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Study on the Efficacy of Selected Antitrematodal Drugs in Naturally Infected Sheep with Fasciolosis

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Abstract: An experimental type of study was designed to evaluate the efficacy of antitrematodals used in Korem and Hashenge areas of Ethiopia in naturally infected sheep with fasciolosis. 28 sheep naturally infected with fasciolosis were purchased and divided randomly into four treatment groups. Those groups were treated with triclabendazole, albendazole, oxyclozanide and a combination of albendazole and oxyclozanide. Pre-treatment *Facsiola hepatica* faecal egg counts per gram of faeces (EPG) were 475, 400, 533 and 317 for triclabendazole, albendazole, oxyclozanide and albendazole-oxyclozanide combination, respectively. After a week post treatment, mean egg per gram of faeces (EPG) egg count reduction test (FECRT) was (0, 100%), (100, 75%), (383, 28.14%) and (117, 63.08%) for triclabendazole, albendazole, oxyclozanide and albendazole-oxyclozanide combination, respectively. Statistical significant difference was recorded for faecal egg count reduction test percent for all the treatment groups for four weeks, but group 1 (triclabendazole) showed 100% efficacy in a single dose after seven days of treatment. However, development of drug resistance has been suspected against albendazole, oxyclozanide and albendazole-oxyclozanide combination.

Key words: Antitrematodals · Efficacy · Fasciolosis · FECRT · Korem and Hashenge

INTRODUCTION

Fasciolosis caused by *Fasciola hepatica* (*F. hepatica*) and *Fasciola gigantica (F. gigantica)*, is one of the most prevalent helminth infections of ruminants in different parts of the world. It causes significant morbidity and mortality and occasionally affects human, thus considered as a zoonotic infection [1, 2]. In general, infection of domestic ruminants with *F. hepatica* and *F. gigantica* causes significant economic losses estimated at over US$ 200 million per annum to the agricultural sector worldwide with over 600 million animals infected [3].

The economic significance of fasciolosis in the highlands of Ethiopia has been reported by several workers [4-6] depicts how important the disease is. Losses due to ovine fasciolosis have been estimated at 48.8 million Ethiopian birr per year of which 46.5, 48.8 and 4.7% were due to mortality, productivity (weight loss and reproductive wastage) and liver condemnation, respectively [7]. Effective control of most trematode infections is based on strategically applied chemotherapy [8], intermediate host control, sanitation and environmental manipulation.

The veterinary clinical case book records of Korem and Hashenge areas of Tigray region showed that ovine fasciolosis was a concern both to the farmers and the veterinarians. No concrete data is available on the efficacy of the most commonly used anthelmintic drugs in the region; hence the study was undertaken to evaluate the efficacy of the commonly used antitrematodal drugs in naturally infected sheep.

MATERIALS AND METHODS

Study Animals and Management: 30 sheep that were naturally infected with fasciolosis, were the study animals. Sheep were purchased from Korem and Hashnege areas of Ethiopia on the basis of clinical signs and fecal examinations and then transported to Mekelle University,

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College of Veterinary Medicine, sheep farm experiment unit. Finally, only 28 animals were included in the study as two died before the experiment. Sheep were managed under semi opened house with feeding and watering facility. Sheep were fed on hay and wheat bran and watered three times a day. However, sheep were not allowed to graze on the field to avoid further infections.

Study Procedure: The sheep immediately after arrival in the college, were tagged with identification numbers. Data recording sheet was prepared to register all the required information. Before the beginning of treatment all animals were subject to fecal examination. The animals were randomly assigned to four different treatment groups (n=7 each) (triclabendazole, albendazole, oxyclozanide and albendazole+oxyclozanide combination). Dosage regimens were based on the manufacturers' recommended dose rates and standard veterinary treatment guidelines [9]. Immediately prior to treatment, fecal examination was conducted and the number of eggs per gram of faeces (EPG) was recorded. Then, animals were received treatment respective of their groups. Exanimations of fecal samples and number of egg per gram of faeces (EPG) from each animal was carried out for 4 weeks with an interval of every other week for two months.

Fecal Examination and Determination of Number of Eggs per Gram of Feces (EPG): Feacal samples were collected directly from the rectum of the animals using two fingers with gloved hand. The faeces collected was taken to Mekelle University, College of Veterinary Medicine, Veterinary Parasitology Laboratory with tightly closed plastic sample tubes. Floatation method; using a mixture of saturated zinc and sodium chloride solution and automatic centrifuge, was used. Fecal examination was done as per Dixon and Westcott [10] procedure. Determination of EPG was made by using McMaster egg counting technique described by Kelly [11].

Determination of Fecal Egg Count Reduction Percent (FECR;%): The fecal egg count reduction test (FECRT) was calculated according to the formula given by Kochapakdee [12], FECR=100 $\times\left(1-\left[\frac{T_2}{T_1}\right]\right)$, Where FECRT is fecal egg count reduction, T1 is arithmetic mean of pretreatment EPG and T2 is the arithmetic mean of post treatment EPG.

Determination of Drug Efficacy: The efficacy of drugs were calculated using the formula given by Kochapakdee [1] and the recommendations of World Association for Advancement of Veterinary Parasitology (WAAVP) guidelines [13]. Accordingly, drugs were considered effective if FECR percent was more than 95% and the lower limit of the 95% confidence was more than 90%, while resistance was suspected when there was less than 95% FECR percent and when the lower 95% confidence interval about the mean was less than 90%.

