

Comparative efficacy of major anthelmintic against naturally infected *Fasciola* species infection in local sheep at Debre-Birhan Agricultural Research Center, Ethiopia

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Abstract

This study aimed to evaluate the efficacy of eight major anthelmintic against *Fasciola* species in naturally infected sheep. In a randomized trial, 90 sheep were divided into 8 treatment groups of 10 animals each and one untreated control group of ten sheep. Prior to treatment, the animals were examined for *Fasciola* eggs. After the examination, they were found positive. On day zero, each group was administered to one of the following eight anthelmintic; Albex 10 %, Expitol, Tribex 10 %, Fascinex (Triclabendazole), Zerofen 10%, Albendazole, Ridafluke, and Tetraclozan. Faecal samples from each animal were examined for *Fasciola* eggs on day zero and then on 7, 10, 14, 21 days and 10 weeks post treatment using the sedimentation technique. Necropsy examination was performed on selected animals for 21 days and 10 weeks of post treatment on selected animals. Ridafluke showed highest efficacy against fascioliasis with 99.6 mature and 94.3 of immature worm count reduction proportion for 21 days post treatment and 91.8 % of adult worm reduction 10 weeks after treatment followed by Tribex 10% solution that showed 96 % and 98.7 % mature and immature worm count reduction, respectively, and 83.6% after 10 weeks post treatments. On the contrary, Fascinex (Triclabendazole) treatment group yielded much lower efficacy with mean 98.3% of mature *Fasciola*

worms being recovered after 10 weeks post treatments. On the other hand, the adult *Fasciola* worm recovery showed high resistance in all broad spectrums anthelmintic treatment groups where Expitol and Tetraclozan showed 64 % and 63.3 % reduction of adult worms. The findings of this study indicated that the treatment group of Ridafluke and Tribex 10% showed highest efficacy and is a primary drug of choice against Fasciolosis in sheep of the study area.

Keywords: Drug Efficacy, *Fasciola* species, Natural infection, Sheep.

Introduction

Sheep are among the predominant livestock species which play an important role in the socio-economic development of the majority of Ethiopian highlands (Gebru et al., 2014; Mesfin et al., 2014). The sheep population in Amhara National Regional State is estimated at 5,722,317 which constitute 23% of the total population of the country (CACC, 2003). Of the total sheep population in the country, 75% of them are raised on the highland regions of the country (Tadesse, 1988). However, the potential of sheep production has not been efficiently exploited by the country due to various sheep diseases especially those caused by helminth parasites (Bekele et al., 1992). Among these diseases, fasciolosis is a serious disease of domestic livestock specially cattle and sheep (Ogunrinade and Adegoke, 1982). Fasciolosis results in significant economic losses through reduced live weight, poor food conversion efficiency, condemnation of liver and death (Gebru et al., 2014). Body weight losses caused by endoparasites in Ethiopia ranged from 3 - 8% and the estimated losses are 48.4 million Ethiopian Birr per year (Gebru et al., 2014; Ngategize et al., 1993). Of the two *Fasciola* species, *Fasciola hepatica* is predominant in highland areas of the country (Zelege et al., 2013; Mulat et al., 2012).

The possible control measures for *Fasciola* infection involves strategic anthelmintic treatment based on epidemiological data in combination with other means of control such as exploitation of resistant breeds, pasture management, improved nutrition and destruction of the snail (Kumar et al., 2009; Tadesse and Getachew, 2002). In Ethiopia,

for decades, the most practical and commonly employed control method is the use of anthelmintics (Baker et al., 1992). The common anthelmintics used for treatment of *Fasciola* infection in the country include Albendazole, Tetraclozan, and Triclabendazole. The sheep farm of Debre Birhan Agricultural Research Center has been seriously affected by the disease with typical clinical signs suggestive of acute fasciolosis. Major clinical signs of ascites, emaciation, bottle jaw and depression have been reported for over 800 sheep mortalities in 2014 (Monthly and quarter report of Debrebirhan Agricultural Research Center and personal communication with the established task force to assess this kind of mortality in very short period).

