

In Vivo Efficacy Evaluation of Three Brands of Ivermectin against Nematodes in Sheep

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Abstract

Background: Nematode parasites are the main problem of sheep production, causing high mortalities, morbidity and production losses. Anthelmintics (AH) mainly albendazole and ivermectin are extensively used to treat parasite infections and their judicious use might extend their AH efficacy. However, extensive use and low-quality AH can cause a reduction of efficacy and facilitates the emergence of AH resistance. This study aimed to test the efficacy of three brands of ivermectin (IVM1), (IVM2) and (IVM3) against gastrointestinal (GI) nematodes in naturally infected sheep.

Methods: The study included 40 sheep, which were divided into 4 groups (each constituted of 10 animals). Groups 1 - 3 were treated with IVM1, IVM2 and IVM3, respectively while group 4 was a control. Faecal samples were collected before treatment on day 0, and 10 days after the treatment. The efficacy of the 3 brands of ivermectin was determined based on the faecal egg count reduction (FECR) test.

Results: The FECR test study showed there was a significant difference ($p < 0.05$) in mean FECR among the treatment groups. Egg count reduction levels of the three tested brands of ivermectin were 93.3%, 98.9%, and 99.8% for IVM1, IVM2, and IVM3, respectively. The larvae study showed that *Trichostrongylus*, *Haemonchus*, *Oesophagostomum* and *Strongyloides* were among the major genera of the parasites identified. Post-treatment faecal culture showed no larva in groups receiving IVM2 and IVM3 while considerable amount larvae were found in groups treated with IVM1.

Conclusion: The study revealed that comparable anthelmintic efficacy was observed in sheep treated with two brands of ivermectin (IVM2 and IVM3) whereas resistance is suspected in sheep treated with a brand of IVM1. Although the anthelmintic efficacy of ivermectin was preserved in the study farm, this study gives a clue about the circulation of substandard-quality brands of ivermectin in the Ethiopian market or decreased efficacy of some of the ivermectin brands as the observed efficacy differences between the three brands could be due to variations in quality. Hence, detail studies are required to clarify the quality and status of the efficacy of AH widely used in different livestock species in Ethiopia.

Keywords: Brands of Ivermectin; Efficacy; FECRT; GI Nematodes; Sheep

Abbreviations

AH: Anthelmintic(s); EPG: Eggs Per Gram of Faeces; FECRT: Faecal Egg Count Reduction Test; FIF: Fairfield Integrated Farm; GI: Gastrointestinal; IVM: Ivermectin; IVM1: Ivermectin 1-China; IVM2: Ivermectin 2-China; IVM3: Ivermectin-Uruguay; L₃: Third Stage Infective Larvae; MAFF: Ministry of Agriculture, Fisheries and Food; WAAVP: World Association for the Advancement of Veterinary Parasitology

Introduction

Small ruminants are widely distributed and have great importance as major sources of livelihood for smallholder farmers and landless rural communities in tropical Africa [1]. In Ethiopia, 85% of the country's population engaged in agricultural activities where animal production forms an integral part of the agricultural system [2]. Ethiopia owns 31.3 million sheep and 32.74 million goats [3]. Despite that, their productivity is low due to a multitude of factors.

Gastrointestinal (GI) parasite infections result in enormous economic losses, especially in areas where ruminants are kept on pasture throughout the year [4]. In tropical countries, 95% of sheep and goats are infected with helminths where such parasites cause stunted growth [5], pre-weaning mortality, and reduction in annual offtake of small ruminants [6]. Nematodes like *Strongyle* species are the major helminth parasites of small ruminants [5]. There are many associated risk factors influencing the prevalence and severity of GI helminths, which include age, sex, weather condition, and husbandry or management practices [7].

