International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(1): 1161-1165

www.veterinarypaper.com
Received: 22-11-2023
Accepted: 26-12-2023

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Sub cellular alterations in natural infestation of intestinal coccidiosis in backyard chicken

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DOI: https://doi.org/10.22271/veterinary.2024.v9.i1p.1110

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 carried out to record the incidence of coccidiosis in chickens from backyard poultry farms in West Medinipur district of West Bengal state and collected sample sent to the Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, 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 which have an effect on many animal and avian species is coccidia. Infections of such organisms result in severe intestinal disease known as coccidiosis that causes weight loss, diarrhoea, urinary tract infection 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. This form is characterized by distribution of lesions all over the length of the gastrointestinal tract, but particularly common on the middle part of the small intestine. 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. The inflammation and damage to the gastrointestinal tract, leading to diarrhoea and subsequent dehydration are important factors for coccidiosis lesions. 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, 14, 21]. Haememorrhagic, 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

In order to record the incidence of coccidiosis in poultry from different backyard poultry farms and collected samples sent to the Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, WBUAFS, Kolkata for confirmatory diagnosis. The results reported in the study were part of a Masters Research project. 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. Paraffin embedded samples were then cut using rotary microtome into thin slices; ribbons of 5 micron and floated in water bath (58 °C) for stretching. When the floated ribbon was properly stretched, the desired portion was placed on a clear glass slide previously coated with Mayer's egg albumin (50 ml glycerol: 50 ml egg white and preservative sodium salicylate 1 gm). Water on the slide was drained off and placed on slide worming plate to allow the paraffin film to dry. Routine haematoxylin and eosin staining procedure was followed to stain the slides containing tissue sections. At first the slide containing tissue sections were kept in xylene for 2 minute to deparaffinize the tissue sections. The deparaffinized tissue sections were then hydrated using descending concentration of graded alcohol (100-70%) each for 2 minutes, and then dipped in distilled water for 2 minute. The hydrated tissue sections were stained with 1% haematoxylin for 3 minutes and washed slowly in running tap water for 5 minutes. The basic dye haematoxylin was used to stain the acidic component of cells such as DNA-rich nuclei. The stained slides were singly dipped in 1:1 HCL: ethanol solution and kept under running tap water for 5 minutes to remove the extra stain. Then, the slides were stained with 1% eosin (counter stain) for 30 seconds. The acidic dye eosin was used to stain the basic component of cells such as cytoplasm. The slides were then dehydrated using ascending concentration of graded alcohol (70-100%) each for 2 minutes. The stained slides were kept in xylene twice for 2 minutes each and were mounted with DPX (Dibutylphthalate Polystyrene Xylene) solution. Finally, mounted slides were examined under microscope and digital photographs of the tissue sections were taken from the stained slides (Aviwioro O.G., 2002) [5].

Results and Discussion

Clinical Findings: Clinical findings have been observed and recorded in all diseased chickens and include 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 through demonstration of post-mortem lesions recorded in dead birds. Post-mortem showed intestinal and caecal coccidiosis lesions (Fig. 1 & 2). In case of intestinal form, external ballooned intestine and petechial hemorrhages could be seen while looking grossly without opening the gut which was similar to the findings of several 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 were observed. 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 post-mortem lesions of intestine

Fig 2: Gross post-mortem 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

On intestinal morphology, reddish-white punctate lesions were found in the distended intestinal wall, especially in the first part of the small intestine.

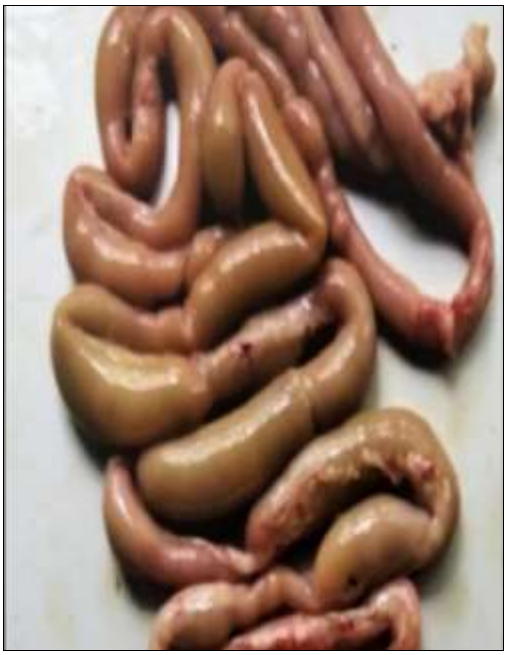

Fig 3: Intestine showing ballooned, thickened and hyperaemic with pin point red spot haemorrhages

The intestinal contents were liquid and mixed with varying amounts of mucus, although some showed streaks of hemorrhage (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 clotted blood and haemorrhages were observed (Fig 4). On opening the caeca, the bloody mass, a characteristics of caecal coccidiosis was 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].