The report has been confirmed by the recovery of flukes from the liver of these animals on postmortem examination. Despite frequent treatment with the commonly available fluckicidal drugs in the country, veterinary professionals on the farm reported the lack of effective therapeutic responses repeatedly (Personal communication with Debrebirhan Agricultural Research Center animal health researchers). Hence, there is a serious concern on the efficacy of the drugs being used on the farm. Although, there are insufficient published reports on the development of *Fasciola* resistance to major anthelmintic in Ethiopia, recent reports from different parts of the world has shown emergence of resistance strains of *Fasciola* to a few commercially available fluckicidal drugs like Triclabendazole and Albendazole (Adam and Johan, 2015; Brockwell et al., 2014; Sargison and Scott, 2011; Moll et al., 2000). Ascertaining the major causes of treatment's failure in the farm is crucial for control of fasciolosis. The control is made in the farm to take necessary measures so as to contain resistant strains in the farm if it is due to resistance. Therefore, this study is designed to investigate the efficacy of major fluckicidal drugs against *Fasciola hepatica* in general and to determine and compare the efficacy of selected drugs on liver fluke in naturally infected local breed sheep at Debre Birhan Agricultural Research Center in particular.

Materials and methods

Study area description

The study was conducted at Debre Birhan Agricultural Research Center. It is located 130 km Northeast of Addis Ababa. The center is situated at an altitude of 2876 masl (meters above sea level), with average annual rainfall for 17 years of 911.2 mm and mean maximum and minimum temperature of 19.67 and -2 degree Celsius, respectively (NMSA, n.d).

Study animal and management

The animals used for this study comprised of 90 local Menz breed male sheep. They were about 6 months of age naturally infected with *Fasciola* spp. The sheep were purchased from small holder farmers at Debre-Birhan and surrounding areas. The sheep was exposed to graze on the infected area of the research station for 2 months. Parasitological examination was done to all animals before grouping them to the treatment category and indoor each group. The sheep were fed with dry hay harvested a year earlier and wheat bran supplement. Water was provided *ad libitum*.

Experimental design

In this randomized trial, 90 sheep were divided into 8 treatment groups of ten animals each and one infected and untreated control group. On day zero, each treatment group was administered with one of the eight anthelmintic based on the company's recommended dose. Albex 10%, Expitol, Tribex 10 %, Fascinex (Triclabendazole), Zerofen 10%, Albendazole, Ridafluke and Tetraclozan were the drugs used for treatment (Table 1).

Coprological examination

Faecal samples were taken directly from the rectum of the studied animals on days 0, 7, 10, 14, 21 and 10 weeks post treatment and were examined (Laiq et al., 2011) using sedimentation technique described by Hansen and Perry (Hansen and Perry, 1994).

PCV values and FAMACHA score

Packed Cell Volume (PCV) values and FAMACHA score were also determined based on the guideline of Bath et al. (1996).

Table 1. List of drugs used with the recommended doze by the producers

Generic Name	Brand Name	Formulation	Country (source of the drugs utilized)
Albendazole	Albex 10%	oral suspension	Ireland
Albendazole (300 mg)	Expitol	bolus	Greece
Triclabendazole	Tribex 10%	oral suspension	Ireland
Triclabendazole (250 mg)	Fasinex	bolus	East Africa
Fenbendazole	Zerofen 10%	oral suspension	Ireland
Albendazole (300mg)	Albendazole QK	bolus	China
Rafoxanide	Radifluk 3%	oral suspension	Ireland
Tetraclozan (900mg, oxyclozanide 450mg, levamisole HCL 450mg)	QK Tetraclozan QK	bolus	China

Necropsy examination

Necropsy examination was conducted on all animals scarified on day 21 and 10 weeks post treatment as well as animals that died during the study period. On day 21 post treatment, 5 animals from each group were randomly selected and sacrificed. The remaining sheep were sacrificed 10 weeks post treatment. Examination for flukes in the liver and gall bladder was conducted as per the procedure described by Hansen and Perry (1994).

