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INTERNATIONAL JOURNAL OF
Veterinary Science and Research

ISSN: 2640-7604 DOI: https://dx.doi.org/10.17352/ijvsr

Research Article

Evaluation of the Efficacy of Triclabendazole in Naturally Infected Sheep with *Fasciola species* at Bonga Sheep Breeding and Improvement Center, South West Ethiopia

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Received: 13 July, 2020
Accepted: 16 December, 2020
Published: 17 December, 2020

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Keywords: Breeding; Efficacy; Fasciola; Improvement; Sheep; Triclabendazole

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Abstract

Fascioliasis is the most important parasitic disease that affects humans and animals all over the world. Triclabendazole (TCBZ) is the most common drug used for the treatment of fasciolosis. This study was conducted in Bonga Sheep Breeding and Improvement Center, Kefa Zone, Ethiopia to evaluate the efficacy of Triclabendazole against *Fasciola* species (*Fasciola hepatica* and *Fasciola gigantica)* in naturally infected sheep. An experimental study design and purposive sampling procedure were employed from the source of sheep population. The study included 20 Bonga sheep breed naturally infected with *Fasciola* species and purposively selected based on their clinical signs, symptoms and faecal egg counts. Twenty (20) sheep of 2-4 years old, irrespective of sex, infested with *Fasciola* species were selected for this experiment and randomly divided into two equal groups (treatment and control group, n=10) each consisted of 10 sheep. Faecal samples were collected from each sheep before and after treatment. McMaster method was used to count faecal eggs. The efficacy of Triclabendazole was determined by Faecal Egg Count Reduction Test (FECRT). Descriptive statistics (means, standard error of mean and reduction percentages) were calculated to manage data. The percentage reduction in mean faecal egg count, after 14 and 21 days of treatment for Triclabendazole were 96.51% and 97.18%, respectively. Generally, this finding indicated that Triclabendazole was found effective against *Fasciola* species in the study area. However, appropriate use of this anthelmintic is credible to prevent future occurrence of resistance.

Introduction

Ethiopia owns about 23.6 million sheep and 23.3 million goats [1] that are mainly kept by smallholder resource poor farmers. In Ethiopia, small ruminants are important sources of income for rural communities whose livelihood is largely based on livestock production [2,3]. However, sheep production in the country is hindered by various factors including animal health constraints, inadequate nutrition, poor husbandry systems and lack of effective veterinary services [4].

Fascioliasis is among the important parasitic diseases in tropical and subtropical countries which limit productivity of ruminants. *Fasciola hepatica* and *Fasciola gigantica* are the two liver flukes commonly reported to cause fascioliasis in sheep [5]. *Fasciola* species infects mammals worldwide, mainly ruminants, but also humans can become infected. In ruminants, and especially in sheep, the infection reduces feed conversion, growth, and meat and milk production. Fascioliasis is a disease that affects the liver parenchyma and bile ducts of numerous animals, including humans, which causes economic losses and

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threatens public health. Moreover, it is one of the major causes of liver condemnations at abattoirs and interferes with fertility and fecundity [6]. Fascioliasis is a very serious disease that produces considerable loss in sheep and cattle worldwide [7]. In Ethiopia, ovine fascioliasis losses annually an estimated ETB of 48.4 million due to mortality, productivity (weight loss and reproductive wastage) and liver condemnation at slaughter [8].

Control and prevention measures of fascioliasis should be done on a preventative rather than curative. Three effective control strategies have been used which are: use of anthelmintic to reduce the number of liver fluke in the definitive hosts and the number of fluke eggs on the pasture, reduce the number of intermediate host and reduce exposure to infection by managing fluke prone areas. Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes [9].

