

## PATHOBIOLOGICAL INVESTIGATIONS IN COCCIDIOSIS AFFECTED RUMINANTS SHOWING GASTROINTESTINAL DISTURBANCES

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### ABSTRACT

The present study was carried out to investigate the pathobiology of coccidiosis in 65 ruminants (38 buffalo, 10 cattle, 5 goat and 12 sheep) having history of gastrointestinal tract disorders which were brought to the Department of Veterinary Pathology, LUVAS, Hisar for post mortem examination. Faecal samples examined from all carcasses by floatation and sedimentation methods revealed presence of *Eimeria* oocysts in 24 (36.92%) cases (3 adult buffaloes and 14 buffalo-calves, one cattle-calf, two adult goat and four sheep). *Eimeria* spp. was also found in association with other parasites as mixed infections in 9 cases. Mixed infection of *Eimeria* was found with *Strongyle* spp. and with *Buxtonella sulcata*. On the basis of morphometry and sporulation, *Eimeria* spp. such as *E. bovis*, *E. zuernii*, *E. bareillyi* and *E. hirci* were identified from the bovine carcasses. Microbiological investigations from heart blood, liver and mesenteric lymph nodes revealed presence of secondary bacterial infections in 13 cases mainly *Escherichia coli*, *Kocuria* spp., *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Sphingomonas* spp., *Enterococcus* spp. and *Salmonella* spp. Main organism found associated with coccidiosis was *E. coli* which was found in 9 cases mainly belonging to O135, O98, O11, O9, O126, O18 serotypes. Pathological studies mainly revealed haemorrhagic as well as severe necro-haemorrhagic enteritis due to development of coccidian parasites in the epithelial cells of the intestine.

**Keywords:** Coccidiosis, Gastrointestinal tract disorders, Pathobiology, Ruminants

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Gastrointestinal tract disorders especially diarrhoea in young ones is a major problem in ruminants and coccidiosis caused by *Eimeria* spp. plays a significant role in calf diarrhoea. *Eimeria* spp. belongs to a protozoan genus that is widely distributed world-wide. The infective stages of the coccidium, the oocysts are thick walled and very resistant which may be viable outside the body under suitable conditions for approximately two years. It plays a crucial role in reducing animal production by lowering the working capacity, growth, body weight and milk yield (Panigrahi *et al.*, 2014). *Eimeria* spp. usually affect young animals of up to 4 months of age, but are also diagnosed in adult animals which in turn act as reservoirs of the infection (Lopes *et al.*, 2013). Out of the 12 species recorded, *E. zuernii* and *E. bovis* are the two most common causes of clinical coccidiosis in calves and young cattle worldwide. The infection is transmitted through the ingestion of sporulated oocysts in contaminated feed, water and licking of contaminated surfaces. This disease is more commonly linked to poor hygiene, higher stock densities and other stress conditions like weaning. The parasite causes damage in the intestine that leads to reduced appetite, reduced body weight, impaired feed conversion, unthriftiness, diarrhoea, dysentery, anaemia and increased susceptibility to other diseases causing huge economic losses to the livestock producers (Panigrahi *et al.*, 2014). Keeping in view of the above facts, the present

investigation was aimed to study the pathobiology of parasitic conditions and morphometrically identification of various *Eimeria* spp. affecting ruminants.

### MATERIALS AND METHODS

Study was conducted on 65 ruminants (38 buffalo, 10 cattle, 5 goat and 12 sheep) having history of gastrointestinal tract disorders which were brought to the Department of Veterinary Pathology, LUVAS, Hisar for post mortem examination.

**Parasitological studies:** Faecal samples were collected from all ruminant carcasses (bovine, ovine and caprine) directly from rectum by using sterile disposable plastic gloves. Detailed history of the cases as consistency of the faeces, age, sex, breed of animals was recorded from the owner and from the requisition form. The samples were placed in labeled sterile vials and preserved at 4 °C in a refrigerator until processing. Faecal materials were placed into Petri dishes with 2.5% potassium dichromate (K<sub>2</sub> Cr<sub>2</sub> O<sub>7</sub>) solution for sporulation of oocyst at laboratory temperature (28 °C). Parasitological examination was carried out by both floatation and sedimentation methods. The slide was covered with cover slip and then examined for the presence of oocysts/ova under a microscope at low and then at higher magnification. The identification of *Eimeria* spp. was performed on the basis of morphological features of the eimerial oocysts i.e. size, shape, color and texture of the oocyst wall, presence or absence of micropyle

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and polar cap (Soulsby, 1982). Microphotography of different ova/oocysts was done with the help of Olympus CX-41 with DP-21.

