitis, pulmonary congestion, splenomegaly, hepatic necrosis and renal congestion. Mortality was highest in birds aged 0-2 weeks (38.1%), followed by those over 10 weeks (26.2%). Histopathology revealed hepatic degeneration and necrosis, fibrinous exudation, splenic lymphoid depletion, interstitial myocarditis and necrotizing enteritis, indicating systemic infection. Bacteriological analysis showed 101 (40.4%) samples positive for *E. coli*, with 59 (58.4%) confirmed through biochemical tests. The study highlights the high prevalence and systemic nature of colibacillosis, emphasizing the importance of combining pathological and bacteriological approaches for accurate diagnosis, along with improved biosecurity and management practices for effective control.

Key words: Broiler, *Escherichia coli*, Fibrinous pericarditis, Fibrinous perihepatitis, Histopathology.

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Introduction

Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is one of the most significant bacterial diseases affecting the poultry industry worldwide. It is responsible for considerable economic losses due to increased mortality, reduced weight gain, poor feed conversion efficiency and decreased overall productivity in broiler chickens (Nolan *et al*., 2013). The disease is widely prevalent in both intensive and semi-intensive poultry production systems, particularly in developing countries where management and biosecurity practices may be suboptimal. *E. coli* is a Gram-negative, facultative anaerobic bacterium that normally inhabits the gastrointestinal tract of birds as a commensal organism. However, certain strains possess specific virulence factors that enable them to act as opportunistic or primary pathogens under conducive conditions (Ewers *et al*., 2007; Dziva and Stevens, 2008). These pathogenic strains, collectively referred to as APEC are capable of causing a variety of localized and systemic infections grouped under the term colibacillosis. The disease commonly manifests as colisepticaemia, characterized by septicaemic spread and involvement of multiple organs including the liver, heart, spleen, lungs, and air sacs (Kabir, 2010).

Clinically and pathologically, colibacillosis presents with a wide spectrum of lesions depending on the route of infection and host factors. The most characteristic gross lesions include fibrinous pericarditis, perihepatitis, air sacculitis and peritonitis, often accompanied by congestion, necrosis and inflammatory exudation (Nolan *et al*., 2013). Histopathological findings typically reveal degenerative and necrotic changes in parenchymatous organs, fibrinous inflammation, lymphoid depletion and infiltration of inflammatory cells, indicating systemic dissemination of the pathogen. These lesions not only impair organ function but also contribute significantly to mortality in affected flocks. The occurrence and severity of colibacillosis are influenced by multiple predisposing factors such as poor ventilation, high stocking density,

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environmental stress, nutritional deficiencies and concurrent infections with viral or mycoplasmal agents (Guabiraba and Schouler, 2015). Young chicks are particularly susceptible due to their immature immune system, although birds of all age groups can be affected depending on the level of exposure and management conditions (Kabir, 2010). The disease often acts as a secondary infection following primary respiratory or enteric insults, thereby complicating disease dynamics in poultry farms.

In recent years, APEC has gained increasing attention due to its zoonotic potential and its role as a reservoir of antimicrobial resistance genes. The emergence of multidrug-resistant *E. coli* strains, including those harbouring plasmid-mediated resistance genes such as *mcr-1*, poses a serious threat to both animal and public health (Liu *et al*., 2016; Mageiros *et al.,* 2021). The potential transmission of resistant strains through the food chain underscores the need for continuous surveillance and judicious use of antimicrobials in poultry production systems. Accurate diagnosis of colibacillosis requires a comprehensive approach involving clinical observation, postmortem examination, histopathological evaluation and laboratory confirmation through bacteriological study. Integration of pathological findings with microbiological confirmation enhances diagnostic precision and facilitates effective disease management. Despite extensive research on colibacillosis, there remains a need for region-specific studies to better understand its prevalence, pathological manifestations and diagnostic patterns under local field conditions. Such studies are essential for developing targeted control strategies and improving poultry health management. Therefore, the present investigation was undertaken to provide detailed clinicopathological and bacteriological insights into colibacillosis in broiler chickens in and around Patna, Bihar, with the aim of contributing to improved diagnosis and control of this economically important disease.

Materials and Methods

The present study was conducted following approval of Institutional Animal Ethics Committee of Bihar Veterinary College, BASU, Patna (Approval No.: IAEC/BVC/2025/29) in and around Patna district, Bihar, India, over a period of six months (July 2025 to December 2025) to investigate the prevalence, bacteriological isolation and pathomorphological characteristics of colibacillosis in broiler chickens.

