

# Field investigation of anthelmintic efficacy and risk factors for anthelmintic drug resistance in sheep at Bedelle District of Oromia Region, Ethiopia

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## Abstract

The study was conducted to investigate the status of anthelmintic (AH) efficacy and associated risk factors for AH resistance in sheep. Questionnaire surveys were undertaken to establish the AH utilization practices of sheep owners at the study area and identify risk factors. Four hundred fourteen sheep were sampled of which 180 sheep were selected based on their higher egg count for the fecal egg count reduction test (FECRT) to assess the efficacy of albendazole, tetramisole and ivermectin against gastrointestinal nematode parasites prevailing in sheep. Fecal culture and postmortem examination techniques were used to identify the species of nematode parasites prevalent before and after treatment. The results show that: 1) albendazole and tetramisole were the most frequently used AH in the area, 2) farmers get the drugs with or without prescription from various sources (10% open markets, 18% private drug stores and 72% government animal health clinics and 3) the prevalence of gastro-intestinal nematode infection in the study areas was 72%. 4) The FECRT revealed all the three tested AH were effective with egg count reduction levels of 96%, 99% and 97% respectively for albendazole, tetramisole and ivermectin. However, post-treatment fecal cultures and postmortem adult worm recovery showed that some *Haemonchus contortus* worms have escaped the treatments. In conclusion, no anthelmintic resistance was detected from clinical cure point of view but the population of *H. contortus* that has escaped the treatments deserves further scrutiny as this parasite is the most prolific and highly pathogenic in sheep.

**Key words:** Anthelmintic efficacy, FECRT, Gastrointestinal Nematodes, Risk Factors, Sheep.

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## Introduction

Among parasitic diseases that are of economic importance to the livestock industry are helminthosis caused by parasites such as *Haemonchus*, *Trichostrongylus*, and *Fasciola* species (Urquhart *et al.*, 1996). They cause production losses and death of animals if left untreated. Treatment of gastrointestinal helminthosis mainly involves commercially available anthelmintics such as the benzimidazole, imidazothiazole and microcyclic lactone groups.

The extensive use of these anthelmintics for the control of helminth infections on grazing livestock has often resulted in the development of resistance that has become a major practical problem in many countries of Africa (van Wyk *et al.* 1997; Waruiru, 1997), Europe (Chartier *et al.* 1998; Coles, 1998, Varady and Corba, 1999), Asia (Chandrawathani *et al.*, 1999), South America (Eddi *et al.*, 1996; Nari *et al.*, 1996) and Australia (Green *et al.*, 1981; Le Jambre *et al.*, 1995). A similar situation has been reported in eastern Ethiopia by Sisay Menkir *et al.*, (2006) where nematodes have shown resistance to albendazole, tetramisole and ivermectin at prescribed dosages in small ruminants. Their long-term utilization, inappropriate handling and under dosage may be some of the reasons for the reduced efficacy and for the increasing development of drug resistance. A study done on the blood feeding parasite, *H. contortus*, has demonstrated the existence of multiple-resistance to repeated applications of benzimidazoles, levamisole and ivermectin (Waruiru, 1997). Highly prolific species such as *H. contortus* with relatively short life expectancy of adult worms have a higher risk of developing diverse resistance-alleles due to spontaneous mutations than the less prolific *Trichostrongylus colubriformis* (Silvestre and Humbert, 2002).

Gastrointestinal helminth infections are very common in many parts of Ethiopia and their control is almost exclusively based on anthelmintic treatment (Dereje Bekele, 2009; Fikru Regassa *et al.*, 2006, Tembely *et al.*, 1997). Bayou Abera (1992) reported the prevalence of helminth parasites in Bedelle wereda (District) and its environs to be around 90% based on fecal examination. *Oesphagostomum*, *Bonostomum*, *Haemonchus* and *Trichostrongylus* species were the major nematodes reported to be found at post-mortem; most animals harboring mixed infections. However, the AH resistance situation in sheep in Bedelle Wereda is not known. Anthelmintics

are utilized in the area since long time and personal observation showed that farmers can get access to these drugs not only from veterinary clinics and drug stores but also from local open markets where misuse of the drugs is highly expected and consequently development of anthelmintic resistance is a possibility. The objective of this study was therefore to assess anthelmintic utilization practices (to identify risk factors for anthelmintic resistance) and field efficacy of three groups of anthelmintics (Albendazole, Tetramisole and Ivermectin) in sheep in Bedelle.

## **Materials and methods**

### Study site and animals

This study was conducted at 3 sites around Bedelle town which is located 480 km west of Addis Ababa (Ethiopia), from November 2010 to April 2011. The area has an altitude ranging between 1,300 to 2000 m. above sea level, and receives mean annual rainfall of 1457mm. The average minimum and maximum temperatures are 11°C and 28°C respectively. Taking the 90% prevalence of helminth parasites reported at Bedelle (Bayou Aberra, 1992), 138 animals were required for the prevalence study (Thrusfield, 2005). Three specific sites were purposively selected based on accessibility and hence the total number of animals considered in the first phase of screening for gastrointestinal nematodes was 414. For the field anthelmintic (AH) efficacy trial, 180 sheep were selected from the 414 based on their higher fecal egg count (FEC) and history of previous treatment (Chartier *et al.*, 1998). Only sheep that had not been treated for the last 8 weeks were included in the anthelmintic resistance test.

