



Cellulitis in broiler chicken due to *Escherichia coli*: A case report

Veterinary Science

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ABSTRACT

In present study, eight broiler carcasses were presented for postmortem examination. On necropsy examination the birds revealed typical perihepatitis, pericarditis lesions of *E. coli* infection. On gross examination out of these 8 birds, 3 birds revealed lesion of cellulitis characterized by thickening and reddish-brown discoloration of skin on head and neck region. Similar symptoms were also present in some illing birds in the flock. Broilers with cellulitis revealed fibrinous exudates in subcutaneous tissue. Microscopic examination of tissue confirmed hyperkeratosis, dermatitis, congestion and infiltration of histiocytes in dermis along with proliferation of fibrous tissue. On the basis of gross lesions and clinical symptoms the samples were collected for microbiological analysis. Isolates were *E. coli* on cultural examination which were further confirmed by Polymerase Chain Reaction.

KEYWORDS

Broiler, Cellulitis, *E. coli*

INTRODUCTION

Cellulitis in chicken was first reported in 1984 by Randall *et al.* in England and referred as subcutaneous tissue inflammation. In various studies, *Escherichia coli* has been found to be the major pathogen isolated from lesions caused by cellulitis (Gomis, Watts, Riddll, Potter and Allan. 1997, Norton, BILgili and McMurtrey.1997, Jeffrey, Chin, Singer. 1999.). Cellulitis in broiler chickens is characterized by a diffuse inflammatory reaction in the subcutaneous tissue that results in complete or partial condemnation of the carcass (Messier, Quessy, Robinson, Deveriese, Hommer and Fairbrother. 1993, Elfqdil, Vaillancourt, Meck and Cyles. 1996). It also cause economic losses due to increased mortality, decreased body weight gain and increased medication cost. The development of the lesions is associated with skin injuries coming in close contact with litter contaminated with *E. coli* because several studies have shown that a scratch is enough for the development of a cellulitis lesion (Macklin, Norton and McMurtrey. 1999).

The case was showing typical pathology of *E. coli* with cellulitis, thus we consider the case as an important pathological evidence of pathogenicity of *E. coli* and thus to be reported.

MATERIALS AND METHODS

Collection of materials

Eight broiler chickens (age=10 days) were brought to the Department of Pathology, Nagpur Veterinary College, Nagpur. The case was accompanied with the history of less feed intake and weakness. The postmortem examination was carried out according to standard necropsy procedure and gross lesions were observed. Morbid tissues of liver, kidney, lung, spleen, heart were collected for histopathology in 10% buffered formal saline immediately after post mortem. These tissues were subjected to processing for making the paraffin blocks. Sections of 4 microns were cut and stained with routine H&E Stain (Luna, 1968).

DNA extraction and PCR

The samples were collected from skin lesions and liver lesions for cultural examination in nutrient broth and then streaked on EMB plates for the isolation and identification of bacteria. The genomic DNA was isolated following the standard method (Sambrook and Russell. 2001). The DNA was subjected to Polymerase Chain Reaction on Gradient Mastercycler (Eppendorf, India) for Avian pathogenic *E. coli* specific *tsh* and *cvi* genes of *E. coli* following primer sequences: *tsh* gene Forward primer 5'ACTATTCTCTGCAGGAAGTC3' and Reverse primer 5' CTTCGGATGTTCTGAACGT 3' and *cvi* gene Forward primer 5' TGGTAGAATGTGCCAGAGCAAG 3' and Reverse primer 3' GAGCTGTTGTAGCGAAGCC 5' were used for amplification of respective genes (Ewers, Janseen, Kiessling, Phillip and Wieler. 2005). The PCR reactions were carried out as per following protocol, PCR master mix 2X (12.5 µl), water (9.5 µl), forward primer 20 pmol (1.0 µl) and reverse primer 20 pmol (1.0 µl). The thermal cyclic conditions used for *tsh* gene and *cvi* gene were initial denaturation at 94 °C for 3 min. Thirty cycles of denaturation at 94 °C for 30 sec, primer annealing at 58 °C for 30 sec, extension at 68 °C for 3 min and final extension at 72 °C for 10 min. PCR product was loaded in agarose gel (1.5% agarose in 0.5X Tris-borate-EDTA buffer, ethidium bromide (0.5 µg/ml) along with standard molecular size marker (100 bp DNA ladder). The gel was electrophoresed (Horizontal gel electrophoresis system, Genaxxy). Amplified product were separated on agarose gel and observed by ultraviolet transilluminator and photographed in a gel documentation system (Syngene, UK). The *E. coli* MTCC 443 strain was used as positive control in present study.

RESULTS and DISCUSSION

The gross observation of the broiler with cellulitis showed thickening and reddish-brown discoloration of skin on head and neck region (Fig. 1), Deposition of the fibrinous layer on subcutaneous layer of skin (Fig. 2), heart, liver and peritoneum was observed (Fig.3).



