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Histopathomorphological alterations in natural infestation of intestinal coccidiosis in backyard chicken

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Abstract
Most of the domestic and wild animals and birds, the Eimeridae is a family that develops within their digestive tract. The following serotypes of Eimeria are recognized as having infected chickens: *E. tenella, E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix* and *E. praecox.* This study was the part of Masters Research project which included the incidence of coccidiosis and pathomorphological alteration in chickens from backyard poultry farms in West Medinipur district of West Bengal and collected samples were sent to the Department of Veterinary Parasitology, WBUAFS, Kolkata for confirmatory diagnosis. The clinical signs observed include greenish, yellowish, brown bloody diarrhoea, inactivity, off fed, weight lost, huddling, drop in feed input, drop in product, emaciation, comb and wattles blench, anemia and unforeseen death. Gross lesions include ballooned and haemorrhagic intestine while histopathological lesions revealed loss of epithelial layers, traffic of blood vessels which indicated dislocation followed by leakage of blood, severe mucosal edema, necrosis of submucosa, loss of villi and pronounced haemorrhages, presence of oocyst within the intestinal villi and lymphoid cells showing hyperplasia. From this, we can conclude that not only clinical signs but also gross and histopathological examinations can be used as assessment tools for coccidiosis.

Keywords: Backyard chickens, coccidiosis, histopathology

Introduction
Coccidiosis is an illness in which, due to the existence of protozoan parasites related to coccidia and its family Eimeridae, it will develop within the intestine of a large number of natural and wild animals and birds. The seven kinds of Eimeria i.e. *E. acervulina, E.brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella* are recognized as being infected in chickens. Although coccidiosis has been observed for a number of years, it is nevertheless considered to be the major parasitic disease affecting poultry production in the whole world and remains an economic problem (Dalloul R and Lillehoj H, 2006) [12]. One of the groups of protozoa that affect many animals and poultry is the coccidiosis. Infection with these organisms leads to a serious intestinal disorder known as coccidiosis that leads to weight loss, diarrhoea, urinary tract infections, and death (Mc Dougald L.R. and Reid W.M., 1997; Moses *et al*., 2015) [23, 25]. The form of avian coccidiosis is divided into gastrointestinal and caecal forms. The *Eimeria necatrix* causes intestinal coccidiosis (Johnson W. T., 1930) [19]. Caecal coccidiosis is a transient illness characterized by diarrhoea, and large caecal haemorrhages caused by *Eimeria tenella* (Gardinar J.L., 1955) [18]. Coccidiosis showed that there is a general distribution pattern as well as an annual variation of infestation levels due to environmental conditions in the rainy season. Due to changes in the Coccidiosis dynamics, it has been noted that overall bird populations have changed with greater diversity, richness of species and uniformity throughout the wet season. There is a higher prevalence of coccidiosis during the wet season than during the dry season, which is attributed to wet bird enclosures with leaky roofs (Carvalho A.A. and Tavares-Dias M., 2017) [8]. The age-related prevalence of coccidiosis was highest in the 31-45 days old group (48%) and lowest in the 0-15 days old group (6%). Coccidiosis has been found to be more prevalent on clay and brick floors compared to concrete floors (Adhikari A, *et al.*, 2008) [2].

