A survey on the occurrence of anthelmintic resistance in nematodes of sheep and goats found in different agro-ecologies in Ethiopia

*Desalegn Lidetu**

National Animal Health Diagnostic & Investigation Center, 04 Sebeta, Ethiopia

Abstract

A survey was conducted on the occurrence of anthelmintic resistance in nematodes of communally grazed sheep and goats belonging to 17 small-holder peasant farmers and to two institutional farms found in different agro-ecologies. The efficacies of albendazole and levamisole were tested by fecal egg count reduction (FECR) tests. Resistance was seen in three out of the 22 flocks belonging to smallholders and was suspected on one farm. The percentage reductions were 67%, 77%, 85%, and 92% with the 95 % confidence intervals below 86%. On Debre-Birhan and Hawasa University farms, the level of resistance to albendazole and levamisole were in a range of 45% to 65%. Resistance was suspected in a flock of sheep on one smallholder farm, where a percentage reduction of 94% and 95% was observed with the lower 95% confidence interval less than 90%. Post-treatment fecal cultures indicated that *Haemonchus* was the resistant nematode to both anthelmintics. This survey indicates that anthelmintic resistance to nematode parasites is developing gradually. Appropriate measures need to be implemented without delay.

Keywords: Anthelmintic resistance, FECR, farms, goats, nematodes, sheep.

Introduction

Resistance is an inevitable consequence of the use of anthelmintics, and the history of parasite resistance to anthelmintics started with the first report on phenothiazine resistance in sheep in the USA by Drudge *et al*. (1957). After five decades, the problem has become an important limiting factor in the control of nematode parasites of ruminants. The geography of resistance is widening and numerous instances of anthelmintic resistance in Africa have been reported, e.g. in Cameroon (Ndamukong and Sewell 1992), Kenya (Maingi *et al*., 1996), Tanzania (Ngomuo *et al*., 1990), Zimbabwe (Boersema and Pandey 1997), and Mozambique (Atanasio *et al*. 2002). In South Africa, anthelmintic resistance has become a major problem with 90% of farms harbor resistant helminth strains (van Wyk *et al.* 1997a, b).

* Mailing address: PO Box 5988, Addis Ababa, Ethiopia. E-mail: deslidetu@yahoo.com

Ethiopia is among the few countries in Africa where anthelmintic resistance was not reported (Anonymous, 2004). Because of among other reasons, the frequent and continuous use of same class of anthelmintic and inappropriate dosage rates it was unlikely that resistance could not occur. In the absence of other alternatives, worm control continues to rely on anthelmintics because of their high performance and, their use is likely to continue in the foreseeable future as the first and foremost line of defense against parasites (Martin, 1985). This study aims to investigate the occurrence of resistance to anthelmintics in selected areas in Ethiopia, by using the fecal egg count reduction test.

Materials and Methods

Study areas

East and North Shewa zones located in the regional States of Oromia and Amhara respectively were identified as the study areas. East Shewa is situated in the Great Rift Valley where the altitude is between 1200-1700 meters above sea level. The Great Rift Valley extends its vast escarpments, cliffs, rivers and plains from the red sea southward through Ethiopia, Kenya, Tanzania, and Malawi to end into the Zambezi river in Mozambique. The valley is some 50-60 Km wide. Several large lakes occur along the study areas. North Shewa is in the highland area where the altitude is more than 2000 meter above sea level (Fig. 1).

In East Shewa the climate is hot and dry with unpredictable rains that vary from year to year. The annual rainfall averages 600 mm and the mean annual temperature is about 26°C. Relative humidity is between 50-80%. Daily minimum and maximum atmospheric temperatures, relative humidity and rainfall data were collected at Metehara Sugar Factory Research Center, Adamitulu Agricultural Research Center and the National Meteorological Services Agency.

