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Research Paper

INCIDENCE OF ESCHERICHIA COLI INFECTION IN TURKEY

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A case of turkey from poultry farm of Nagpur Veterinary College was presented for the postmortem examination at Department of Pathology. On detail postmortem examination the heart revealed myocarditis, while liver showed congestion and slight enlargement. Microscopically, liver showed necrosis and diffuse leucocytic infiltration while heart showed myocarditis with infiltration of heterophills. On the basis of gross lesions samples were collected for microbiological examination. Samples recovered E.coli which were further confirmed by polymerase chain reaction.

Keywords: E.coli, Turkey, PCR

INTRODUCTION

Avian pathogenic Escherichia coli strains are associated with diverse diseases, mainly extra intestinal *E.coli* are responsible for the great losses in poultry industry (Gross, 1994). In addition to the negative economic impact, avian pathogenic *E.coli* is also considered a major source for spreading antimicrobial resistance to other bacteria mainly through their plasmids and exchange of genetic material (Gyles, 2008). In present case, *E.coli* infection in turkey is not generally reported. Considering it we thought to put it on record.

MATERIAL AND METHOD

The turkey carcass was submitted to detail post mortem examination at Department of Pathology, Nagpur Veterinary College, Nagpur. The post mortem examination was carried out according to standard necropsy procedure. Gross lesions on various vital organs including Heart, Lung, Liver, Kidney and Spleen were noted. Morbid tissues from these organs were collected in 10% formal saline. These tissues were further processed to form paraffin blocks. Sections of 4 micron were taken and detail histopathological examination was carried out to study the microscopic lesions (Luna, 1968).

DNA EXTRACTION AND PCR

According to the sings and gross lesions, Samples were collected from heart blood for bacterial isolation in nutrient broth and were subsequently cultured on EMB agar for the isolation and identification of causative agent. The genomic DNA was isolated by following standard

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protocol (Sambrook and Russell, 2001). The DNA was subjected to Polymerase Chain Reaction on Gradient Mastercycler (Eppendorf, India) for Avian pathogenic *E.coli* specific *cvi* and *tsh genes of E.coli* following primer sequences: *tsh* gene Forward primer 5'ACTATTCTCTGCAGGAAGTC 3' and Reverse primer 5' CTTCCGATGTTCTGAACGT 3' and *cvi* gene Forward primer 5' TGGTAGAATGT GCCAGAGCAAG 3' and Reverse primer 3' GAGCTGTTTGTAGCGAAGCC 5' for amplification of respective genes (Ewers *et al.*, 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 conditions used in thermal cycler 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, Genaxy). Amplified product were separated on agarose gel and observed by ultraviolet transilluminator and photographed in a gel documentation system (Syngene, UK).

RESULT AND DISCUSSION

The gross examination of heart revealed (Figure 1) myocarditis. Gross examination of liver (Figure 2) reveled enlargement with congestion (Figure 2) while spleen reveled mild enlargement. The gross observation in this case resembles to the earlier findings of *E.coli* infection in chicken. (Kumar *et al.*, 2004; and Bhalerao *et al.*, 2013).

Figure 1: Heart Showing Myocarditis

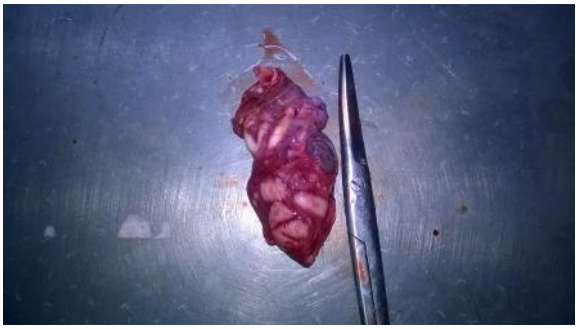


Figure 2: Liver Showing Congestion and Enlargement

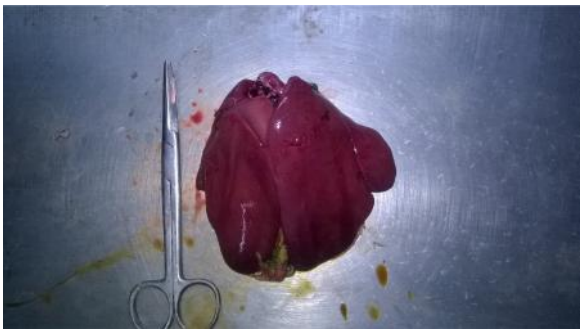
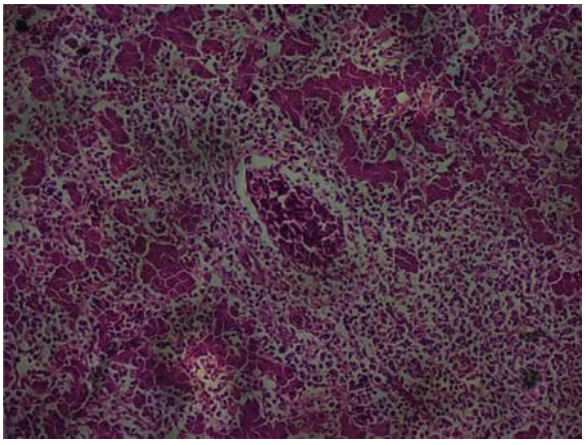


