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Studies on Clinical, Gross, Histopathological and Biochemical Parameters in Broiler Birds Suffered from *Eimeria necatrix* Infection in Aizawl District of Mizoram, India

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Abstract: Studies were undertaken to evaluate the clinical, gross, histopathological and some biochemical parameters in broiler birds suffered from *E. necatrix* infection in a local broiler farm in the Aizawl district. Clinically, 20 birds out of 100 no. (aged between 4-6 weeks) showed loss of appetite, unthriftiness and bloody diarrhoea with mucus. Postmortem examination revealed anaemic pale musculature, distended small intestine with petechial haemorrhages. Similar changes were also observed in some areas of the large intestine and caecum. Histopathological examination showed severe haemorrhagic enteritis with necrosis and disintegration of glandular epithelial cells. Several schizonts were observed in the epithelial cells along with merozoites, infiltrating neutrophils and eosinophils. Biochemically, the infected broilers presented hypoglycaemia associated with a reduction in liver glycogen level. Biochemical serum analysis of coccidia infected chickens also showed a significant increase ($p < 0.01$) in alanine amino transferase levels (2.9-245.2) and there were also changes in total protein and cholesterol level in the serum.

Key words: Clinical, gross, histopathological and biochemical parameters, *E. necatrix* infection

INTRODUCTION

Coccidiosis is an enteric disease, primarily causing severe enteritis/typhlitis with haemorrhages, tissue damage and some metabolic disturbances (Kalra *et al.*, 1996; Panda *et al.*, 1997; Deger *et al.*, 2002). Avian coccidiosis is classified into caecal and intestinal forms. Caecal coccidiosis is an acute disease characterized by diarrhoea and massive caecal haemorrhages. *Eimeria necatrix* has been reported as the most common pathogenic species causing intestinal coccidiosis in the domestic poultry among all the *Eimeria* species (Johnson, 1930). In this form, lesions are distributed throughout the length of the intestine, predominantly in the middle portion of the small intestine. Present study was carried out to determine the pathology and some selected biochemical parameters in broiler chicken naturally infected with *E. necatrix*.

MATERIALS AND METHODS

A total 20 broiler birds showing bloody mucoid diarrhoea with mortality in a poultry farm located in Aizawl district of Mizoram were clinically examined and symptoms, morbidity and mortality were recorded. Postmortem examination was conducted and the gross lesions were recorded. Fresh faecal samples were also collected for subsequent sporulation with 2.5% Potassium dichromate solution for detail morphological study of oocysts. 2-3 ml of blood was collected from naturally

infected birds, allowed to clot and then serum was separated for biochemical and enzymatic studies.

For histopathological changes, morbid tissues from the intestine and caeca with lesions were collected and fixed in 10% formal saline solution. Tissues were then dehydrated in absolute alcohol, cleared in xylene, embedded in paraffin for preparation of fine blocks in paraffin wax. Sections were made at 5 μ m and slides were stained with Haematoxylin and Eosin stains.

RESULTS AND DISCUSSION

Affected birds in the age group of 4-6 weeks showed depression, anorexia and bloody mucoid diarrhoea. Out of 20 affected birds from total 100, 10 died due to *E. necatrix* reaching mortality upto 50%. Coccidiosis was ascertained through demonstration of oocyst in mucosal scrapping from caeca, faeces and postmortem lesions recorded in dead birds. The principal lesions were found in the small intestine, the middle third of which was the most seriously affected (Fig. 1). Small, white, opaque foci were found here. Mucosal surface revealed severe haemorrhages. The intestine was extremely ballooned and petechial haemorrhages could be easily seen while looking grossly without opening the gut. However, discrete haemorrhagic spots were also observed on the mucous membrane of intestine when opened. Intestinal lumen was filled with intestinal content mixed with blood. Intestinal wall was oedematous, thickened, showing



Fig. 1: Severe haemorrhagic enteritis in broiler birds affected with *E. necatrix*



Fig. 2: Sporulated oocyst of *E. necatrix*. (40 x)



Fig. 3: Severe haemorrhagic enteritis showing schizonts, merozoites with infiltration of neutrophils and eosinophils (H and E, 10X)

necrosis and sloughing of intestinal epithelium. The caeca were not severely affected. However mucosal scraping from caeca revealed unsporulated oocyst. Similar findings were recorded earlier by Tyzzer (1929), Johnson (1930) and Davis (1963). Morphometry of sporulated oocyst (Fig. 2) further confirmed that the broiler chickens were suffered from *Eimeria necatrix*. The length and diameter of sporulated oocyst was measured as 14.74 μm x 14.45 μm .

