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Pathology of Colibacillosis and Molecular Detection of its Pathogen in Chickens in Gazipur District

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ABSTRACT

Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is an important cause of morbidity, mortality and resultant economic losses in poultry industry worldwide. Prevention of colibacillosis is becoming challenging due to the emergence of virulent and multidrug resistant strains. Early detection of colibacillosis is crucial in formulating preventive and therapeutic strategies in commercial chickens. This study aimed to characterize pathology and causal agent by molecular methods in chickens. Tissue samples were collected and fixed in 10% neutral buffered formalin, swabs were collected from dead chickens and cloaca of sick chickens were enriched in tetrathionate broth. The bacteria were cultured on different culture media, stained with Gram's stain, allowed to ferment different sugars, and subjected to gene amplification by PCR and histological studies. The bacteria showed metallic sheen on EMB agar, were pink color short rod to coccoid in shape, and were single or paired with Gram's staining. The bacteria fermented maltose, mannitol, dulcitol, dextrose, arabinose and dextrose with production of acid and gas but did not ferment inositol. Postmortem examination revealed congestion and consolidation of lungs, perihepatitis and multifocal necrosis in liver, and hemorrhage and mucus in duodenum. The heart displayed pericarditis with thickening of pericardium, multifocal coagulation necrosis with infiltration of heterophils and lymphocytes in the periportal area of liver.

Introduction

Chickens are the most popular amongst different poultry species around the globe. Owing to their relatively low fat and cholesterol content, chicken meat is considered a healthy protein of animal origin for all age group of people irrespective of cultural belief. Chicken ranks to be the cheapest among the meats consumed globally (Bostami and Selim, 2023). Poultry is an important sub-sector of livestock and contributes 1.6% in national GDP, and approximately 6 million people work in this sector to earn livelihood in Bangladesh (BBS, 2022-23). The chicken accounts for almost 36% of the whole animal

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protein sources in Bangladesh (Rahman, 2023). It has been reported that the demand of poultry meat is increasing steadily in Bangladesh, per capita meat intake increased from 120 g/day/head to 137.38 g/day/head and egg consumption increased from 104 number/year/head to 134.58 number/year/head in the year 2022-2023 from year 2013-2014 (BBS, 2022-23). However, poultry industry is facing challenges with flock health issues associated with infectious diseases including colibacillosis, mycoplasmosis, salmonellosis, mycotoxicosis and Newcastle disease and hindering expansion of poultry farming in Bangladesh (Rahman, 2023). Colibacillosis, the most common bacterial disease in chickens, possesses major health problem in chicken industry worldwide including Bangladesh (Khaton, 2008). So far 28 strains of pathogenic *E. coli* have been identified (Joseph et al., 2023). All avian species and every age groups of birds are affected with *E. coli* infection. Avian pathogenic *E. coli* (APEC) strains belong to the phylogenetic group linked with extra-intestinal pathogenic *E. coli* (ExPEC) and show a wide serological diversity among strains (Schouler et al. 2012). Though serotype O2 and O78 causes 80% of APEC globally (Dziva and Stevens, 2008), their prevalence varies amongst farms and geographical locations (Barbieri et al. 2015; Younis et al. 2017). The mortality of chickens by APEC is less than 10% for non-virulent strains, 10-29% for intermediate strain and greater than 29% for virulent strains (Larive and Partners, 2020). Poor navel health, damage of mucosa due to viral infections and immunosuppression are thought to be some predisposing factors for colibacillosis in chickens. It causes economic losses both in broiler and layer in terms of morbidity, mortality, decreased egg productivity and chick quality (Nolan, 2022). APEC produces different types of lesions in chickens including colisepticemia, coligranuloma, peritonitis, osteomyelitis, salpingitis, omphalitis, swollen head syndrome, yolk sac infection, arthritis and synovitis (Kabir, 2010). Prevention and controlling of colibacillosis is becoming challenging due to the emergence of virulent and multidrug resistant strains (Johnson et al. 2006). *E. coli* is identified by cultural and staining properties, biochemical tests, gene detection and sequencing. However, works on characterization of colibacillosis from field outbreak is rare in Gazipur district, a poultry rearing hub in Bangladesh. Therefore, this study aimed to characterize pathology, cultural, staining and biochemical properties and detection of gene of *E. coli* from natural outbreak of colibacillosis in Gazipur district.