**Pathological studies:** During post mortem examination, all the organs and tissues were examined critically to detect gross changes during postmortem examination and the lesions so observed were recorded. Representative tissue pieces from organs, which revealed lesions were collected in 10 per cent buffered formalin for histopathological examination as per standard paraffin embedding method with routine haematoxylin and eosin stain (Luna, 1968).

**Microbiological studies:** For microbiological studies, bacterial isolations were attempted from heart blood and liver (Cruikshank, 1973) and confirmatory identification was done by using Vitek 2 system (Bio Merieux, Inc. Hazelwood, MO, USA).

### RESULTS AND DISCUSSION

Detailed history of the cases revealed that out of 65 cases, 24 (36.92%) cases were affected with coccidiosis as depicted in Table 1. The consistency of faeces was found to be soft to watery in 13 cases while it was firm in two cases. Most of the carcasses in the present study were female. This might be due to the fact that females are reared more by farmers than males for commercial purpose. Parasitological examination of faecal examination revealed that *Eimeria* spp. alone infection was found in 15(23.1%) cases including buffalo-calves (11), adult buffaloes (2), cattle-calf (1) and adult goat (1). *Eimeria* spp. infection was also found in association with other parasites (*Strongyle* spp. and *Buxtonella* spp.) in 9 cases.

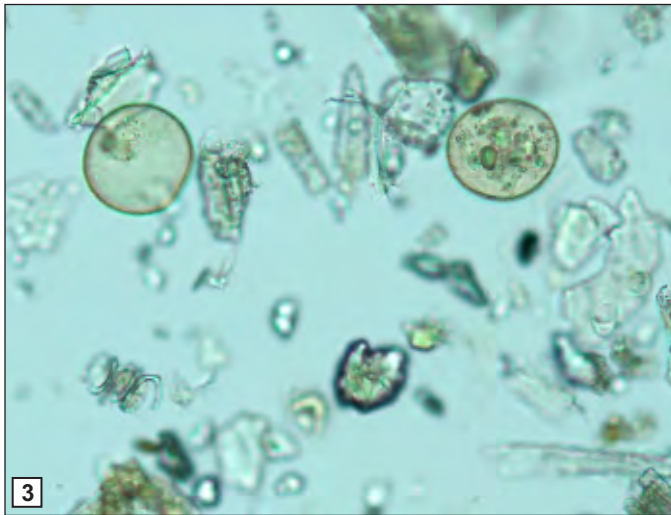
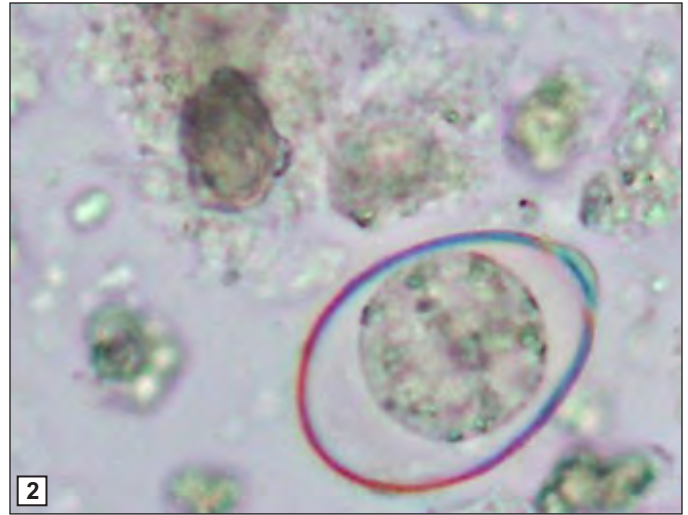
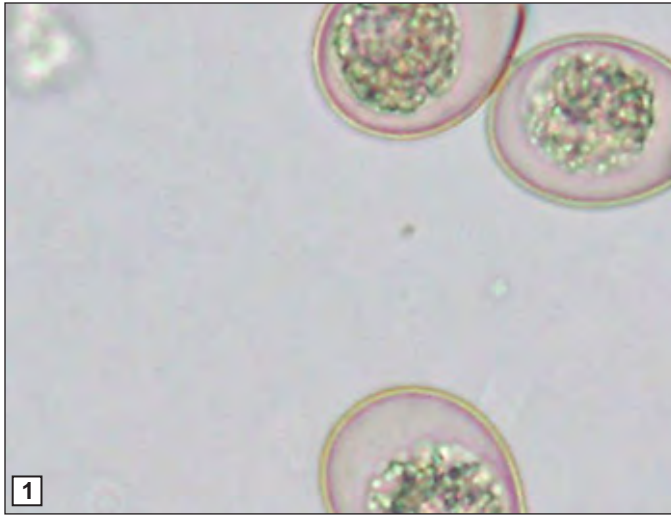
Out of 9 cases of mixed infection, *Eimeria* spp. was found in association with *Strongyle* spp. in 6 (25%) cases 2 each affecting buffalo-calves and adult sheep and one case each in adult buffalo and adult goat. In 3 (12.5%) cases, the *Eimeria* spp. was found associated with *Buxtonella sulcata* (2 cases in adult sheep and one case in buffalo-calf). *Eimeria* spp. identified from bovine carcasses on the basis of morphometry and sporulation were *E. bovis*, *E. zuernii*, *E. bareillyi* and *E. hirci*. Faecal examination smears showing oocysts/ova of different parasites are shown in Fig. 1, 2, 3 and 4.