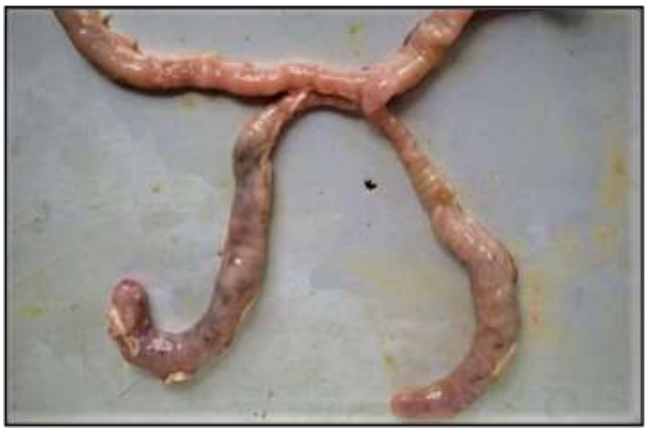

Fig 4: Caecal pouches showing thickened distended with blood clots, blood and reddish brown contents

Microscopical lesions

Histopathologically, extensive damage to the absorptive epithelium of the intestine was observed in the affected part. The villi were stunted, and sloughing off and Eimeria oocyst decreased of villi height (Fig 5). The sporadic epithelial hyperplasia and hypertrophy were also seen. 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, 14], Michel and Hodges (1971) [24].

The superficial layers of mucosa appeared desquamated and had homogenous eosinophilic staining. In many of the internal glands, considerable enlargements of the epithelial cells with developmental stages of parasites were observed (Fig 6). Inflammatory cells predominantly eosinophils, macrophages and lymphocytes were found extensively infiltrating especially around the glands with damaged epithelial cells. The cellular infiltrations were also observed 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 almost all cases of caecal coccidiosis, the enlargement of the caecum and the appearance of clotted blood in the area, along with haemorrhagic on the caecal wall, inflammation, dilatation, necrotic patches of the caecum with consolidation of the caecal contents. 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].

In case of caecal forms, the histopathological lesions were revealed to have lost epithelial tissue, narrowed blood vessels and signed for disruption followed by leakage of blood, severe mucosal oedema, and necrosis of submucosa, loss of

villi and marked haemorrhage and lymphoid hyperplasia. In addition, Eimeria oocyst showed up on chicken caecum and intestine (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].

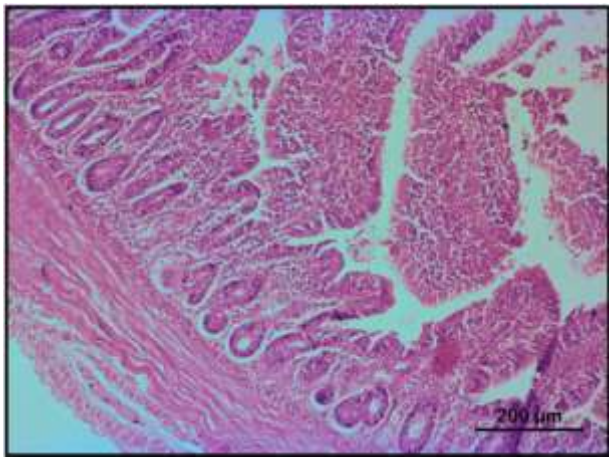

Fig 5: Development stages of *Eimeria* species in the epithelial cells of intestine (H & E X 100).

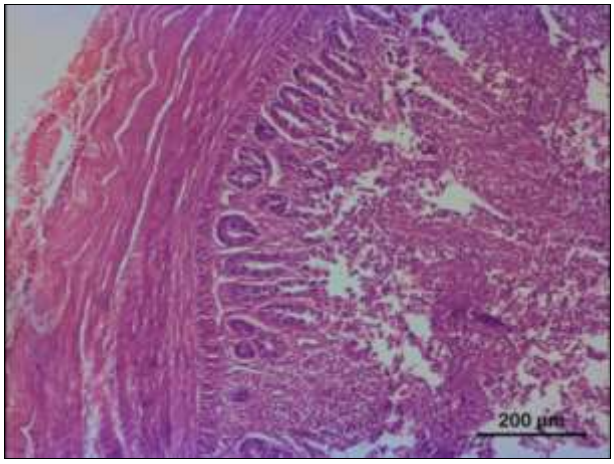

Fig 6: Intestine showing desquamation of necrotic villi of sloughing off from the lining epithelial layers (H & E X100).