Materials and methods

Ethics statement

All animal experiments described in this manuscript were conducted in accordance with the principles of care and handling of experimental animals approved by the ethical committee of the Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU/ AREC/ 2023/26).

Collection of samples

A total of 30 dead chickens (20 broilers and 10 layers) died of suspected colibacillosis were dissected, gross lesions were recorded and tissue samples from liver, lungs, spleen, intestine, heart and kidney were collected aseptically for culture and in 10% neutral buffered formalin for histopathology. Cloacal swabs from 20 clinically sick chickens, 10 broilers of 5-week-old and 10 layers of 35-week-old, were collected aseptically in tetrathionate broth (TB) from the same flock (Joseph et al. 2023). The tissues and swab samples collected were brought to the Pathobiology Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706 for further analysis.

Isolation of bacteria

The bacterial population were increased by incubating the samples collected in TB at 37℃ for 24 h. Thereafter, cultures were streaked on Nutrient agar followed by subculturing on Triple sugar iron (TSI) agar, Brilliant green agar (BGA), Eosin methylene blue (EMB) agar, Salmonella Shigella (SS) agar, MacConkey (MC) agar and then incubated at 37℃ for 24 h for cultural studies (Joseph et al. 2023). Single colony of bacteria was further sub-cultured on EMB agar until pure cultures were attained.

Cultural and biochemical test

The cultural characteristics such as shape, size, surface texture, edge and elevation and color, opacity of bacteria on different media were recorded. Gram's staining was used to determine the size, shape and arrangement of bacteria as described previously (Zinnah et al. 2007) and motility test was performed by hanging drop technique (Haider et al. 2003). Biochemical test