In all of these species, it was reported to be the most common pathogenic and chronic disease in domestic poultry. Severe lack of clotting may be observed in the acute form. Most infected flocks show a significant decline in their consumption of food and water due to mild or severe exposure, which will have an effect on birds as they tend to be agitated and prone to huddles and weight decrease observed (Barde J.I. *et al*, 2012) [7]. Weight loss may occur when the mucosa of the gastrointestinal tract is damaged as a result of reduced absorption. Ulcerations, loss of pigmentation in the gastrointestinal tract may also be observed at the end of infection. (Conway D.P. and Mckenzie M.E., 1991; Edgar S.A., 1992; Lillehoj H.S. and Trout J.M., 1993) [9, 15, 21]. Haemorrhagic, malabsorption, diarrhoea and decrease in weight gain are the most clinically significant manifestations of coccidiosis (Moses *et al*., 2015) [25]. Coccidiosis continues to be an important problem around the world, because of difficulties in diagnosis. It may be difficult to differentiate between species by morphological characteristics of the oocyst and requires trained personnel (Soulsby E.J.L., 1982) [29]. Clinical signs, coprology and pathomorphological analyses as well as pathomorphological analysis may be used for the diagnosis of coccidiosis (Conway D.P. and McKenzie M.E., 2007) [10]. The significance of the pathological findings is important, based on macroscopic and histology damage to the intestines. This study therefore aims at determining the incidence of coccidiosis by means of conventional and histopathology methods.

Materials and Methods

This study was the part of Masters Research project which included the incidence of coccidiosis and pathomorphological alterations in chickens from backyard poultry farms in West Medinipur district of West Bengal and collected samples were sent to the Department of Veterinary Parasitology, WBUAFS, Kolkata for confirmatory diagnosis. A clinical history and symptoms have been observed. Post-mortem examinations have been carried out and lesions observed, followed by a new analysis of faecal contents using the technique described below to assess oocysts and sporozoites (Adams *et al.*, 1971) [1].

Histopathological studies: The collected samples for histomorphological analysis were kept in 10% neutral buffers formalin for 48 hours of fixation. After fixation the tissue samples were kept for washing under slow running tap water overnight to remove excess formalin from the samples. The tissue samples were then passed through ascending grades of acetone for dehydration (70-100%). Samples were kept in each concentration of acetone for 1 hour. It was done to remove the excess water from the samples. The dehydrated samples were then kept in benzene (absolute) for 1 hour to make the samples clear and transparent. After clearing the samples were passed through three¬ liquid paraffin baths (temperature 56 °C) each in 1 hour for impregnation and finally the samples were embedded in melted paraffin using metal moulds. The paraffin-embedded samples were then cut with a rotary microtome into thin slices; 5 microns and float in a water bath (58 °C) to stretch. When the floating ribbon was properly stretched, the desired portion was placed on a transparent slide that had been previously coated with Mayer's egg albumin (50 ml glycerol: 50 ml egg white and sodium salicylate preservative 1 g). The water on the slide is drained and placed on a worm plate to allow the paraffin film to dry. Conventional haematoxylin and eosin staining procedures are performed to stain slides containing tissue sections. Initially, slides containing tissue sections were kept in xylene for 2 min to deparaffinize the tissue sections. Deparaffinized tissue sections were then hydrated using a decreasing alcohol concentration (100-70%) per section for 2 min, followed by immersion in distilled water for 2 min. The hydrated tissue sections were stained with 1% haematoxylin for 3 min and washed slowly under running water for 5 min. Basic staining Hematoxylin has been used to stain the acidic component of cells such as DNA-rich nuclei. Stained slides are embedded separately in 1:1 HCl: Ethanol solution and hold under running water for 5 minutes to remove further stains. Then, the slides were stained with 1% eosin (reverse dye) for 30 s. Eosin acid stain was used to stain basic cell components such as cytoplasm. The slides were then dehydrated using an increasing graded alcohol concentration (70-100%) each for 2 min. Stained slides were kept in xylene twice for 2 min each and mounted with DPX (Dibutylphthalate Polystyrene Xylene) solution. Finally, the mounted slides were examined under the microscope and digital photographs of tissue sections were taken from the stained slides (Aviwioro O.G., 2002) [5].

Results and Discussion

Clinical Findings: Clinical findings were recorded form all affected chickens which were observed greenish, yellowish, and brownish bloody stools, lack of activity, lack of feed, weight loss, crowding, reduced feed intake, reduced production, wasting, pale crown and wattles, and anemia. The report was found similar with previous reports (Gardinar J.L., 1955) [18].