Data Analysis: The data was analyzed using JMP-5 statistical for one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Fasciolosis is an economically important disease in Korem and Hashengae area of Ethiopia. Different types of antihelmintics have been introduced to the region to control the disease but still the rate of morbidity and mortality produced by the disease hasn't significantly reduced. Anthelmintic efficacy and drug resistance surveys have not been fully conducted and resistance to anthelmintics hasn't yet been well established.

Pretreatment Fecal Examination: Before the beginning of treatment fecal samples were collected for all sheep to recheck the presence of *Fasciola* eggs. All sheep in the entire treatment groups were identified as positive before the beginning of the treatment. *Fasciola* eggs recovered during fecal examinations were differentiated from *Paramphistomum* eggs by using 1% methylene blue and the morphological characteristics of the tow parasites were very similar with those described by Soulsby [14]. The parasites were detected as *F. hepatica* based on the morphological characteristics illustrated by Dunn [15]. *F. hepatica* is also the most species predominantly isolated in Hashenge and Korem based on case book record and personal communication with veterinary personnel working in the areas.

Egg per Gram of Feces (EPG): Determination of EPG was made using McMasters egg counting technique. The highest EPG recorded during pretreatment was 1400 and the lowest was 100. In the different groups the arithmetic means of the pre treatment EPG were 475, 400, 533 and 317 for triclabendazole, albendazole, oxyclozanide and albendazole-oxyclozanide combination, respectively

Table 1: Egg count per gram faeces (EPG) pre and post treatment and faecal egg count reduction test (FECRT) in naturally infected sheep with fasciolosis

FECRT	Triclabendazole	Albendazole	Oxyclozanide	Albendazole+ Oxyclozanide
Week 1				
Numbers of sheep	7	7	7	7
Mean EPG (pre treatment)	475[a]	400[a]	533[a]	317[a]
Mean EPG (post treatment)	0[b]	100[b]	383[b]	117[b]
FECRT (% Reduction)	100	75	28.14	63.09
Week 2				
Number of sheep	7	7	7	7
Mean EPG (Pre treatment)	475[a]	400[a]	533[a]	317[a]
Mean EPG (Post treatment)	0[b]	180[b]	33[b]	33[b]
FECR (% Reduction)	100	55	93.81	89.59
Week 3				
Number of sheep	7	7	7	7
Mean EPG (Pre treatment)	475[a]	400[a]	533[a]	317[a]
Mean EPG (Post treatment)	0[b]	280[b]	0[b]	33[b]
FECRT (% Reduction)	100	30	100	89.59
Week 4				
Number of sheep	7	7	7	7
Mean EPG (Pre-treatment)	475[a]	400[a]	533[a]	317[a]
Mean EPG (Post treatment)	0[b]	280[b]	167[b]	0[b]
FECRT (% Reduction)	100	30	68.67	100

[a-b]means with different superscript in the same column for each week was statistically significant($p<0.05$)

(Table 1). Considering EPG ≤ 500 being "low", EPG from 500 to 2000 being "moderate" and EPG > 2000 being "High" [16], in the present study 19 sheep were with low EPG and the rest 9 sheep were with moderate EPG. In no animal was the EPG more than 2000. The relatively lower EPG may be due to the consequence of the mass anthelmintic treatment campaign in the area three months before the purchase of sheep.

Fecal Egg Count Reduction: All the drugs used in the experiment are known to have a good pharmacologic activity against adult f*asciola* parasites [17], but after a week post treatment, mean EPG were 0, 100, 383 and 117 for triclabendazole, albendazole, oxyclozanide and albendazole-oxyclozanide combinations. The fecal egg count reduction test indicated that 100, 75, 28.14 and 63.08% for triclabendazole, albendazole, oxyclozanide and albendazole-oxyclozanide combination, respectively. A statistically significant ($p<0.05$) difference was observed between different weeks post treatment and between pre and post treatment egg count reduction percentages (Table 1). Triclabendazole was the most efficient with 100% fecal egg count reduction followed by albendazole (75%), albendazole-oxyclozanide combination (63.09%) and oxyclozanide (28.14%) after 7 days of treatment. In case of albendazole-oxyclozanide combinations, the faecal egg count reduction percent after week of post treatment was 63.08%, this has became 0% at the end of 4th week (Table 1), but it took a very long treatment regimens to cure animals. There was a decrease in the percentage of post treatment fecal egg count reduction starting from the second week in case of albendazole and from the fourth week in case of oxyclozanide (Table 1). As described by Fetterer and Rew [18], this may be due to the development of drug resistance and reduction in the efficiency of these drugs against the juvenile stages of liver fluke.

Determination of Drug Efficacy: The efficacy of the drugs was determined by the principles of Kochapakdee [12] and Wood *et al.* (13). In the current study, among the treatment groups triclabendazole was effective as the FECR percent was more than 95% and the lower limit of the 95% confidence was more than 90% (Table 1). This was in accordance with the suggestions of several researchers indicating triclabendazole as the most efficient and a drug of choice for fasciolosis [19]. However, anthelmintic resistance was indicated for albendazole, oxyclozanide and albendazole-oxyclozanide combination as FCRT was less than 95% with lower 95% confidence interval about the mean was less than 90% for these drugs. This was in agreement with the principles described by Wood *et al.* [13]. This could also be due to a very long and extravagant use of the anthelmintics in the area as has been stated by Fawcett [20].

As conclusion, in the present study, triclabendazole was suggested to be the most effective to treat fasciolosis in these areas. It was also noticed that at forth week certain level of efficiency was observed by albendazole-oxyclozanide combinations, but it may aggravate drug resistance as it took a long time treatment regimen to be effective.

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