Case Report

ISOLATION, IDENTIFICATION AND MOLECULAR DETECTION OF *ESCHERICHIA COLI* FROM AN OUTBREAK OF COLIGRANULOMA IN WHITE LEGHORN LAYER CHICKEN

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ABSTRACT

Coligranuloma is an economically important bacterial disease caused by Escherichia coli and is characterized by nodular granulomas in liver, mesentery and walls of intestine. Three adult white leghorn layer birds of 24-week-old were brought for necropsy examination from Jeelakarragudem village of West Godavari district. Upon necropsy, the birds revealed typically hard, yellow, nodular granulomas in the mesentery and on the wall of the intestines with few tiny nodular lesions on the liver. Impression smears of the nodules showed large number of gram-negative rod-shaped organisms. The inoculums from the nodular lesions when streaked on to MacConkey agar and EMB agar resulted in characteristic rose-pink and metallic sheen colonies respectively. PCR targeting the E. coli 16S ribosomal DNA resulted in an amplicon of 585 bp. Based on the necropsy lesions, staining and cultural characteristics and PCR, the disease was confirmed as Coligranuloma.

Keywords: Colibacillosis, Coligranuloma, *E. coli*, Layer chicken, PCR

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Escherichia coli is a gram-negative, rod-shaped bacterium normally found in the intestines of poultry andother animals. Although most of them are non-pathogenic, a limited numbersresult in infections (DebRoy and Maddox, 2001).Though a large number of *E coli* are maintained in the poultry house environment through faecal contamination, an initial exposure to pathogenic *E coli* may occur in the hatchery from infected or contaminated eggs. Systemic infections usually requires predisposing environmental factors like poor air quality and other environmental stresses or infectious causes like mycoplasmosis, infectious bronchitis, Newcastle disease, haemorrhagic enteritis, and turkey bordetellosis (Charlton, 2000).

Colisepticaemia, egg peritonitis, yolk sac infection, and coli granuloma (Hjarre's disease) are the well-recognised

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clinical outcomes of *E. coli* infection and are collectively grouped under the heading 'Colibacillosis' (Calnek *et al*., 1997). Coligranuloma is one of the important forms of colibacillosis in chickens, turkeys and partridges. They occur sporadically in individual, adult birds and the condition is of interest due to the similarity of its lesions to those of tuberculosis and *Tetratrichomonas gallinarum* (Landman and van Eck, 2017).

In India, there has been a lot of work done on colibacillosis, but the work done on coligranuloma is minimal and reported mostly in broilers. The present study is one such attempt to confirm, coligranuloma in layer chicken by conventional (Gram's staining, cultural techniques) and molecular methods (PCR).

Around 24-week-old bird carcasses were brought for the necropsy with the history of no apparent clinical signs.

Staining and cultural methods

The tissue samples of intestines, nodular lesions, liver were collected and the impression smears were stained with Gram's stain (McLeod *et al*., 1981) to demonstrate the bacteria. The collected tissue samples were also used for isolation of the interested bacteria as per standard microbiological methods (Anon., 1984) with different media (Nutrient broth, MacConkey agar, Eosin Methylene Blue agar) and the resulted colony characteristics were recorded.

Polymerase Chain Reaction (PCR):

For PCR confirmation of the isolated *E. coli*, oligonucleotide primers, ECO-f and ECO-r primer targeting *E. coli* 16S ribosomal DNA gene were used (Amit-Romach *et al*., 2004).

Primers	Primer sequence (5'-3')	PCR amplicon (bp)
ECO-f	GACCTCGGTTTAGTTCACAGA	585
ECO-r	CACACGCTGACGCTGACCA	

The culture samples i.e., in nutrient broth, (2ml) were centrifuged at 10,000 rpm for 10 min. and the pellet resuspended in 0.5 ml of TE Buffer, washed two times and the final pellet was resuspended in 100 µl of TE Buffer. From this suspension 5 µl of the sample was used for PCR in a total volume of 50 µl (containing 5µl of 10X PCR reaction buffer, 100 mM primers, 110mM dNTPs and 1µl of Taq Polymerase from Applied Biosystems, USA). The PCR amplification cycle included 96 ° C for 10 min followed by 35 cycles of 94 °Cfor 2 min., 55 °C for 1 min. and elongation at 72 °C for 2 min. The PCR amplicons were electrophoresed in a 1 % agarose gel prepared in Tris AE buffer, pH 8.3 and the results visualized in a UV transilluminator.