Post-mortem Lesions (Gross Pathology): Coccidiosis was determined by demonstrating postmortem lesions noted in dead birds. Post-mortem revealed lesions of intestinal coccidiosis and caecum (Figures 1&2). In the intestinal form, external bowel distention and petechiae can be observed by macroscopic examination without colostomy, which is similar to the results of some authors (Tyzzer E.E., 1929; Johnson W.T., 1930; Davies S.F.M., 1963) [30, 19, 13]. In case of caecal coccidiosis, enlargement of caecum with clotted blood, haemorrhages and reddish brown contents. In catarrhal type of caecal coccidiosis, threr were petechial spots in serous surface. Caecal walls were thickened, congested, necrosed and ulcerated in different places. After caecum expansion, blood clots indicative of caecal coccidiosis were detected by a number of authors (Raillet F. and Lucet M., 1891; Fantham H.B., 1910; Tyzzer E.E., 1929; Long P.L., 1973; Moses *et al*., 2015) [26, 16, 30, 22, 25].

Fig 1: Gross PM lesions of intestine

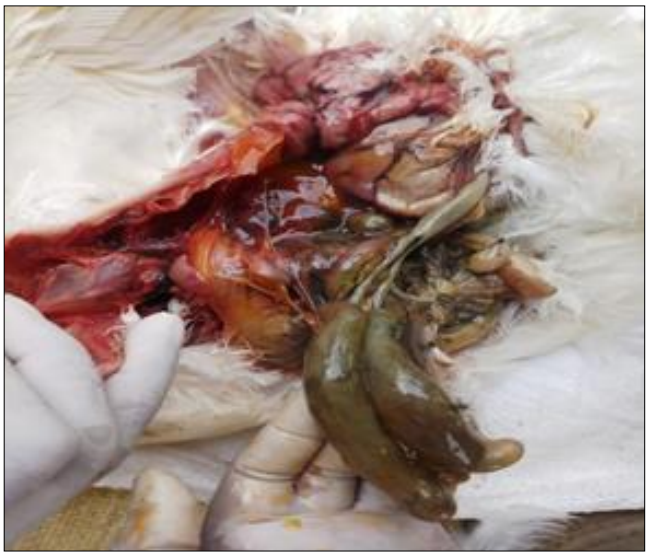

Fig 2: Gross PM lesions of caecum

Pathomorphological Studies

Pathomorphological studies are of great importance to distinguish normal and healthy gross structural abnormalities at macroscopic and microscopic levels. The purpose of histopathological examination is to detect small changes in tissue structure caused by disease (Culling, 1963) [11].

Intestinal Coccidiosis

Macroscopic lesions

In terms of intestinal morphology, red-white dots were seen in the distended intestinal wall, especially the first part of the small intestine.

