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Gross and Histopathological Changes Associated with Coccidiosis in a Poultry Farm in Amritsar

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ABSTRACT

Coccidiosis is caused by protozoan parasite belonging to family Eimeriidae and in poultry; most species belongs to genus Eimeria which produces lesions in various sites in the intestine. This could lead to severe damage in the intestinal tract eventually causing death of affected birds. The present study was carried out in Instructional livestock farm complex (ILFC) poultry farm of Khalsa veterinary college in birds greater than 6 weeks of to study the gross and histopathological changes associated with coccidia affected birds. All birds were belonging to White leg horn breed (WLH). Faecal samples were analysed by wet smear and flotation technique and intestinal tissues were collected for further examination. Out of 145 samples analysed 43 were found to be positive for Eimeria species infection. Grossly caeca was filled with blood tinged contents and showed ballooning. Other intestinal portions showed few pin pointed and diffused haemorrhages. Histopathological examination of intestine revealed inflammatory cells, haemorrhages along with coccidia eggs. The presence of coccidia could lead to great economic losses in poultry farm. Thus the farm management authority was advised to check for wet litter, adopt proper hygienic practices and reduce the stocking density of birds.

HIGHLIGHTS

- Eimeria infections are mainly found only in intestines.
- They are characterized by accumulation of blood in caeca, clotted blood with tissue debris and oocyst in mucosal core.

Keywords: Coccidia, Eimeria, Flotation technique, Histopathology, White leg horn breed, Poultry

The poultry industry has tremendous role in national economy as it provides animal protein (meat and eggs) and manure to crops (Antonissen *et al.*, 2016). With boost in poultry trade, poultry diseases are one of the major constraints causing huge loss to the industry. Among all the diseases, coccidiosis is the most dreadful parasitic diseases of poultry that impacts commercial market every year. It is caused by Eimeria species of protozoa. Eimeria infects intestinal tract causing reduced feed intake, absorption of nutrients and impaired immunity (Francesca *et al.,* 2020). Mortality is higher in young ones than older birds. Birds between ages of 3 weeks to 18 weeks are most susceptible (Dakpogan and Salifou, 2013). The life cycle of Eimeria species involves two or more generation of an asexual development known as schizogony, then merogony which is followed by sexual phase gametogony resulting in the formation of oocysts (Fatoba and Adeleke, 2018). The infected and recovered birds both shed oocysts and contaminate the surroundings (feed, water, litter). Fresh oocysts are not infective until they get favourable environment for the sporulation. Sporulation takes

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usually 1- 2 days under optimal environmental conditions (Chapman *et al.*, 2014). These sporulated oocysts are most resistant and can survive for longer period of time. Farmers are often encouraged to ensure adequate bio- security by avoiding wet litter which provides optimum environment for oocyst sporulation and reduce the stocking density of birds. Pathogenicity of disease varies from bird to bird depending upon the host genetics, age of host, nutritional factors, and species of Eimeria. Nine coccidian (Eimeria) species are identified as causative agent among poultry but only seven of them have been reported to be pathogenic (Arabkhazaeli *et al.*, 2013). *Eimeria necatrix* (E. necatrix) and *Eimeria tenella* (*E. tenella*) are the most pathogenic Eimeria species. *Eimeria acervulina* (*E. acervulina*), *Eimeria maxima* (*E. maxima*) and *Eimeria mivati* (*E. mivati*) are moderately pathogenic while *Eimeria brunetti* (*E. brunetti*) is pathogenic but uncommon. *Eimeria mitis* (*E. mitis*), *Eimeria praecox* (*E. praecox*) and *Eimeria hagani* (*E. hagani*) are non-pathogenic species (Bhatia, 2016). Most species develop in epithelial cells of intestinal villi (Ali *et al.*, 2014). *Eimeria tenella* infections are found only in caeca and are identified by accumulation of blood in caeca, clotted blood with tissue debris and oocyst in caecal core while *Eimeria necatrix* produces lesions in anterior and middle portions of intestine, small white spots with intermingled red spots in serosal surfaces. Clinical signs in birds suffering from coccidia ranges from decrease growth rate, severe diarrhea and high mortality rate.

MATERIALS AND METHODS

The study was conducted in ILFC, Khalsa Veterinary College poultry farm in between 15 August 2022 to 30 September 2022. A total of 145 suspected birds were analysed for coccidian infestation. 43 birds were found to be positive for coccidial infection out of which 35 died. Post mortem examination was conducted in dead birds and gross lesions were recorded. Samples were further kept on 10% Formalin for further processing.

