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Efficacy of amprolium against coccidiosis of goats in central Kashmir

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Abstract
The present work was undertaken to evaluate the anticoccidial efficacy of amprolium against *Eimeria* species of goats in central Kashmir. For this purpose, 30 kids harbouring *Eimeria* spp. infection (OPG above 6000) were randomly selected and divided into 2 groups (Group A and Group B), each comprising of 15 animals. Group A received amprolium@ 100 mg/kg body weight/day orally for 7 days and Group B was kept as untreated infected control. Faecal samples from rectum were directly collected on '0' day before treatment and on 8th, 14th, 21st and 28th day after starting treatment. It was found that the drug was 87.88% effective on 8th day, 91.02% on day 14, 89.53% on day 21st and 92.20% on 28th day, respectively. Faecal oocyst count was below 1000 in 13 animals of treated group by 28th day of the study period (end of trial).Animals having diarrhoea had normal faeces on 8th day and onwards. Identification of oocysts revealed the presence of *E. arlongi*, *E. caprina*, *E. christenseni* and *E. ninakohlyakimovae*.

Keywords: Amprolium, *Eimeria*, Goats, Efficacy, Coccidiosis, Kashmir.

1. Introduction
India has a vast resource of livestock and poultry, which play a vital role in improving the socio-economic conditions of rural masses. Parasitism is one of the foremost impediments to livestock productivity inflicting economic losses through mortality, morbidity, reduced feed conversion ratio, inefficiency of production and by the way of costs incurred upon their treatment and control [1]. Among the parasitic diseases, coccidiosis is probably the most important disease of veterinary importance and is clinically associated with dehydration, reduced growth, anaemia and death in young animals [2, 3]. The disease is worldwide in distribution and is caused by many species of *Eimeria*, which invade the cells of intestinal epithelium [4]. It is one of the most important diseases of sheep and goats [5, 6, 7]. throughout the world. Goats from all ages and breeds are susceptible, however, in kids of 1-4 months age, it is one of the most common cause of enteritis leading to diarrhoea and mortality may reach upto 58% [8] while the rest of the herd might act as carriers, continually contaminating the environment with oocysts which serve as a source of re-infection and of new infections for young kids [9]. Permanent scarring of the colon due to coccidial infection can result in chronic poor development and/or slow growth in some kids [6].
Prevention of reduced productivity and losses depends on maintenance of hygiene and effective treatment to reduce the level of environmental contamination by infective oocysts. Although environmental control must be part of the herd health approach, judicious use of anti-coccidial drugs is necessary to ensure adequate control of this disease and reducing the risk of infection to young ones. Also, the administration of anti-coccidials at weaning and shipping is required, when stress is likely to cause disruption of the host-parasite equilibrium. Most drugs used for control of coccidiosis have suppressive effect on the early developmental stages [10]. Amprolium which is effective against the earlier stages of infection [11] has been previously investigated in small ruminants [12, 13, 14]. It is structurally related to thiamine, and its antiparasitic activity is thought to be related to competitive inhibition of active thiamine transport into the parasite. It is freely soluble in water so can be mixed with drinking water, administered orally, or added to the feed of affected animals. Although amprolium is approved in many countries for the treatment of *Eimeria* infections in animals, there is limited data available on its efficacy against coccidiosis in goats in this part of the globe.
Anti-coccidial products form the basis of coccidiosis control practices in animals at present. Regular evaluation of the efficacy of these products is advisable, as it will provide information

on the oocysts reappearance period and the resistance status in the coccidian population. In the absence of adaptation of other alternative control measures, it is necessary to conserve the efficacy of currently available anti-coccidial drugs. With the result it becomes imperative to check their efficacy from time to time, so that a proper rotational strategy is devised to delay the development of resistance. In Jammu and Kashmir only two studies on the use of anti-coccidials in goats have been carried out in Jammu region [12, 15] and no such study has been carried out in Kashmir valley so far. Therefore, the current study was undertaken to evaluate the efficacy of amprolium against natural infection of coccidiosis in goats.

2. Materials and methods

2.1 Study area

This study was conducted on goats at Mountain Research Centre for Sheep and Goat (MRCSG), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shuhama, Srinagar located in central Kashmir. No coccidiostats had been used in any of the study animals during the initial examination.

2.2 Study design

First, faecal collection and analysis was performed to quantify the number of *Eimeria* oocysts per gram of faeces to identify and allocate animals randomly into control and experimental groups. For the study purpose, 30 kids of 3 to 6 months age naturally suffering from coccidiosis (OPG above 6000) were randomly assigned to 2 groups each comprising 15 animals identified with ear tag numbers. First group (group A) was treated with amprolium @ 100 mg /kg body weight/ day orally for 7 days and the other group (group B) was kept as untreated infected control (Table-1). Amprolium used in this study was an oral formulation. The current study was approved by the Animal Ethical Committee of SKUAST Kashmir as per requirement.