During postmortem examination, the livers were categorized based on the degree of gross pathological lesions: Lightly affected: if quarter of the organ is affected; Moderately affected: if half of the organ is affected and two or more bile ducts are hyperplastic; Severely affected: if almost the entire organ is affected and the right lobe is often atrophied as described previously by Ogunrinade and Adegoke (1982). The presence of more than 50 flukes per liver was considered as high pathogenicity (Soulsby, 1982).

Statistical analysis

Efficacy of selected anthelmintic treatments in sheep was calculated using the geometric mean of worm count in groups of treated and untreated animals, respectively (Coles, 2005). $E = [1 - (T/C)] * 100$, where E = percentage efficacy; C = mean number of worms in the control group; T = mean number of worms in the treated group. The mean parasite count between treatment and control groups and among different treatment groups were compared using ANOVA.

Results

Coprological examination

From a total of 90 faecal samples examined on day zero, all found positive for *Fasciola* spp eggs. On day 21 post treatment, 90-100% *Fasciola* eggs were recovered from all broad spectrums (including adult *Fasciola* spp) anthelmintics treated and control groups. Of the groups treated with fasciolicides Fascinex (Triclabendazole) and Tribex 10% groups yielded 90 %, and 44 % positive for *Fasciola* eggs, respectively. No eggs were identified from the Ridafluke treatment group after 21-day post treatment. After 10 weeks post treatment, all broad-spectrum treatment groups showed 90-100 % positive for adult *Fasciola* whereas fasciolicides groups showed only 22 %.

Worm Count Reduction of fasciolicides treatment groups

The test was conducted 21 days and 10 weeks post treatments using the formula ($WCR\% = [(MC - MT) / MC] * 100$). Table (2) shows the result of worm count reduction where Ridafluke and Tribex 10 % yielded high reduction than the Fascinex group.

The results of the treatment group Ridafluke showed highest efficacy against fasciolosis followed by Tribex. However, Fascinex (Triclabendazole) yielded much lower efficacy level with mean 98.3 and 22.2 mature and immature *Fasciola* worms recovered, respectively (Figure 1). From the Fascinex (Triclabendazole), treatment group one sheep died 8 weeks post treatment. The sheep showed severe clinical signs of fasciolosis with ascites and adult *Fasciola* worms were recovered from the liver and gall bladder.

Table 2 Worm Count Reduction Percent (WCR %) in fasciolicides treatment groups

Treatment	21 days post treatment		10 weeks post treatment	
	Mature	Immature	Mature	Immature
Tribex (Group III)	96%	98.7%	83.6%	98.3%
Fascinex (Group IV)	65%	71.4%	49.5%	22.2%
Ridafluke (Group VII)	99.6%	94.3%	91.8%	98.3%

A statistically significant difference was observed between the recovered adult worms from the three groups treated with fasciolicides (Tribex 10%, Fascinex and Ridafluke) on day 21 ($P = 0.0205$) and 10th weeks post treatment ($P = 0.004$). Similarly, statistically significant difference was observed between Tribex and Fascinex and Ridafluke vs. Fascinex ($P \leq 0.05$) whereas it was no statistically significant difference between Tribex 10% and Ridafluke ($P \geq 0.05$) (Table 2).

