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Pathology and Molecular Detection of Avian Colibacilosis in Commercial Chickens of Nagpur Region

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ABSTRACT*: E. coli* is one of the most important pathogenic agent affecting chickens which costs the poultry industry resulting into high economic losses due to increased mortality. During present study, mortality due to Colibacillosis in nine commercial chicken flocks belonging to Nagpur region of Maharashtra was noticed. Characteristic lesions perihepatitis, pericarditis, air saculitis and pneumonia were observed. Pink colonies on MLA, metallic sheen on EMB confirmed the *E. coli* infection. Clinical samples of liver collected from nine commercial chicken flocks were further confirmed as *E. coli*. by PCR amplification of 500bp of *ecp* gene. Pathological lesions, cultural characteristics along with PCR amplification of *ecp* gene confirmed *E. coli* infection in commercial chickens.

KEYWORDS: Colibacillosis, commercial chickens, molecular diagnosis, pathology,

INTRODUCTION

Poultry industry during the past two decades has been one of the most dynamic and ever expanding sectors in the world. Multifocal growth in the poultry population with changed husbandry practices resulted in prevalence of many infectious diseases. *Escherichia coli* (*E. coli*) is one of the most important pathogenic agent affecting chickens, which costs the poultry industry resulting into high economic losses due to increased mortality, decreased weight gain and increased medication costs and feed conversion ratio. *E. coli* is commonly seen in a microbial flora of the intestine of healthy birds. Though most isolates are nonpathogenic, about 10 to 15% of intestinal coliforms are pathogenic [1].

Escherichia coli is rod-shaped, Gram-negative, non-acid-fast, non-spore-forming bacillus that grows aerobically and belongs to the family *Enterobacteriaceae*. Avian pathogenic *Escherichia coli* strains are known as APEC and are associated with diverse diseases, mainly extra intestinal, being responsible for great losses in the poultry industry [2]. In addition to the negative economic impact, avian pathogenic *E. coli* (APEC) is also considered a major source for spreading antimicrobial resistance to other bacteria mainly through their plasmids and exchange of other genetic material [3].

Routinely laboratory diagnosis of *E. coli* is done by conventional culture methods which are time consuming and not specific. Recently polymerase chain reaction (PCR) technique is used for the diagnosis of *E. coli* infection in poultry. PCR assay have demonstrated their utility as screening tools for *E. coli* in poultry. The *ecp* gene of *E. coli* has been proved as a suitable PCR target, with potential diagnostic application [4]. Hence, present investigation was undertaken to confirm *E. coli* infection by PCR technique in commercial chickens of Nagpur region which showed the symptoms and gross lesions suggestive of Colibacillosis.

MATERIALS AND METHODS

Mortality due to Colibacillosis in commercial chicken farms with capacity of 1000-5000 birds was observed in Nagpur region of Maharashtra. Dead birds suspected for *E. coli* were brought to Department of Pathology, Nagpur Veterinary College, Nagpur for necropsy.

Gross pathology: Dead birds were subjected to detailed post mortem examination and gross pathological lesions were recorded.

Histopathology: Tissue samples of liver, heart and lung were collected in 10% buffered formalin and processed for histopathological study by paraffin embedding technique. Sections were cut at 5-6 µ thickness and stained with routine haematoxylin and eosin (H and E) staining [5].

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Bacterial isolation: Loopful of samples from liver were inoculated immediately in buffer peptone water and incubated at 37˚C for 18 -24 hrs under aerobic condition. Loopful from the broth of each sample was streaked onto MacConkey's agar (MLA) and Eosin Methylene blue (EMB) agar. The inoculated plates were incubated at 37°C for 24 hours [6].

Detection of *E. coli* by PCR: Tissue samples of liver was also collected from the birds belonging to nine commercial chicken farms which showed gross lesions suspected of Colibacillosis and preserved at - 20ºC for detection of *ecp* gene of *E.coli* by PCR. Bacterial DNA from tissue homogenate was extracted using HiGenoMB® genomic DNA Purification Kit (Himedia) as per the manufacturer's instructions. The *ecp* gene of *E. coli* from field samples were detected by using the forward primer 5' TGGTAATTACCGACGAAAACGGC 3' and reverse primer 5' ACGCGTGGTTACAGTCTTGCG 3' to amplify 500 bp fragment of *E. coli* [4]. For amplification, 3µl of DNA was incubated in total volume of 20 µl reaction mix containing 10 µl PCR master mix (2x), 1µl of each forward and reverse primer (10 pmol) and 5 µl of nuclease free water. PCR was carried out following initial denaturation at 95°C for 5 min and then 30 cycles at 95°C for 45 sec, 60°C for 45 sec, and 72°C for 90 sec and a further extension at 72°C for 10 min. The PCR products were separated in 1.5% agarose-gel and visualized in Geldoc (Biorad).

RESULTS AND DISCUSSION

Cultural characteristics of bacterial isolates: On MLA, pink coloured colonies indicative of lactose fermenter organisms were observed. On EMB, deep purple colonies with green metallic sheen were noticed (Fig.1). All the nine chicken farms were found positive for *E. coli* infection on the basis of cultural examination. Cultural characteristics of MLA and EMB observed during the present study were in accordance with the previous researcher [7].