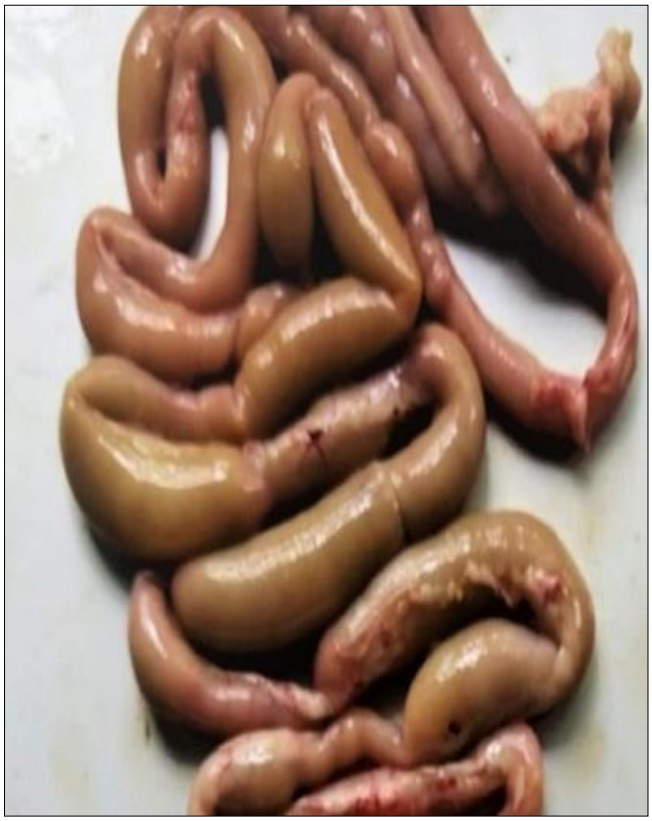

Fig 3: Intestine showing ballooned, thickened and hyperaemic with pin point red spot haemorrhages

Intestinal contents are watery and mixed with varying amounts of mucus, although some have hemorrhagic streaks (Fig 3). The central part of the gastrointestinal tract was swollen and petechiae were seen through the serosa. The lining of the intestine was hard and engorged, with sharp red spots that bleed regularly. The lesions, which looked like those of a few scientists, had been detected when examined without opening the gastrointestinal tract (Tyzzer E.E., 1929; Johnson W.T., 1930; Davies S.F.M., 1963) [30, 19, 13].

Caecal Coccidiosis

Macroscopic lesions

Gross lesions of caecal coccidiosis included distention of caecal pouches with blood clots, blood and reddish brown contents in haemorrhagic type of infection (Fig 4). In catarrhal type of caecal coccidiosis, there were petechial spots in serous surface. Caecal walls were extensively thickened, engorged with blood clotted mass, congested, necrosed and ulcerated lesions found that is similar to the reports of several researchers (Raillet F. and Lucet M., 1891; Fantham H.B., 1910; Tyzzer E.E., 1929; Long P.L., 1973; Moses *et al*., 2015) [26, 16, 30, 22, 25].

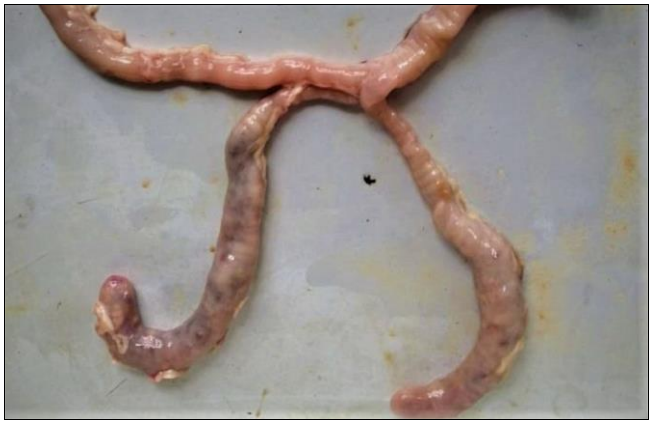

Fig 4: Caecal pouches showing thickened distended with blood clots, blood and reddish brown contents

Microscopical lesions

Histopathologically, observed that the absorptive epithelium of intestine were extensively damage in the affected part. The villi were stunted, and sloughing off and Eimeria oocyst decreased of villi height (Fig 5). In case of intestinal forms, lesions were found in the form of complete detachment of the mucosal layer from sub-mucosal layer and heavily infiltrated

with macrophages, plasma cells and lymphocytes as described by Shukla *et al.* (1990) [27], Levine (1942) [20], Davies (1956, 1963) [13], Michel and Hodges (1971) [24].