### Questionnaire survey

Questionnaire survey was performed on the 414 sheep owners in the area (average: 1 owner/2 sheep sampled). The questioner consisted of two main sections. The first section dealt with AH utilization for sheep and aimed at establishing whether animals were dewormed, conditions for deworming, frequency of deworming, and the sources of the drugs. The participating farmers were also requested to rank their preference for various AH and to

indicate the criteria used in the selection of AH. The second section of the questionnaire aimed at establishing efficacy of the AH. The farmers were requested whether there were improvements in clinical signs and/or in body conditions after treating their animals.

#### Evaluation of anthelmintic efficacy

Anthelmintic efficacies were measured on 180 sheep using the fecal egg count reduction test (FECRT) followed by fecal cultures and postmortem worm recovery. Test sheep were selected according to their pretreatment fecal egg count results (above 150 eggs), individually identified and randomly allocated into three treatment and a control groups (I-IV) at each site. The drugs used for the tests are indicated in Table 1.

Table 1. Anthelmintic's used in the field efficacy evaluation against sheep nematodes

Trade name	Generic name	Manufacturer	Dose(mg/kg BW)	Route
Albenjung-S	Albendazole	Daehan, Korea	7.5	Per os
Doxamine	Tetramisole	Daehan, Korea	15	Per os
Ivermic	Ivermectin	Microsules, Uruguay	0.2	Sc*

\*Sc: Subcutaneous

#### Fecal egg count reduction test

Animals in the groups I-III were treated with one of the three anthelmintics: albendazole, tetramisole or ivermectin according to the manufactures recommendation and based on the heaviest animals in the group. Animals in group IV remained untreated as controls until the end of the study. Fecal samples were collected from the rectum of each animal on day zero immediately before dosing, and on days 7 & 14 post treatment for the FECRT. Fecal egg count (FEC) was performed using the modified McMaster technique (MAFF, 1977; Raynaud, 1970). The mean FEC of each

treatment group expressed as egg per gram of feces (EPGt) was calculated and compared to that of the control group (EPGc). Percentage Reduction (R) was determined using the formula  $R = [100(1 - \text{EPGt}/\text{EPGc})]$  (Coles *et al.*, 1992; Bartley *et al.*, 2006).

### Larval culture and adult worm recovery

Pre- and post-treatment larval cultures were performed on pooled fecal samples for each treatment group. Third stage larvae collected by a Baerman technique were examined using a stereomicroscope and identified according to established descriptions given by MAFF (1977). At the end of the study (day 21 post treatment), 9 animals from the treated groups and three animals from the non-treated group were humanely killed for gastrointestinal parasite recovery and species identification (MAFF, 1977). Briefly, the contents and washings of the abomasum and the small and large intestines were separately collected, sieved and immediately examined for the presence of worms with a hand lens and a stereomicroscope. When worms were found, further morphological characterization was made under a binocular light microscope.

### Data analysis

Data was analyzed using descriptive statistics (SPSS statistical software). Questionnaire survey results were compared using  $X^2$  statistics and levels of significance were determined at  $P < 0.05$ . The efficacy of AH was evaluated based on the reduction in FEC. The arithmetic mean, percentage of reduction and 95% upper and lower confidence limits (CL) were calculated. Resistance is declared only when the percentage of reduction is less than 95% and the lower 95% CL is less than 90%.

## Results

### Questionnaire survey responses

The questionnaire survey indicated Albendazole was the most commonly used drug followed by Tetramisole. All sheep owners responded that they

use anthelmintic only when animals show symptoms like poor body condition, diarrhea or coughing. Where the anthelmintic was not prescribed by animal health personnel, most of the respondents indicated that they selected their drug of choice by color from what was available in the local markets. The survey indicated that farmers prefer green color/Albendazole followed by white/Tetramisole, and the difference is statistically significant ( $P < 0.05$ ). Significantly higher percentage of sheep owners use anthelmintics prescribed by animal health personnel than those who buy them from private drug stores or open markets ( $P < 0.05$ ). The frequency of treatment with anthelmintics was, on average, twice per year. Significantly higher number of the farmers also indicated that their animals had shown improvements in clinical signs and body condition after AH treatment (Table 2).

Table 2. Responses of farmers to questionnaire survey on anthelmintic utilization practice

Questionnaire focus	Responses
When to use AH:	Reduced body condition, diarrhea, coughing etc...
Commonly used AH:	Albendazole 58%, Tetramisole 26% , Tetraclozan 16%
Reason for AH choice:	By price (8%), color (42%), prescriptions by vet. clinics (50%)
Sources of AH:	10% open markets/shops, 18% private veterinary pharmacies, 72% government veterinary clinics
Treatment frequency:	Average twice per year (range: 0-4 times)
Response to treatment:	71% improvement in clinical signs and body conditions and 29% little or no improvement

AH: anthelmintic

### Gastrointestinal nematode prevalence

The prevalence of gastrointestinal nematodes of sheep during the study period was 72%. Coproscopic examination by McMaster Method revealed an average EPG of 270 (100 to 1050). The results of the pretreatment composite fecal cultures of all treatment groups of sheep showed that *Haemonchus* spp. was the dominant nematode (Table 3). Others such as *Osophagostomam* spp.

