PATHOLOGICAL STUDIES ON NATURAL CASES OF AVIAN COLIBACILLOSIS IN HARYANA STATE

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ABSTRACT

Pathological studies were undertaken on natural cases of poultry carcasses to study the incidence and pathological lesions of Escherichia coli infection. Colibacillosis was noticed in different age groups but maximum mortality was evident in birds of 3-4 weeks of age. E. coli was isolated from blood and liver samples in 86.6% cases. Gross pathological changes included congestion in various organs, accumulation of fibrin on the liver and heart. Histopathologically there was fibrinous pericarditis, myocarditis, fibrinous perihepatitis, hepatitis and fatty changes in hepatocytes, interstitial pneumonia, necrosis and depletion of lymphocytes in spleen and enteroitis. It is concluded that pathogenic E. coli in natural cases caused systemic lesions in chicks and also resulted in immunosuppression.

Key words: Escherichia coli, chicken, pathological lesions, immunosuppression

During last few decades, intensive rearing has become a method of poultry husbandry that has predisposed birds for various infectious diseases and thus poultry industry has suffered great losses. Escherichia coli is a commensal organism residing in the intestinal tract of poultry, and often turns pathogenic under adverse conditions. Colibacillosis is a complex syndrome characterized by multiple organ lesions like airsacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis or yolk sac infection. Avian pathogenic E. coli (APEC) causes significant morbidity and mortality leading to multimillion-dollar losses to poultry industry (Barnes, 2008). The present study was undertaken to elucidate pathological lesions in field cases of E. coli infected broiler chicks. The investigation on the field cases included postmortem examination, isolation of E. coli organism and histopathology.

MATERIALS AND METHODS

The study was conducted on 30 broiler carcasses (1-8 weeks of age) suspected of E. coli infection which were brought to the Departments of Veterinary Pathology and Veterinary Public Health and Epidemiology for postmortem examination during the period from August to October, 2010. The carcasses were from different parts of Haryana state. Blood samples from heart and pieces of liver, spleen and intestine were collected for bacteriological examination.

Isolation and Identification of E. coli: The isolation and identification of the organism was done as per method described by Cruickshank et al. (1975). The tissue pieces of liver, spleen, intestine and heart blood of affected birds were inoculated on modified Mac Conkey’s lactose agar (MLA) and kept at 37°C. After 24 hours of incubation, the colonies were purified and smears were prepared for Gram staining. Pink colonies on MLA were also cultured on Eosin Methylene Blue (EMB) agar and the cultures with typical metallic sheen were subjected to various biochemical reactions such as indole production, Voges-Proskauer test, methyl red test, urease and citrate utilization and sugar fermentation tests for the confirmation of E. coli cultures.

Gross Changes: During postmortem examination all organs of the dead birds were examined critically to detect gross lesions.

Histopathological Examination: Portions of various organs were collected in 10% buffered formalin for histopathological examination. The formalin fixed tissues were processed and embedded in paraffin wax (melting point 60-62°C). Paraffin sections were cut at 4µm thickness and stained with routine haematoxylin and eosin stain (Luna, 1968).

RESULTS AND DISCUSSION

Detection of E. coli: Out of 30 cases, E. coli was isolated from 26 cases from heart blood and liver i.e. 86.6% cases indicating systemic infection. From spleen and intestine, the organisms were isolated in 11 and 4 cases,
respectively. It is worth to mention that systemic lesions as evidenced by pathological studies were noticed in these cases. Similar results have been reported by Khaton et al. (2008) who also isolated E. coli organisms from 87% samples including liver from 30 dead birds.

**Pathology:** Pathological observations in the present study in which E. coli organisms were isolated revealed thickening of pericardium, epicardium and hepatic area just above the surface of liver due to accumulation of fibrinous exudate. These lesions were consistently present in 2-4 weeks old birds. In liver, the fibrinous exudate was in form of layer/film which in 95 % cases was separated easily from the hepatic surface i.e. hepatic capsule. In 27% cases, fibrinous exudate was also present in abdominal cavity particularly on serosal surface of gastrointestinal tract including gizzard and mesentery. On the other hand, fibrinous exudate surrounded heart and in severely affected chick, it was not easy to remove from the heart indicating adhesions between pericardium and epicardium. Air sacs were cloudy with deposition of fibrinous mass particularly in the birds more than 3 weeks of age. Heart, liver, lungs, spleen and kidneys were found to be congested in about 95% cases. In 25% cases, there was presence of small necrotic foci on the liver and ascites characterized by excessive accumulation of straw colored fluid in the abdominal cavity giving a pot bellied like appearance to the abdomen.