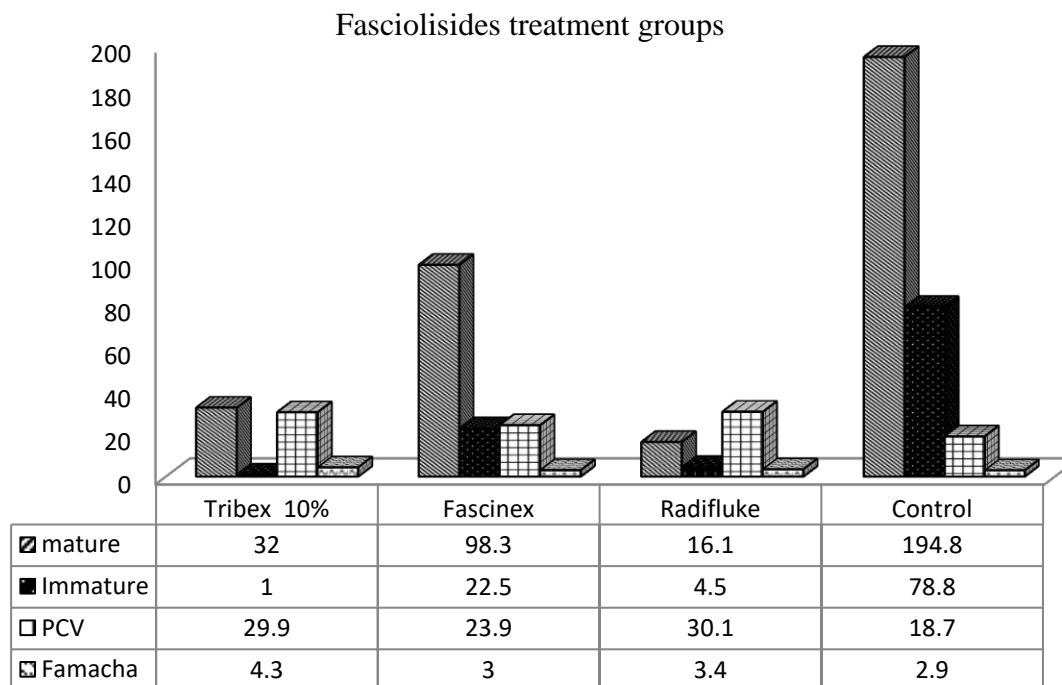


Figure 1. Mean of PCV, FAMACHA score and *Fasciola* worms recovered from the treatment and control groups.

FAMACHA score and PCV values showed lower score in the control group than the treatment groups (FAMACHA score in the controls at day 14 and day 21 should not decrease, expected to be pallid/whitened but the indoor feeding with supplement prevent new infection). Higher score from the fasciolicides groups were obtained, i.e. statistically significant difference was observed on 14 and 21 days post treatment between the three fasciolicides ($P \leq 0.05$) (Table 3). The mean of the adult worm recovered indicated more than 50 flukes per liver which can be considered as high pathogenicity.

Table 3. Famacha score, PCV and fasciola worm recovered from fasciolisides drugs group. Results expressed as mean \pm standard deviation.

			Tribex 10%	Fasinex	Ridafluke	Control
Mature treatment	21-day	post	5.5 \pm 6.4***	57.5 \pm 36.7**	0.5 \pm 1.0***	123.2 \pm 62.4
Mature fluke treatment	10 th weeks	post	52.8 \pm 34.3***	155.8 \pm 65.3	31.8 \pm 10.5***	266.4 \pm 108.6
Immature treatment	21-day	post	1.0 \pm 2.0***	22.5 \pm 14.9	4.5 \pm 8.34***	75.8 \pm 18.5
FAMACHA score on day	0		3.5 \pm 0.7	3.1 \pm 0.6	3.3 \pm 0.5	3.1 \pm 0.7
	14		4.0 \pm 0.9**	3.2 \pm 0.4**	3.6 \pm 0.5**	2.9 \pm 0.5
	21		4.2 \pm 0.8***	3.0 \pm 0.7**	3.8 \pm 0.5***	2.5 \pm 1.0
PCV on day	0		28.8 \pm 6.9	26.9 \pm 6.6	28.7 \pm 4.5	27 \pm 4.5
	14		31.1 \pm 7.3***	26.4 \pm 5.4	28.7 \pm 2.8	24.5 \pm 4.8
	21		31.8 \pm 8.3***	24.9 \pm 6.1	28.9 \pm 3.3**	20.7 \pm 8.8

*** and **indicated the statistically significant difference between the 3 fasciolisides and control group ($P \leq 0.05$) similarly for the FAMACHA score and PCV between the 3 fasciolisides and control ($P \leq 0.05$).

NB: Data after 7 days were taken only for Famacha and PCV to evaluate if any change happen 7 days post treatment but was not significant difference with day 0.