The correct time to use anthelmintics based on weather and climate conditions. Drugs play a crucial role in the control of fascioliasis. More frequent treatments are necessary if you use drugs that are only effective against advanced mature flukes aged 12-16 weeks or older. Using TCBZ based flukicides, the most effective drug against both early mature and adult liver flukes. The best control measures may be achieved if this drug use three times yearly. Namely; August/September: to prevent pasture from contamination and to eliminate adult flukes came from autumn and winter. January/February: to completely remove of flukes picked up during late spring and early summer. April/May: to remove flukes picked up during summer and early autumn [10]. Several drugs have been proposed for the treatment of fascioliasis mainly bithionol, praziquantel and benzimidazole (BZD) family of anthelmintics such as Triclabendazole (TCBZ) and Albendazole (ABZ) [11].

In Ethiopia, Fascioliasis infections in sheep are controlled by application of Albendazole and Triclabendazole. However, drug efficacy can be negatively affected by various factors such as under dosing, resistance arising from the exclusive use of drugs of the same mode of action for long periods of time, the use of substandard quality drugs and inappropriate use of the drugs. Misuse and smuggling of anthelmintics in many forms, such as illegal trading in open markets and irrational administration, are widespread in the study area and most parts of the country. This is due to an absence of strong regulatory system on the use of anthelmintics use. In addition, methods that can preserve and prolong the efficacy of anthelmintics, and prevent the emergence of anthelmintics resistance, are very low the study area and all over the country [12,13].

Despite the use of Triclabendazole against fasciolosis for a considerable period of time, there is a scarcity of information on the current efficacy and resistance of this anthelmintic in Southwestern Ethiopia. Thus, the objective of this study was to evaluate the efficacy of Triclabendazole in naturally infected sheep under field condition in the study area.

Material and methods

Study area

The study was conducted at Bonga Sheep Breeding and Improvement Center in Kefa Zone; Southwest Ethiopia, Southern Nations Nationalities and Peoples Regional State, which is located 449 kilometers South West of Addis Ababa. The Zone has a latitude and longitude of 7°16'N36°14'E with elevation of 1500-2500 above sea level. It receives an annual rain fall of 1500-2000 mm with annual mean temperature of 6°C (minimum), 15.75°C (medium) and 25.50°C (maximum). The agro-ecological description includes 15% lowland, 75% midland and 10% highland [14].

Study animals and treatments

Sheep which have been breed in Bonga Sheep Breeding and Improvement Center were purposively selected to evaluate Triclabendazole efficacy against *Fasciola* species. Animals which have not been treated in the previous 8 to 12 weeks were considered for the study and grouped into control and treatment groups. A control (untreated) group had been used to allow for monitoring of natural changes in faecal egg counts during the test period. Generally, each animal under the study was identified by ear tag and were randomly allocated into two groups (ten in each). The first group was treated with Triclabendazole while the second group was left untreated (control) (Table 1).

The drug was available in the local market purchased from retailer pharmacy with visual inspection for trade name, manufacturer's name and logo, manufacturer's full address, medicine strength, batch/lot number, manufacturing and expiry dates, storage information, leaflets/package insert, uniformity of color, and uniformity of size. The quality of the drug were evaluated by Ethiopian veterinary drug authority for the presence of required active ingredient, and then animals were treated with 10 mg/kg of Triclabendazole dosage as per the recommendations of the manufacturers according to the weight of animal (Table 2).

Table 1: Baseline characteristics of sheep from Bonga Sheep Breeding and Improvement Center (N=20) selected for efficacy test of Triclabendazole.

	Positive Control Group (n=10)	Treatment Group (n=10)	Total (N=20)
Age (in years)			
Mean	3.1	3.1	3.1
Minimum	2	2	2
Maximum	4	4	4
Sex			
Male	4	2	6
Female	6	8	14
EPG (Arithmetic Mean)	**440.00**	**455.00**	**895**
Minimum	300	300	600
Maximum	550	600	1150
EPG(Geometric Mean)	**433.41**	**439.24**	**872.65**

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Table 2: Detail information on the drug used in the faecal egg count reduction test for field efficacy trial.