To determine the therapeutic efficacy of the drug, faecal samples were collected from each animal of these groups on "0" day before treatment and on day 8th, 14th, 21st and 28th after starting treatment and parasitic load quantification i.e, OPG (oocyst per gram) was determined by modified McMaster technique.

Briefly, the McMaster count was performed by adding 2 g of crushed faeces to 28 ml of floatation solution and mixed using magnetic stirrer dispersing oocysts into the solution and an aliquot pipetted onto a grid slide (Chalex LLC, USA) where the number of oocysts were counted.

2.3 The faecal oocyst count reduction test (*in vivo* test)

The percent efficacy of the drug was calculated from the arithmetic mean of the group OPGs as per the formula given below [16].

$$\text{Efficacy of drug treatment \%} = \frac{\text{Mean pre-treatment OPG- Mean post-treatment OPG}}{\text{Mean pre-treatment OPG}} \text{X} 100$$

2.4 Speciation or species identification

On day 0, pooled faecal samples obtained from kids were thoroughly mixed with an aqueous 2.5 % (w/v) potassium dichromate solution, placed in thin layers in petridishes at 27±2°C for 5-7 days to allow for sporulation. Then, using a sodium chloride floatation technique, oocysts were concentrated and evaluated at 40x magnification to determine the species based on characteristics as previously described in literature [17, 18, 19]. An ocular micrometer was used to measure the size of sporulated oocysts.

Table 1: Complete schedule of drug trial in goats suffering from coccidiosis

Group	Number of animals	Drug used	Dosage	Route of Administration
A	15	Amprolium	100mg/kg bd.wt	Orally for 7 days
B	15	Control	-	-

2.5 Statistical analysis

The results were subjected to standard statistical analysis using appropriate statistical softwares [20].

3. Results

3.1 Efficacy of amprolium against coccidiosis in goats

Identification of oocysts revealed the presence of *E. arlongi*, *E. caprina*, *E. christenseni* and *E. ninakohlyakimovae*. Group A animals treated with amprolium were having pre-treatment mean oocyst per gram (OPG) of 8500.0±570.2 on Day '0'. However, on day 8th, 14th, 21st and 28th after starting treatment, the mean OPG decreased to 1030.0±116.7, 763.3±108.6, 890±108.9 and 663.3± 100.0, respectively. It was found that the drug was 87.88 percent effective against *Eimeria* species on 8th day, 91.02 percent on day 14, 89.53 percent on day 21st and 92.20 percent on 28th day after starting treatment (Table-2; Fig.1&2).All the animals with the exception of 2 kids, had faecal oocyst count below 1000 by 28th day of the study period. On faecal score basis, diarrhoea in one animal and loose stools in 2 other animals had also subsided on day 8th and onwards.

In the control group (group-B), mean OPG of faeces for *Eimeria* species was 8426.7±488.1 on '0' day which increased to 12763.0±501.7, 16543.0±610.9, 15547.0±600.6 and 18993.0±509.4 on 8th, 14th, 21st and 28th day, respectively (Table-2 Fig. 2).

Table 2: Oocyst per gram counts (OPGC) of two groups (mean ±SE) and efficacy of amprolium in goats against coccidiosis

Days	Mean OPG of treatment group and percent efficacy (Group A)			Mean OPG of control group (Group B)	
	OPG (Mean ±SE)	Range	Efficacy	OPG (Mean ±SE)	Range
0	8500.0 ±570.2	6200-13450	-	8426.7±488.1	6500-12650
8	1030.0±116.7	250-1900	87.88%	12763.0±501.7	9800-16500
14	763.3±108.6	0-1500	91.02%	16543.0±610.9	13000-20150
21	890±108.9	0-1450	89.53%	15547.0±600.6	10500-20200
28	663.3±100.0	0-1300	92.20%	18993.0±509.4	14500-22000

