

GROSS MORPHOLOGICAL, CYTOPATHOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON INTESTINAL COCCIDIOSIS IN GOAT

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Received: 31.05.2022; Accepted: 21.07.2022

ABSTRACT

The present investigation was aimed to find out the occurrence of coccidiosis and evaluate the cytological and histopathological alterations in intestine of goats in Jaipur region of Rajasthan. In this study, a total of 504 samples of intestine were collected from goats, irrespective of age breeds and sex. Out of 504 samples, 98 representative samples of intestine were processed for subsequent parasitological, cytological and histopathological examinations. The results of the study revealed an overall occurrence of coccidiosis as 19.44%. Macroscopically, small, whitish, non-pedunculated nodules were observed in the mucosa of intestine. In cytopathology, impression smears from the intestinal mucosa showed the presence of oocysts, merozoites and schizonts along with degenerative changes, necrotic cells and mononuclear cell aggregations. Histopathologically, sections of intestine from the nodular areas revealed mild to moderate inflammatory reaction with the presence of developmental stages of *Eimeria* spp. in the epithelium of affected intestinal villi and crypts. This study indicated for the prompt diagnosis of coccidiosis in goat production of semi-arid zone of Rajasthan such as Jaipur. This study may open the way up for using cytopathological technique as a reliable and useful diagnostic procedure for evaluation of infectious diseases viz. coccidiosis not only in goats but also in other animal practices.

Keywords: Coccidiosis, Cytohistopathological, Goat, Intestine, Occurrence

How to cite: Meena, S., Dadhich, R., Sharma, S., Meena, D.S., Saini, S., Sharma, S.K. and Rathore, A. (2023). Gross morphological, cytopathological and histopathological studies on intestinal coccidiosis in goat. *The Haryana Veterinarian* 62(1): 38-41.

Infectious diseases are a global problem and considered as a major obstacle in the health and product performance of livestock specially goats (Nath *et al.*, 2014). Gastro-intestinal parasitic infection is a serious constraint in small ruminant that may greatly reduce the animals productivity levels (Benavides *et al.*, 2015). Intestinal coccidiosis is one of the most important parasitic diseases of small ruminants worldwide. *Eimeria* spp. infection can cause weight losses in goats and severe cases can lead to the death of the affected animals, resulting in economic losses to the goat industry (Tafti and Mansourian, 2008). The disease is more serious in 4-6-months old kids and lambs and also in any age when animals are kept under conditions of intensive husbandry. Stress factors such as weaning, inclement weather, dietary changes, traveling and regrouping have important roles in coccidiosis in small ruminants. In goats, sixteen species of *Eimeria* have been described from different parts of the world, of which, *E. christenseni*, *E. arloingi*, *E. caprina* and *E. ninakohlyakimovae* are reported as the most pathogenic species (Kheirandish *et al.*, 2014). Coccidia can invade and destroy intestinal cells of the hosts, causing intestinal pathology (Uzal *et al.*, 2016).

The availability of a variety of rapid, safe and cost-effective techniques and stains often places cytopathology in the forefront of diagnostic evaluations, including the diagnosis of infectious diseases (Sood *et al.*, 2008).

Although major advances have occurred in the cytological diagnosis of various organ pathologies, gastrointestinal (GI) cytology has not gained popularity and is only occasionally practiced in the form of brushings and washings. Touch imprint cytology is a rapid, simple and inexpensive method of diagnosis of GIT lesions (Vijayanarasimha *et al.*, 2014).

In Rajasthan, so far very few efforts have been made to study cytological and histopathological features of intestinal coccidiosis in goats. Therefore, looking to the immediate need of understanding the various cytohistopathological features of intestinal coccidiosis and for providing timely diagnosis, the present research work was proposed in goats.