Family Name	Generic Name	Trade Name	Manufacturer	Dose mg/kg	Route of Administration	Lot. Number	Manufactured Date	Expired Date
Benzimidazole	Triclabendazole	Fasinex	Ashish Life Science PVT limited, India	10mg/kg	Bolus/ oral	Kd-600	April 2017	March 2021

The history of the farm indicated that all animals were received regular treatments with anthelmintics 3 up to 4 times per year. Additionally, the farm also experienced to treat with anthelmintics based on individual animal exhibiting clinical parasitism. The records in the farm indicated that the types and sources of anthelmintics used on the farm included mainly Albendazole (500 mg) and Ivermectin (1%), which all were available on the local markets.

Study design and methodology

Field experimental study design was conducted to investigate the efficacy of Triclabendazole against *Fasciola* species in naturally infected sheep of governmental Bonga Sheep Breeding and Improvement Center, through Faecal Egg Count Reduction Test (FECRT).

Sampling techniques and selection of study animals

Purposive sampling technique was used as sampling technique to select the experimental study animals on the basis of age, size and body weight uniformity as well as faecal egg count where by animals were selected at Bonga Sheep Breeding and Improvement Center and randomly allocated into two equal groups (treatment and control groups, n=10).

Total 20 naturally infected Bonga breed sheep of 2 up to 4 years of age with uniform size and weight (25-28 kg on average estimation) were used for the study (Table 1). The sheep which have been breed in Bonga Sheep Breeding and Improvement Center were purposively selected to evaluate Triclabendazole efficacy against *Fasciola* species. Only animals with faecal *Fasciola* species egg counts equal or higher than two eggs per gram (≥ 2 epg) will be included in the study. Animals which have not been treated in the previous 10 to 12 weeks were considered for the study.

Faecal sample collection and examination

After taking all the relevant information, faecal samples were collected directly from the rectum of sheep. Before collection of the faecal samples, possible hygienic measures including wearing of hand gloves and the faecal sample holding containers were clean to avoid contamination. About 3 gram of faecal sample was collected from each animal in the farm. Each sample was kept in a separately labeled (species, age, tag number, date) screwed capped universal bottle and the samples were sent to Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) parasitology laboratory aseptically. The transportation of the sample was done by using ice box with ice until reaching the laboratory and the samples were stored in fridge under 4°C for one day.

Faecal samples were processed for microscopy examination after sedimentation method. Measure out approximately 3gm of faeces and crush by using pestle and mortar. Then add about 40 ml tap water. Disperse the faeces in the water vigorously with pestle. Swirl and pour mixture quickly through tea strainer into the beaker after that the filtered was put in to the test tubes for 10 minutes in order to sediment the sample. Stain the sediment by adding a drop of 1% methylene blue solution to differentiate *Fasciola* egg from Paramphistomun eggs. Transfer a drop of stained sediment to a slide; cover with a cover slip and examine the sediment under microscope, starting with the 10x objective lens [15,16].

Determination of the number of egg per gram of faeces (EPG)

To count EPG, each faecal sample was analyzed individually with flotation method by using saturated zinc sulfate fluid, and automatic centrifuge was used. The egg per gram of each sample of the treatment and control groups were counted by using McMaster technique described by Kelly. Immediately prior to treatment, faecal 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 faecal samples and number of egg per gram of faeces (EPG) from each animal was carried out at 14 and 21 days.

Determination of triclabendazole efficacy

The faecal egg count reduction test (FECRT) was employed to evaluate the efficacy of Triclabendazole. The efficacy of Triclabendazole was tested according to World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for efficacy evaluation of anthelmintics. 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%. Thus, the percentage reduction of mean egg excretion on the 14 and 21 days post-treatment was calculated according to the formula given by Kochapakdee *et al.* [17] as follows:

$FECR\% = 100*(T_1-T_2)/T_1$, Where:

FECRT = Faecal Egg Count Reduction;

T_1 = Arithmetic Mean of Pretreatment EPG, and

T_2 = Arithmetic Mean of Post-treatment EPG

Ethical considerations

Ethical clearance and approval to conduct the research was granted by the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), School of Veterinary Medicine, Ethiopia.