The superficial layers of mucosa appeared desquamated and homogenous eosinophilic staining. In many of the glands, considerable enlargements of the epithelial cells with developmental stages of parasites i.e. oocysts (Fig 6). Inflammatory cells were infiltrated with eosinophils, macrophages and lymphocytes especially around the glands with damaged epithelial cells and in between the muscle fiber of the intestinal wall (Fig 7). The musculature showed evidence of oedema and instance eosinophilic staining which were described by Fernando and McCraw (1973) [17], Babu *et al.* (1976) [6], Attar (1982) [4], Shukla *et al.* (1990) [27], Ahmad *et al*. (2000) [3].

In caecal coccidiosis, the enlargement of the caecum with clotted blood, along with haemorrhagic on the caecal wall, catarrhal inflammation, congestion, dilatation, necrotic patches of the caecum. Loss of blood vessel congestion, oedema, epithelial lesions, and necrosis of the caecal mucosa and loss of villi were histopathological manifestations of caecal coccidiosis (Fig 8) such similar findings were observed by Soomro *et al.* (2001) [28]. Loss of epithelial tissue, mucosal oedema and necrosis of submucosa, sloughing of villi and marked haemorrhage, lymphoid hyperplasia with Eimeria oocyst (Fig 9). Intestinal morphology showed lesions with complete detachment of the mucosal layer from the sub-mucosal layer. In addition, detachment of villi and Eimeria oocysts, decreased villi height, cancerous necropsy and lipolysis were observed in the chicken intestine (Fig 10). Such similar findings were observed by previous researchers' (Long P.L. and Joyner L.P., 1984; Conway D.P. and McKenzie M.E., 2007) [22, 10].

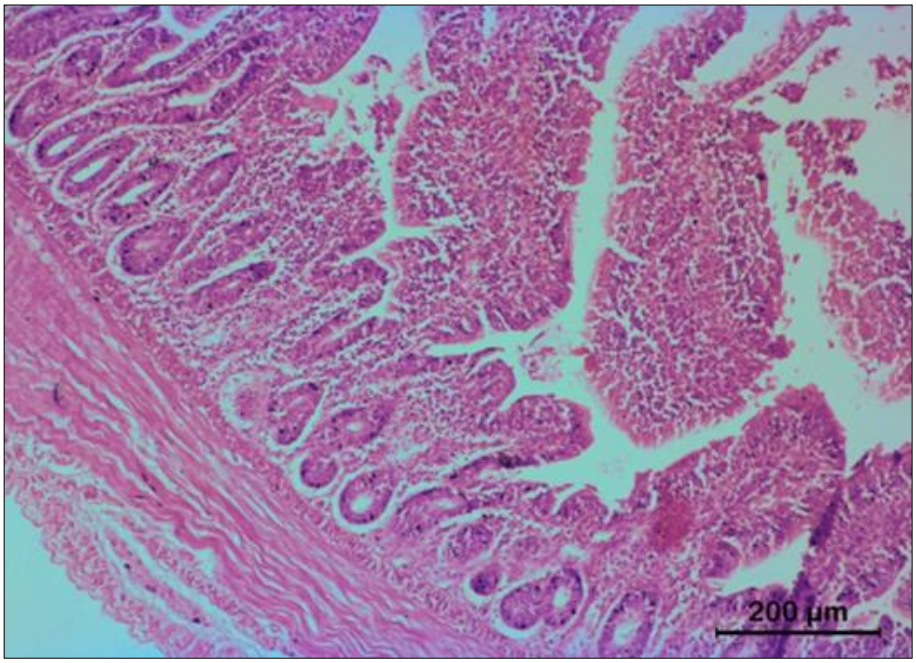

Fig 5: Development stages of *Eimeria* species in the epithelial cells of intestine (H & E X 100)