Present investigation revealed coccidiosis as the major infection followed by mixed parasitic infection (*Eimeria* spp., *Strongyle* spp. and *Buxtonella* spp.) in affected ruminants. The buffalo-calves were found to be more predominantly affected with coccidiosis than adult buffaloes. However, ovine and caprine showed mixed parasitic infection (*Eimeria* spp., *Strongyle* spp. and *Buxtonella* spp.) in most of the cases where adults were found to be affected more as compared to lambs. Similar to our findings, Rehman *et al.* (2011) and Maharana *et al.* (2016) also reported the gastrointestinal parasites in bovines mainly as *Strongyle* spp., *Eimeria* spp. and *Buxtonella sulcata* with higher infection of *Eimeria* spp. in buffalo-calves. More or less same findings were also reported in respect of the identification of the gastrointestinal parasites by Sushma *et al.* (2016). Likewise Priti *et al.* (2008) also reported higher infection of *Eimeria* spp. in case of the female buffalo-calves. Rehman *et al.* (2011) further stated that ground-fed animals were at higher risk to *Eimeria* infection than trough fed animals. deMacedo *et*

Table 1

Faecal examination for parasitic infections in ruminant carcasses affected with gastrointestinal tract disorders (n=24)

Species (No.)	<i>Eimeria</i> alone	% age	Mixed Parasitic conditions	% age	Grand total	% age
Adult buffalo (17)	2	8.33	1 ( <i>Eimeria</i> spp. + <i>Strongyle</i> spp.)	4.17	3	12.50
Buffalo-calves (21)	11	45.83	1 ( <i>Eimeria</i> spp. + <i>Buxtonella</i> spp.) 2 ( <i>Eimeria</i> spp. + <i>Strongyle</i> spp.)	12.50	14	58.33
Total (38)	13	54.17	4	16.17	17	70.83
Adult cattle (4)	0	0	0	0	0	0
Cattle-calves (6)	1	4.17	0	0	1	4.17
Total (10)	1	4.17	0	0	1	4.17
Goat (3)	1	4.17	1 ( <i>Eimeria</i> spp. + <i>Strongyle</i> spp.)	4.17	2	8.33
Kids (2)	0	0	0	0	0	0
Total (5)	1	4.17	1	4.17	2	8.33
Sheep (8)	0	0	2 ( <i>Eimeria</i> spp. + <i>Strongyle</i> spp.) 2 ( <i>Eimeria</i> spp. + <i>Buxtonella</i> spp.)	16.67	4	16.67
Lambs (4)	0	0	0	0	0	0
Total (12)	0	0	4	16.67	4	16.67
Grand Total (65)	15	62.50	9	37.50	24	100



**Fig. 1 to 4.** (1) Faecal smear showing Eimerial oocysts (*E. bovis*) in buffalo-calf, (2) Faecal smear showing *Eimeria* spp. oocyst (Adult goat), (3) Faecal smear showing oocyst of *Buxtonella sulcata* (Adult sheep), (4) Faecal smear showing ova of *Strongyle* spp. (Adult sheep)



**Fig. 5 to 7.** (5) Photograph showing reddish mucosa of the intestine associated with mixed infection of *Eimeria* spp. and *Strongyle* spp. in case of adult goat; (6) Photograph showing necrotic intestinal mucosa having white round raised spots in case of buffalo-calves; (7) Photograph showing necrotic intestinal mucosa having white round raised spots in case of buffalo-calves.