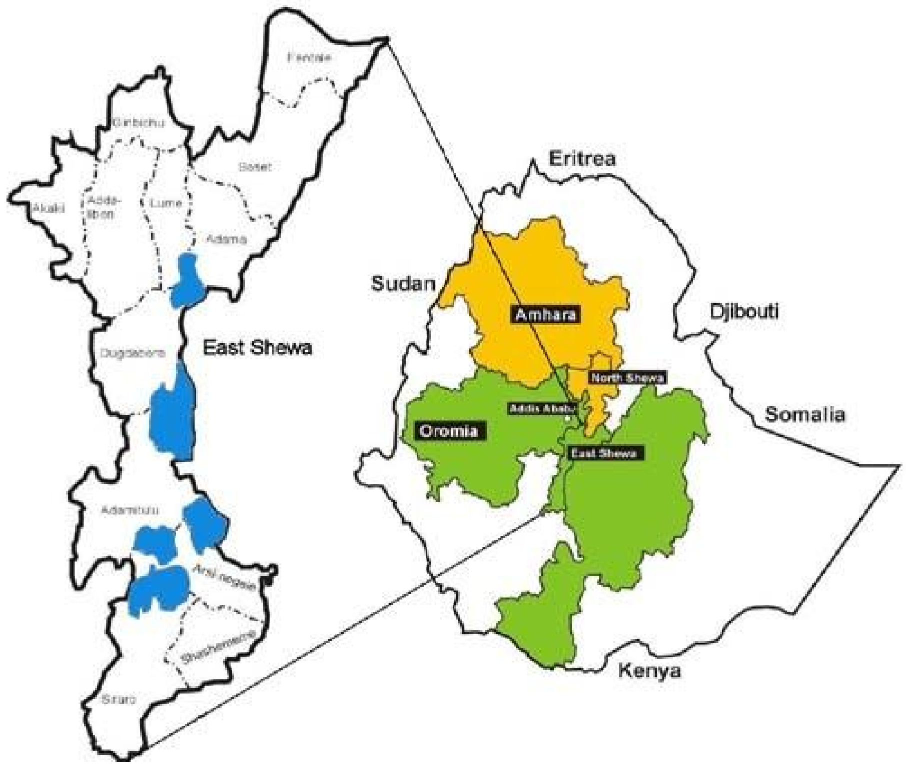

Figure 1. Map of East Shewa showing sub-districts where the study sites located. North Shewa is not expanded as only one sub-district was studied.

The climate in north Shewa is characterized by a long, cool rainy season (June-September) that accounts for 75% of the annual rainfall, a short, rainy warm season (February-May) and a dry, cold season (October-January). The annual rainfall averages 960 mm and the mean maximum temperature ranges from 14-23°C and the mean minimum from 7-18°C. The climate data for north Shewa was obtained from the National meteorological services agency.

A systematic sampling procedure was followed to select the study sites. Firstly, a list of all accessible districts found in both zones was prepared. The districts were selected from this list. Similarly a list of peasant farmer's villages that met certain criteria, such as accessibility by vehicle all year round, availability of veterinary clinics, animal health representatives from the local Peasant Association, farmers' villages where questionnaire survey on the management of worms took place, willingness of peasant farmers to participate in the current study, the availability of more than 15 sheep or goats on the farm with a nematode egg count of more than 500 per gram of feces, and the assurance that animals in the flock were not treated in the last 8-10 weeks, was prepared. Twenty two smallholder farms in 17 localities and two institutional farms (Debre-Birhan sheep breeding and improvement center in north Shewa, and Hawasa University (the then Debub University) goat farms in east Shewa were selected. Thus, a total of 24 farms were involved in this study (Table 1).

Study animals

Sheep and goats belonging to the peasant farmers in East Shewa were of the local breeds and they were of the East African type (Galal, 1983 cited by Gatenby, 1986). The indigenous goats at Hawasa University were of mixed local breeds. For ease of reference we kept the name "rift valley goats," as they were identified by the university farm. In general the goat breeds that were used in this study were the rift valley goats, and their crosses with Toggenburg or Anglo-Nubian, and pure Toggenburgs. Ten, fifteen, or more goats (<12 months old) were identified to be included in the study. The animals were divided into different breed groups, namely local rift valley goats, crossbred with Toggenburgs or Anglo-Nubians, and pure Togenburgs, all of them with the same worm population on the same farm to detect differences in the extent of resistance.

At Debre Birhan in north Shewa, flocks of Menz sheep breed, exotic sheep breed namely Awasi and their crosses with Menz which were mostly (<12 months old) were used in the study. No goats were included in the study as there were no goats in the selected study sites. On every peasant farm up to 15 sheep or goats with their worm eggs per gram (epg) counts more than 500 were divided into three treatment groups, the albendazole, levamisole and non-treated positive control groups. Animals were randomly allocated to each of the groups and each animal was identified by an ear tag with a code number.