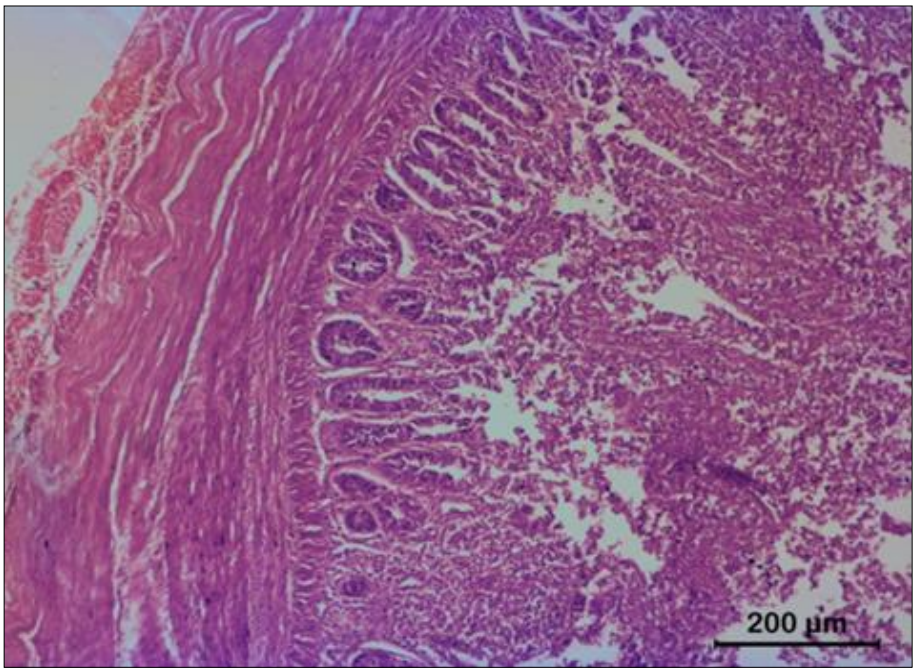

Fig 6: Intestine showing desquamation of necrotic villi of sloughing off from the lining epithelial layers (H & E X100)

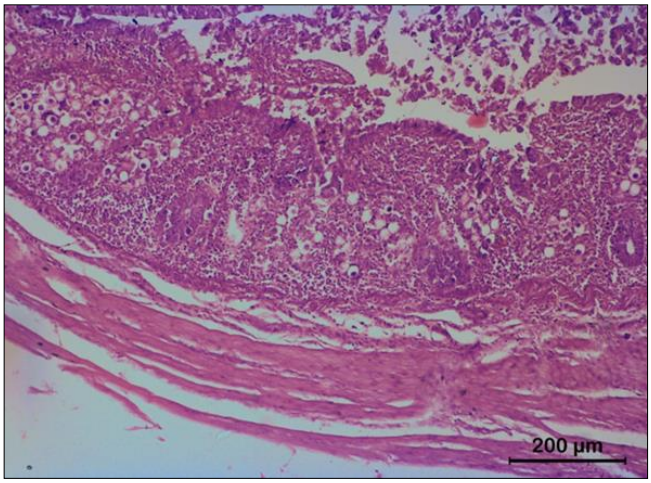

Fig 7: Intestine showing atrophied and shortened of villi, proliferation of connective tissues and infiltration of mononuclear cells, macrophages and lymphocytes (H & E X100)

