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Coccidiosis in goats: Pathological observations on intestinal developmental stages and anticoccidial efficacy of amprolim

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ABSTRACT

In an outbreak of coccidiosis at a goat farm having 200 animals of different age groups (0-3 months, 4-6 months and 7-9 months), kept under stall fed conditions, mortality of 2 kids aged 2-3 months in a span of 2-3 days was seen. The kids were having a history of severe diarrhoea, anorexia and general weakness. Standard qualitative and quantitative coprological examination of randomly collected faecal samples from 60 goats of different age groups revealed that 58 (96.66%) were infected with coccidian oocysts. Among positive samples, 25(43.10%) were heavily infected (OPG=5000-1,23,000), 22(37.93%) had a moderate (OPG=1000-5000) and 11(18.96%) had a mild (OPG=100-1000) infection. Significant difference ($P<0.05$) observed in the mean OPG between the 3 age categories with highest infection in kids with the age group of less than or equal to 3 months,followed by 4-6 months and 7-9 months. Mixed infection of five *Eimeria* species, namely *E. arloingi, E. ninakohlyakimovae, E. christenseni, E. hirci* and *E. alijevi* was seen and *E. arloingi* was most predominant species among them. Systematic necropsies of naturally died kids of coccidial infection revealed small whitish non-pedunculated nodules in the small intestine. Histopathologically, these nodules revealed papillary hyperplasia of the mucosal epithelium with mild to moderate inflammatory reaction with the presence of developmental stages of *Eimeria* including trophozoites, schizonts, microgamonts, macrogamonts and oocysts in the epithelium of affected intestinal villi and crypts. The affected animals were successfully treated with amprolium @ dose rate of 2g/40kg body weight. Significant reduction in the oocysts count ($P< 0.01$) 7 days post treatment 610.52±201.17 was seen compared to pre treatment values (10685.96±3128.22).

Key words: Amprolium,Coccidiosis, *Eimeria* spp., Histopathology, Kids.

INTRODUCTION

Coccidiosis, an important stress induced enteric protozoan parasitic infection affecting several animal species including small ruminants worldwide (Daugschies and Najdrowski, 2005). It is caused by different species of enteropathogenic *Eimeria,* an apicomplexan protozoan which develops in the small and large intestine, causing more pathogenic effects in young animals. Though the disease is present in clinical form mostly in younger animals; however, adults may also be affected severely, at times (Taylor *et al*. 2007). In comparison to the clinical entity, subclinical coccidiosis is the most common form of the disease and, since it cannot be readily identified, have a significant impact on the flock's health and production efficiency. Such chronically infected animals shed oocysts of *Eimeria* spp. in the faeces and serve as foci of infection to other animals. Ingestion of sporulated oocysts may lead to severe infection in healthy susceptible young stock. In the small intestine, sporulated oocysts release sporozoites and invade to intestinal epithelial cells, the resulting developmental stages lead to loss of electrolytes, nutrients, malabsorption and enteritis (Jubb *et al*. 2007; McGavin and Zachary, 2011). Kids or lambs affected with clinical form of the disease shows the symptoms of bloody diarrhoea, dehydration, fever, inappetance, weight loss, poor and retarded growth and death in severe cases. Among the twelve species of Eimeria infecting the goat, the commonest ones are *E. arloingi* followed by *E. crandalis, E. parva, E. ninakohlyakimovae, E. intricata, E. christenseni, E. ahsata, E. hirci and E. parbhaniensis*. Among all E. *arloingi* are the most pathogenic species in goats (More *et al*. 2015). The coccidian parasites often lead to destruction of epithelial cells of intestine and interfere with intestinal microflora as well. Coccidiosis is mainly suspected when animals are kept under poor hygienic conditions and the mortality is mainly evident during weaning period (Chartier and Paraud, 2012).