Liver classification for gross lesions

Livers were categorized based on the degree of gross pathological lesions. Accordingly, from Tribex 10 % treatment group, 20 and 35.6 % liver found recovered (unaffected) and lightly affected, respectively and 22.2 % were moderately and severely affected. Where as from Ridafluke treatment group, about 50 % of liver found unaffected (recovered), the remaining 37.5 % and 12.5 % were lightly and moderately affected, respectively. According to histopathology test and necropsy, signs of liver regeneration were observed

in both Ridafluke and Tribex 10 % treatment groups, from Fascinex (triclabendazole) treatment group 30 % and 70 % found moderately and severely affected, respectively (Table 4). From albex 10 % group, about 88.9 % of livers were severely affected. In the other broad-spectrum anthelmintic treatment groups, more than 90 % of livers were found severely affected.

Table 4. Liver gross lesion classification

Treatment group	unaffected	Lightly affected	Moderately affected	Severely affected
Tribex 10%	20 %	35.6 %	22.2 %	22.2 %
Ridafluke	50 %	37.5 %	12.5 %	-
Fascinex (Triclabendazole)	-	-	30 %	70 %

Effect of Broad-spectrum anthelmintics on adult *Fasciola* worms

The results of Worm Count Reduction Percent (WCR %) of the broad-spectrum anthelmintic treatment groups showed resistance of the *Fasciola* to all treatments, Expitol and Tetraclozan demonstrated 64 % and 63.3 % reduction respectively, whereas the rest anthelmintics (Albendazole, Zerofen and Albex 10 %) yielded ≤ 56 % reduction of the adult *Fasciola* worm. One sheep from Albex group, died 9 weeks after treatment and 2 sheep from Control group at 19 days and 8 weeks from the time when commencement of the experiment. All sheep showed severe signs of fasciolosis, adult *Fasciola* worms were recovered from the livers, although several adult flukes were also found in the gall bladders (Figure 2).

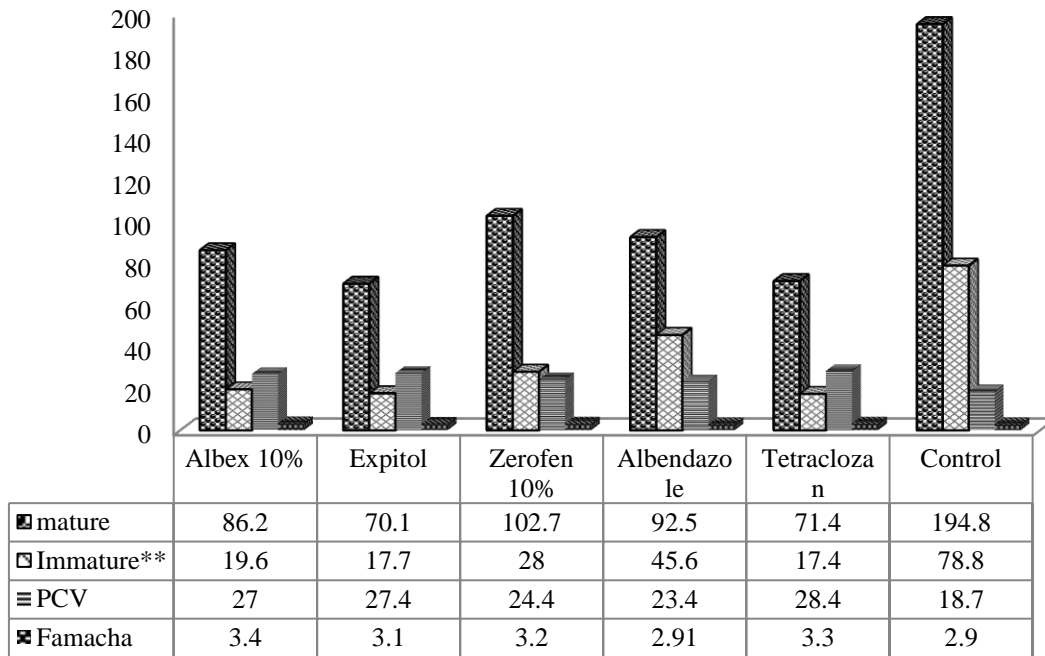


Figure 2. Mean of PCV values, FAMACHA score and *Fasciola* worms recovered 10 weeks post treatment