Wet smear and Flotation

Fresh faecal samples were collected by using a spatula from freshly voided faces and directly from the cloaca of selected chicken and then the spatula were washed after each sample collection in order to avoid contamination. Each faecal sample was placed in a prelabeled bottle and then transported to the KCVAS Parasitology Laboratory for faecal examination. Samples were immediately stored in the refrigerator at 4° C until processed. Samples were blended by a mortar and pistol, and then the floatation technique was applied using sodium chloride solution to harvest oocysts. Processed solution was poured through a strainer into a beaker then into a 15 ml centrifuge tube. Centrifuge tube was covered with a cover slip and allowed to stand for 10 minutes. The cover slip was than removed and placed on a slide and was examined at 10x and 40x magnifications to identify the oocysts (Conway and Mckenzie, 2007).

Histopathological examination:

Dead birds were subjected to necropsy examination. Various changes in the visceral organs were recorded and photographed. Representative tissue pieces (approximately 0.5 cm each) were collected from multiple sites of intestine and immediately fixed in 10% formalin for 48-72 hours with 2-3 changes of formalin. After fixation tissue samples were trimmed to 1.5 mm thickness and given overnight washing under running tap water. The tissue samples were then dehydrated by passing through ascending grades of ethyl alcohol, cleared in xylene and embedded with paraffin wax (melting point 58°C) for block making. The sections were cut at 4-5 µm thickness and stained by Haematoxylin & Eosin (H&E) stain as per standard procedure (Luna, 1968).

RESULTS AND DISCUSSION

A total of 145 samples were analysed for the presence of coccidia infection in ILFC, Khalsa Veterinary College, Amritsar poultry farm during 15 August 2022 to 30 September 2022. Total positivity rate was found to be 29.65%. 35 birds suffering from coccidiosis died during the period. The details of farm is mentioned in Table 1.

On the basis of flotation technique, faecal samples collected from the farms were analysed and eimerian oocysts containing 43 samples were labelled as positive (Fig. 1).

Pathomorphological alterations: The gross pathology and histopathological changes with respect to the present study included the following:

Table 1: Details of Poultry Farm

Farm name	Total number of birds/ Samples analysed	Total number of birds/ Samples found positive	Total number of birds died	Percentage of positive samples	Percentage of birds died
ILFC Farm, KCVAS Amritsar	145	43	35	29.65%	24.13%

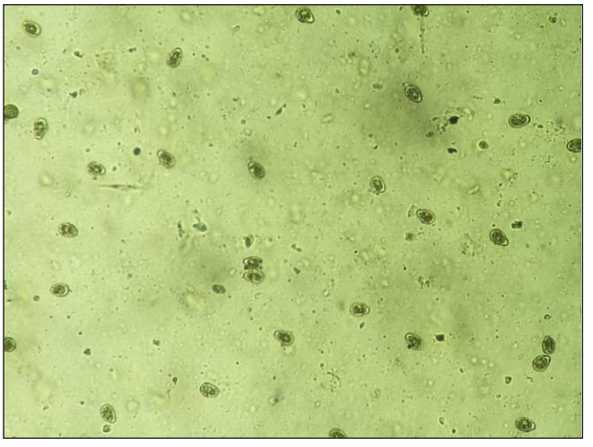

Fig. 1: Flotation method: Presence of coccidian oocysts in faecal sample of affected birds

Fig. 2: Balooned intestine along with haemorrhages in intestinal lumen

Fig. 3: Haemorrhages in caeca

Fig. 4: Mucus along with bloody exudate in intestinal lumen

Gross lesions

35 birds died during the study period which was earlier found positive for coccidiosis through flotation method. Post-mortem examination of dead birds revealed lesions in intestinal and caecal region. Grossly, extremely ballooned intestine, caeca along with hemorrhages in intestinal mucosa were seen (Fig. 2 & 3). Mucus and bloody exudate was also observed in the intestinal lumen (Fig. 4). There was presence of haemorrhagic enteritis in whole portion of intestine (Fig. 5). Liver was found to be pale with infarcts (Fig. 6). These findings regarding the gross pathology are in accordance with (Fantham, 1910; Tyzzer, 1929; Long *et al.*, 1975). These scientists also found that clotted blood, hemorrhages and ballooned intestine were common findings in birds suffering from coccidiosis.

