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INCIDENCE OF ESCHERICHIA COLI IN NATIVE INDIAN CHICKENS - A CASE REPORT

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ABSTRACT Carcases of eight 4 days old native Indian chickens were presented to Department of Pathology, Nagpur Veterinary College, Nagpur. Postmortem examination was conducted and gross lesions were noted. On the basis of gross observations, samples from yolk sac were collected for microbiological examination. They were streaked on Eosin Methylene Blue Agar, MacConkey agar, Brilliant Green agar, Blood agar which showed colonies resembling to E. coli which were further confirmed by Polymarase Chain Reaction (PCR).

KEYWORDS : E.coli, Native Indian chickens, PCR

INTRODUCTION:

Escherichia coli cause number of diseases in chickens, ultimately leading to economic losses due to reduced body weight and mortality. Most of E. coli strains are non-pathogenic but some of them which can establish themselves outside of the intestine lead to diseases. E.coli serotypes which cause systemic diseases in birds are called avian pathogenic E.coli (APEC) (Gross, 1994). Significant increase in appearance of drug-resistant strains of E. coli isolated from poultry has complicated the problem (Gyles, 2008). Yolk being the rich sources of nutrients helps in multiplication of bacteria and histological disturbances at naval hamper absorption of yolk. Inadequate incubation conditions resulting in excessive water retention and slowly-healing navels and 'tags' of yolk at the navel on hatching also contribute to the problem. In present case, E.coli infection led to yolk sac retention in kadaknath chicks. On molecular detection it was observed that the E.coli stains carries cvi gene which plays important role in drug resistance. Desi birds are considered as sturdy and disease resistance. Considering this, prevalence of drug resistance E.Coli in desi birds is serious condition, thus author thought to put it on record.

Materials and Method:

Post-mortem Examination:

Carcases of eight 4 day old kadaknath chickens were presented to Department of Pathology, Nagpur Veterinary College, Nagpur. Detail post mortem examination was conducted according to standard necropsy procedure. The gross observations were noted. On the basis of gross observation the samples were collected for microbiological examination.

Isolation and Identification:

Yolk sac contents were propagated in nutrient broth and incubated overnight at 37oC. These samples were aseptically cultured on selective media– Eosin Methylene Blue Agar, MacConkey agar, Brilliant Green agar, Blood agar. All the inoculated media were incubated at 37oC and inspected for growth after 24hr. Further Gram's staining was performed on the smear prepared from isolated colony. Identification was done on the basis of staining, colony morphology and cultural characteristics.

DNA extraction and PCR

The isolates were identified by PCR. The genomic DNA was isolated by following standard 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'ACTATTCTCTGCAGGAAGTC3' and Reverse primer 5' CTTCCGATGTTCTGAACGT 3' and cvi gene Forward primer 5' TGGTAGAATGTGCCAGAGCAAG 3' and Reverse primer 3' GAGCTGTTTGTAGCGAAGCC 5' for amplification of respective genes (Ewers, Janseen, Kiessling, Phillipp 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 conditions used in thermal cycler for tsh gene and cvi gene were initial denaturation at 94 0C for 3 min. Thirty cycles of denaturation at 94 0C for 30 sec, primer annealing at 58 0C for 30 sec, extension at 68 0C for 3 min and final extension at 72 0C 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

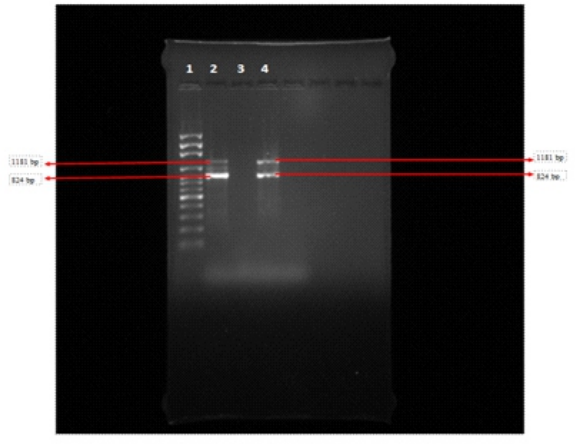
On gross examination, the main lesion was yolk sac retention (Fig. 1). The observations were similar to the earlier reports(Amare, Amin, Shiferaw, Nazir and Negussie. 2013).The bacterial growth on EMB agar revealed oval shaped flat colonies with metallic sheen, on

MacConkey agar pink colonies were formed due to lactose fermenting property of E.coli. The colony characteristics observed were similar to earlier reports (Quinn, Markey, Carfer, Donnelly and Leonard. 2002; Swayne, Glisson, Wood, Pearson, Reed.1998). The colonies were confirmed as E.coli on the basis grams staining which revealed pink colour rods.

Fig. 1 Gross picture showing retention of yolk sac

The isolated DNA was subjected to Polymarase Chain Reaction. The primer specific to cvi and tsh gene were amplified to the product size of 1181 bp and 824 bp respectively confirming it as an Avian pathogenic E.coli (Fig. 2). The results were similar to earlier results (Ewers, Janssen, Kiessling, Philipp and Wieler. 2004).

Considering the results which were indicative of presence of cvi gene responsible for antibacterial resistance in native Indian chickens, authors think that this case is important to put on record.



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isolates

Lane 1- Showing Ladder
Lane 2- Test sample showing tsh (824 bp) and cvi (1181 bp) gene
Lane 3- Negative control
Lane 4- Positive Control

Reference

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