*Trichostrongylus* spp. and *Bonostomum* spp. were also found to a lesser extent. No significant difference was observed in the prevalence of helminth infections between all the four groups at the beginning of the study.

Table 3. Confirmation of nematode parasite species by fecal culture before treatment

<i>L3-pretreatment (fecal culture)</i>	<b>Treatment groups</b>			
	<b>Control</b>	<b>Albendazole</b>	<b>Tetramisole</b>	<b>Ivermectin</b>
<i>Haemonchus</i> spp. (%)	71	69	73	70
<i>T. colubriformis</i> (%)	17.6	21.5	16	19.1
<i>Oesophagostomum</i> spp. (%)	8.3	6.5	6.7	7
Others	3.1	3	4.3	3.9

#### Anthelmintic efficacy/FECR test

Percentage reduction in fecal egg counts and their confidence limits in the efficacy evaluation of albendazole, tetramizole and ivermectin revealed that all the three drugs were effective from clinical cure point of view (Table 4). Treatment has significantly reduced the egg per gram of feces (EPG) when compared to that in the untreated control group ( $P < 0.01$ ). In the post treatment faecal cultures of all the three treatment groups, only third stage larvae of *Haemonchus* spp. were detected while other species of nematodes (68% *Haemonchus*, 21.9% *Trichostrongylus*, 6% *Oesophagostomum* and 4.1% others) were still there in the untreated control groups. The results of postmortem parasite recovery also showed that the gastrointestinal nematode parasite present in all the treated groups was *Haemonchus* spp. while other species of nematodes were still prevalent in the untreated control group.

Table 4. Results of the fecal egg count reduction test after treatment of sheep with Albendazole, Tetramisole and Ivermectin

Measurements	Treatment groups			
	Control	Albendazole	Tetramisole	Ivermectin
Mean EPG pretreatment	984	867	914	892
Mean EPG post-treatment	752	33	11	25
% reduction in EPG	23.3	95.6	98.5	96.7
95% UCL	-	97.6	99.6	98.8
95% LCL	-	93.6	98.3	94.5
<b>Interpretation</b>	-	<b>Effective</b>	<b>Effective</b>	<b>Effective</b>

EPG: egg per gram of feces, UCL: upper confidence limit, LCL: lower confidence limit

## Discussion

In this study, the result of the questionnaire survey indicated that Albendazole was the most widely used anthelmintic followed by Tetramisole. This finding is in agreement with reports from southern Ethiopia by Bersisa Kumsa and Ajebu Nigussie (2008). Although, higher percentage of farmers give anthelmintics to their sheep only by prescription, 10% response on using drugs purchased from open markets suggests that misuse of the commonly used anthelmintics is prevalent in the area. This observation agrees with those from previous works by Arece *et al.*, (2004). The presence of the three genera of parasites in and around Bedele worda agrees with the findings of Bayou Abera (1992). The dominance of *H. contortus* among the parasites identified may be due to the fact that *Haemonchus* is a highly prolific parasite with high degree of pasture contamination than the other genera (Reviewed in Getachew Terefe *et al.*, 2007). This finding is also in line with the work of Nebiyu Thomas *et al.*, (2007).

The evaluation of FECRT showed that the three tested anthelmintics were effective against the parasites identified. This finding is in accordance with studies carried out on gastrointestinal nematodes of sheep kept under



extensive management by small holder farmers in Ethiopia (Bersissa Kumssa and Nigussie Ajebu, 2008; Sissay Menkir *et al.*, 2006, Yacob Hailu *et al.*, 2009). This could be associated to low frequency of anthelmintic treatment. On the contrary, the absence of resistance could also be explained by the low sensitivity of the FECRT to detect levels of resistance below 25% (Martin *et al.*, 1989).

The FECRT detects clinical cure rather than the total elimination of the parasites (Coles *et al.*, 1992). In this respect, the finding of *H. contortus* after treatment with the anthelmintics 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 (Alvarez-sanchez *et al.*, 2006; Keyyu *et al.*, 2002; van wyk *et al.*, 1997). This could potentially result in selective perpetuation of resistant isolates of the parasite consequently posing future risk of drug resistance as anthelmintic resistance is mostly inherited (Silvestre and Cabaret, 2002).

In conclusion, the present study showed that helminth parasites are highly prevalent in sheep in Bedelle Woreda. The drugs most commonly used in the area were Albendazole and tetramisole. The fact that anthelmintics can easily be accessed from illegal sources implies that this risk factor for AH resistance must be addressed through expansion of veterinary extension programs. Despite the clinical cure of nematodosis by the three anthelmintics tested, the only nematode escaping treatment was *Haemonchus* spp. It is recommended that the efficacy of the above two drugs and the response of *Haemonchus* spp. to treatment be periodically monitored.

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