The economic cost is considerable, in terms such as poor growth performance, decrease in productivity, mortality, morbidity, and the cost of prevention and treatment. These losses can be linked to reduced production, in the case of moderate infection without clinical signs or direct consequences of diarrhea on the growth of the animals and

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on mortality, in the case of clinical coccidiosis (Chartier and Paraud, 2012; Foreyt, 1990). The sporadic reports on clinical and subclinical coccidiosis from all around the globe including India result in direct and indirect economic losses. This paper reports a first clinical outbreak of coccidiosis from Punjab state in goats resulting into fatality of goat kids. Further the gross and histopathological observations and anticoccidial efficacy of amprolium has been evaluated.

MATERIALS AND METHODS

An outbreak of coccidiosis at goat farm with 200 animals of different age groups (0-3 months, 4-6 months and 7-9 months) kept under stallfed conditions, was reported with mortality of 2 kids aged 2-3 months in a span of 2-3 days with a clinical symptoms of severe diarrhea, anorexia and general weakness. Based on the history the cause for death was suspected due to coccidiosis. Faecal samples were collected randomly from 60 goats of different age groups and were first subjected to standard qualitative faecal sample examination by using direct smear method and floatation technique for detection of coccidian oocysts. The coccidian parasites were identified on basis of the morphological features of oocysts described by Soulsby (1982). For quantitative faecal sample examination, standard McMaster's technique was used to calculate oocysts per gram (OPG) of faeces (Soulsby, 1982). Morphometric study was also conducted to know about the major species of *Eimeria* involved in the pathology (Eckert *et al*. 1995).

Systematic necropsies were performed on the two kids died of natural coccidial infection. The gross pathological lesions were recorded. After thorough gross examination, small representative pieces (approximately 0.5 cm thickness) of intestines were collected and fixed in 10% neutral buffered formalin. The collected tissue samples were preserved in 10% neutral buffered formalin.Routine tissue sectioning and haematoxylin and eosin staining was performed (Singh and Sulochana 1996). Sections were thoroughly examined under light microscope for various histopathological changes.

Infected 58 animals were treated with amprolium @ dose rate of 2g/40kg body weight for 5 days and faecal samples of all the animals were collected 7 days post treatment and processed qualitatively and quantitatively as per standard procedure for the estimation of efficacy of amprolium in reduction of OPG count.

Data analysis: The collected data was analyzed using Statistical Analysis System (SAS for Windows, Version 9.4, USA). One-way ANOVA was used to test the association between OPG values and the age category studied. A statistically significant association between the variables and the infections was considered to exist if the P-value was <0.05.

RESULTS AND DISCUSSION

In this outbreak of coccidiosis out of 60 animals, 58 were infected with coccidian occysts with an overall prevalence of 96.66%. Among positive samples, 25 (43.10%) were heavily infected (OPG=5000-1,23,000), 22 (37.93%) had a moderate (OPG=1000-5000) and 11 (18.96%) had a mild (OPG=100-1000) infection. There was a significant difference (P<0.05) in the mean OPG between the 3 age categories with the kids of less than or equal to 3 months of age showing highest OPG followed by 4-6 months and 7-9 months age old animals (Table 1). Mixed infection of five *Eimeria* species, namely *E. arloingi, E. ninakohlyakimovae, E. christenseni, E. hirci* and *E. alijevi* was seen and *E. arloingi* was most predominant species among them. Due to heavy infection multiple species of *Eimeria* were seen in a single field (Fig 1). The mortality was observed in kids of 0-3 months of age showing typical and severe clinical signs of coccidiosis having higher OPG. Yadav *et al*. (2007) observed that coccidiosis resulted into mortality and severe ill health among 16 Beetal kids, housed with 25 adult goats in Jammu. Under modern production system when kids are born into potentially heavily contaminated environment with sporulated oocysts often result into severe disease and fatality (Taylor *et al*. 2007). High mortality rate in kids may be attributed to under-developed and lower immunological resistance towards coccidian infection in kids as compared to adult animals. Further, stocking rate and togetherness of young and adults in intensive system of management exposes the young animals to infection and reinfection (Singla 1995; Sharma *et al*. 2009; Rehman *et al*. 2011).