Several anthelmintics (AH) with different modes of action are available on the market for the control of helminthosis in both livestock and human, of which albendazole, ivermectin, and related drugs are most effective [8]. Furthermore, owing to a lack of sound management strategies against helminths of livestock in Ethiopia, control of adverse effects of nematodes in the grazing system relies almost exclusively on the use of AH drugs [9]. Ivermectin, a semisynthetic derivative of avermectin that contains large macrocyclic lactone fermented product of the microorganism *Streptomyces avermitilis* [10], is effective against GI nematodes as well as ectoparasites [11]. The remarkable effectiveness of this drug over a long period has decreased the motivation for AH drug discovery programmes [12]. However, intensive and indiscriminate use of this drug to suppress parasitic infection in livestock has resulted in the rapid selection of resistant strains [9].

In Ethiopia, there is no policy about the usage of AH in livestock and as a result misuse and smuggling of AH such as illegal trade on open markets and irrational administration are widespread [13]. There are also reports on the AH resistance of GI nematodes in livestock in the country; albendazole, tetramisole and ivermectin resistance in goats in the Eastern part [14], low efficacy of tetramisole in goats in Southern Oromia [15]. Furthermore, due to the lack of modern veterinary services, particularly in rural areas, livestock owners often use easily accessible prescription drugs without prescription papers, to treat various livestock diseases. Besides, most of the livestock owners choose and purchase veterinary drugs based on their color [16,17], which could indicate the branded drugs having public trust for their better efficacy. Similarly, clinicians and other animal health experts prefer to use some brands of ivermectin among various brands available in the local market, claiming that variations in clinical efficacy. Despite the extensive use of ivermectin, there is a paucity of information about the efficacy of ivermectin in sheep, and it is believed that rotational alternate AH therapy could reduce the development of resistance by the helminth parasites. In this study, we compared the efficacy of three frequently used brands of ivermectin against GI nematodes at the sheep farm, namely, Fairfield Integrated Farm (FIF) located in Bishoftu, central Ethiopia that practicing rotational alternate AH therapy for the last 7 years.

Materials and Methods

Study area

The study was conducted from November 2014 to May 2015 at Fairfield Integrated Farm (FIF) located in Bishoftu town, 47 km East of Addis Ababa. Bishoftu is located at 9°N latitude and 40°E longitudes at an altitude of 1,850 m above sea level and situated in central highlands of Ethiopia. It has an annual rainfall of 866 mm of which 84% falls in the long rainy season (June to September). The mean annual maximum and minimum temperature is 26°C and 14°C respectively with 61.3% annual mean relative humidity [3].

Study animals

A total of 40 local breeds of sheep of both sexes from FIF were used for this study. The sheep (fat-tailed indigenous breed) purchased from Arsi were kept under an extensive production system, where they were freely grazing on pastures and water was provided ad libitum, but there is no additional concentrate feeds supplement. Suckling lambs, pregnant sheep, old animals (> 4 years) and sheep treated with AH during the three months preceding this study were excluded. Each animal was individually identified with a numbered ear tag. All sheep were returned to the same pastures so that they were subject to the same parasite challenge.

The information obtained from the farm record revealed that AH mainly albendazole, tetramisole and ivermectin were alternately used three times a year to control GI nematode infection since the establishment of the farm. Decisions on what drug to use were largely determined by availability, and alternation of anthelmintic type was deliberately practiced on the farm. During the current study period, a total of 60 sheep and more than 40 dairy cows (Holstein Friesian breed) were grazing on a permanent natural pasture of the farm.

Experimental design

The sheep were divided randomly into four experimental groups (each constituted of 10 animals). Consideration was made for animal's age (categorized as adult: > 12 months; young: 6 - 12 months) to include in each group. Groups 1, 2 and 3 were treated with three different brands of ivermectin IVM1, IVM2, and IVM3, respectively while group 4 was a control.