Fig. 5: Haemorrhagic enteritis

Fig. 6: Pale, fragile liver with infarcts

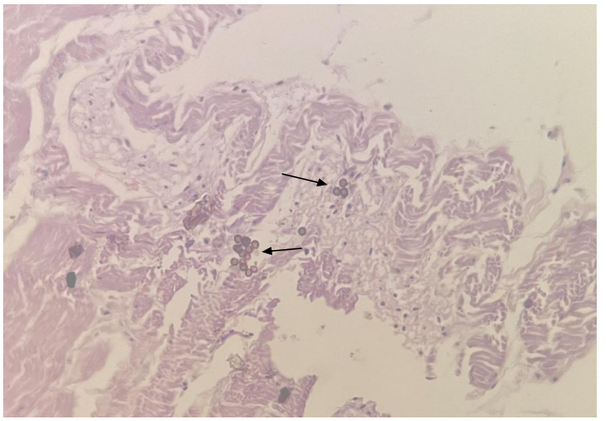

Fig .7: Presence of oocysts (arrow) in the submucosa of intestine. H & E×400

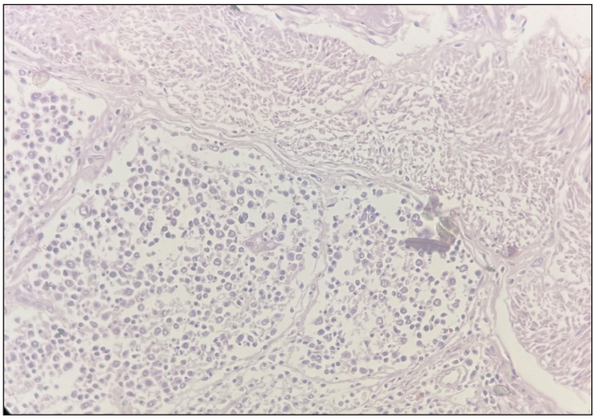

Fig. 8: Infiltration of heterophils and mononuclear cells along with some developing schizonts in muscularis mucosa. H & E×400

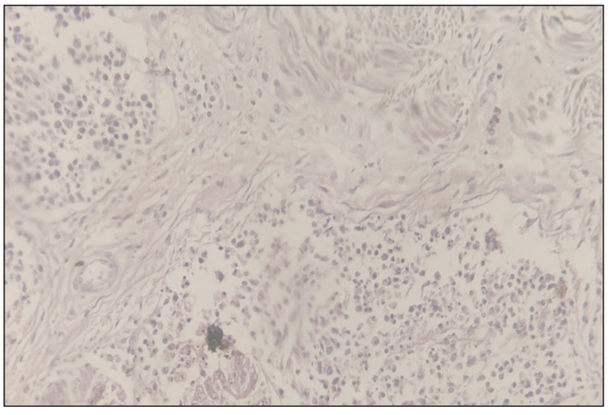

Fig. 9: Mild fibrosis of sub mucosa along with inflammatory cells. H & E×400

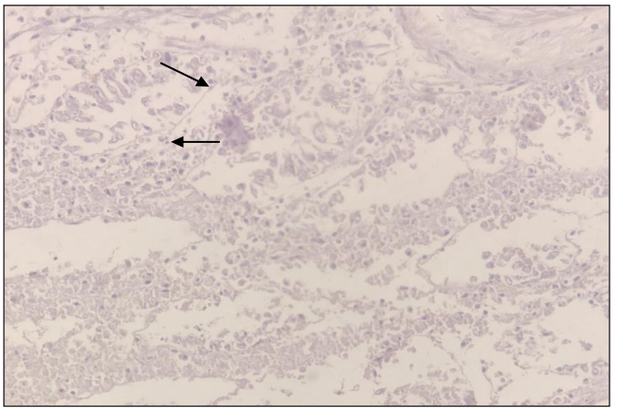

Fig. 10: Edema, congestion (arrow) along with degeneration of sub mucosal glandular epithelium. H & E×400

Histopathological changes

Histopathology of intestinal samples collected from morbid birds revealed presence of developing and developed oocysts in the epithelial cells of submucosal glands of caecum with massive infiltration of heterophils and mononuclear cells (Fig. 7, 8). Presence of developing schizonts with desquamation of enterocytes, mononuclear cells infiltration and mild fibrosis was also observed (Fig. 9). Massive congestion of sub-mucosa along with fibrosis, edema and degeneration of sub-mucosal glandular epithelium along with few red blood corpuscles was seen (Fig. 10). Also, intestine showed desquamation and sloughing of enterocytes leading to necrosis of intestinal villi with MNC infiltration. Similar changes were observed by (Soomro *et al.*, 2001 ; Sood *et al.*, 2009)

CONCLUSION

Based on the findings it can be concluded that gross observations include extremely ballooned intestine and caeca along with haemorrhages and watery ingesta mixed with mucus and blood. Also, haemorrhagic enteritis is prominent feature found in coccidiosis. Histopathologically it is evident that various changes are observed in portions of caeca and small intestine. Coccidial oocysts with massive infiltration of heterophils and mononuclear cells are commonly observed in lamina propria of intestine. Massive congestion of submucosal blood vessels, fibrosis with oedema along with various stages of schizogony and gametogony are also observed. As there is high damage to enteric system of bird, the bird eventually becomes dull, depressed, off feed showing severe bloody diarrhea. Mortality rate can reach upto 60% if appropriate measures are not adopted. Thus it becomes important in farms to adopt proper hygienic practices and reduce the stocking density of birds. Antibiotics must be used for all poultry birds of the flock in affected farm so as to prevent the further spread of disease.

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