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Statistical analysis

While collecting faecal samples from study animals, all data were recorded with pre-designed format and entered in to computer using Microsoft excel spread sheet. Data were recorded in Microsoft excel spreadsheet and analyzed by descriptive statistics (means, median, standard deviation, standard error and etc.). All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 statistical software. Arithmetic means of pre-treatment and post treatment faecal egg counts of control and treated groups were used to calculate the percentage efficacy of Triclabendazole by using faecal egg count reduction test. The FEC reduction percentage (FECR) was calculated using Kochapakdee, et al. [17] formula FECRT% = $100*(T_1-T_2)/T_1$; where FECRT is faecal egg count reduction, T_1 is arithmetic mean of pretreatment EPG, and T_2 is the arithmetic mean of post-treatment EPG at 14 and 21 days.

Results

Percentage reduction in number of eggs

Data represented in Table 3 showed that the faecal egg counts was significantly reduced ($P<0.05$) in sheep treated with Triclabendazole. The percentages of reduction were 96.51% and 97.18% at 14 and 21 days of treatment, respectively, as compared with those of positive control group. However, there were strict differences ($p=0.00$) in the net faecal egg count between infected (treatment) and control groups on the post-treatment.

The data presented here showed that a single dose of Triclabendazole was effective against *Fasciola* species infection in sheep. The pre-treatment, post-treatment faecal egg count, mean, standard error of mean and the percent reduction in the faecal egg counts are presented in Tables 4,5.

Discussion

Currently, fascioliasis is mainly treated using Triclabendazole because of its ability to act in both immature and mature stages. This drug has been used since the 1980s and it has shown good activity against liver flukes for many years [18]. However, in recent years *Fasciola* species has been reported to present resistance to Triclabendazole in various countries [19].

In this study, the efficacy of Triclabendazole bolus against *Fasciola* species infection in naturally infected sheep was investigated in Bonga sheep breeding and Improvement Center, southwest Ethiopia. A total of 20 sheep grouped into two equal categories (treated and controlled) were selected to evaluate the efficacy of Triclabendazole against *Fasciola* species infection.

In the current investigation, the percentage reduction in faecal egg counts and their confidence limits in the efficacy evaluation of Triclabendazole were evaluated. A decrease in faecal eggs count was found to be more in cases treated with Triclabendazole than that of untreated group. The result showed that the mean faecal egg count reduction value of Triclabendazole at 14 and 21 days were 96.51% and 97.18%, respectively. Consequently, faecal egg count reduction test indicated that Triclabendazole resistance was not found. As per World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines [20], resistance is considered if the percentage reduction in faecal egg counts is less than 95% and /or the lower 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected. The high efficacy of Triclabendazole against immature flukes might ensure successful treatment of acute fascioliasis. Excellent results have been achieved with the same drug in treatment of acute fascioliasis in sheep, cattle, goats and other experimental animals with 90-100% efficiency [21].

Fourteen (14) and twenty one (21) days after treatment, we observed a statistical difference between control and treatment groups; the faecal samples from all animals in the control group were positive for *Fasciola* eggs (mean = 455), whereas the samples from treated animals had egg counts reduced significantly with $P = 0.00$ which is $P<0.05$. The reason for good efficacy of Triclabendazole bolus in the present study was probably due to recent introduction and low frequency of treatment. Therefore, majority of trematode parasite populations in sheep remained unexposed to anthelmintic selection, thus remaining susceptible. This is in agreement with previous study conducted at Mekelle University by Taddese, et al. [22] who reported that Triclabendazole has 100% efficacy against *Fasciola* infection. However, Triclabendazole resistance was reported in European countries such as Netherland, Britain, Russia, and Scotland due to extensive and prolonged use of Triclabendazole [23].

Table 3: Faecal egg counts for control (infected group) and treated group with Triclabendazole expressed in means ± standard error.