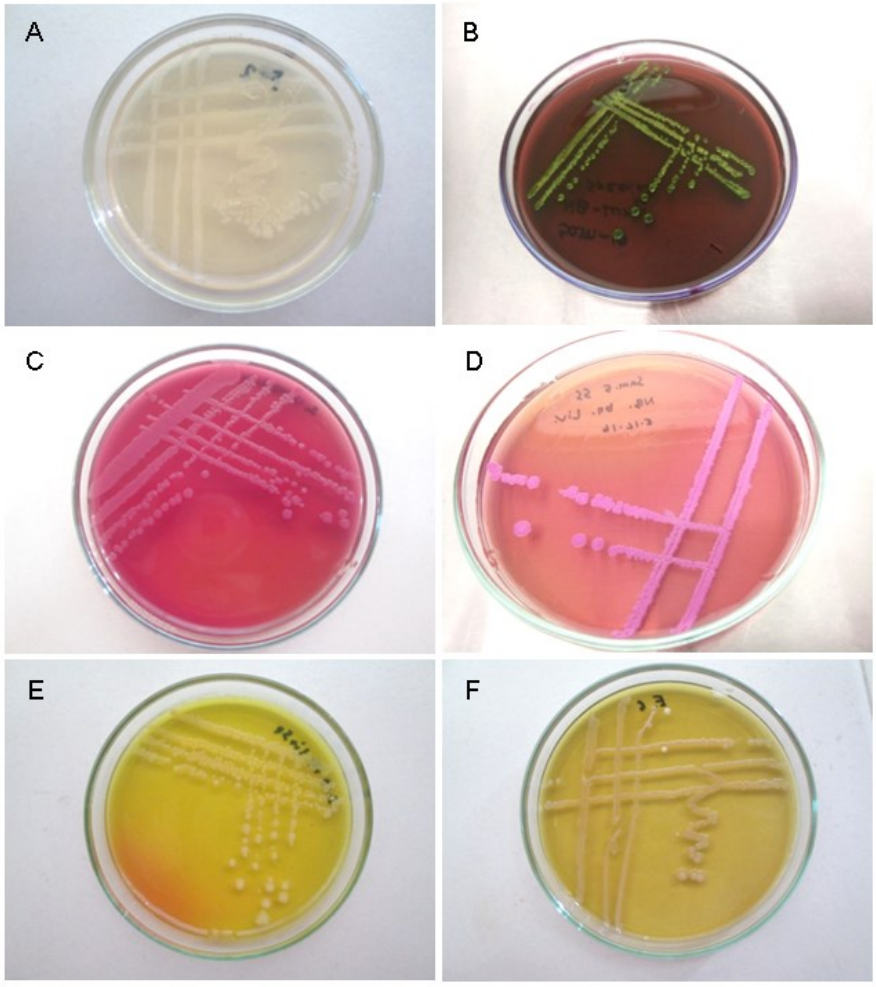

Fig. 1. The organisms showed round and white colonies on Nutrient agar (A), bright pink smooth and raised colonies on MacConkey agar (B), yellowish green colonies surrounded by an intense yellow green zone on Brilliant Green agar (C), pink colonies on SS agar (D), transparent colonies on TSI agar (E) and greenish colonies with metallic sheen on EMB agar (F) after overnight incubation.

was performed by adding 0.2 mL of nutrient broth culture of pure isolated colonies into the test tube containing sugars namely glucose, sucrose, lactose, mannitol and maltose and incubated at 37℃ for 24 h. Color change from red to yellow indicated the acid production and bubbles in the inverted Durham's tube indicated gas accumulation (Khaton et al. 2008).

Polymerase chain reaction (PCR)

DNA was extracted from *E. coli* isolate using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) and extracted DNA was quantified by using spectrophotometer (DU-640, Beckman, Germany) on the basis of optical density ratio at 260: 280 nm (Haider et al. 2013). The oligonucleotide sequence targeting 16S rDNA gene were 27F (5´-AGAGTTTGATCMTGGCTCAG-3´) and 1492R (5´-TACGGYTACCTTGTTACGACTT-3´). Thirty microliter of PCR reaction mixture containing buffer 3.00 µL, 10mM dNTP 0.60 µL, 5mM forward primer 1.00 µL, 5mM reverse primer 1.00 µL, Taq DNA polymerase 0.25 µL and ultrapure water 22.15 µL was used for amplification (Mastercycler™ 9600, eppendorf, Germany). Briefly, Initial denaturation was at 94ºC for 3 minutes, followed by denaturation at 35 cycles for 94ºC for 1 minutes, annealing at 55ºC for 45 sec and extension at 72ºC for 1 min and hold for 4ºC.

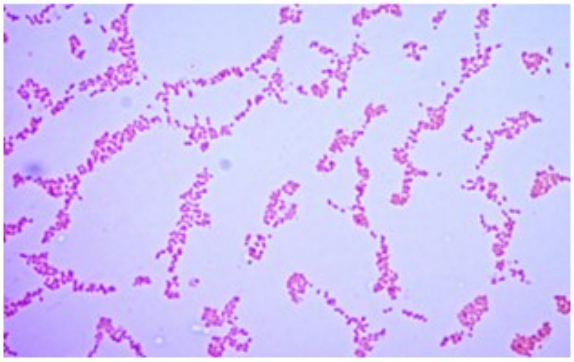

Fig. 2. The isolates of *E. coli* were pink color short rod to coccoid in shape with Gram's stain.

Agarose gel Electrophoresis

Amplified products were analyzed by electrophoreses on 1.5% agarose gel containing 5 µg per ml ethidium bromide with a 100 bp ladder as molecular weight marker (Haider et al. 2013). Bands were observed under UV light on a transilluminator (Labortechnic, Germany).