Necropsy revealed typical hard, yellowish, nodular granulomas in the mesentery (Fig.1) and wall of the intestines (Fig. 2) with few nodular lesions on the liver (Fig. 3). The granulomas usually develop when the immune system fails to eliminate, amongst others, disease-causing microorganisms and diverts towards sequestering, often resulting in well-demarcated focal lesions mainly consisting of cells belonging to the mononuclear phagocyte system and necrotic/ caseous debris at the core of the granuloma. The nodular lesions observed in this study is similar to the observations made by Vegad and Katiyar (2003).

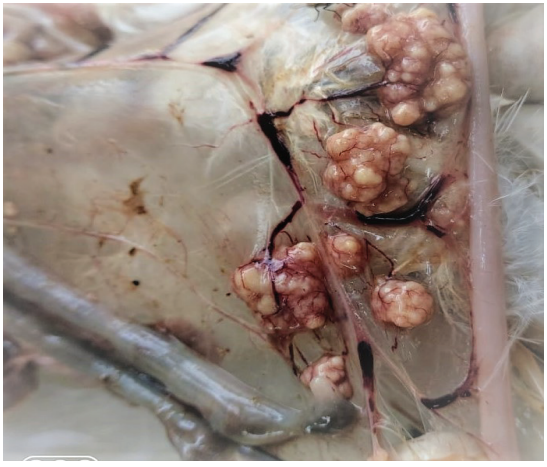

Fig. 1. Mesentery showing multiple nodular granulomas

Fig. 2. Serosa of intestines showing nodular granulomas of varying sizes

A presumptive diagnosis was made based on large number of gram-negative rod-shaped organisms found in the impression smears of dissected nodules (Fig. 4). Chauhan (2003) also observed similar results from the impression smears collected from Coligranuloma affected birds

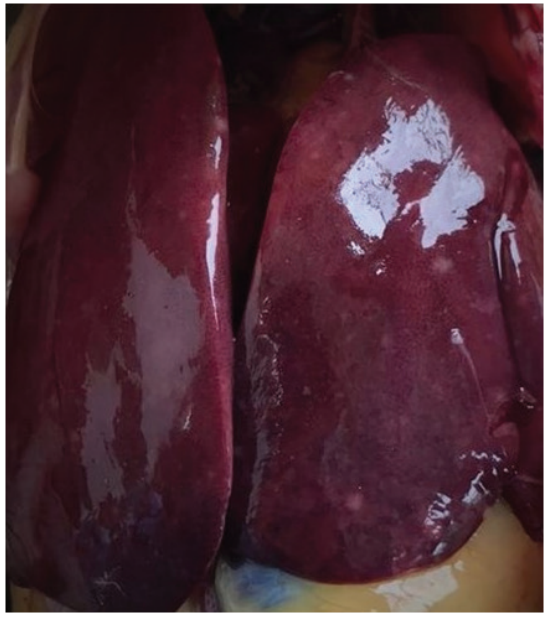

Fig. 3. Liver showing multifocal whitish nodules

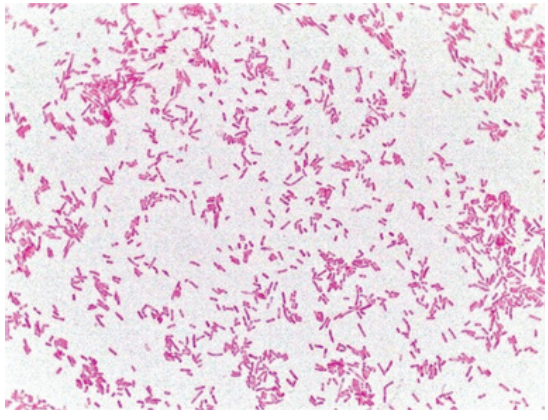

Fig. 4. Impression smear showing gram-negative rod-shaped organisms (20x)