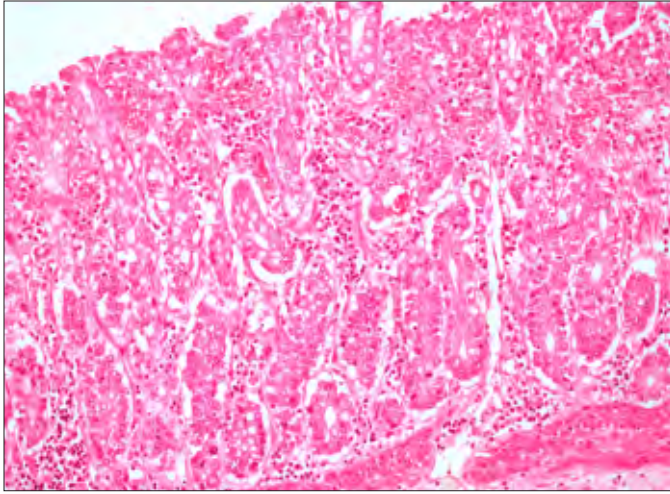


Fig.8. Photomicrograph of intestine showing necro-hemorrhagic enteritis with congestion, hemorrhages of mucosal and submucosal blood vessels along with infiltration of mononuclear cells mainly lymphocytes in mucosa (H&E 200X) (Adult goat, *Eimeria* spp., *Strongyle* spp., *E.coli* infection)

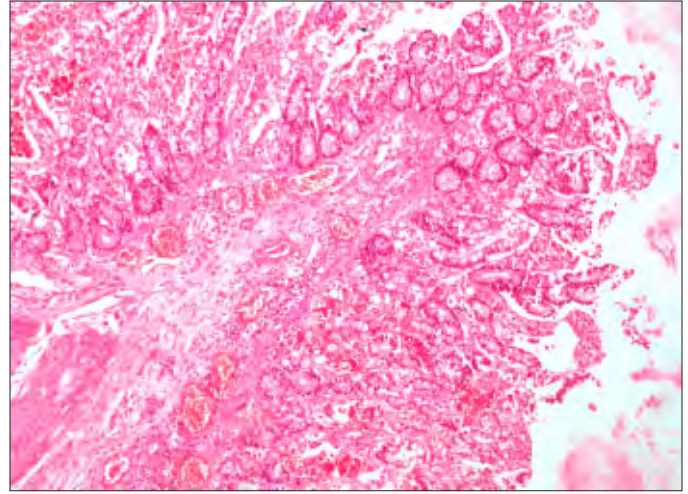


Fig.9 Photomicrograph showing necro-hemorrhagic enteritis characterized by congestion, hemorrhages in mucosa and submucosa and infiltration of mononuclear cells inflammatory cells along with presence of coccidial oocysts. (H&E 100X) (Buffalo calf)

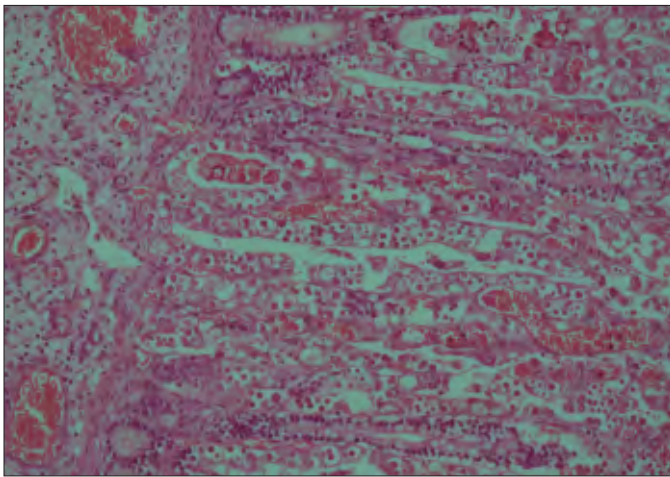


Fig.10. Photomicrograph showing necro-hemorrhagic enteritis characterized by congested, hemorrhages in mucosa and submucosa, infiltration of mononuclear cells inflammatory cells along with presence of coccidial stages (H&E 200X & 400X) (Buffalo calf, *E.coli*, *Kocuria kristinae*)

