SHORT COMMUNICATION

Identification, Isolation and Pathological Changes Associated with Natural Cases of Avian Colibacillosis in Assam

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Abstract

The present study deals with the pathological changes associated with natural cases of avian colibacillosis in Assam for a period of one year (January to December 2022) at Department of veterinary Pathology of the College in Khanapara, Assam. Among the 1,449 birds presented for the post-mortem throughout the year from both commercial layer and broiler flock, mortality was 6.48% (94/1449) due to avian colibacillosis. The incidence of colibacillosis was recorded higher in the months of July (40%) and August (27%), and the least in April (4.2%). Molecular confirmation of the isolates was done using 16S rRNA. Gross lesions mostly included deposition of fibrinous exudate on the pericardial surface and pericardial thickening associated with pericarditis, apart from lesions in liver and kidneys. Histopathologically, heart showed heavy infiltration of mononuclear phagocytic cells in myocardial fibres resulting in severe myocarditis and in the epicardium, fibrin deposition was present mixed with infiltrating cells.

Key words: 16S rRNA, Avian colibacillosis, Breed, Colony morphology, Month, Pericarditis.

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Introduction

Poultry sector is the fastest growing sector which contributes a share of 1% GDP in India. Our country is currently producing 129.60 billion eggs and 9.29 million tonnes of chicken meat. Among all the bacterial diseases the septicaemia is one of the important causes of death in poultry especially chicken and imparting substantial economic loss to poultry industry in India as well as across the globe. Among the avian bacterial diseases avian colibacillosis is the most common bacterial diseases in the poultry industry all over the world because of high mortality and morbidity it causes (Rahman *et al*., 2004). Avian colibacillosis is the major infectious pathogen in birds caused by the family Enterobacteriaceae, the Gram-negative, non-spore forming, facultative anaerobes (Kakooza *et al*., 2021). Colibacillosis and salmonellosis are the most commonly occurring avian diseases that are communicable to humans (Kabir, 2010). According to Sola-Gines *et al.* (2015), colibacillosis in the poultry sector is primarily caused by avian pathogenic *E. coli* (APEC). Chickens of all ages can contract colibacillosis. *E. coli* was considered a secondary pathogen to other pre-disposing factors such as viral infection, stress, ammonia, etc. until current thought that APEC can also be a primary pathogen. Although pathological investigations of natural colibacillosis are well documented (Tonu *et al*., 2011; Bhalerao *et al*., 2013; Srinivasan *et al*., 2014), information on pathological changes associated with natural colibacillosis in chickens in Assam is scanty, hence investigated in this study.

Materials and Methods

The present study on Avian Colibacillosis was conducted at the Department of Veterinary Pathology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22 (Assam, India) from January to December 2022 on 1,449 birds from both commercial layer and broiler flock, irrespective of breed, age, sex, presented for post-mortem. Gross lesions were recorded and the collected clinical samples were inoculated in Brain heart infusion (BHI) broth for enrichment and incubated at 37°C for 16-18 h. The inoculum was further steaked on MacConkey agar and EMBA agar, then incubated at 37°C for

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16-18 h (Quinn *et al.*, 2011). The characteristic colonies were then selected for biochemical characterization using HiMedia IMViC kit.

Molecular characterization was performed by amplification of 16S rRNA by PCR with published primers (Table 1) and as described by Agung (2019) with slight modification in thermocycling parameters, which included initial denaturation at 95°C for 3 min, followed by denaturation at 94°C for 45 sec, annealing at 52°C for 1 min, extension at 72°C for 1 min, 35 cycles each and final extension at 72°C for 10 min. The 50 µL amplified PCR product was sent for sequencing at XXIIDT, Malaysia. The sequence thus obtained was blasted in NCBI nucleotide GenBank with reference strains.

Table 1: Primers used for 16S rRNA

Sl. No	Primer	Sequence (5′-3′)	Amplicon Size (bp)	Reference
1	*16S rRNA*-27F	AGAGTTTGATCCTG-GCTCAG	1465	Agung (2019)
	16S rRNA-1492R	CTACGGCTACCTT-GTTACGA		

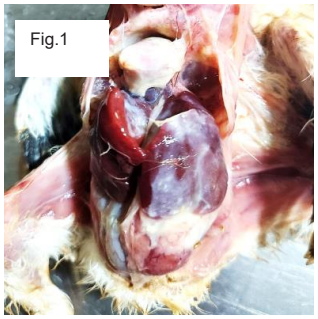
The affected tissues were collected in 10% neutral buffered formalin for histopathological examination as per procedure of Luna (1968).

Results and Discussion

During the study, the mortality rate of 6.48% (94/1449 birds) due to colibacillosis was recorded with the monthly lowest frequency in April (4.2%), May (9.5%), June (18%), and higher in July (40%), and August (27%), Among the flocks, the mortality was highest in flock of Brown layer (30%), followed by BV-300 (22.3%), and the least in local breed (9.4%) like Daothigir (9.4%), Dahlem red (8.5%), Kamarupa (7.4%), Rainbow red (6.5%), and Golden layer (6.5%), which were maintained under intensive system. The higher mortality recorded in the month of July and August might be due to the hot-humid climate of Assam, which predisposes the bird to this colibacilosis and the incidence most commonly occurred in Brown layer, BV-300, and local breeds like Daothigir, Dahlem red, Kamarupa, Rainbow red, and Golden layer in descending order.