Sample Collection and Necropsy Examination

A total of 250 deceased broiler chickens suspected of colibacillosis were collected from different poultry farms and subjected to detailed post-mortem examination at the Department of Veterinary Pathology of the College in Patna. Systematic necropsy of each bird was performed under aseptic conditions. Gross pathological lesions were carefully recorded with emphasis on characteristic findings such as fibrinous perihepatitis, fibrinous pericarditis, airsacculitis, pulmonary congestion, splenomegaly, hepatic necrosis and renal involvement. Representative tissue samples from liver, heart, spleen, intestine and kidneys were collected for further laboratory investigations.

Bacteriological Examination

For bacteriological analysis, a total of 250 samples comprising 188 visceral organ samples and 62 swab samples (34 cloacal and 28 tracheal) were collected aseptically. The samples were processed for isolation of *Escherichia coli* following standard microbiological procedures (Quinn *et al*., 2011). Briefly, samples were initially inoculated onto Nutrient Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for primary culture and incubated at 37°C for 24 h. Subsequently, isolates were streaked onto selective and differential media including MacConkey Agar and Eosin Methylene Blue (EMB) Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Colonies showing characteristic lactose fermentation (pink colonies) on MacConkey agar and green metallic sheen on EMB agar were considered presumptive for *E. coli*.

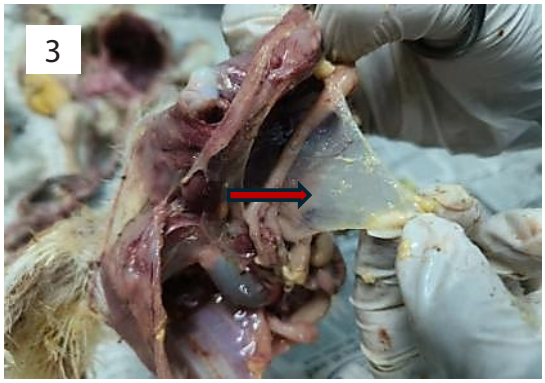
Presumptive isolates were subjected to Gram staining as per standard procedures (Quinn *et al*., 2011), revealing Gram-negative short bacilli. Motility was assessed using the hanging drop method which demonstrated characteristic diffuse growth indicative of motile organisms. Biochemical characterization of isolates was carried out using standard tests including IMViC reactions, Catalase test, Urease test, Triple Sugar Iron (TSI) agar test and Carbohydrate fermentation tests to differentiate *E. coli* from other enteric bacteria.

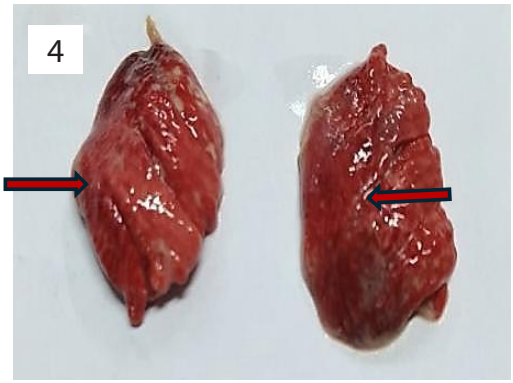
Histopathological Examination

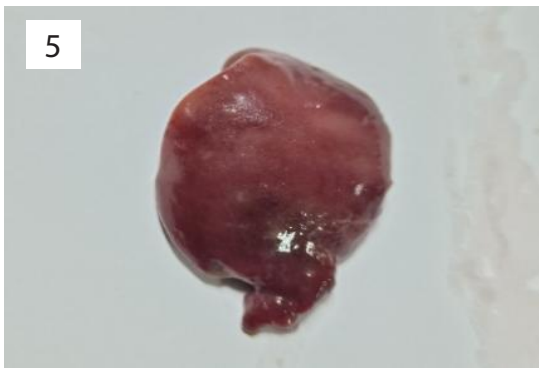
Representative tissue samples were fixed in 10% Neutral Buffered Formalin (NBF) for 24-48 h. Fixed tissues were processed using routine paraffin embedding techniques. Sections of 4-5 µm thickness were prepared using a rotary microtome and stained with Haematoxylin and Eosin (H&E) following standard procedures (Luna, 1968). The stained sections were examined under a light microscope to evaluate histopathological alterations in different organs associated with colibacillosis.