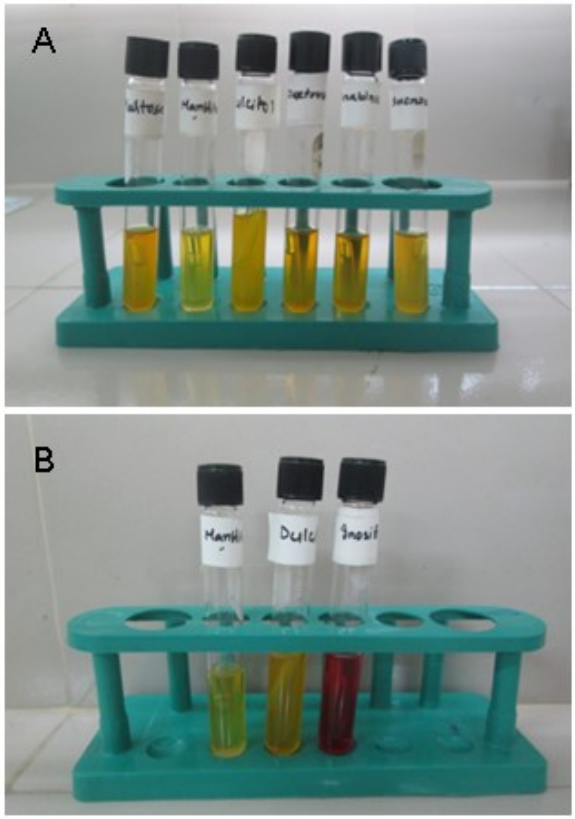

Fig. 3. Isolated *E. coli* fermented maltose, mannitol, dulcitol, dextrose, arabinose and dextrose with acid and gas production (A). Mannitol and dulcitol were fermented and Inositol was not fermented by isolated *E. coli* (B).

Histopathological examination

The formalin fixed tissues were trimmed and re-fixed in 10% neutral buffered formalin (pH 7.4) for overnight. Tissues were placed in 70% ethanol, dehydrated through a series of graded ethanol, cleared in xylene and embedded in paraffin. Tissues sectioned at a thickness 5 µm were stained routinely with hematoxylin and eosin (HE) and mounted with Canada Balsam (Haider et al. 2003). Images were grabbed by using Photographic Microscope System (ZEISS AxioCam ERc 5s).

Results

Cultural, biochemical and staining properties

The organisms showed round and white colonies on Nutrient agar (Fig. 1A), bright pink smooth and raised colonies on MacConkey agar (Fig. 1B), yellowish green colonies surrounded by an intense yellow green zone on Brilliant Green agar (Fig. 1C), pink colonies on SS agar (Fig. 1D), transparent colonies on TSI agar (Fig. 1E) and greenish colonies with metallic sheen on EMB agar (Fig. 1F). Staining of bacteria showed short rod to coccoid shaped, single or paired Gram-negative bacilli (Fig. 2). The organisms fermented basic sugars such as dextrose, maltose, mannitol, arabinose, sucrose, lactose and dulcitol (Fig. 3A) with production of acid and gas. Mannitol and dulcitol were fermented and Inositol was not fermented by isolated *E. coli* (Fig. 3B). Acid production was indicated by the accumulation of bubbles in the inverted Durham's tube and color changes from reddish to yellow on TSI slant culture. The isolated bacteria did not ferment inositol. Active motility was observed on hanging drop slide prepared with broth culture.

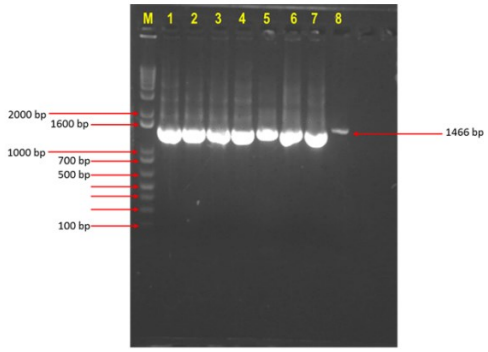

Fig. 4. Amplified DNA showed an amplicon size of 1466 bp on agarose gel electrophoresis.

Table 1. Prevalence of *E. coli* from cloacal swabs of apparently healthy layer chickens

Samples/Swabs	Number of samples examined	Number of positive isolates	Overall prevalence (%)
Total swabs	50	40	80

Table 2. Cultural prevalence of *E. coli* collected samples in different organs

Sl. No.	Organs of swabs	Total swabs	Positive	Cultural Prevalence (%)
1.	Liver	30	20	66.67
2.	Spleen	30	15	50.00
3.	Lungs	30	25	83.33
4.	Intestine	30	26	86.67
5.	Cloaca	20	11	55.00
6.	Total	140	97	69.29