Clinical signs reported were decreased feed intake, weakness, emaciation, difficulty in movement, restlessness, ruffled feathers, weight loss and abnormal droppings. On post-mortem examination, the characteristic changes found on heart included deposition of fibrinous exudates on the pericardial surface and pericardial thickening associated with pericarditis (Fig. 1 & 2). Liver was mildly enlarged, congested with distinct deposition of fibrinous exudates on the surface (Fig. 3). Kidneys were enlarged with congestion and few white necrotic areas (Fig. 4). As all these cases were suspected for bacterial infection, were further processed for bacterial detection and isolation of *E. coli*. A total of 94 (6.48%) isolates of *E. coli* were bacteriologically positive from 1,449 birds affected tissue samples, like liver, spleen, pericardium examined. The isolates on MacConkey agar showed rose pink lactose fermenting pin point colonies and on EMB agar greenish metallic sheen colonies after overnight incubation, which confirmed it as *E. coli*. Biochemical tests of colonies were positive for indole methyl red and negative for Vogues Proskauer, citrate utilization test. The mortality rate as well as gross and colony characteristics observed in present study concurred well with the earlier reports on avian colibacillosis from different parts of the country (Rahman *et al.*, 2004; Vijayalingam *et al.*, 2007; Kalita *et al.*, 2010; Bhalerao *et al.*, 2013; Srinivasan *et al.*, 2014; Hasan *et al.*, 2017; Chandravathi *et al.*, 2024).

Amplification of 16S rRNA gene of positive isolates by PCR showed a band at 1465 bp on agar gel electrophoresis (Fig. 5). The isolates were confirmed to be avian pathogenic *E. coli* based on 16S rRNA gene sequencing, as reported by earlier researchers (Lauro *et al.*, 2009; Tonu *et al.*, 2011; Agung 2019). The sequence obtained when blasted showed similarity with *Escherichia coli* sequences in NCBI GenBank. Histopathologically, heart showed heavy infiltration of mononuclear phagocytic cells in myocardial fibres resulting in severe myocarditis and in the epicardium, fibrin deposition was present mixed with infiltrating cells (Fig. 6A & 6B). Hepatic parenchyma had multifocal coagulative necrotic areas of hepatocytes combined with dilatation of sinusoidal spaces with mild haemorrhage in some area. In renal parenchyma there were extensive haemorrhages along with the degeneration and necrosis of tubular epithelium. Histpathological changes observed in present study were similar to those reported earlier on avian colibacillosis from different parts of the country (Rahman *et al.*, 2004; Ghosh *et al.*, 2006; Kabir, 2010; Jha *et al.*, 2012; Bhalerao *et al.*, 2013; Srinivasan *et al.*, 2014; Chandravathi *et al.*, 2024).



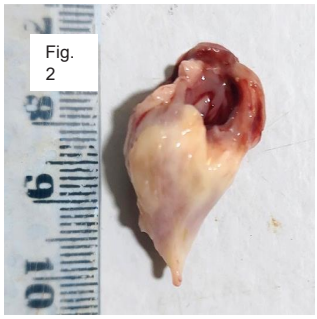


Fig. 1 & 2: Show deposition of fibrinous exudates on the pericardial surface and pericardial thickening associated with pericarditis.

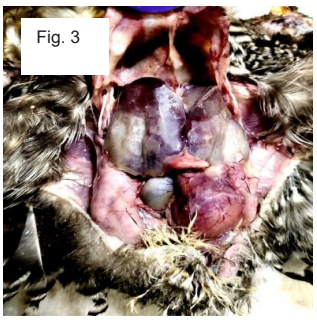


Fig. 3: Mildly enlarged congested liver with distinct deposition of fibrinous exudates on the surface

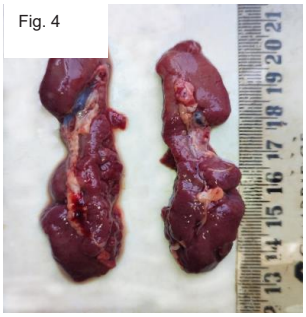


Fig. 4: Enlarged kidneys with congestion and few white necrotic areas.

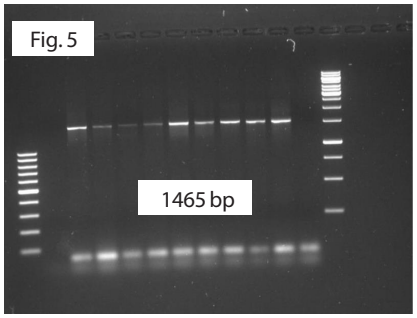


Fig. 5: Amplification of 16S rRNA gene of positive isolates by PCR showed a band at 1465 bp on agar gel electrophoresis.

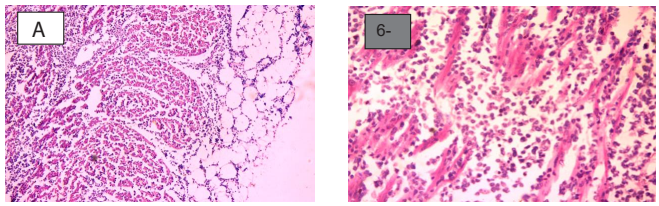


Fig. 6: Photomicrographs showing (A) fibrinous pericarditis with infiltration of inflammatory cells (H&E x10), and (B) heart showing infiltration of polymorphic lymphocytes in between of myofibrils (H&E, x40).

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