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Efficacy of Commonly Used Anthelmintic Drugs in Naturally Infected Sheep and Goats in Central Oromia, Ethiopia

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Abstract: The study was conducted to investigate the efficacy of commonly used anthelmintics (Albendazole, tetramisole and ivermectin) against gastrointestinal nematodes of naturally infected sheep and goats in central Oromia. About 40 sheep and 40 goats were divided into 4 groups of 10 animals each: The 1st group treated with albendazole, the 2nd with tetramisole, the 3rd with ivermectin and the 4th served as the untreated control group. Faecal samples were collected on day 0 before treatment and again day on 10 post treatment. Efficacy for each anthelmintic was determined by the Faecal Egg Count Reduction Test (FECRT). Assessment was also made on handling of these drugs by questionnaire to 50 randomly selected farmers in 6 peasant associations. The study indicated the efficacy of albendazole was 100% in both sheep and goats; ivermectin was 100% effective in goats but 95.7% in sheep where as efficacy of tetramisole was 96.6 and 97% for sheep and goats, respectively indicating a good state of efficacy of these anthelmintics. Nevertheless, there was complaint of anthelmintic drug treatment failure that could be due to improper treatment. A questionnaire survey conducted to gather information on control practices of gastrointestinal nematodes of small ruminants' revealed lack of basic awareness among owners about the correct use and efficacy of anthelmintics. In addition, it indicated that farmers in the study area apply many practices that may lower the efficacy of anthelmintics and favor the emergence of anthelmintic resistance. Therefore, this study discusses on threats of anthelmintics resistance development due to incorrect drug use and handling practice observed during the study and identified important small ruminant parasite profile in the study area.

Key words: Efficacy, albendazole, tetramisole, ivermectin, nematode, anthelmintics resistance, FECRT

INTRODUCTION

In Ethiopia, small ruminant production and productivity are affected by diseases, inadequate nutrition and poor management system. Several studies in different parts of Ethiopia have emphasized that gastrointestinal parasites are one of the most prevailing problem of small ruminants causing mortalities, morbidity and production losses (Bekele *et al*., 1992; Tembely *et al*., 1997; Shimelis *et al*., 2011).

The control of parasitic helminthes in domestic animals relies largely on the use of few classes of anthelmintic drugs. As a consequence anthelmintic resistance is becoming a serious problem world wide (Urquhart *et al*., 1996; Prichard *et al*., 2007; Ihler, 2010; Shalaby, 2013). Resistance to anthelmitics in sheep and goats is rapidly increasing particularly in warm and humid climatic regions due to frequent dosing (Anderson and Waller, 1985; Desie and Amenu, 2010). Thus, continuous assessment of efficacy of the available anthelmintics has paramount importance as there are overwhelming complaint of treatment failure and resistance report in different parts of the world. Recently, some African countries reported small ruminant parasite drug resistance, for example: Kenya (Gatongi *et al*., 2003), Tanzania (Keyyu *et al*., 2002), Mozambique (Atanasio *et al*., 2002), Zambia (Gabrie *et al*., 2001), South Africa (Van Wyk *et al*., 1999), Zimbabwe (Mukaratirwa *et al*., 1997), Cameron (Ndamukong and Sewell, 1992) and Nigeria (Mbah *et al*., 1992).

In Ethiopia, there are few reports on the resistance of gastro-intestinal nematodes to anthelmintics in livestock, this includes tetramisole resistance in sheep in SNNP region (Asmare, 1997), albendazole resistance in dairy cattle (Addis, 1998) and albendazole, tetramisole and ivermectin resistance in goats in Eastern region (Sissay *et al*., 2006), low efficacy of tetramisole in goats in Southern Oromia (Kumsa *et al*., 2010). In mid-highlands of Ethiopia like Debre Zeit, livestock production is mainly supplementary to the major crop production. Currently,

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farmers are complaining about treatment failure using anthelmintics. However, there is neither study anthelmintic resistance nor assessment of the handling of the drugs was conducted. Therefore, the present study is designed to assess the use, source and handling of anthelmintics and investigate the efficacy of commonly used anthelmintics to speculate about the contributing factors and risk of anthelmintic drug resistance in the study area.

MATERIALS AND METHODS

Study area: The study was conducted from October, 2006 through April, 2009 in central Oromia, East Shoa Zone in 6 peasant associations around Debre Zeit which is located at 45 km South East of Addis Ababa. The area is situated at 9°N latitude and 4°E longitude, at an altitude of 1850 m above sea level in the central high lands of Ethiopia (NMSA, 1999). Farmers in the vicinity of Debre Zeit town practice a mixed crop-livestock farming system and a cereal legume cropping system (Zeleke *et al*., 2005).