Table 3. Results of different sugar fermentation tests of isolated *E. coli*

Sl. No.	Sugar	Acid	Gas
1.	Dextrose	+ve	+ve
2.	Lactose	+ve	+ve
3.	Maltose	+ve	+ve
4.	Sucrose	+ve	+ve
5.	Dulcitol	+ve	+ve
6.	Arabinose	+ve	+ve
7.	Inositol	-ve	-ve
8.	Mannitol	+ve	+ve

Polymerase chain reaction (PCR)

The extracted DNAs amplified using 27F-1492R primer sets targeting 16S rRNA demonstrated 1466 bp products from the bacteria of each field samples (Fig. 4). Usually, diagnosis of colibacillosis is done by isolation and identification of *E. coli* which depend upon the culture of the organism using different selective media and biochemical tests. PCR, on the other hand, is considered highly sensitive and specific for the detection of pathogenic bacteria from clinical samples.

Gross lesions

The necropsy examination revealed congestion and consolidation of lungs. The intestine showed profuse mucus, congestion and hemorrhagic enteritis, hemorrhage was also evident in the cecal tonsil. The air sacs were cloudy and opaque, and the livers were with whitish thick capsule indicating air saculitis and perihepatitis (Fig. 5A), respectively. In some cases, the livers showed necrotic foci and

greenish hue. The spleens were enlarged and showed severe congestion with thickening of capsule (Fig. 5B). The pericardium was cloudy with moderate thickening indicative of pericarditis.

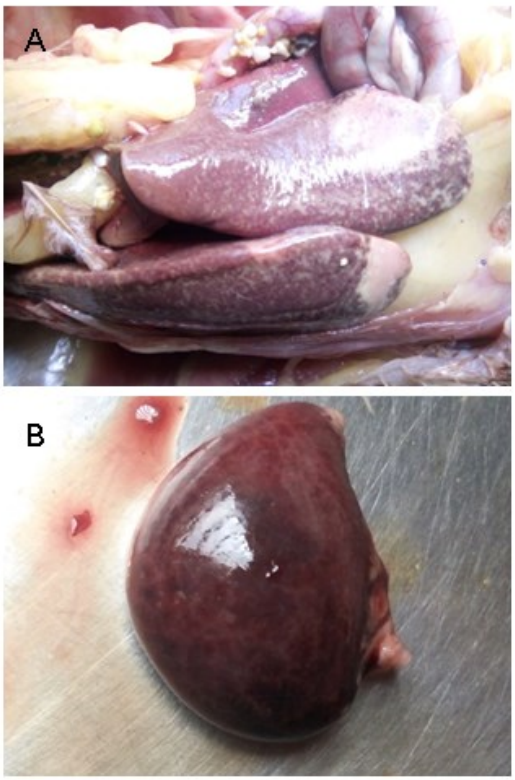

Fig. 5. Gross lesions of colibacillosis. Liver shows multifocal necrosis and perihepatitis (A) and hemorrhage and necrotic foci in spleen (B).

Microscopic lesions

Histopathological examinations of heart showed pericarditis characterized by thickening of pericardium and infiltration of macrophages (*Fig. 6A*). In duodenum, there was severe infiltration of leukocytes, mainly involving heterophils and lymphocytes in the submucosa (Fig. 6B). Livers exhibited multifocal coagulation necrosis along with infiltration of heterophils and lymphocytes in the periportal area (Fig. 6C). The lungs displayed severe congestion, infiltration of heterophils and lymphocytes in the wall of the bronchus as well as in the peribronchial alveoli (Fig. 6D). Spleen revealed scattered pyknosis of lymphocytes and proliferation of RE cell.