Histopathological examination of the affected intestine showed severe haemorrhagic enteritis and typhilitis. The entire lamina propria revealed severe haemorrhages, necrosis and disintegration of glandular epithelial cells. Several schizonts were observed in the epithelial cells along with merozoites, infiltrating neutrophils and eosinophils (Fig. 3). Similar changes were also observed by (Soomero *et al.*, 2001).

The serum glucose, cholesterol, total protein, albumin, total bilirubin, AST and ALT were estimated using diagnostic kits procured from M/s. Merk Specialities, Mumbai, in a UV-Vis double beam spectrophotometer, Spectroscan 2600 (Chemito make). The estimated values along with the reported normal values and their method of estimation are given in Table 1.

The infected birds presented hypoglycemia ($p < 0.05$). This finding is in agreement recorded by Padmavathi and MuraliDharan (1986) in *E. tenella* infection and Freitas *et al.* (2008) in *Eimeria acervulina* infection. The result did not agree with previous records by Basit *et al.* (1998) in experimental *E. necatrix* infection in chickens and by Hirani *et al.* (2007) in broiler birds affected with coccidiosis. The low levels of plasma glucose possibly activated the pancreatic secretion of glucagon, which mobilized liver glycogen to compensate glucose homeostasis, as suggested by Allen and McMurtry (1984) who observed high glycogen levels during the first week after the infection with *E. acervulina* oocysts. According to Freeman (1970), anorexia and intestinal tract inflammation inhibit glucose absorption, resulting use of liver glycogen reserves and in severe infections, hypoglycaemia due to inhibition of liver glycogenolysis. The infected birds showed significant reduction ($p < 0.05$) of serum globulin. Nayak and Rai (1986) and Basit *et al.* (1998) have also recorded similar findings.

Marked reduction in values of total serum protein and albumin was recorded which may be contributed by nutrient mal absorption, liver changes and marked haemorrhagic enteritis. Reduced level of Serum cholesterol was recorded ($p < 0.05$) which may be due to failure in synthesis by the liver and intestine, which also synthesizes cholesterol (Machado, 2002). As cholesterol is an important component of other molecules, it is possible that its reduction caused by parasitism affects the synthesis of steroid hormones, Vitamin D and bile salts.

Table 1: Biochemical values estimated from *E. necatrix* affected birds

Biochemical parameter	Methods of estimation	Normal value	Test sera
Glucose	GOD-POD	130-270 mg/dl	25.82 mg/dl
Cholesterol	CHOD-PAP	125-200 mg/dl	101.89 mg/dl
Total Protein	Biuret	3.44 gm/dl	2.84 gm/dl
Albumin	Bromocresol Green	1.6-2 gm/dl	1.51 gm/dl
Globulin		2.3-3.3 gm/dl	1.33 gm/dl
A:G ratio		1.14	0.88
Total Bilirubin	Jendrassik and Grof's	0.18 mg/dl	0.09 mg/dl
SGOT/AST	Reitman and Frankel	33.00 IU/dl	32 IU/dl
SGPT/ALT	Reitman and Frankel	4.42 IU/dl	8 IU/dl

The disease coccidiosis due to *E. necatrix* produced a significant increase in Alanine Amino Transferase (ALT) levels which is indicative of hepato cellular damage. Similar observation was recorded by Akpavie (1998). No significant variation was observed in Serum Glutamic Pyruvic Transaminase (SGPT) level. SGPT is not exclusively synthesized in the liver and as reported by Borugere-Picoux *et al.* (1987), SGPT has limited value for biochemical diagnosis in birds. As expected total bilirubin level in the serum was reduced possibly due to hepato-cellular damage on account of *Eimeria necatrix* infection.

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