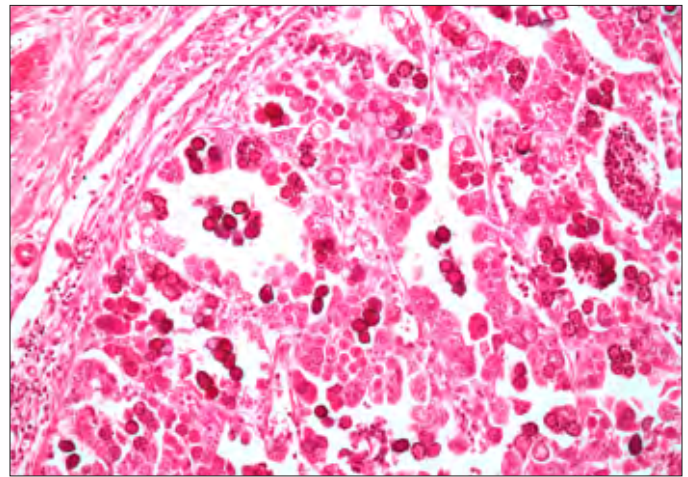


Fig.11. Photomicrograph showing necro-hemorrhagic enteritis characterized by congested, hemorrhages in mucosa and submucosa and infiltration of mononuclear cells inflammatory cells along with presence of coccidial stages (H&E 1000X)

*al.* (2019) also reported 70.07% positive cases for *Eimeria* oocysts in small ruminants and correlated high positivity to the poor hygienic sanitary conditions, which may be considered an aggravating factor for the spreading of the coccidiosis. Meena *et al.* (2016) also postulated that crowded conditions associated with poor sanitation leads to faecal-oral transmission of large numbers of coccidial organisms (Zachary and McGavin, 2012). This may be due to the fact that high humidity and moderate temperature facilitate the survival and sporulation of the oocysts.

A significantly higher proportion of calves were infected with coccidia than other age groups. Similar to our results regarding age-wise more incidence among buffalo-calves was also reported by Nain *et al.* (2017). According to Gunathilaka *et al.* (2018), the susceptibility and pathogenicity of gastrointestinal infections are greater in

young animals than the matured ones. The causes for variations in the prevalence of parasites at different age groups are difficult to explain, but they might be due to an immunological status of the animals, difference in the grazing area, and management conditions (Regassa *et al.*, 2006). Bilal *et al.* (2019) also stated that as the immunity in calves is lower than the adult cattle, they might be more susceptible to coccidian infections. Similar to our findings in sheep and goats, Chartier and Paraud (2012) also stated that adults were most frequently affected than young ones in case of small ruminants and further suggested the reason that the adults might have a huge epidemiological burden, especially in conditions of stress such as parturition, transportation, food and climate changes besides high density stocking.

Gross pathological changes were found mainly in

intestine in most of the coccidiosis affected cases and revealed reddish mucosa with white necrotic patches (Fig. 5, 6 & 7). Histopathological examination revealed necro-hemorrhagic enteritis characterized by desquamation of the mucosal epithelium with congestion, hemorrhages and necrosis along with presence of all the developmental stages of coccidian parasites in the epithelial cells of the intestinal gland in most of the cases (Fig. 8, 9, 10 & 11). In the present study, cases affected with coccidiosis showed fusion and necrosis of the intestinal villi, presence of macrogamonts with eosinophilic nuclei and microgamonts with basophilic nuclei with infiltration of lymphocytes in the intestinal tissues. All these findings were in accordance to that of Singh *et al.* (2008), Gazyagci *et al.* (2015) and Satish *et al.* (2019). Satish *et al.* (2019) also reported coccidiosis in small ruminants and revealed congestion and white round raised spot in the intestine along with development of different stages of *Eimeria* spp. in the mucosa of the intestine. Catarrhal enteritis, granulomatous enteritis and suppurative enteritis observed in few cases might be due to association with other bacterial infections.

Microbiological investigations from heart blood, liver and mesenteric lymph nodes revealed that out of 24 cases affected with coccidiosis, 13 cases revealed presence of other opportunistic pathogenic bacteria mainly *Escherichia coli*, *Kocuria* spp., *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Sphingomonas* spp., *Enterococcus* spp. and *Salmonella* group. *Escherichia coli* (9) were found in maximum number of cases mainly belonging to O135, O98, O11, O9, O126, O18 serotypes. Most of the newly isolated bacteria associated with coccidiosis in the present study belong to opportunistic pathogens from Gram-negative, non-fermenting bacteria group which have the ability to infect animals and humans with immune compromised conditions.

Parasitic diseases caused by intestinal parasites particularly coccidiosis decreases the livestock production, enhance the susceptibility to bacterial and viral diseases and cause losses due to condemnation of carcasses and organs, as well as cost of treatment and veterinary care. The prevalence rate and clinical diseases caused by parasitic infections vary due to different environmental and managemental factors in different areas. Attention needs to be paid to the managing procedures of herds like washing premises with disinfectants and separation of calves from infected animals.

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