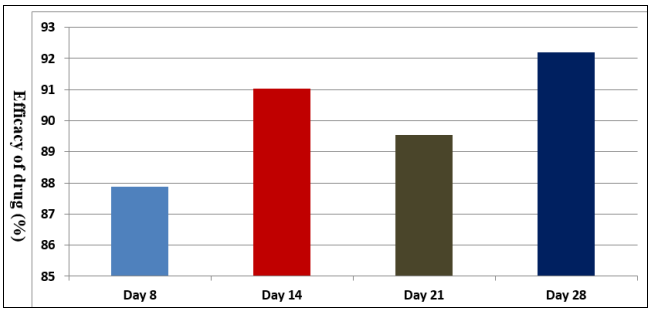

Fig 1: Efficacy of amprolium on treatment group observed on different days

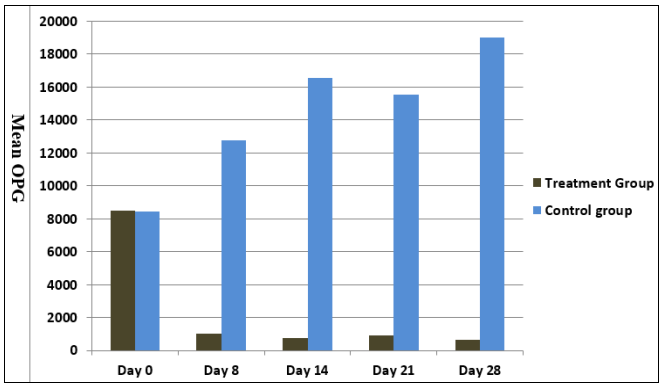

Fig 2: Mean OPG counts of two groups observed on different days

4. Discussion and conclusion

This study investigated treatment of animals with greater than 5000 eimerian oocysts per gram of faeces as OPG over 5000 is considered significant in ruminants [21]. In the present study, amprolium was found to be 87.88, 91.02, 89.53 and 92.20 percent effective against *Eimeria* species on 8th, 14th, 21st and 28th day after starting treatment in goats. Our findings are in close proximity with a study [22] which reported 76.92%, 90.38% and 92.31% efficacy on 7th, 14th and 21st day, respectively with amprolium @ 100mg/kg body weight for 3 days per os. Similar findings were recorded in another study [23] which found significant reduction in faecal oocyst count on 7th day post-treatment with amprolium against *Eimeria* spp.@ 50mg/kg body weight, however there was minimal to no efficacy of amprolium when dosed at 10 mg/kg over a 5 day period. A study in Ludhiana [14] also observed significant reduction in the oocyst count 7 days post-treatment with amprolium @ 2g/40kg body weight. In one trial, 18 goats ranging from 6 months to 1 year of age with naturally occurring coccidiosis were treated with either 100 mg/kg amprolium (added to the feed for 7 days), 2mg/kg salinomycin (added to the feed for 7 days), or cotrimoxazole (60 mg/kg) which was given orally twice a day for 3 days. Faecal analyses for oocysts per gram were done at several time points and the results indicated that amprolium was the most effective of the three treatments [24]. A case report of clinical coccidiosis in kids at Jammu revealed that amprolium at 100 mg/kg for 7 days was an effective treatment showing efficacy of 86.95 percent on day 7 and 100 percent on day 14 and 28 after starting treatment [15]. A larger study compared several different treatments in Pashmina kids with coccidiosis. Amprolium given at 20 mg/kg on day 1, followed by 10 mg/kg for an additional 4 days was found to have 93% efficacy [25]. Similarly, it has been reported that amprolium @100 mg/kg body weight in goats for four days or longer results in a rapid reduction in oocyst output and clinical recovery [17]. On contrary, very little response on kids after treating with amprolium@50mg/kg body weight has also been reported [13]. Faecal oocyst count reduction value of 99.60 percent against *Eimeria* species in Pakistan when treated with amprolium was also observed [26].

Of the many species of *Eimeria* that are known to infect goats, three are considered most pathogenic, *E. christenseni*, *E. ninakohlyakimovae*, and *E. arlongi* [27]. The identified species from the goats in this study were *E. arlongi*, *E. caprina*, *E. christenseni* and *E. ninakohlyakimovae*. Clinical signs of coccidiosis including diarrhoea and weight loss and histological evidence of proliferative enteritis in the colon have been found in goat kids experimentally infected with *E. ninakohlyakimovae* oocysts [28]. *E. arlongi* has been reported in outbreaks of coccidiosis in goats and disease associated with this species has also been experimentally reproduced [29].

On the basis of the present study mean oocyst counts remained much lower than pre-treatment counts for at least 3 weeks post-treatment. A similar pattern of decreased oocyst excretion was observed in goats when treated with toltrazuril. Significantly lower oocyst counts were found upto 28 days in goats following treatment with toltrazuril [12, 30] up to 21 days after treatment with furazolidone, sulfadimidine and amprolium [26] and upto 28 days following treatment with amprolium [31]. On contrary, oocyst count remained high with ponazuril treatment upto 28 days [31]. Differences in *Eimeria* species, age of the animal, and stage of disease at treatment may have resulted in the variability observed between studies. It may be concluded that amprolium was effective in treating goat coccidiosis indicating that amprolium may still be used in coccidiosis control programmes against goats in central Kashmir. Coccidia are ubiquitous parasites and the most obvious initial source of infection are does. Coccidial oocysts shed by adult females then cycle through kids leading to a rapid built up of oocysts and environmental contamination. If hygiene is improved, it is possible that the faecal oocyst counts would remain low for a longer duration of time. Therefore, effective control programmes must include changes in management hygienic standard and should not rely on medication alone.

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