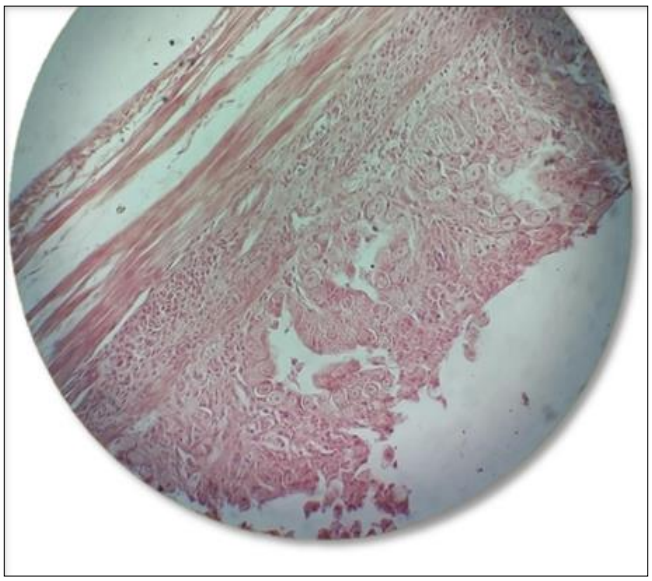

Fig 8: Caecum showing inflammatory cells predominantly macrophages and lymphocytes with extensive vacuolization in the glandular epithelial cells (H & EX 100)

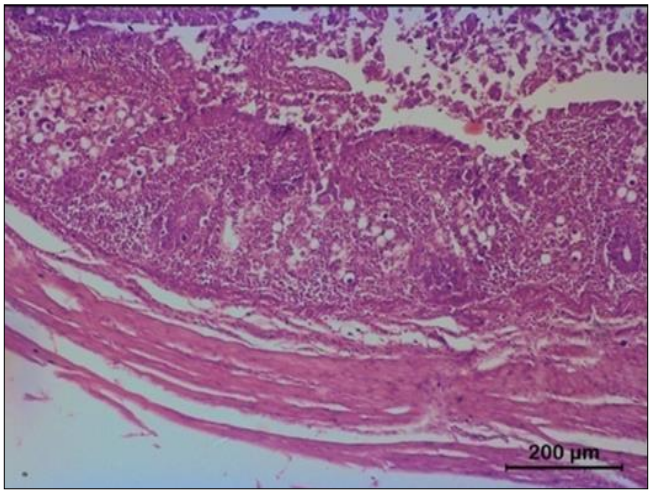

Fig 9: Caecum showing different grades of developmental schizonts and infiltration of inflammatory cells especially mononuclear cells, macrophages and lymphocytes around the epithelial cells of glands (H & E X 100)

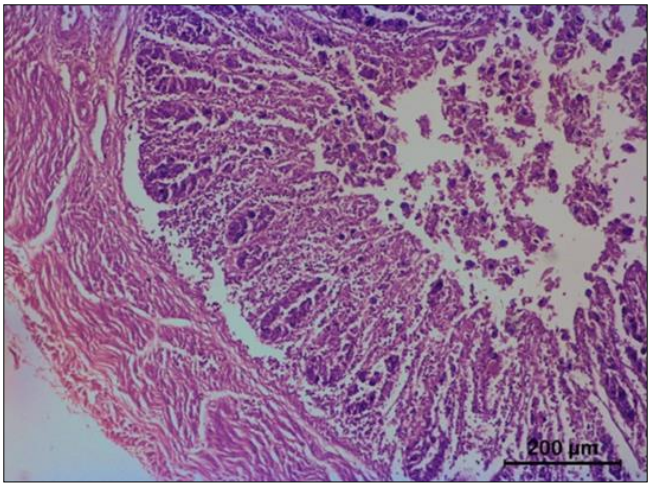

Fig 10: Caecum showing desquamation of necrotic villi and sloughing of epithelial lining and infiltration of eosinophils, lymphocytes and macrophages (H&E X 100)

Conclusion

Clinical signs were observed greenish, yellowish or brownish watery diarrhoea followed by bloody tinge droppings, depression and droopiness, anorexia, ruffled feathers, weight loss, hunched posture, reduced food intake, and decreased production, weakness, anaemia resulting death. The gross lesions were ballooned and haemorrhagic intestine, while histopathology included eroded of epithelial linings, congested blood vessels suggestive of subsequent haemorrhage, severe oedema as well as sub mucosal necrosis. It was found that villi had been lost and prominent hemorrhages were present, with the presence of oocytes. Intestinal villi and lymphocytes were shown to have hyperplasia. It is concluded that, as a tool of diagnostics for coccidiosis, clinical signs, gross examination and histopathology may be applied.

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