Study animals: Total 80 small ruminants (40 sheep and 40 goats) of both sexes of randomly selected small holder farmers in six peasants association were used for the study. The suckling lambs and kids, pregnant females, very old animals (>5 years) and animals treated with anthelmintics or traditional medicine were excluded from the study. In addition, each animal was individually identified with a numbered ear tagged and eggs per gram (epg) of each animal were determined.

Experimental design: The study comprises questionnaire survey on indicators of anthelmintic application, source, method of application and handling of anthelmintics and parasitological study to identify the prevailing helmenths and efficacy study on the commonly used anthelmintics, such as albendazole, tetramisole and ivermectin.

A questionnaire survey was conducted on randomly selected 50 small ruminant owner farmers in 6 randomly selected peasant associations in the study area. Information to assess anthelmintics usage, source, time and method of administrations and educational back grounds of the farmers were all collected and compiled. Small holder farmers in six peasants association were randomly selected with subsequent purposive selection of sheep and goats in their flock. Then, fresh fecal samples were taken directly from the rectum of the animals for 3 consecutive days and mean eggs per gram (epg) of each animal were determined using modified McMaster techniques with a sensitivity of 50 epg as described in MAFF (1979), Soulsby (1986) and Coles *et al*. (1992). Then, 40 sheep and 40 goats with average epg >200 (Presidente, 1985) were randomly selected and allotted to one untreated control group and three treatment groups with 10 animals in each group. Then, the treatment group animals were treated with three commonly used anthelmintics drugs, namely; albendazole, tetramisole and ivermectin based on manufacturers dose recommendation after the body weight of each animals were determined. Albendazole and tetramisole were administered orally using balling gun whereas ivermectin was administered via subcutaneous injection route. Mean Packed Cell Volume (PCV) of the animals were assessed before and after anthelmintics treatment in all the groups after taking blood sample from ear vein in capillary tubes. Then after 10 days of post treatment, faecal samples were taken again and the epg was determined (Soulsby, 1986). Pooled fecal samples both from treated and untreated (control) groups of animals were also cultured to see the nematodes in pre and post treatment in the study area as described in MAFF (1979) and the larvae were identified for respective groups as discussed in Van Wyk *et al.* (2004).

Anthelmintics: All the evaluated anthelmintics were imported into the country by registered companies that are licensed to distribute veterinary drugs. All the drugs were used within their expiry date and stored as per instructions of the manufacturers. The details of anthelmintics used in the FECRT are given in Table 1.

L_3 identification: About 5 g rectal fecal samples from each treated and untreated (control) groups of animals were pooled and incubated at 26°C for 7 days to assess the nematodes in pre and post treatment in the study area as described in MAFF (1979). The L_3 were recovered using Baermann technique and then L_3 were counted and identified for respective groups according to the morphological keys as discussed in Van Wyk *et al.* (2004).

Table 1: Detailed information of anthelmintic drugs used in the FECRT for efficacy evaluation

Trade name	Generic name	Manufacturers	Country of origin	Dose/kg body weight (mg kg^{-1})	Mode of administration
Albendazole 300 bolus	Albendazole	Star Laboratories Pvt. Ltd.	Lahore, Pakistan	7.5	Oral
Bitimisole	Tetramisole	Brilliant Industries Ltd.	Hyderabad, India	22.5	Oral
Vermic	Ivermectin	Laboratorio Centrovet Ltd.	Santiago, Chile	0.2	SC injection

FECRT = Faecal Egg Count Reduction Test; SC = Subcutanoeus

Efficacy evaluation: The efficacy of anthelmintics was evaluated based on the reduction in fecal egg count in the feces post treatment. The Fecal Egg Count Reduction Test (FECRT) is calculated according to the procedure described by Presidente (1985):

$$FECR = (1-T_2/T_1 \times C_1/C_2) \times 100$$

Where:

T_1 = Pre treatment EPG in anthelmitic treated group
T_2 = Post treatment EPG in anthelmintic treated group
C_1 = Pre treatment EPG in control group
C_2 = Post treatment EPG in control group

According to the method of Coles *et al.* (1992), resistance is present if only one of the following criteria is met:

- The percentage reduction in egg counts is <95%
- The 95% confidence level is <90%

Data analysis: The data, thus obtained was stored in excel microsoft and analyzed by descriptive statistics using SPSS version 20. Chi-square (χ^2-test) was used to assess the difference in mean epg of the helminthes egg count and PCV before and after treatment.

