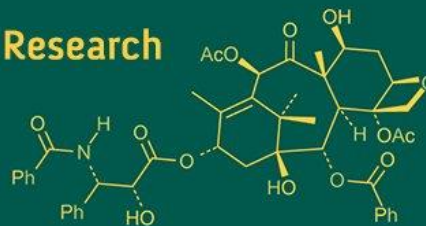


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Pathological and bacteriological investigation of colibacillosis in broiler chickens: A case report

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Abstract

Avian pathogenic *Escherichia coli* (APEC)-induced colibacillosis is one of the most common bacterial infections affecting poultry and causing significant financial losses. In the present study, colibacillosis cases in broiler chickens were reported, with emphasis on the macroscopic lesions and identification of bacterial etiology. Gross pathology included fibrinous pericarditis, perihepatitis, and a "bread and butter" appearance of the liver with a thick fibrinous layer. Bacterial etiology was identified as *Escherichia coli* based on its growth characteristic on MacConkey lactose agar, eosin and methylene blue agar, and triple sugar iron agar.

Keywords: Avian pathogenic *Escherichia coli*, colibacillosis, broiler chickens, gross pathology

Introduction

Escherichia coli is a Gram negative facultative anaerobic bacterium that colonizes the lower intestine of mammals and birds soon after birth where it coexists in harmony with the host and aids in the synthesis of essential vitamins (Sarowska *et al.*, 2019) [12]. They are motile, catalase-positive, oxidase-negative, citrate-negative, nitrate-reducing and carbohydrate fermenters (Al Humam, 2016) [2]. While the majority of *Escherichia coli* strains are not harmful, some serotypes can infect poultry and cause disease. *E. coli* infections in poultry flocks have been documented more frequently in recent years, highlighting the pathogen's expanding importance (Schouler *et al.*, 2012) [13]. The two primary categories of pathogenic *E. coli* are intestinal pathogenic *E. coli* (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC), which are distinguished by the organs in which they typically exhibit their virulence (Kaper *et al.*, 2004) [7]. Among disease-causing strains, avian pathogenic *E. coli* (APEC) holds particular importance in the broiler industry because it leads to colibacillosis, a condition responsible for considerable economic losses through bird mortality and reduced performance. The disease can appear in different forms, ranging from yolk sac infections (omphalitis) and septicemia in both chicks and adult birds, to salpingitis in layers. Respiratory involvement, especially airsacculitis, is very common in both broilers and layers, while lesions such as perihepatitis and pericarditis in other internal organs are also frequently observed (Kaper *et al.*, 2004; Nolan *et al.*, 2013) [7, 10]. The most susceptible age group for avian colibacillosis in broiler chickens is 4-6 weeks (Dho-Moulin *et al.*, 1999) [6]. Contaminated feed and water are significant sources of the disease because *E. coli* can persist for longer periods in dry and dusty conditions. Fecal contamination of eggs, where the bacteria can enter through the shell and infect the developing chick, is another important mode of transmission (Khoo *et al.*, 2010; Matin *et al.*, 2017) [8, 9]. The present case report describes the occurrence of colibacillosis in broiler chickens, including associated gross pathological lesions and confirmation of bacterial etiology.

Materials and Methods

A thorough and systematic necropsy was performed on carcasses presented to the Department of Veterinary Pathology, School of Veterinary Sciences, Abhilashi University. The gross lesions were closely examined and recorded. The liver sample was aseptically collected in the sterile container to identify and confirm the bacterial etiology and after processing it was inoculated on brain heart infusion (BHI) agar and MacConkey's lactose

(MLA) agar, incubated for 24 hours at 37 °C. The discrete lactose-fermenting colonies (pink colonies) were chosen and then sub-cultured onto Eosin Methylene Blue (EMB) agar and incubated for 24 hours at 37 °C. On EMB agar, colonies that displayed the distinctive metallic green sheen were regarded as presumed *Escherichia coli*. The growth characteristics of bacteria on triple sugar iron (TSI) agar were also observed. Gram staining, catalase test, and oxidase test were also performed.

Results and Discussion

The liver of the affected birds had a distinctive "bread and butter" appearance during necropsy because of a thick layer of fibrinous material that covered the whole organ (Fig. 1). Grossly, the liver was black, swollen, and friable. In addition, the surface of the liver also had focal necrotic regions. Besides, lesions indicative of septicemia were also visible, including perihepatitis and fibrinous pericarditis with an adherent white pericardial sac (Fig. 2). The deposition of fibrinous exudate over the liver and heart surface observed in this case is in accordance with the description by (Apostolakis *et al.*, 2021) [3], Kumar *et al.* (2013) [10] and Renu *et al.* (2012) [11]. The concurrence of our observations with previous reports confirms the