Faecal collection and coproscopy

Faecal samples were collected from each animal for a coproscopic examination on day 0 (before treatment) and day 10 post-treatment. Fresh faecal samples were taken directly from the rectum of animals and placed into universal bottles, labelled with the individual ear tag numbers. The samples were examined for parasite eggs using a saturated salt solution as a flotation fluid within 4 hours of collection at the veterinary parasitology laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University. Mean eggs per gram (EPG) for each animal was determined using modified McMaster techniques as described in Coles, *et al.* (1992 and 2006) [18,19]. Then 40 sheep with average EPG greater than 200 [20] were randomly assigned to four groups (one control group and three treatment groups with 10 animals in each). Larvae recovered from the samples were also identified. Three different brands of ivermectin (IVM1, IVM2, and IVM3) available in veterinary clinics found in Bishoftu town, were subcutaneously administered to animals in the treatment groups based on the dose recommended by the manufacturers (1% Ivermectin solution with a dose of 0.02ml/kg of animal body weight). The manufacturers' country of the brands of ivermectin was IVM1 and IVM2 from China whereas IVM3 was from Uruguay). Ten days post-treatment, faecal samples were collected again, the EPG was determined, and the type of the nematode egg was identified [21].

Larval identification

Pooled faecal samples both from treated and untreated (control) groups of sheep, pre- and post-treatment were also cultured to identify the prevailing nematodes on FIF as described by MAFF (1979) and the larvae were identified for respective groups as discussed in Van Wyk, *et al.* (2004) [22].

Efficacy evaluation

The efficacy for each brand of ivermectin was determined according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for efficacy evaluation of anthelmintic [18,19]. The faecal nematode egg count reduction percentage (FECRT%) was calculated using the following formula:

$$FECRT\% = \left(1 - \frac{T_{10}}{C_{10}}\right) \times 100$$

Where, T₁₀ and C₁₀ are the arithmetic mean EPG in the treated (T) and untreated control (C) groups on post-treatment day 10, respectively. According to the method described in Coles., *et al.* (1992) [19], resistance is present if only one of the following criteria is met: (i) The percentage reduction in egg counts is < 95% or (ii) The 95% confidence level is < 90%.

Ethical approval and consent to participate

The study was granted ethical approval from the College of Veterinary Medicine and Agriculture Institutional Research and Review Committee. The animals used for the research were also kept under the natural grazing (extensive farming) system and their welfare was maintained in compliance with the international guidelines described by Animal Welfare Act 2006 [23]. The animal care was also made as recommended by Olfert., *et al.* (1993) [24].

Statistical analyses

The differences in mean EPG between different sex, age and treatment groups were tested by the nonparametric Kruskal Wallis test. Association between mean EPG and body weight was checked by the Spearman correlation test. The level of significance was set at a P-value of < 0.05. All statistical analyses were performed using SPSS 20 (Statistical Package for the Social Sciences version 20).

Results

Faecal examinations

The means of EPG for animals in the control group before and after treatment were 5,652 and 6,304 but mean EPG greatly reduced in the treatment groups (Table 1). The mean EPG for group 1 reduced from 5,120 to 420, for group 2 from 5,692 to 70, and for group 3 from 5,710 to 10.

Study group	Pre-treatment		Post-treatment		FECRT (%)
	Mean ± SE EPG	95% CI for mean	Mean ± SE EPG	95% CI for mean	
IVM1	5120.0 ± 1410.81	1928.51;8311.49	420.0 ± 286.279	-227.61; 1067.61	93.3
IVM2	5692.0 ± 1752.22	1728.20;9655.80	70.0 ± 51.747	-47.06; 187.06	98.9
IVM3	5710.0 ± 1506.02	2303.14;9116.86	10.0 ± 10.00	-12.62; 32.62	99.8
Control	5652 ± 744.39	2259.30;9044.70	6304.0 ± 1925.148	1949.01; 10658.99	NA
P = 0.948			P = 0.000		

Table 1: Effects of different brands of ivermectin on faecal egg count (EPG) in sheep.
 CI: Confidence Interval; EPG: Egg Per Gram of Faeces; FECRT: Faecal Egg Count Reduction Test;
 IVM: Group of animals randomly assigned and treated with brands of ivermectin, NA: Not Applicable;
 SE: Standard Error; %: Percent.