Discussion

The study was conducted for isolation, identification and characterization of the causal agent of colibacillosis and characterization of its pathology in naturally infected broiler and layer chickens. All isolates of *E. coli* produced pink colonies on MacConkey agar and greenish colonies with metallic sheen on EMB agar. Similar results were reported by different authors (Haider et al. 2013; Ashraf et al. 2015; Matin et al.2017). On BG agar, it showed yellowish green colonies surrounded by an intense yellow green zone (Zinnah et al. 2007). Morphology of *E. coli* as evidenced by Gram's staining were pink colored, short rod to coccoid shaped, single or paired Gram-negative bacilli and on hanging drop slide all isolates were found to be motile (Jakaria et al. 2012). In the present study all of the isolates of *E. coli* fermented dextrose, maltose, mannitol, arabinose, sucrose, lactose and dulcitol which are in agreement with the findings reported earlier (Matin et al. 2017). Importantly, the isolates did not ferment inositol. The reason might be due to slight shared antigenic variations among the isolates of *E. coli*. The extracted DNAs were amplified by using 27F-1492R primer sets targeting 16S rRNA and found 1466 bp products comparing with 100 bp ladder as evidenced by agarose gel electrophoresis (Westhuizen and Bragg, 2012). Usually, diagnosis of colibacillosis is done by isolation and identification of *E. coli* which depends upon the culture of the organism using different selective media and biochemical tests. PCR, on the other hand, is considered as highly sensitive and specific for the detection of pathogenic bacteria from clinical samples (Cohen et al. 1993).

Gross appearances showed in the lungs and intestines were congestions, consolidations and hemorrhages which were also extended to cecal tonsil. Air saculitis and congested and thickened liver capsule were also recorded. Presence of these lesions were variable in all dead birds and these changes could be due to the vascular damage caused by *E. coli* endotoxin (Dutta et al. 2013; Srinivasan et al. 2014).

Histopathological evaluation exhibited intense damage of the pericardium and moderately inflammation of duodenum. The observed inflammatory and necrotic lesions in the liver, lungs and spleen of the affected birds were due to vascular injury by endotoxin released from *E. coli* (Abalaka et al. 2017).