Egg Counts	Positive Control (Infective Group)	Treatment Groups	
		At 14 days of post treatment	At 21 days of post treatment
Mean	440±24.495	17±3.661	10.8±2.511
Percentage Reduction	-	**96.51%**	**97.18%**

Table 4: Faecal egg counts in pre and post treatment of sheep at Bonga sheep breeding and Improvement Center.

Measurements	Pre-treatment	Post-treatment	
		At 14 days	At 21 days
Mean EPG Pre-treatment	455.0	-	-
Mean EPG Post-treatment	-	17.00	10.80
Percent Reduction in EPG	-	96.51%	97.18%
95% Upper Confidence Limit	521.28	25.28	16.48
95% Lower Confidence Limit	388.72	8.72	5.12
Interpretation	-	**Effective**	**Effective**

Table 5: Descriptive statics of the faecal egg counts pre and post treatment with Triclabendazole (treatment group, n=10).

	Pre-treatment	14 days Post Treatment	21 days Post Treatment
Median	475.00	13.50	10.00
Mode	500	8	5
Range	300	35	25
Sum	4550	170	108
Standard deviation	**92.646**	**11.576**	**7.941**

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As pointed out earlier, previous study on antitrematodal drugs conducted at Mekelle University reported the faecal reduction percentage of Triclabendazole was showed that 100% efficacy. However, in the present study the faecal reduction percentage was 96.51% and 97.18% at 14 and 21 days post treatment, respectively. This difference might be due to the difference in the quality of the drugs and seasonal variations of the studies, since the parasite burden affected by season. When we came to the difference between the percentage of faecal egg count reduction at 14 and 21 days, it was due to lack of eggs in the gall bladder for some weeks which it needs sufficient time to pass out all eggs form the gall bladder and be excreted [22].

In general, this study demonstrated that Triclabendazole at 10 mg/Kg, PO (orally) acts efficaciously against *Fasciola* species in naturally infected sheep. In other words, the percentage reduction in faecal egg counts and their confidence limits in the efficacy evaluation of Triclabendazole revealed that the drug was effective from clinical cure point of view. Although the study was not designed to evaluate the safety or pharmacokinetics of this dosage of Triclabendazole in sheep, the animals did not show any signs of toxicity. Hence, from this study we concluded that Triclabendazole had good efficacy against *Fasciola* species infection in naturally infected sheep at Bonga sheep breeding and Improvement Center.

Conclusion

In general, the current study result showed that the faecal egg count reduction percentages Triclabendazole against *Fasciola* species infection in naturally infected sheep in the study area were 96.51% and 97.18% at 14 and 21 days of post-treatment, respectively. In conclusion, this finding indicated that Triclabendazole was found to be effective against *Fasciola* species infection (conversely, *Fasciola* species infection in sheep was susceptible to Triclabendazole) in Bonga Sheep Breeding and Improvement Center. Thus, to maintain and prolong the lifespan and efficacy of Triclabendazole, the following recommendations are forwarded: Strict supervision on the use and application of Triclabendazole should be implemented. Professionals and livestock owners should be well aware about Triclabendazole and its effectiveness. Regular monitoring for anthelmintic resistance in livestock production communities is essential to keep a track of their efficacy. More research works should be implemented on Triclabendazole efficacy and its resistance in Ethiopia largely.

Acknowledgements

Authors would like to express their deepest gratitude to God for his permission to do all daily activities as well. Authors also have no enough words to express their great full & pretty thanks, sincere appreciation and great respect for Bonga Sheep Breeding and Improvement Center members for their valuable advice, encouragement and support during sample collection. Most importantly, authors would like to express their deepest appreciation for families, for their endless help, love and moral devotion in every situation. Finally, special thanks go to all friends, for their valuable comments and encouraging ideas that greatly aid for the completion of this research.

Authors' contributions

Warkaw Merachew: Collected the relevant data, performed the laboratory investigation and drafted the manuscript.

Motuma Debela: Coined the title, analyzed the data and approved the drafted manuscript.

Tewodros Alemneh: Revised the manuscript, searched & selected journals, and got published the manuscript online to be available worldwide for the entire clock.

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