Prevalence of coccidiosis across the country have been well documented as climatic conditions are most conductive for sporulation and survival of coccidian oocysts throughout the year (Kumar *et al*. 2001; Khillare and Narladkar, 2014; Singh *et al*. 2017) and stress conditions are responsible for flaring up of infection resulting into a form of outbreaks resulting in to mortalities.

Pathological studies: During the present study, two goat kids aged 3 months died with history of mucoid to haemorrhagic yellow watery diarrhea, anorexia, emaciation, poor growth and death. Coccidial-induced enterocyte hyperplasia results in nodule formation and thickening of the intestinal wall can cause reduction in food absorption,

Table 1: ANOVA for the association between age and OPG count.

Age (month)	Mean	Standard error	Standard deviation	Lower 95% CL	Upper 95% CL	F	df	P value
0-3 months	25060.00	6867.72	30713.38	10685.69	39434.31			
4-6 months	14371.05	3983.73	17364.68	6001.55	22740.56	4.33	2	0.0178
7-9 months	5966.67	1767.10	8097.86	2280.57	9652.77			

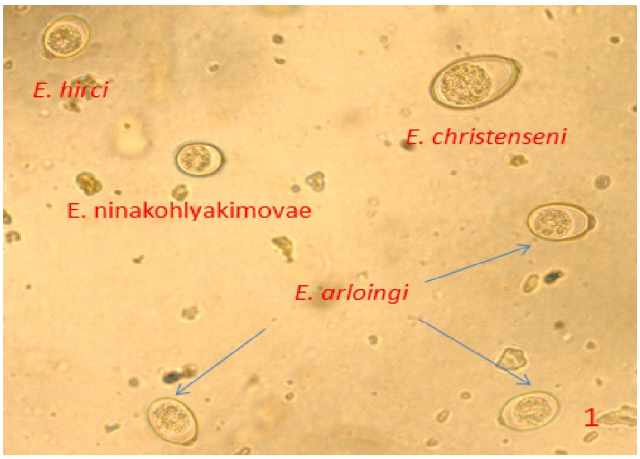

Fig 1: Mixed infection of *Eimeria* species with*E. arloingi, E. ninakohlyakimovae, E. christenseni* and *E. hirci.*

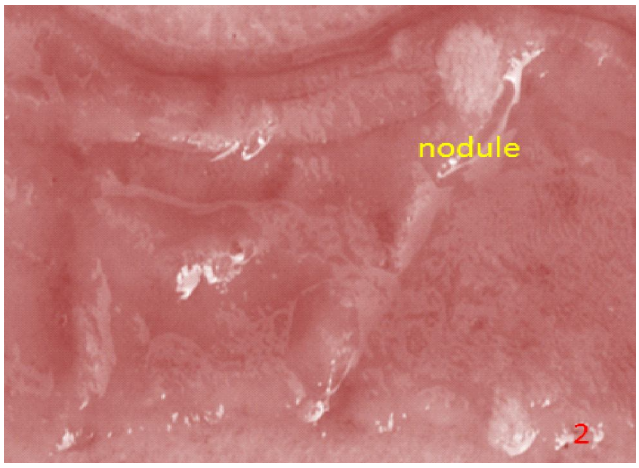

Fig 2: Thickening of the jejunal mucosa and presence of scattered whitish non-pedunculated nodules in the intestine.

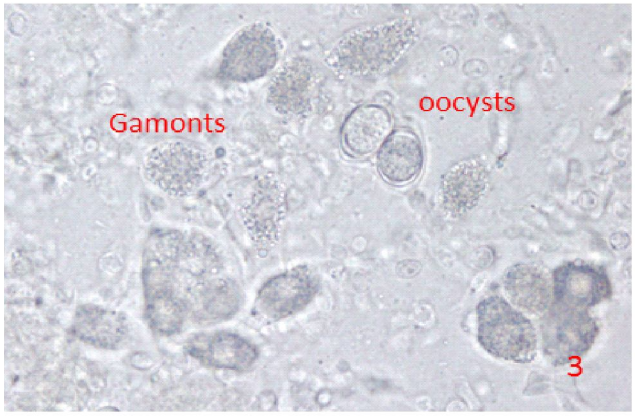

Fig 3: Microscopic examination of wet smear of the affected mucosa shows coccidia oocysts resembling *E. arloingi* in the epithelial tissue.