Fig.1



Fig.2



Fig. 3

Fig.1. Thickening and reddish brown discoloration of skin of head and neck. Fig.2 Fibrinous exudation in sub-cutaneous tissue. Fig.3 Fibrinous pericarditis and perihepatitis in chicks.

Deposition of the fibrinous layer on heart, liver and peritoneum in broilers challenged with *E. coli*. was observed in earlier studies conducted by (Bhalerao, Gupta, and Mamta kumar. 2013, Kumar, Jindal Shukla, Asrani, Ledoux and Rottinghauns. 2004) We also recorded similar lesions in chicks, which are typical lesions of *E. coli* infection in broilers.

Histopathological examination of the skin revealed hyperkeratosis, excessive accumulation of exudates, infiltration of inflammatory cells in dermis of skin (Fig. 4), the fibrin shreads accumulation was also evident. Adipose tissue revealed severe congestion. Section of liver revealed accumulation of thick fibrinous layer around liver. There was evidence of inflammatory cells in the fibrinous layer. Sinusoidal congestion was consistent finding. Hepatocytes revealed varied degree of degenerative changes (Fig. 5),

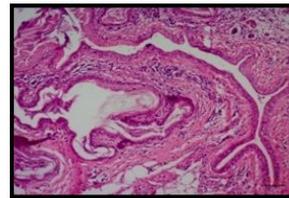


Fig.4

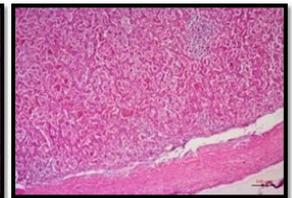


Fig.5

Fig.4 Section of skin showing congestion, hyperkeratosis, cellulitis and dermatitis. Fig.5 Peri-hepatitis with infiltration of inflammatory cells and sinusoidal congestion.

Microscopic findings of our study like hyperkeratosis, dermatitis, congestion and infiltration of histiocytes in dermis along with proliferation of fibrous tissue were quite similar to observations to previous studies, these studies reported subcutaneous fat fragments associated with avian cellulitis, with dermis thickening (Norton et al.,1999. Jeffrey et al 1999) hyperkeratosis, hyperplasia of the epidermis(Messier et al.,1993). Liver tissue revealed hepatitis, peri-hepatitis, sinusoidal congestion and infiltration of inflammatory cells were similar histopathological changes observed in pigeons died due to *E. coli* infection (Dutta,Borah, Satmah, Gangil, 2013)

The genomic DNA isolated from isolated bacteria were subjected to PCR. The primers specific for the *tsh* and *cvi* gene amplified the product size of 824 bp and 1181 bp respectively confirming them as Avian Pathogenic *E. coli* (Fig 6).

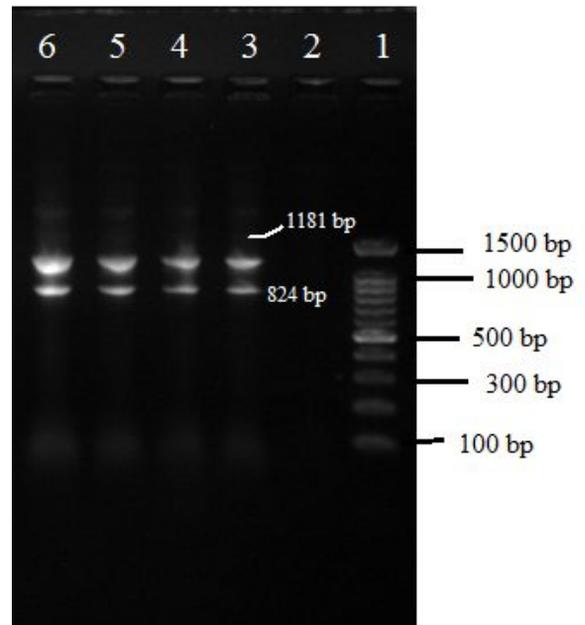


Fig. 6 Agarose gel electrophoresis of PCR product of *tsh* and *cvi* gene

- Lane 1- 100 bp DNA ladder
- Lane 2- Negative control
- Lane 3- Positive control *E. coli* (MTCC 443)
- Lane 4- Isolate from liver lesion positive for *tsh* and *cvi* gene
- Lane 5- Isolate from cellulitis lesion positive for *tsh* and *cvi* gene
- Lane 6- Isolate from cellulitis lesion positive for *tsh* and *cvi* gene

Different strains of *E. coli* are primarily classified by the specific virulence genes. *In vitro* amplification of DNA by PCR method is a powerful tool in microbiological diagnostics (Malorny, Hoorfer, Bunge, Helmuth, 2003). In this study, virulence genes of Avian Pathogenic *E. coli* were targeted which includes *cvi* and *tsh* genes. The Avian Pathogenic *E. coli* specific *tsh* and *cvi* genes were detected in both isolates from cellulitis and colibacillosis lesion by PCR using specific primer confirms that causative pathogen of both cellulitis and colibacillosis were Avian Pathogenic *E. coli*.

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