MATERIALS AND METHODS

Source and Collection of Samples: In the present study, a total of 504 samples of the intestine were collected from goats of either sex, irrespective of age and breeds. Out of 504 samples, 98 representative samples of intestine were processed for subsequent parasitological, cytological and histopathological examinations. All three techniques were applied for every single case of goat carcasses. The entire work was carried out in the Department of Veterinary Pathology, PGIVER, Jaipur. The organ/tissue samples for this study were collected from various slaughter houses, carcasses of goat submitted for post-mortem examination to department of Veterinary Pathology, any mortality at

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Veterinary Clinical Complex, PGIVER, Jaipur and samples received from the field veterinarians in the department of Veterinary Pathology for the routine histopathological examination were included in this study.

Gross Examination: All the samples were examined grossly for alterations in morphology in terms of shape, size, colour, consistency, odour, location and type of the lesions in individual part of the intestine.

Cytological Examination: For cytological examination following techniques were used.

Impression Smear/Touch Imprint Cytology: Impression smears from tissues were collected at necropsy. At least four slides were prepared from each case which was used for histopathological examinations, by using the technique described by Tribe (1965) and Tseng *et al.* (1999). The prepared smears were allowed to air dry. After drying, smears were immediately fixed using methanol. Then imprints were stained with Giemsa stain (Hi Media Laboratories, India) and thoroughly examined under light microscope for various cytopathological alterations.

Wet mount Faecal Cytology: Wet mount faecal cytology was performed by collecting a small amount of faecal samples from the cases which were used for cytological and histopathological examinations. All faecal samples were first subjected to standard qualitative faecal sample examination by using direct smear method for detection of coccidian oocysts. The coccidian parasites were identified on the basis of the morphological features of oocysts described by Soulsby (1982).

Histopathological Examination: After thorough gross examination, representative pieces (approximately 0.5 cm thickness) of intestines were collected and fixed in 10% neutral buffered formalin. The formalin fixed tissues processed for paraffin embedding by acetone and benzene technique (Lillie, 1965). The tissue sections of 4-5micron thickness were cut and stained with haematoxylin and eosin staining method (Suvana *et al.*, 2008) for histopathological evaluation. Sections were thoroughly examined under light microscope for various histopathological changes. In addition, special stains such as Periodic Acid Schiff (PAS) technique for coccidia were also performed (Culling, 1974).

RESULTS AND DISCUSSION

Occurrence of coccidiosis:

In this study, intestine from 504 goats were examined and 98 samples were found to be infected with coccidiosis. Various prevalence rates of *Eimeria* infection (Coccidiosis) in goats have been reported in various parts of the world. The present findings exhibited the occurrence of *Eimeria* infection in goats (19.44%) was closely related

to the previous studies (Sorathiya *et al.*, 2017) as 20 percent, and as 25.8% (Matsepe *et al.*, 2021) in Lesotho, South Africa while lower as compared to that reported by several authors as 96.66% (Kaur *et al.*, 2018) in Ludhiana, as 86.71% (Sharma *et al.*, 2017) in Mathura (UP) India, as 60% (Mohamaden *et al.*, 2018) in Egypt and as high as 90.96% (Singh *et al.*, 2020) in Makhdoom, Mathura, India. However, it was greater than that noticed in few reports as 12.74% (Sisodia *et al.*, 1997) in sheep, Western Rajasthan, India. Occurrence of coccidiosis across the country has been well documented as climatic conditions are most conducive for sporulation and survival of coccidian oocysts throughout the year (Singh *et al.*, 2017). Possibly oocysts from previous flock were present and suitable conditions like high moisture contents, warmth of 25-30°C and stress conditions are responsible for flaring up of infection resulting into a form of outbreaks resulting into mortalities (Kaur *et al.*, 2018).