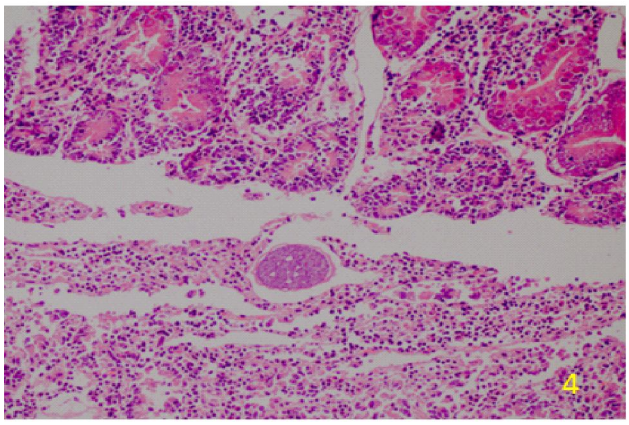

Fig 4: Mixed inflammatory reaction containing lymphocytes, plasma cells, macrophages and eosinophils in the lamina propria were seen.

emaciation, serous atrophy of fat, diarrhea, and dehydration (Hashemnia *et al*.2012). In the present study, at necropsy, gross lesions were observed mostly in the distal part of the jejunum and ileum. Macroscopic lesions were thickened mucosa with scattered small whitish non-pedunculated nodules (Fig. 2). Microscopic examination of wet smear from the formalin fixed mucosa shows coccidia oocysts in the epithelial tissue resembling *E. arloingi* (Fig. 3). Histopathological examination of the non-pedunculated nodules revealed papillary hyperplasia of the mucosal epithelium with mild to moderate inflammatory reaction mainly infiltration of lymphocytes, plasma cells and eosinophils in the lamina propria (Fig. 4). In these early histopathologic 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. The other developmental stages of *Eimeria* viz. microgamonts, macrogamonts and oocysts were seen in the epithelium of affected intestinal villi and crypts (Fig. 5, 6). In case of *E. arloingi* the developmental cycle involves large schizonts in the endothelium of the lacteals in villi in the upper small intestine (Fig. 7). The schizogony in intestinal epithelium induced necrosis, haemorrhages and subsequent hyperplasia due to second generation schizonts eventually developed into papillary projections of reactive epithelium (Fig. 8). Infiltration of inflammatory cells especially lymphocytes and eosinophils can be prominently seen in advanced lesions (Hashemnia *et al.* 2012). The mature macrogametocytes had a central nucleus with eosinophilic peripheral wall forming bodies. The microgamonts were round and had peripherally located nuclei (Fig. 5,6). Oocysts were oval and had two layers wall and were seen in the mucosa and occasionally in necrotic-hemorrhagic exudates in the intestinal lumen observed in the present study (Fig. 6 and 3). Though histopathologic findings including proliferation of intestinal villi during different stage of coccidial life cycle in epithelial cells have also been studied previously (Khodakaram Tafti and Mansourian, 2008; Radad and Khalil, 2011; Hashemnia *et al*. 2015), however,in the present paper all the stages are demonstrated more clearly in H & E stained histopathological sections.

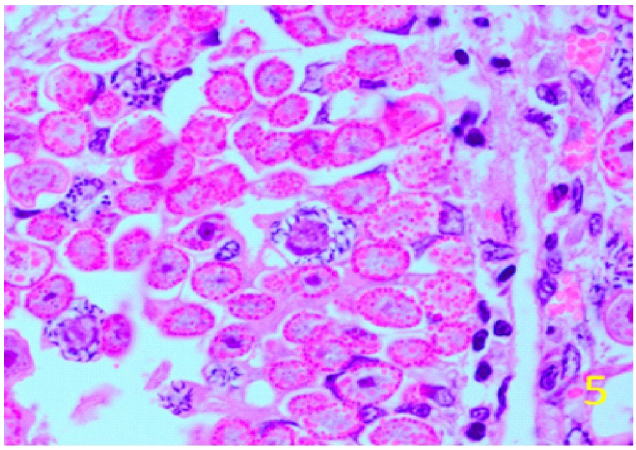

Fig 5: Presence of different developmental stages of the *Eimeria* including trophozoites, microgamonts and macrogamonts with eosinophilic peripheral granules