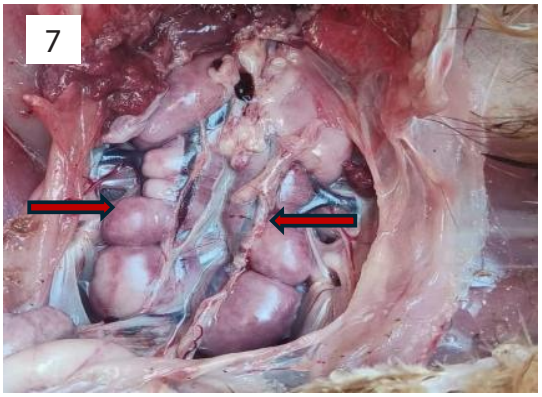
Results and Discussion

A total of 250 broiler chickens suspected of colibacillosis were examined, out of which 101 cases (40.4%) showed characteristic lesions suggestive of colibacillosis. Among these, 59 isolates (58.4%) were biochemically confirmed as *Escherichia coli*, indicating a considerable occurrence of the disease in the study area. This prevalence reflects the significant role of colibacillosis as an important bacterial disease in commercial poultry farms, particularly under intensive rearing systems where environmental stress and poor management practices facilitate disease transmission.









Gross lesions - Images showing **Fig. 1.** Thick fibrinous layer over liver indicative of severe fibrinous perihepatitis, **Fig. 2.** Thick layer of fibrin over heart of broiler chicken, **Fig. 3.** Congested lungs with multifocal haemorrhages along with mild fibrin, **Fig. 4.** Thickened and cloudy air sacs, **Fig. 5.** Congested spleen with necrotic foci and mild fibrin, **Fig. 6.** Yellowish exudate over intestinal mucosa of broiler chicken, **Fig. 7.** Swollen and mottled kidney of broiler chicken, **Fig. 8.** Congested trachea with fibrinous exudate.

Age-wise Distribution

Age-wise analysis revealed the highest incidence in the 0-2 weeks age group (38.1%), followed by 3-5 weeks (26.2%), 5-7 weeks (16.7%), 7-10 weeks (11.9%) and above 10 weeks (7.1%). The higher susceptibility of younger birds may be attributed to their immature immune system, inadequate development of protective intestinal microflora and increased environmental stress during early life. These findings were in agreement with earlier reports describing higher mortality and prevalence in young chicks (Khatun *et al.,* 2025).

Gross Pathological Findings

Gross examination revealed lesions typical of colisepticaemia (Table 1), with fibrinous perihepatitis (57.5%) and fibrinous pericarditis (52.5%) as the most prominent findings. These were characterized by thick fibrinous deposits over the liver (Fig. 1) and heart (Fig. 2). Other lesions included air sacculitis (47.5%) (Fig. 3), pulmonary congestion (43.8%) (Fig. 4), fibrinous peritonitis (37.5%), splenomegaly (Fig. 5), hepatic necrosis, intestinal exudation (Fig. 6), renal congestion (Fig. 7) and haemorrhagic tracheitis (Fig. 8) indicating generalized polyserositis. These findings were consistent with previous reports (Shah *et al*., 2019; Ahmad *et al*., 2022), confirming that fibrinous inflammation of serosal surfaces is a hallmark of septicaemic *E. coli* infection. The generalized congestion observed may be attributed to endotoxin-mediated vascular damage (Srinivasan *et al.*, 2014).

Table 1: Occurrence of Colibacillosis on the basis of Post-mortem lesions among broiler chicken (n=250) in and around Patna district

Gross Lesion	No. (%) in population
Fibrinous perihepatitis	92 (57.5)
Fibrinous pericarditis	84 (52.5)
Airsacculitis	76 (47.5)
Fibrinous peritonitis	60 (37.5)
Pulmonary congestion	70 (43.8)
Haemorrhagic tracheitis	34 (21.6)

Histopathological Findings

Histopathological examination revealed marked alterations in multiple organs. The liver showed fibrin deposition (Fig. 9), capsular fibrosis with inflammatory cell infiltration, hepatocellular degeneration, focal to multifocal necrosis, fatty changes. The heart exhibited thickened pericardium, fibrinous pericarditis (Fig. 10) and interstitial edema with mild fibrosis and infiltration of mononuclear cells. The intestines revealed epithelial desquamation (Fig. 11) and enteritis with villous distortion (Fig. 12). Kidneys showed tubular degeneration, necrosis with interstitial inflammatory infiltration and increase in pericapsular space around nephrons (Fig. 13). The spleen showed capsular fibrosis (Fig. 14). These findings were in close agreement with earlier reports (Srinivasan *et al*., 2014; Riaz *et al*., 2016; Surjagade *et al*., 2020), confirming the systemic nature of colibacillosis. The hepatic and serosal lesions may be associated with endotoxin-induced vascular injury and increased permeability.

