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

Prashant M. Sonkusale¹, Shubhangi R. Warke², Chaitanya S³, Sukirti Sharma⁴, Mrunal V. Kamble⁵, Bhushan M. Khati⁶

¹-⁶Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur

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].
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 sacculitis, 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, perportal leucocytic aggregates, multiple hemorrhages and fibrinous exudates with large number of heterophilic cells 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 heterophils 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.
Conflict of Interests
There is no conflict of interest.

REFERENCES
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)