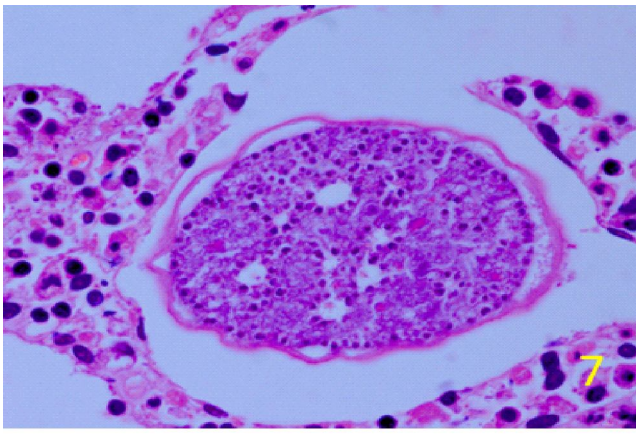

Fig 7: The presence of mature schizont in the endothelium of the villi

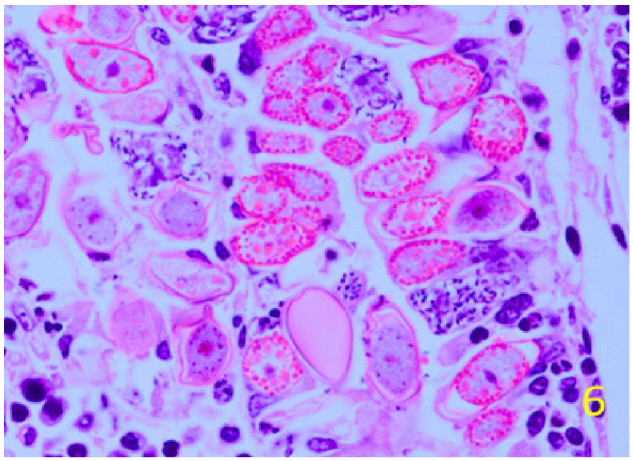

Fig 6: Numerous macrogametocytes and a few oocysts in enterocytes

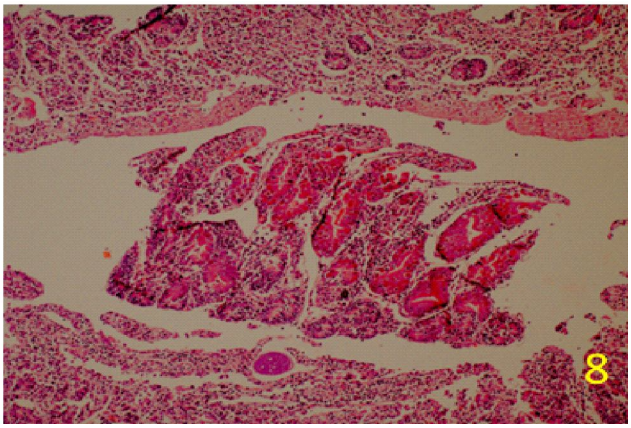

Fig 8: Necrosis of villus epithelial cells along with hemorrhage and also hyperplasia of enterocytes due to schizonts

Anticoccidial efficacy of amprolium: The affected animals after treatment with amprolium @ dose rate of 2g/40kg body weight for 5 days were looking clinically recovered from the infection. Faecal examination 7 days post treatment for coocidial oocysts indicated significant reduction (610.52±201.17) in the oocyst count (P< 0.01) as compared to pre treatment values (10685.96±3128.22). The specific dosage regimen of amprolium, which is effective against the later stages of infection (Chartier and Paraud, 2012) was selected for treatment based on the previously investigated favourable reports in goat coccidiosis (Iqbal *et al*. 2013; Young *et al*. 2011).

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