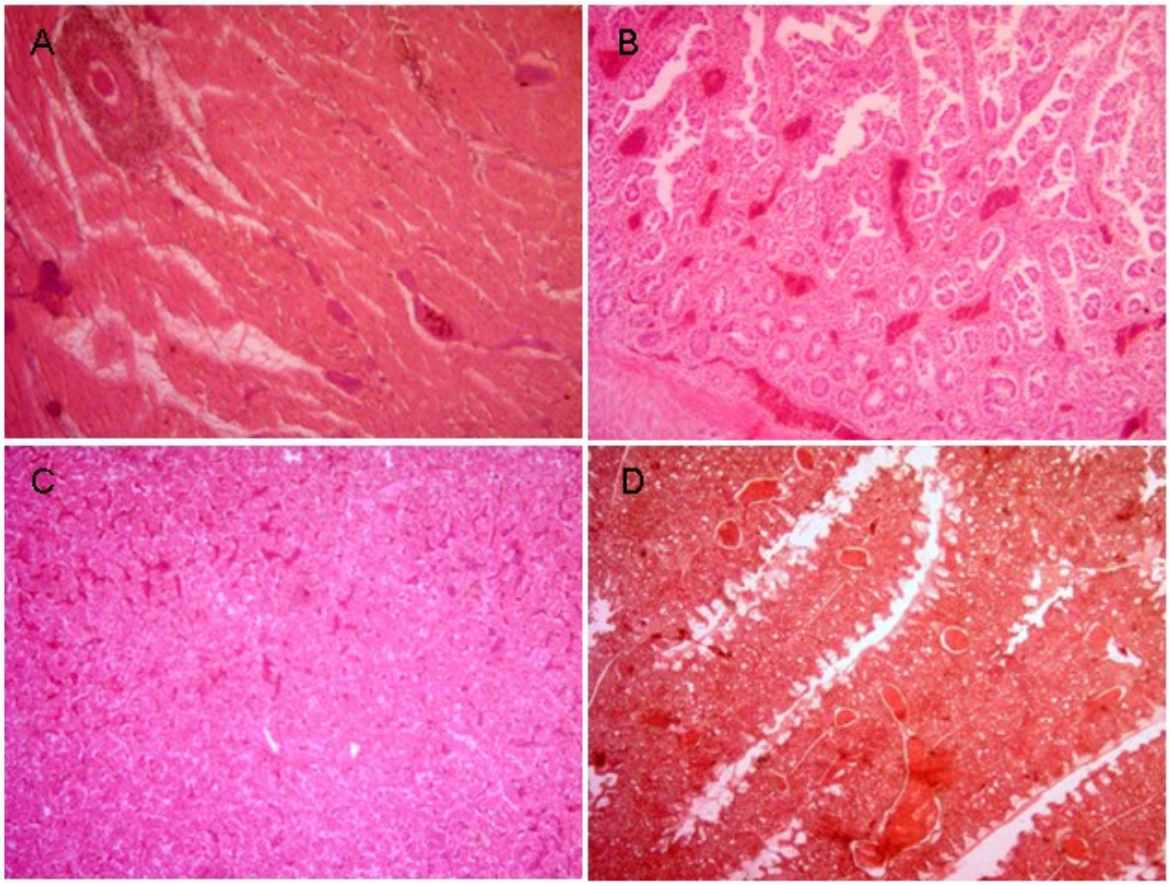

Fig. 6. Microscopic section of heart showing focal infiltration of mononuclear cells (A). Infiltration of leukocytes, mainly involving heterophils, lymphocytes and macrophages in the submucosa of duodenum (B). Livers section showing multifocal coagulation necrosis of hepatocytes, infiltration of heterophils, lymphocytes and macrophages mainly in the periportal area (C). The lungs showing severe congestion, infiltration of heterophils, macrophages and lymphocytes in the wall of the bronchus as well as in the peribronchial alveoli (D).

The coagulative necrosis of liver with focal lesions could be due to tissue hypoxia associated with vascular damage (Myers et al. 2012). Comparable focal necrosis and cellular infiltrations were documented earlier in the liver and heart of birds that died of colibacillosis (Srinivasan et al. 2014). Similarly, congestions of central veins and sinusoids with cellular infiltrations around the periportal area were described in the liver of broiler chickens suffering from colibacillosis (El-Ghany and Madian 2011).

Conclusion

Colibacillosis is considered one of the leading causes of chick mortality in poultry industry in Bangladesh. To boost up the poultry industry a rapid and reliable diagnosis and strategic management is a must. Early diagnosis of colibacillosis based on the cultural and staining properties, postmortem findings and confirmation PCR could be the choice for effective management of poultry farms. Findings of this work will assist field veterinarian and practitioner to suggest the poultry farmers for productive management of their farm in a better way.

Authors' Contribution

Conceptualization, MGH; Formal analysis TC; Methodology, TC and MGH; Investigation, TC, MAHNAK and MGH; Writing- original draft preparation, MGH and ANMAR; Writing-reviewing and editing, MGH and ANMAR; Supervision, MGH. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interests.

References

Abalaka SE, Sani NA, Idoko IS, Tenuche OZ, Oyelowo FO, Ejeh SA and Enem SI (2017). Pathological changes associated with an outbreak of colibacillosis in a commercial broiler flock. Sokoto Journal of Veterinary Sciences 15 (3): 10–14.

Ashraf AAET, Samirm AAEA, Ebtisamm M and Doaam AEM (2015). Prevalence of *E. coli* in broiler chickens in winter and summer seasons by application of PCR with its antibiogram pattern. Benham Veterinary Medical Journal 29(2): 253–261.

Barbieri NL, Oliveira AL, Tejkowski TM, Pavanelo DB, Matter LB, Pinheiro SR, Vaz TM, Nolan LK, Logue CM, Brito BG and Horn F (2015). Molecular characterization and clonal relationships among *Escherichia coli* strains isolated from broiler chickens with colisepticemia. Foodborne Pathogen Disease 12: 74–83.

BBS (2022-23). P denotes Provisional; Prepared by Dr. Hossan Md. Salim, Planning Section, Department of Livestock Services (DLS).

Bostami ABMR and Selim ASM (2023). Effect of medicinal plant byproduct combinations on carcass traits and meat quality attributes in broiler chickens. 12th international poultry seminar, 14-15 March 2023, Radisson blu Water Garden, Dhaka, Bangladesh. Pp. 77–81

Cohen ND, Neibergs HL, Mcgruber ED, Whitford HW, Behle RW, Ray PM and Hargis BM (1993). Genus specific detection of Salmonellae using the polymerase chain reaction (PCR). Journal Veterinary Diagnostic and Investigation 5: 368–371.