RESULTS AND DISCUSSION

Questionnaire survey: The questionnaire indicated that about 88% of the interviewed animal owners could not read and understand the information on the leaflets of the drugs. This indicates that the farmers could not get the dose rate and expiry date indicated on the leaflets. It was noted that 86% of the farmers have been practicing traditional therapy. About 80% of the farmers reported that they used albendazole as the most widely used anthelmintic and 20% used tetramisole as a second most commonly used drug in the area. Most of the interviewed farmers rely on clinical manifestation, such as diarrhea (86%), nasal discharge (60%), inappetence (16%) and loss of body condition (16%) to treat their sheep and goats using anthelmintics. Moreover, 88% of the interviewed farmers were not satisfied with the response of therapy while some of them treated their sheep and goats only for fattening purpose. Furthermore, most of the farmers (58%) administer albendazole bolus (300 mg) for single therapy whereas 24% gave half a bolus regardless of the body weight of the animals.

During the study, body weight of sheep and goats included in the study was taken which was in the range of 13-38 kg with average weight of 26.7 kg, thus the study showed that the drugs were given overdose for albendazole as compared to the manufacturers' instruction. According to the manufacturers instruction, the currently available albendazole is a 300 mg for 60 kg and for tetramisole 600 mg for 40 kg of body weight of the animals. During the survey body weight of 50 sheep and 50 goats were taken which ranged from 13-38 kg with average weight of 26.7 kg. Accordingly, the drugs were given over dose by the farmers. The lack of response in the earlier case could be associated with inaccurate diagnosis of the cases. It is possible that these animals suffer from diarrhea, nasal discharge and other symptom complained due to other possible causes. Moreover, there could be rapid re-infection and inefficiency of the drugs to clear arrested or larval stages of the parasites. As the parasite profile of the study animals indicated, there is multiple infections which indicates high contamination of the pasture with different species of the nematodes. This finding is in consent with reports in the same country on small ruminants and dairy cows (Addis, 1998; Sissay *et al.*, 2006; Kumsa *et al.*, 2010).

Table 2: Nematodes profile in sheep and goats before and after anthelmintic therapy

Animal species	Pre treatment	Post treatment
Sheep	*Trichostrogylus* sp.	*Trichostrogylus* sp.
	Oesophagostomum sp.	*Strongloides* sp.
	Haemonchus sp.	
	Bunostomum sp., *Strongloides* sp.	
	Cooperia sp., *Trichuris* sp.	
	Paramhistomum sp.	
Goats	*Trichostrogylus* sp.	*Trichostrogylus* sp.
	Haemonchus sp.	*Strongloides* sp.
	Oesophagostomum sp.	
	Strongloides sp., *Cooperia* sp.	

These farmers were buying Albendazole (green colour) and tetramisole (yellow) from illegal private drug vendors (62%), government drug shops (22%) and legal private drug vendors (26%). It was understood during the survey that the farmers were forced to buy the drugs from such persons because of insufficient supply of the drugs in government clinics and inaccessibility of legal private or government drug shops in their locality. It was also noted that none of the drug vendors and the farmers were good at obeying the manufacturer's drug handling, storage and dosage recommendations. Based on information obtained in the present study anthelmintics are prone to misuse and the likelihood of development of resistance to the drugs is high.

Faecal examinations: The parasitological study indicated that trichostyrongylus, haemonchus, oestophagostomum and strongyloides were the major parasites identified by their larvae (Table 2), indicating multiple parasitic infections in sheep and goat of the study area.

Table 3: Mean±SE and 95% CI of epg in sheep and goats before and after anthelmintic treatment

		epg				
		Pre treatment		Post treatment		
Animal species	Treatment groups	Mean±SE	95% CI	Mean±SE	95% CI	ER (%)
Goat	ABZ	2040±373.0	1196.2, 28883.8	0.0	-	100.0
	IVM	1120±345.4	1901.4, 338.700	0.0	-	100.0
	TMS	1740±435.7	754.4, 2725.70	40±22.1	-10.0, 90.0	96.6
	C	1770±499.3	640.5, 2899.50	1200±271.6	585.6, 1814.4	0.0
Sheep	ABZ	2150±577.0	844.7, 3455.30	0.0	-	100.0
	IVM	730±107.5	486.8, 973.200	20±20.0	-25.2, 65.2	95.7
	TMS	2180±567.4	896.4, 3663.60	30±21.3	-18.2, 78.2	97.0
	C	2020±387.1	1144.3, 2895.70	1280±221.0	780.1, 1780.0	0.0

ABZ = Albendazole; C = Control; CI = Confidence Interval; epg = Egg per gram of faeces; ER% = Percent of egg reduction; IVM = Ivermectin; PCV = Packed Cell Volume; SE = Standard Error; sp. = Species; TMS = Tetramisole

Post treatment fecal culture showed no larva in albendazole and ivermectin treatment group in both sheep and goats while one sheep and one goat showed larvae in tetramisole treatment group.