The organisms were readily isolated from the lesions and identified by aerobic incubation overnight at 37ºC directly on different selective agar that resulted in rose-

pink coloured colonies on MacConkey agar (Fig. 5) and the characteristic metallic sheen on EMB agar (Fig. 6).As *E. coli* are very strong lactose fermenters, the colonies on MacConkey agar are bright pink and on Eosin methylene blue agar (EMB), the colonies have a unique and characteristic metallic sheen appearance. These observations were in accordance with Levinson and Jawetz (2000).

Fig. 5. MacConkey agar showing rose-pink lactose fermenting colonies of *E. coli*

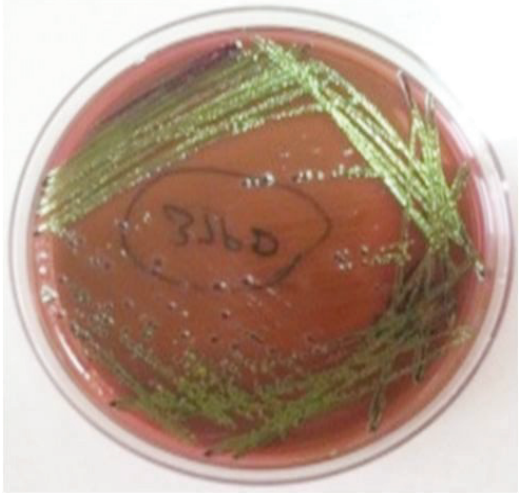

Fig. 6. EMB agar showing characteristic metallic sheen confirming *E. coli*

In this study, the isolated *E. coli* organisms were grown in nutrient broth for overnight at 37°C, and was amplified by PCR with the ECO-f and ECO-r primers targeting *E. coli* 16S ribosomal DNA. We obtained a 585 bp amplicon that was visible upon agarose gel electrophoresis (Fig. 7). The similar results were also found by Amit-Romach *et al*. (2004). Carli *et al*., (2001) opined that PCR helps in accurate diagnosis of the disease by confirming the causative agent.

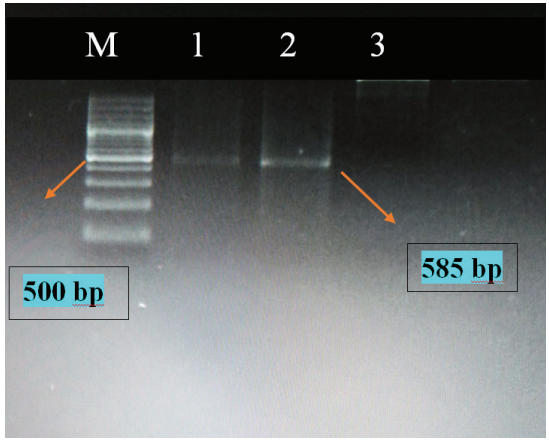

Fig. 7. PCR of E. coli isolated from granuloma lesions in colibacillosis

(M- 100 bp ladder; Lane 1- Test sample; Lane 2- Positive sample; Lane 3- Negative control. Amplicon size: 585 bp)

Based on the necropsy lesions, staining and cultural characteristics and PCR, the disease was confirmed as Coligranuloma caused by *Escherichia coli*. Early screening and accurate diagnosis of the disease by conventional and molecular methods can help in preventing and controlling the mortality in chicken there by reducing the economic losses. Further investigation should be focused on

serotyping and detection of genes of *E. coli* that are responsible for pathogenicity of the organism.

CONFLICT OF INTEREST

There is no known conflict of interests in the present study.

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