Efficacy evaluation

The AH efficacy evaluation of different brands of ivermectin was conducted using the FECRT method; the interpretation of which was carried out according to the WAAVP recommendations [25]. According to Coles., *et al.* (1992), FECRT results below 95% strongly suggests the presence of AH resistance or the 95% confidence level is less than 90%. The present AH efficacy evaluation indicated that the sheep in groups 1, 2 and 3 were treated with IVM1, IVM2, and IVM3 1% subcutaneous injection and the rates of reduction of EPG were 93.3%, 98.9% and 99.8% on the 10th day post-treatment, respectively. There was a significant difference ($p < 0.05$) in mean faecal egg count reduction among the treatment groups. Furthermore, treatment has significantly reduced the EPG compared to the untreated control group ($p = 0.000$).

The association of EPG count with sex, age, and body weight was also determined. The analysis showed there was no significant difference between male and female animals before treatment ($p = 0.331$) and after treatment ($p = 0.939$). Similarly, there was no significant difference in EPG count among age (from 6-48 months) of individual animals both before ($p = 0.209$) and after ($p = 0.416$) treatment. However, there was significant difference between age groups (young: ≤ 12 months; adult: > 12 months) before treatment ($p = 0.038$) but not after treatment ($p = 0.840$). Furthermore, this study showed that there was an inverse correlation between body weight and mean EPG in pre-treatment faecal samples ($p = 0.001$), while it was not significant after treatment ($p = 0.464$) (Table 2).

Study group	Body weight (mean)	EPG of pre- treatment (mean)	EPG of post-treatment (mean)
IVM1	22.05	5120	420
IVM2	18.1	5692	70
IVM3	22.45	5710	10
Control	21.45	5652	6304
p-value		(0.001)	(0.464)

Table 2: Relationship of mean body weight and mean EPG before and after ivermectin therapy.

IVM1: Group of animals randomly assigned and treated with ivermectin brand 1;

IVM2: Group of animals randomly assigned and treated with ivermectin brand 2; IVM3: Group of animals randomly assigned and treated with ivermectin brand 3; EPG: Egg Per Gram of Faeces.

The parasitological study showed that *Trichostrongylus*, *Haemonchus*, *Oesophagostomum*, and *Strongyloides* were among the major genera of the parasites identified based on the recovered larvae, indicating multiple parasitic infections in sheep of the study area. Protozoa oocysts of *Eimeria* were also observed in samples. Post-treatment faecal culture showed no larva in groups receiving IVM3 while a considerable number of larvae were found in groups treated with IVM1 and only *Haemonchus* was identified from IVM2 (Table 3).

Pre-treatment	Post- treatment
<i>Bunostomum species</i>	<i>Haemonchus spp.**</i>
<i>Haemonchus spp.</i>	<i>Trichostrongylus spp.#</i>
<i>Oesophagostomum spp.</i>	Others: <i>Eimeria species</i>
<i>Strongyloides spp.</i>	
<i>Trichostrongylus spp.</i>	
Others: <i>Eimeria species</i>	

Table 3: Helminth profile of sheep before and after ivermectin therapy.

Note: All parasites listed in the table were identified before treatment from all groups; **The parasite was identified only from groups of sheep treated with brand of ivermectin IVM1 and IVM2; # the parasite was identified from group of sheep treated with brand of ivermectin IVM1.

Discussion

Gastrointestinal nematodes are highly prevalent in sheep that freely grazing on pasture [17]. AH has been used to control the nematode parasites for decades. However, improper use of a limited group of drugs for a long period of time may favor the development of resistance [26]. Hence, assessing the status of AH resistance is possibly the most important step in establishing and maintaining effective parasite control of nematode parasites in livestock, especially in sheep. This can be done *in vivo* using the FECRT, or *in vitro* using a variety of assays such as larval development assay [27]. The FECRT is generally accepted as the test of choice but it requires a large number of suitable animals [28,29]. The FECRT detects clinical cure rather than the total elimination of the parasites [19]. In the current study, the FECRT was used to evaluate the efficacy of different brands of ivermectin found on the local market.