Efficacy evaluation: The anthelmintic efficacy was conducted using FECRT method the interpretation of which was carried out according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations (Coles *et al*., 1992). According to Coles *et al*. (1992), FECRT results below 95% strongly suggests the presence of anthelmintic resistance or the 95% confidence level is <90%. The present study indicated that albendazole brought 100% reduction of egg output in both sheep and goats, ivermectin 100 and 95.7% in goats and sheep, respectively whereas tetramisole caused 96.6 and 97% reduction in goats and sheep, respectively (Table 3). Furthermore, the study indicated increment of PCV in all treated animals (Table 4) though it was not statistically significant ($p>0.05$).

Thus, the study revealed that there is no evidence of resistance to albendazole that brought 100% reduction in EPG in both sheep and goats examined. This could be due to the fact that the drugs were given over dose by farmers as discussed before, even though laboratory diagnosis of parasitosis was not conducted. The present finding is in agreement with previous researchin Ethiopia. For instance, Ogaden isolates of *H. contortus* were susceptible to Albendazole (Kumsa and Wossene, 2006), *H. contortus* and *Trichstrogyles* sp., from East Harraghe (Sissay *et al*., 2006) and strongyle and *Tricuris* sp. isolates from rift valley were susceptible to the same drug. However, this result is inconsistent with findings from Southern part of the country where experimental infection of *H. contortus* revealed resistance to albendazole (Eguale *et al*., 2009)

Table 4: Mean±SE and 95% CI of PCV in sheep and goats before and after anthelmintic treatment

		PCV			
		Pre treatment		Post treatment	
Animal species	Treatment groups	Mean±SE	95% CI	Mean±SE	95% CI
Goat	ABZ	28.6±1.5	25.2, 32.0	30.3±1.6	26.7, 33.9
	IVM	27.6±1.1	25.1, 30.1	31.3±1.0	29.0, 33.6
	TMS	26.3±1.1	23.8, 28.8	29.6±0.9	27.6, 31.6
	C	28.1±1.4	24.9, 31.3	29.0±1.3	26.1, 31.4
Sheep	ABZ	26.8±1.4	23.6, 29.7	32.3±1.2	29.6, 35.0
	IVM	26.9±1.6	23.3, 30.5	29.9±1.4	26.7, 33.1
	TMS	27.9±1.3	25.0, 30.8	30.0±1.1	27.5, 32.5
	C	26.0±1.5	22.6, 29.4	28.9±1.3	26.0, 31.8

ABZ = Albendazole; C = Control; CI = Confidence Interval; IVM = Ivermectin; PCV = Packed Cell Volume; SE = Standard Error; sp. = Species; TMS = Tetramisole

There was also no resistance to tetramisole which caused 96.6 and 97% egg count reduction in goats and sheep, respectively which could be attributed the unknowingly overdosing of the drug. However, compared with albendazole the lower efficacy in teramisole against nematodes of small ruminants in this study might be due to several factors like poor quality drugs due to its low bioavailability, misuse and inappropriate treatment by owners. The finding is also consistent with that of Sissay *et al*. (2006). Likewise, the study revealed that ivermectin brought 100% reduction in egg count in goats that is consistent with Kumsa *et al*. (2010) and 95.7% in sheep, hence it is concluded that there is no resistance to this drug in both species of animals. During the survey, it was noted that ivermectin is only available in injection form in study area and as opposed to other anthelmintics, consequently, it was not prone to be directly purchased from open market and relatively not misused.

CONCLUSION

A questionnaire survey was undertaken to assess anthelmintics usage, source, time and method of administrations and efficacy evaluation of commonly used

anthelmintics (albendazole, tetramisole and ivermectin) was conducted using FECRT. The result revealed that in spite of the inappropriate use of the anthelmintics with regard to proper diagnosis of the cases, poor handling and storage conditions, there was no resistance to these drugs as the drugs have good efficacy. This could be due to the overdosing practice by the farmers. However, the study underlined that there is high threat of emergency of anthelmintic resistance as the farmers practice the treatment without good knowledge of the drugs, the disease condition and doses.

RECOMMENDATIONS

Therefore, it is recommended that the appropriate institution, such as the Drug Administration and Control Authority (DACA) and Ethiopian veterinary service agency should act accordingly to circumvent the existing threat by imposing appropriate drug control and administration policy.

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NOMENCLTURE

CL = Confidence Limit
epg = Eggs per gram of faeces
FEC = Faecal Egg Count
FECRT = Faecal Egg Count Reduction Test
L_3 = Third stage infective larvae
PCV = Packed Cell Volume
WAAVP = World Association for the Advancement of Veterinary Parasitology

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