** 21 days post treatment

Discussion

In Ethiopia, Fasciolosis is one of the major constraint factors for ovine production development by inflicting direct and indirect loss at different parts of the country. The high incidence of fasciola species in the area is in agreement with previous report by Zeleke et al. (2013). Yemisrach and Mekonnen (2012) also reported low incidence of fasciolosis than our study. However, high prevalence was reported from Sheno (Mahidere et al., 2017) in the marshlands of Debrebirhan Agricultural Research Center (DBARC). High fasciola infection was reported during the long rains period. However, the suitability of the area is favorable for the replication of intermediate host (snail) in different seasons (Monthly and yearly report of DBARC). Maintenance of sheep's population on this marshy grazing land for years with frequent use of anthelmintic drugs led to the drug resistance and high occurrence of acute and chronic fasciolosis.

Previous studies from Australia (Overend and Bowen, 1995), Ireland (Lane, 1998; O'Brien, 1998) and Scotland (Mitchell et al., 1998) have indicated the resistance of liver fluke to Triclabendazole (TCBZ) in sheep. A study from the Netherlands reported the resistance of *Fasciola hepatica* to TCBZ (Moll et al., 2000). Conversely, the current study demonstrated the significant efficacy of Ridafluke and Tribex 10% against *Fasciola* species. The lower efficacy of the broad spectrum anthelmintic against adult *Fasciola* is comparable with the findings of Moll et al. (2000); Coles (2005); Sergison and Scott (2011); Shokier et al. (2013); Brockwell et al., (2014). Our result recovered more than 50 flukes (adult fasciola worm) per liver from triclabendazole treatment group indicating high pathogenicity as reported by Soulsby (1982). Rahmeto et al. (2010) used 55 fluke by liver as the mean fluke burden in the affected livers and found 11.6% severely affected liver which is lower than our findings 70 % were severely affected from Fascinex (triclabendazole) treatment group and 30 % were moderately affected.

The prevalence of fascioliasis among sheep recorded in this research work can be attributed to the climatic conditions of this location. The location is favorable for the survival of the intermediate hosts, the snail which prefers swampy areas with slowly moving water. This study showed high incidence of triclabendazole resistance which may be a major threat for the livestock production and producers of the study area that can lead researchers for the need of alternative drugs.

Conclusion

The study concluded that Ridafluke and Tribex 10% should be used as primary chosen drugs against Fasciolosis of sheep in the study area. Considering the magnitude of fluke burdens, losses due to high mortality, carcass weight reduction and liver condemnation, fasciolosis appears to be a major parasitic disease in the study area. In controlling fasciola species, anthelmintic treatments were performed over the years. However, it was not effective. The findings of this study demonstrated the necessity for a comprehensive control program against this parasitic disease. The control program was made based on

detailed epidemiological studies. These findings suggest that the resistance to triclabendazole may be an emerging problem of the study area

Recommendation

Short term recommendation

Strategic application of chemotherapy with a combination of a selected nematocidal and flukicidal drugs should be implemented. To this end, there is a need of vector control using molluscicides on possible transmission sites, pasture and environmental management (burning transmission sites, clearing vegetation and paddock) etc.

Debre Birhan Agricultural Research Center (DBARC) Station Long term recommendation

Detailed epidemiological studies should be conducted for sound fasciolosis control strategies at DBARC, Parasite strain characterization and recommendations to prevent drug resistance, Snail reduction. This can be made by drainage and building dams at a marshy and low laying area; Keeping the animals off from marshy areas or fencing of these dangerous sites and eliminate pond waters from the center

Ethical considerations

This research proposal was approved by the Amhara Regional Agricultural Research Centre Annual Review and the Institutional Review Board of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University. International guiding principles on the use of animal for experimental research was followed during the course of the study.

Acknowledgement

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