Dutta P, Borah MK Sarmah R and Gangil R (2013). Isolation, histopathology and antibiogram of *Escherichia coli* from pigeons (*Columba livia*). Veterinary World 6(2): 91–94.

Dziva F and Stevens MP (2008). Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathology 37(4): 355–66.

El-Ghany WAA and Madian K (2011). Control of experimental colisepticemia in broiler chickens using sarafloxacin. Journal of Life Science 8(3): 318–328.

Haider MG, Hossain MG, Chowdhury EH, Hossian MS, Das PM and Hossian MM (2003). Prevalence of enteric bacteria isolated from cloacal swabs in Sonali chickens. Progressive Agriculture 14 (1&2): 67–72.

Haider MG, Chowdhury EH, Sharif SMK and Hossain MM. (2013). Pathogenesis of Pullorum disease (PD) in chickens by local isolate of *Salmonella* Pullorum in Bangladesh. SARRC Journal of Agricultural 11(2): 01-16. Dhaka, Bangladesh.

Jakaria ATM, Islam MA and Khatun MM (2012). Prevalence, characteristics and antibiogram profiles of *Escherichia coli* isolated from apparently healthy chickens in Mymensingh, Bangladesh. Microbes and Health 1:27–29.

Joseph J, Jenning M, Barbieri N, Li Zhang, Adhikari P, and Ramachandran R. (2023). Characterization of Avian Pathogenic *Escherichia coli* Isolated from Broiler Breeders with Colibacillosis in Mississippi, USA. *Poultry*, 2023, *2*(1), 24-39;

Johnson TJ, Wannemeuhler YM, Scaccianoce JA, Johnson SJ and Nolan LK (2006). Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring among extraintestinal pathogenic *Escherichia coli*. Antimicrobiological Agents and Chemotherapy 50: 3929–3933.

Kabir SML (2010). Avian Colibacillosis and Salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environment Research Public Health 7: 89–114.

Khaton R, Haider MG, Paul PK, Das PM and Hossain MM (2008). Colibacillosis in commercial chickens in Bangladesh. The Bangladesh Veterinarian 25(1):17-24.

Larive BV and Light Castle Partners Ltd. (2020). Poultry sector study Bangladesh. Prepared for: Embassy of the Kingdom of the Netherlands in Bangladesh. NEA Pp:1–78.

Nolan LK (2022). MSD Manual, Colibacillosis in Poultry. Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, USA.

Matin MA, Islam MA and Khatun MM (2017). Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Veterinary World 10(1): 29–33.

Myers RK, McGavin MD and Zachary JF (2012). Cellular adaptations, injury and death morphological, biochemical and genetic bases. In: Pathological Basis of Veterinary Diseases (Zachary JF, MD McGavineditors). First edition. Elsevier Mosby, St. Louis, Missouri, USA. Pp 1–59.

Rahman M (2023). WPSA-BB-Newsletter. Pdf. Volume-14-Issue-I-September-2023.https://wpsa-bb.com/wp-content/uploads/ 2024/01/

Schouler C, Schaeffer B, Bree A, Mora A, Dahbi G, Biet F, Oswald E, Mainil J, Blanco J and Moulin-Schouleur M (2012). Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes. Journal of Clinical Microbiology 50: 1673–1678.

Srinivasan P, Balasubramaniam GA, Murthy TR and Balachandran P (2014). Pathomorphological studies of polyserositis in commercial caged layer chicken. Asian Pacific Journal of Tropical Medicine 7(1): 313–320.

Westhuizen WA and Bragg RR (2012). Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avian Pathology 41(1): 33–40.

Younis G, Awad A and Mohamed N (2017). Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. Veterinary World 10: 1167–1172.

Zinnah MA, Bari MR, Islam MT, Hossain MT, Rahman MT, Haque MH, Babu SAM, Ruma RP and Islam MA (2007). Characterization of *Escherichia coli* isolated from samples of different biological and environment sources. Bangladesh Journal of Veterinary Medicine 5(1&2): 25–32.