Figure 3: Liver Showing Congestion, Necrosis and Diffuse Infiltration of Inflammatory Cells



Histopathological examination of liver revealed hepatic necrosis and diffuse infiltration of the macrophages and heterophills (Figure 3). Lung

Figure 4: Lung Showing Congestion, Pneumonis and Infiltration of Inflammatory Cells

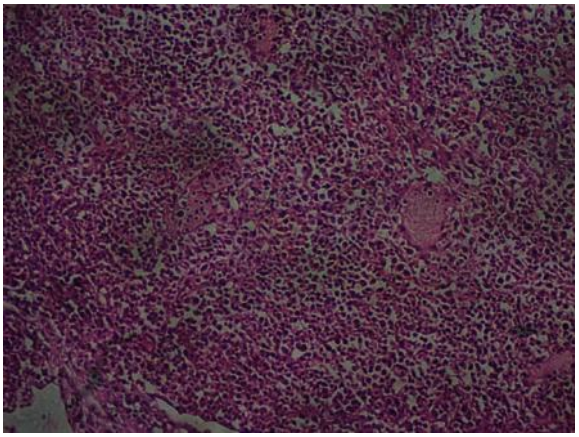
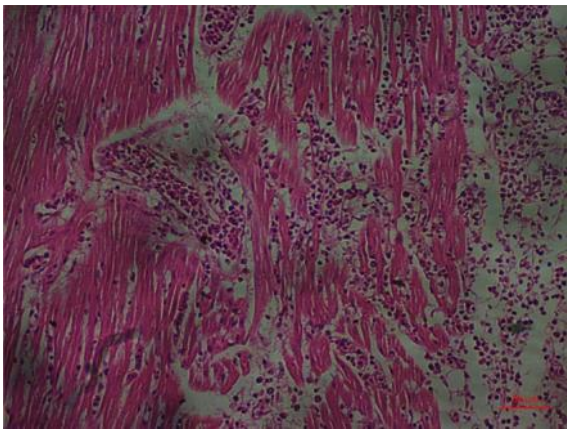


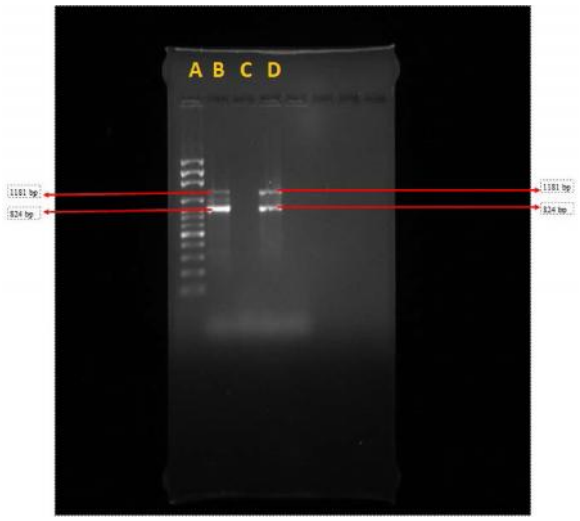
Figure 5: Heart Showing Myocarditis and Infiltration of Inflammatory Cells



showed severe pneumonia and infiltration of inflammatory cells (Figure 4). The changes in heart were evident showing myocarditis and infiltration of inflammatory cells (Figure 5). The lesions observed in this case were similler as that of reported earlier (Tonu *et al.*, 2011; Hassan *et al.*, 2012; and Bhalerao *et al.*, 2013).

The bacterial growth on EMB agar revealed oval shaped flat colonies with metallic shin. The isolated DNA was subjected to Polymarase chain reaction. The primer specific to *cvi* and *tsh* gene

Figure 6: Gel Documented Image Showing cvi (1181 bp) and tsh (824 bp)



Note: A - Ladder, B - Sample, C - Negative Control, D - Positive Control.

were amplified the product size of 1181 bp and 824 bp respectively confirming it as an Avian pathogenic *E.coli.* (Figure 6).

Incidence of *E.coli* infection in turkeys is rare. Considering the importance of *E.coli* infection and its rare occurrence in turkey, we thought to put it on record.

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