The pre FECRT nematode profile of the study animals showed there was multiple GI parasitic infections in sheep. *Strongyle* species predominated over other GI helminths which may indicate high contaminations of the pasture with different species of the nematodes. This finding agrees with previous studies [9,16,17,30,31]. The finding of *Haemonchus* and other *Strongyle* after treatment with IVM1 brands of ivermectin may mean that few of these parasites have escaped or resisted the treatment and the animal can continuously shed eggs of these worms. This finding supports the reports of previous studies [17,32-34]. This could potentially result in selective perpetuation of resistant isolates of the parasite consequently posing the future risk of drug resistance as AH resistance is mostly inherited [35].

The inverse relationship we observed between the high EPG values for pre-treatment faecal samples and body condition score in the study animals may suggest that GI nematodes caused significant effects on growth young small ruminants, which is line with studies done in Kenya [36] and Ethiopia [37].

The result of the current FECRT study showed that only two of the tested three brands of ivermectin have good clinical efficacy against nematodes in sheep (IVM2 \approx 99.8% and IVM3 \approx 98.9%). This could indicate the probability of the occurrence of AH resistance on the farm was very low. This finding agrees with previous researches conducted in Ethiopia by Regassa, *et al.* (2013) [17] in central Ethiopia (95.7%), Kumsa, *et al.* (2010) at Ziway (95.7%) [15]; Getachew, *et al.* (2013) at Bedelle (97.0%) [38]; Desie and Amenu (2010) at Wolaita (98.3%) [39]; Melaku, *et al.* (2013) at North Gondar (96.69%) [40] and Ayalew, *et al.* (2014) in and around Jigjiga (100%) [41]. The good efficacy of the drug might be due to alternate AH use practiced on the farm by an animal health professional. However, one of the three brands of ivermectin (IVM1) showed relatively poor efficacy (93.3%). This could be due to the low quality of IVM1 (active pharmaceutical ingredient/API/or poor bioavailability/slow release from the site of injection to systemic circulation/as suggested by researchers elsewhere [42,43].

Although ivermectin will continue to be used to combat nematode infection in the area for the foreseeable future, the study underlined that there is a risk of development of AH resistance as widespread utilization of AH is common [13] and substandard /poor quality/ medicines are circulating in Africa [42]. For now, there is no need for a radical change in the method of nematode control in the farm as indicated by Šimpraga, *et al.* (2015) [44]. However, the AH drug's efficacy must be preserved through judicious and planned treatment time in order to avoid the development of drug resistance [21,39].

Conclusion

In this study, multiple GI helminth infections were detected in sheep. Strongyles and *Haemonchus* larvae were completely cleared in the sheep treated with IVM2 and IVM3, while IVM1 did not achieve complete clearance after the treatment. This suggests that IVM1 might have inferior quality or the parasites might have developed resistance to this brand. Generally, the efficacy of the two brands of ivermectin tested in this study was high, indicating the absence of resistance to these brands in the study place. While our current findings show high

efficacy of the ivermectin brands that are available on Ethiopian markets, alternate use of other AH with different mechanisms of actions coupled with regular quality surveillance is suggested to minimize the risk of the development of helminth resistance against ivermectin.

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Conflict of Interest

The authors declare that they have no competing interests.

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Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

TBT, FR, and AFB conceived the research idea. EW, HW, MT, BW, and TBT carried out the experimental work. EW, DA and TBT drafted the manuscript. AFB, FR, and TBT provided valuable information about laboratory work and the design of the study. AFB and TBT revised the manuscript. TBT coordinated and supervised the study. All authors agreed on the results and conclusions and approved the final version of the manuscript.

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