Gross pathology: Birds affected with Colibacillosis showed gross lesions of fibrinous perihepatitis and pericarditis, air saculitis, lung congestion, pneumonia, peritonitis and omphalitis (Fig. 2). Gross lesions noticed during study are in agreement with researchers [8, 9] who also reported pericarditis, perihepatitis and congestion in various organs of broilers which were challenged with *E. coli*.

Histopathology

Liver: showed vacuolar degenerative changes in cytoplasm of hepatocytes along with congestion in portal vein. Focal to multifocal areas of necrosis, periportal leucocytic aggregates, multiple hemorrhages and fibrinous exudates with large number of heterophills over the surface of liver were also evident. Similar lesions were also observed by researcher [10] in which liver showed dilatation of central and portal veins, moderate to marked congestion and multifocal areas of necrosis infiltrated with mononuclear cells in chickens died of Colibacillosis. Fibrinous exudates with large number of heterophills over the surface of liver in pigeons which died due to *E. coli* infection was also reported [11].

Lung: varying degrees of congestion, hemorrhages and infiltration of heterophils, lymphocytes and macrophages in the wall of bronchi and peribronchial alveoli were noticed. Lesions observed in present study were also recorded by other researcher during Colibacillosis in birds [10, 12].

Heart: The microscopic lesions noticed in the heart revealed congestion, hemorrhages, infiltration of inflammatory cells, fibrinous pericarditis along with degenerative changes and areas of focal necrosis in myocytes. Similar lesions of myocarditis, infiltration of inflammatory cells and fibrinous pericarditis was also observed in birds died due to Colibacillosis [9, 13].

Polymerase chain reaction: Tissue samples of liver collected from nine commercial flocks were confirmed as *E. coli* infection by PCR assay. Amplification of *ecp* gene of *E.coli* revealed 500 bp product for all nine commercial flocks (Fig. 3). These findings are in accordance with previous researcher [14, 15] who detected *E. coli* from chickens by PCR assay. Routine PCR test in conjunction with traditional identification methods could be effective in providing a more accurate profile for prevalence of *E. coli* in poultry flocks. Poor samples quality and delayed transport media make the cultural diagnosis difficult and tedious. Hence, nucleic acid based techniques are considered as the best alternative tools for easy and rapid confirmatory diagnosis of Colibacillosis.

CONCLUSION

Pathological lesions, cultural characteristics along with PCR amplification of *ecp* gene suggested the outbreak of *E. coli* in commercial chickens. *Ecp* gene based PCR for detection of *E. coli*. is simplest and less expensive method and is advantageous when compared with conventional cultural methods.

Contribution of Authors

The authors contributed equally.

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Conflict of Interests

There is no conflict of interest.

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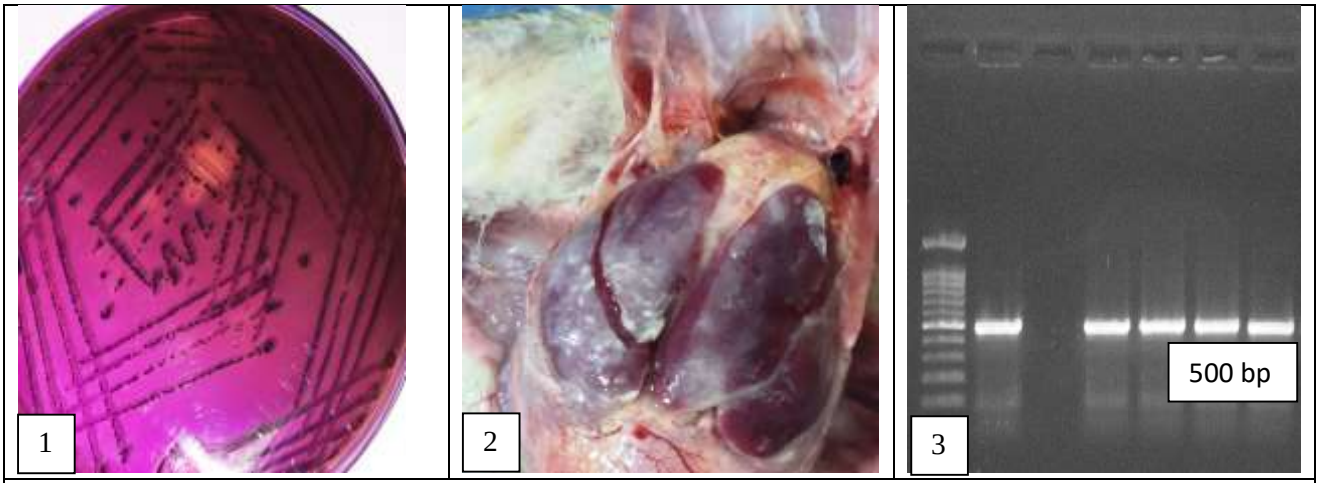

Fig. 1. EMB agar showing deep purple colonies with metallic sheen. **2.** Bird showing perihepatitis and pericarditis. **3.** Agarose gel photograph showing amplified PCR products of *E. coli* Lane 1: 100bp DNA ladder, Lane 3: Negative control, Lane 2,4,5,6 &7: positive field sample (500bp of *ecp* gene of *E.coli*)

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