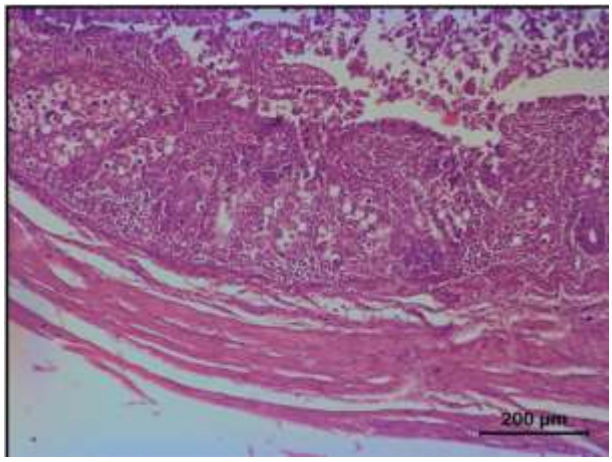

Fig 7: Intestine showing atrophied and shortened of villi, proliferation of connective tissues and infiltration of mono nucleus cells, macrophages and lymphocytes (H & E X100).

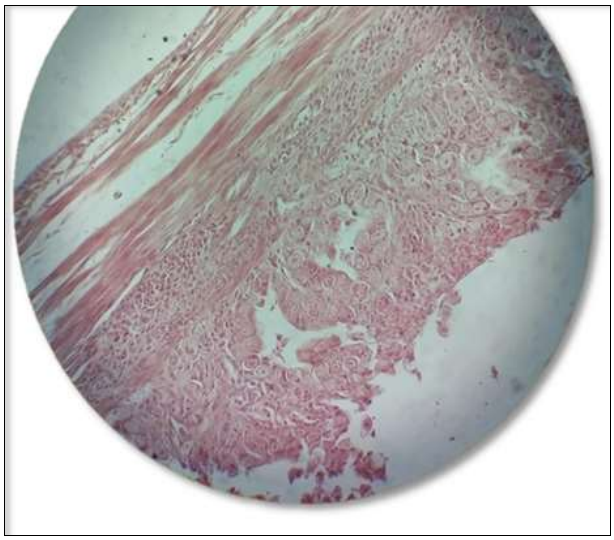

Fig 8: Caecum showing inflammatory cells predominantly macrophages and lymphocytes with extensive vacuolization in the glandular epithelial cells (H & E X 100)

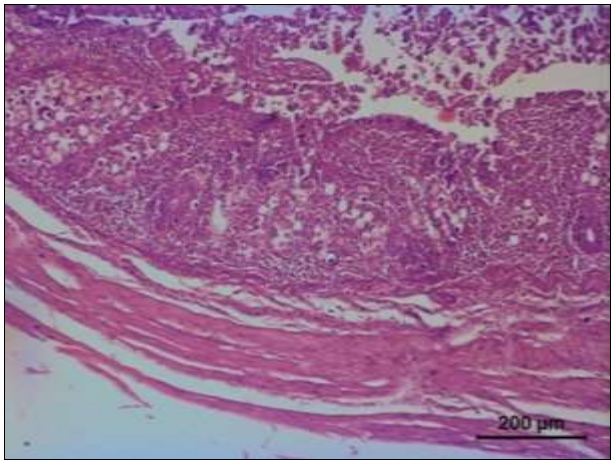

Fig 9: Caecum showing different grades of developmental schizonts and infiltration of inflammatory cells especially mononuclear cells, macrophages and lymphocytes around the epithelial cells of glands (H & E X 100)

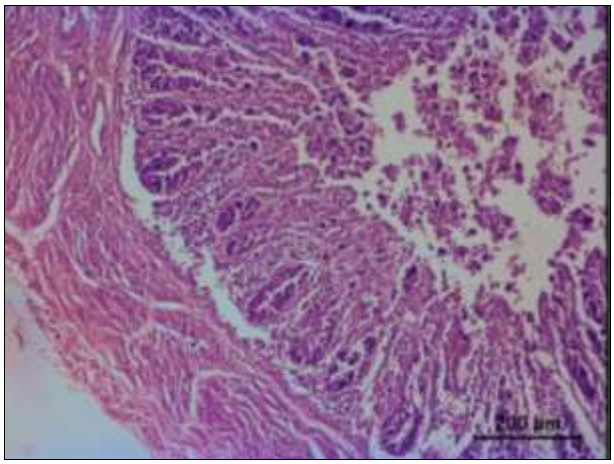

Fig 10: Caecum showing desquamation of necrotic villi and sloughing off epithelial lining and infiltration of eosinophils, macrophages and lymphocytes around the damaged epithelial cells (H&E X 100)

Conclusion

Clinical signs were observed greenish, yellowish or brown blood stools, lack of activity, starvation, weight loss, hunched posture, reduced food intake, decreased production, weakness,

pale comb and wattles, anaemia and sudden death. The gross lesions were intestinal ballooned and haemorrhage, while histopathology included loss of epithelial tissue, blood vessels congestion suggestive of disease with 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.

Acknowledgement
The authors thanks to the Dean, Faculty of Veterinary and Animal Sciences and the Head, department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, Kolkata for providing necessary facilities during the time of research.

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