Bacteriological and Biochemical Findings

Cultural examination revealed characteristic growth of *E. coli*, producing pink lactose-fermenting colonies on MacConkey agar (Fig. 15) and green metallic sheen colonies on Eosin Methylene Blue (EMB) agar (Fig. 16). Gram staining showed Gram-negative bacilli arranged singly or in pairs (Fig. 17). Motility testing demonstrated diffuse growth radiating from the line of inoculation, confirming motility (Fig. 18).

Biochemical characterization revealed a typical IMViC pattern (++---), with positive indole, methyl red and catalase tests and negative Voges-Proskauer, citrate and urease tests. TSI agar showed acidic slant and butt without hydrogen sulfide production. Carbohydrate fermentation tests showed acid and gas production in glucose and lactose while adonitol was not fermented. These findings were in accordance with previous studies (Khaton *et al*., 2008; Tonu *et al*., 2011; Panchal *et al.,* 2020; Choudhari *et al*., 2020), confirming the identity of *E. coli*.

Conclusion

The present study demonstrates that colibacillosis is a systemic, septicaemic disease characterized by polyserositis and multi-organ involvement. The higher prevalence in young birds, along with characteristic gross and histopathological lesions and confirmatory bacteriological findings, emphasizes the importance of early diagnosis and integrated disease management. Variations in prevalence compared to earlier studies may be attributed to differences in management practices, environmental conditions and biosecurity measures. Overall, the findings highlight that improved farm hygiene, strict biosecurity and timely diagnostic intervention are essential to control colibacillosis and reduce economic losses in poultry production.

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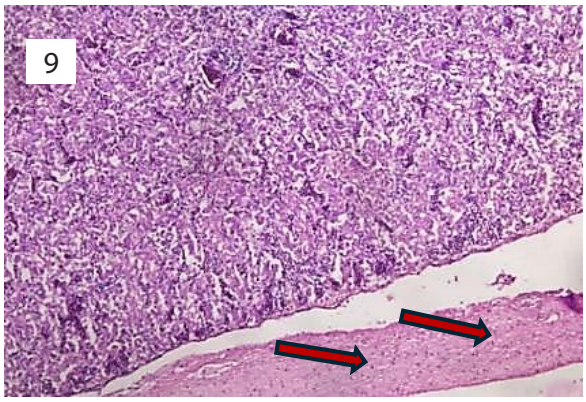