consistency of fibrinous inflammatory lesions in colibacillosis. *E. coli* was successfully identified and confirmed from the liver tissue based on growth characteristics on MacConkey lactose (MLA) agar, eosin and methylene blue (EMB) agar, and triple sugar iron (TSI) agar. On MLA agar, discrete dry pink lactose-fermenting colonies were observed (Fig. 3). On EMB agar, colonies exhibited the characteristic metallic green sheen (Fig. 4). On TSI agar, the bacterial isolate was able to ferment all three sugars (both slant and butt were yellow) with gas production, but no H₂S production (Fig. 5). These colonial characteristics of bacteria on MLA, EMB agar, and TSI agar are considered classical hallmarks of *E. coli* and serve as a reliable means of its presumptive identification (Cheesbrough, 1985) [5]. The isolated bacteria were found Gram negative, catalase positive, and oxidase negative. The observations of this study are in agreement with the findings of Abd El-Mongy *et al.* (2017) [1], who also reported lactose-positive pink colonies on MacConkey agar and metallic green sheen on EMB agar while isolating *E. coli* from poultry and emphasised that these colony characteristics provide a simple and dependable diagnostic tool for the confirmation of avian colibacillosis.

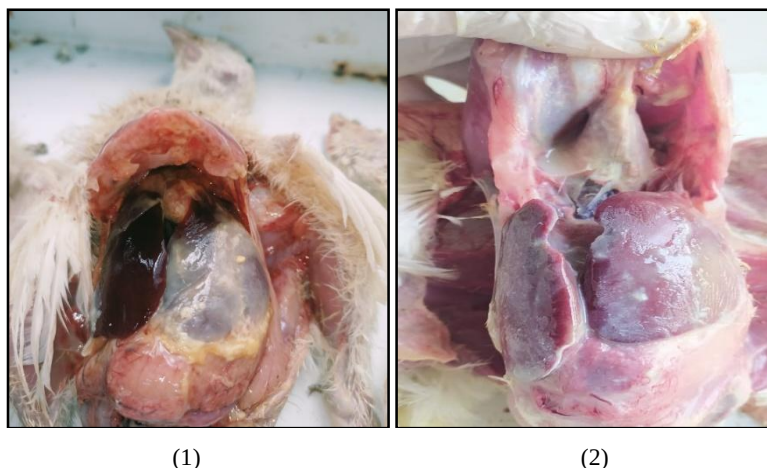


Fig 1-2: Broiler chicken affected with colibacillosis showing dark red liver with fibrinous deposits over the liver and heart.

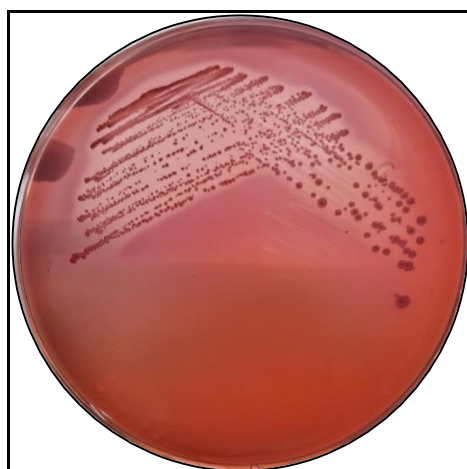


Fig.3. Pink colour colonies produced by bacteria on MLA agar

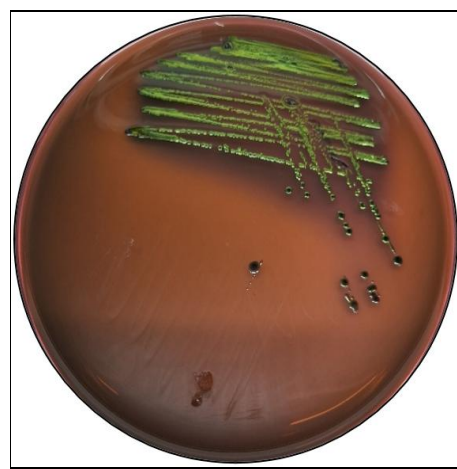


Fig 4: Metallic green sheen produced by bacterial colonies on EMB agar



Fig 5: Uninoculated TSI agar (Left) and reaction of isolated bacteria on TSI agar (Right) showed Slant: Yellow, Butt: Yellow, Gas production (TSI agar pushed upward due to Gas production indicated by black arrow), no H₂S production

Conclusion

The present investigation confirms colibacillosis as the cause of mortality in broiler chickens based on characteristic gross lesions along with successful bacteriological identification of *E. coli*. The presence of fibrinous perihepatitis, “bread and butter” liver, and fibrinous pericarditis, coupled with the cultural identification of lactose-positive pink colonies on MacConkey agar, metallic green sheen colonies on EMB agar, and characteristic reaction on TSI agar was highly suggestive of the disease.

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