Cytohistopathological studies: In the present study, at necropsy, gross lesions were observed mostly in the distal part of the small intestine and caeca. Macroscopic lesions included congestion and thickened mucosa along with the presence of scattered, small, whitish and non-pedunculated nodules (Fig. 1). Histopathological examination of the non-pedunculated nodules revealed enteritis characterized by mild to moderate infiltration of lymphocytes, plasma cells and eosinophils in the lamina propria (Fig. 2). In these early histopathological lesions, the presence of intracytoplasmic developmental stages of the parasite such as immature to mature schizonts were also observed. The most prominent microscopic lesion was proliferative enteritis. Few normal crypts of Lieberkühn with the majority of the crypts invaded with the asexual developmental stages (Figs. 3 and 4). The intestinal epithelium showed necrosis and haemorrhages (Fig. 5) and hyperplasia eventually developed into papillary projections of reactive epithelium. Gross changes like thickened and congested mucosa with scattered, small, whitish, non-pedunculated nodules and histopathological findings such as proliferation of intestinal villi during different developmental stages of coccidian life cycle in epithelial cells, have also been studied previously by several authors (Tafti and Mansourian, 2008; Radad and Khalil, 2011; Hashemnia *et al.*, 2015; Kaur *et al.*, 2018; Satish *et al.*, 2019). On microscopic examination of wet mount faecal smears from the rectal samples, showed multiple species oocysts of *Eimeria* with *E. arloingi*, *E. ninakohlyakimovae*, *E. ahsata* and *E. hirci* in a single field (Fig. 6). The similar findings also described by Kaur *et al.* (2018) also postulated that stocking rate and togetherness of young and adults in intensive system of management exposes the animals to