Histopathological changes in the liver and heart were of subacute type. There was large amount of fibrinous exudate on the surface of liver consisting of heterophils and lymphocytes (Fig. 1). It appeared that hepatic capsule was affected. However, inflammatory cells, fibrin and degenerative changes in hepatocytes were evident in liver parenchyma. Fibrinous pericarditis was the most common lesion, though it varied in degree in different age groups. Accumulation of severe fibrinous exudate was noticed in pericardial sac. It is worth to mention that it was not possible to differentiate between epicardium and pericardium, so involvement of epicardium could not be excluded. These results indicate that in heart both pericardium and epicardium were affected whereas in the liver only hepatic capsule was affected. These differences might be due to histological differences between hepatic capsule and epicardium (Eurell and Fappier, 2007). Hepatic capsule is composed of fibrous connective tissue with poor cellular elements and blood capillaries whereas epicardium is rich in cells and blood capillaries. Therefore, the adhesions between hepatic peritoneal sac and hepatic capsule might not be so strong and thus fibrinous layer accumulated on the surface of liver could easily be removed but not so in case of heart. Fibrinous inflammation observed in liver and heart due to colibacillosis might be due to bacterial endotoxins and vascular injury (Truscott et al., 1974; Thomson, 1978). The fibrinous exudate also accumulated in abdominal cavity probably due to involvement of peritoneum, mesentery and gastrointestinal serosa indicating that colibacillosis also caused serositis.

Respiratory lesions observed in 80% cases of the present study were fibrinous air-sacculitis, interstitial/ fibrinous bronchopneumonia along with congestion and perivascular edema (Fig. 2). The lesions were mainly noticed in the birds of more than 3 weeks of age. Respiratory lesions in colibacillosis are usually associated with mixed infections with other infectious agents such as Mycoplasma, infectious bronchitis and Newcastle disease virus (Gross, 1984).

There was necrosis and depletion of lymphocytes in the white pulp and reticuloendothelial cells proliferation at some places in spleen (Fig. 3). Bursa of Fabricius in chicks of more than 2 weeks of age revealed haemorrhages, atrophy and depletion of lymphocytes in bursal follicles and thickening of interfollicular space due to fibroblast proliferation and mononuclear cells infiltration. Necrotic foci and depletion of lymphocytes in spleen observed in field cases of colibacillosis could be attributed to chick-lethal toxin (CLT) of E. coli (Truscott et al., 1974) and as a result differentiation between white and red pulp was not possible. Regarding depletion of lymphocytes in bursa of Fabricius it could be associated with infectious bursal disease (IBD) virus vaccine as the chicks were vaccinated against IBD. Furthermore IBD vaccine has been reported to facilitate colibacillosis in field cases (Nakamura et al., 1990). Nevertheless, E. coli infections have been reported to cause immunosuppression in poultry (Nakamura et al., 1985; McGruder and Moore, 1998).

Intestines revealed enteritis characterized by desquamation of epithelial mucosa of villi, and infiltration of heterophils and lymphocytes in the mucosa. Enteritis has also been reported by other workers (Goyal et al., 2004; Roy et al., 2004; Gangane et al., 2006). Overall pathological lesions of colibacillosis as observed in field cases of the present study were more or less similar to those reported by other workers in the natural cases.
REFERENCES


Fig.1. Liver showing perihepatitis and hepatitis characterized by infiltration of heterophils and lymphocytes (H.&E. X400)

Fig.2. Lung showing pneumonia and perivascular edema (H.&E. X100)

Fig.3. Multiple necrotic foci causing depletion of lymphocytes in the white pulp of spleen in a chick (H.&E. X100)

On the basis of results of the present study it can be concluded that birds naturally infected with E. coli revealed systemic lesions and E. coli appeared to cause immunosuppression since there was depletion of lymphocytes in lymphoid organs.

(Susantha et al., 1997; Barnes and Gross, 1997).