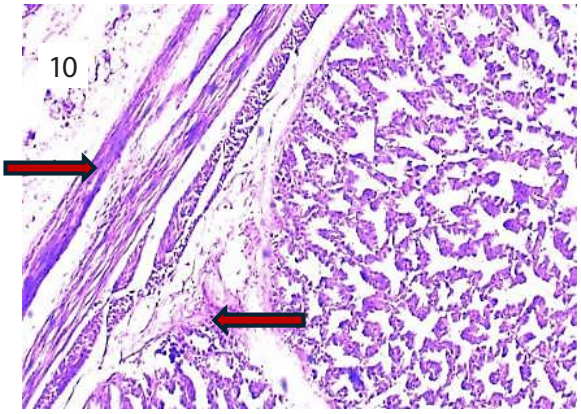

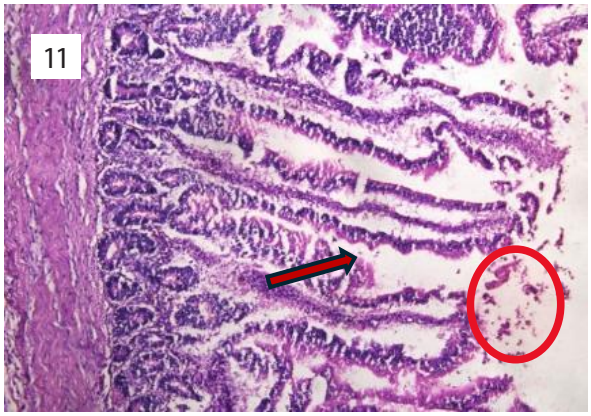

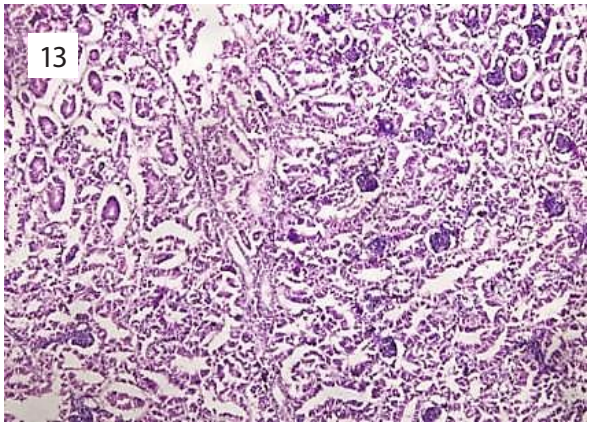

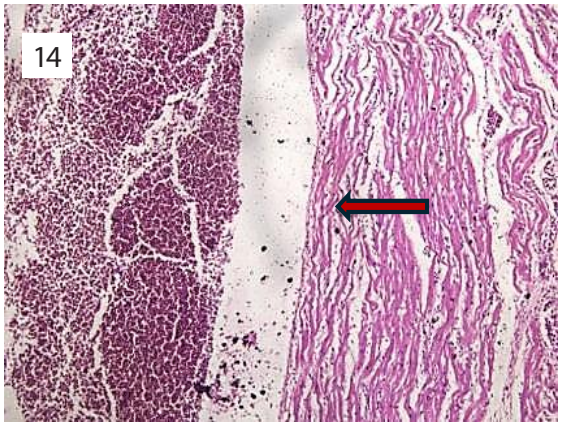

Histopathology: Fig. 9. Liver of broiler (H&E, x10) showing fibrinous perihepatitis having large amount of fibrin deposits, **Fig. 10.** Heart of broiler (H&E, x10) showing fibrinous pericarditis with infiltration of mononuclear cells, **Fig. 11.** Intestine of broiler (H&E, x10) showing desquamation, necrosis and infiltration of mononuclear cells, **Fig. 12.** Intestine of broiler (H&E, x10) showing severe villous distortion with epithelial necrosis and inflammatory cell infiltration, **Fig. 13.** Kidney of broiler (H&E, x10) showing increase in pericapsular space around nephrons along with infiltration of mononuclear cells, **Fig. 14.** Spleen of broiler (H&E, x10) showing capsular fibrosis.

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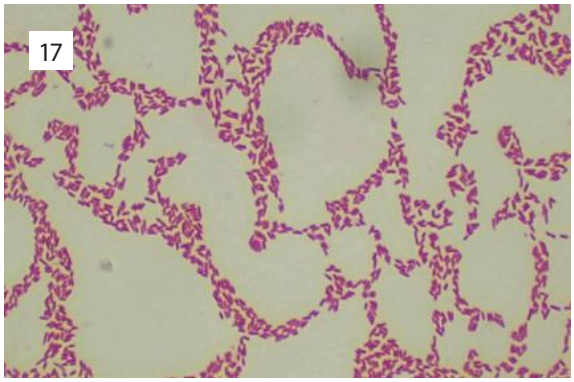

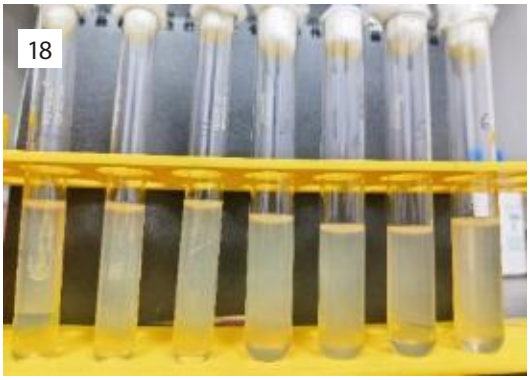

Fig. 15: Pinkish colored lactose fermenting colonies of *E.coli* on MacConkey agar., **Fig. 16.** Characteristic Green Metallic Sheen colonies of *E.coli* on EMB agar, **Fig. 17.** Microscopic examination of the Gram-stained smear demonstrated short, pink-stained Gram-negative bacilli (x100), **Fig. 18.** Motility test demonstrating diffuse growth radiating from the line of inoculation.

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