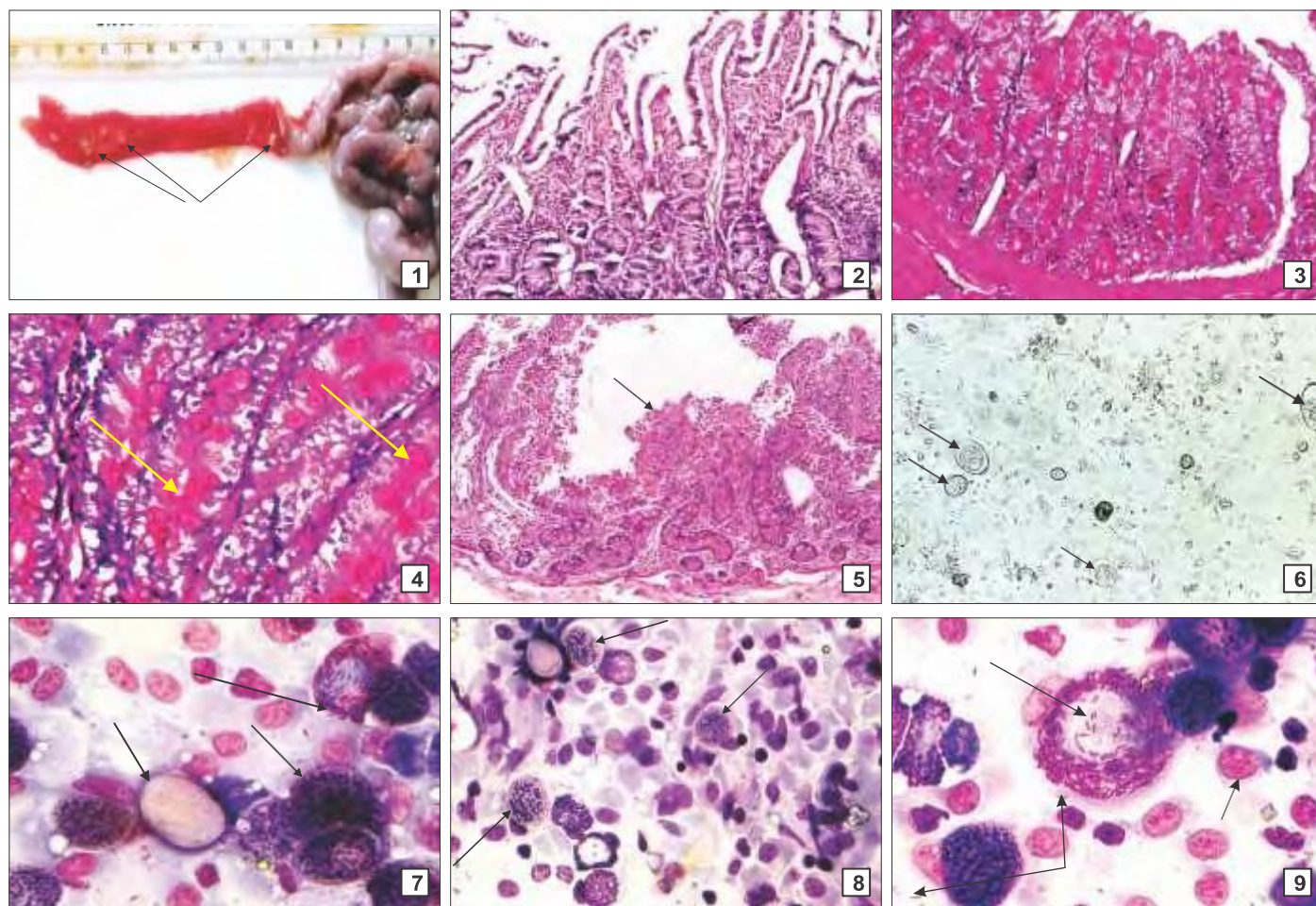


Fig. 1-9. (1) Photograph, showing congested and thickened mucosa along with scattered, small, whitish and non-pedunculated nodules. (2) Photomicrograph of intestine depicting enteritis, characterized by mild to moderate infiltration of lymphocytes, plasma cells and eosinophils in the lamina propria. (H&E, 100X) (3) Photomicrograph of intestine showing crypts of Lieberkühn invaded with the asexual developmental stages of *Eimeria* spp. (PAS, 100X) (4) Photomicrograph of crypts of Lieberkühn showing intracytoplasmic coccidian stages such as immature to mature schizonts. (PAS, 400X) (5) Photomicrograph of intestine depicting mucosal epithelium with inflammatory reaction and necrotic-hemorrhagic exudates in the lumen. (H&E, 100X) (6) Direct faecal wet mount smear from the rectum, showing multiple species of *Eimeria* oocysts with *E. arloingi*, *E. ninakohlyakimovae*, *E. ahsata* and *E. hirci* in a single (400X). (7) Impression smears from the intestinal mucosa depicting the presence of oocyst, merozoites, schizonts and macrogametocyte along with the, degenerative and necrotic changes. (Giemsa's stain, 1000X) (8) Impression smears from the intestinal mucosa showing the many macrogametocytes having a central nucleus with eosinophilic peripheral wall forming bodies in enterocytes and detached epithelia cells and few lymphocytes in background. (Giemsa's stain, 400X) (9) Touch imprint from the intestinal mucosa showing the schizont containing merozoites in an enterocyte, crescent shape merozoites, necrotic cells and lymphocytic infiltration in background. (Giemsa's stain, 1000X)

infection and re-infection due to poor hygienic conditions (Chartier and Paraud, 2012).

In cytopathology, impression smears from the intestinal mucosa depicted the presence of many oocysts (two layered wall), merozoites and schizonts along with the degenerative changes, necrotic cells and mononuclear cell aggregations (Fig. 7). The mature macrogametocytes had a central nucleus with eosinophilic peripheral wall forming bodies (Fig. 8). The microgamonts were round and had peripherally located nuclei. Touch imprint from the intestinal mucosa also depicted the mature schizont contained merozoites in an enterocyte, crescent shape merozoites, necrotic cells and lymphocytic infiltration in background (Fig. 9). Though cytopathological findings

including different stages of coccidian life cycle in epithelial cells of intestine have been studied previously in goats (Garba *et al.*, 2020) and other species such as dog, cat and rabbit (Manjunatha *et al.*, 2019) but, in the present study all the developmental stages of *Eimeria* spp. in goats are demonstrated more clearly in Giemsa stained impression smears.

CONCLUSION

Present study concluded that occurrence of intestinal coccidiosis in Jaipur district was found to be high in goats. Cytological alterations and pathomorphological changes reported in this study can be used by field veterinarians as guidelines for clinical evaluation and

disease severity assessment. Based on present results, utilizing the science of cytopathology particularly impression smear or touch imprint cytology is cost effective, rapid, simple and accurate. It can be used not only in the routine diagnosis of neoplastic growths but also for infectious diseases *viz.* coccidiosis.

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