Table 1. Location and number of sheep and goats in the anthelmintic resistance survey

Number.	Survey zone	Location of smallholders	Number of Farms	Number of Sheep	Number of Goats
1	East Shewa zone	Shashemene	1	-	35
2		Meki	2	26	26
3		Zewai	2	30	30
4		Dugdabura	1	-	23
5		Wolenchiti	2	-	26
6		Fentale	1	22	20
7		Alemgena	1	-	30
8		Arsi negele	1	25	35
9		Boset	1	-	22
10		Chabi	1	30	35
11		Modjo	2	35	35
12	North Shewa none	Karafino	1	27	-
13		Yato	1	37	-
14		Chacha	1	45	-
15		Faji	1	30	-
16		Sululta	1	20	-
17		Sheno	2	45	-
18	DSBIC	DSBIC1	1	45	-
19	HU	HU	1	0	197
TOTAL			24	417	514

1Debre Birhan Sheep Breeding & Improvement Center; Hu = Hawasa University, - = No Animals Sampled

Anthelmintics

Albendazole (albenol) and levamisole were selected for this study, to represent the benzimidazoles and imidazothiazoles. The anthelmintics that had been used mostly frequently and for six consecutive years were used. The anthelmintics were identified based on the findings of a questionnaire survey where the drugs did not change for over four or more years in the study area (Desalegn, 2005). The anthelmintics used in the present study were suspensions for oral use and administered to the animals with 10 or 20 ml plastic syringes. One ml albenol contains 100 mg albendazole. The recommended dose equals 1ml/20kg body weight. Double of the recommended dose was given to goats. A dose of 5 mg kg^{-1} of albendazole and 7.5 mg kg^{-1} levamisole were given to each animal.

The dose rates for sheep were based on the manufacturer's dose rates, while goats were given one and a half to two times' higher dose because of pharmacokinetic differences (Gillham and Obendorf, 1985). Each animal was drenched according to its actual weight in kg.

Determination of fecal egg count

Faecal egg count reduction test (FECRT) was used to determine changes in fecal egg count using the method of Coles *et al*. (1992), as advocated by the World Association for the Advancement of Parasitology (WAAVP). Feces were collected from the rectum of each sheep and goat and placed in clean specimen bottle. Fecal samples were kept in a cool-box and transported to the laboratory at Sebeta. Samples were examined the same day using a modified McMaster technique (Hansen and Perry, 1994). Fecal samples were recollected 14 days from the same animals after the first treatment. For an anthelmintic to be fully effective no worms should survive following the time taken to empty the intestine(3 days for LEV, 8 days for BZs, MLs 14-17 days), *i.e*. allowance has to be made for temporary suppression of egg production and timing are based on best guesses (Coles *et al*., 2006). Pre-treatment samples with fewer than 100 epg were omitted from this experiment.

The use of arithmetic means in the method of Coles *et al*. (1992) has been considered to be a more stringent criterion reflected in a more conservative estimation of therapeutic activity of drugs (Vercruysse *et al*., 2001). The method of Presidente (1985) was used calculating the geometric means as it is considered appropriate estimate of central tendency and has less potential for misinterpretation. The method of Presidente (1985) was used merely to observe the distinctions of the methods. The following formulas were used to assess FECRT.

1. $FECRT\% = 100 \times (1-[T_2/C_2])$ - Coles *et al*. (1992) use arithmetic means, 95% confidence level is provided; resistance is present if the percentage reduction is less than 95% and the lower 95% confidence limit for the reduction is less than 90%. In this formula the T_2 and C_2 designate the counts after treatment, using the arithmetic means.

2. $FECRT\% = 100 \times (1-[T_2/T_1]\ [C_1/C_2])$ - Presidente (1985) uses logarithmic transformation of egg counts to stabilize variances. Efficacy is corrected for changes that occur in the control group by the equation listed. Resistance is present if the percentage reduction is less than 90%. In this formula the T and C were the geometric means for the treated and control groups and the subscripts 1 and 2 designate the counts before and after treatment.

Larval identification and counts

Pooled samples were collected from each group on the day of first treatment and again 14 days after treatment. To identify the species of nematodes present, cultures were made and up to 100 larvae identified with the aid of the descriptions of Reinecke (1983), van Wyk *et al*. (1997b) and van Wyk *et al*. (2004).

Results

Tables 2 and 3 show the fecal egg count reduction percentages for the 22 smallholder and the two institutional farms, which were calculated before and after treatment, according to the methods proposed by the WAAVP (Coles *et al*., 1992). Albendazole was tested on 21 smallholder farms, while levamisole was tested on six farms. Of the 22 farms only six of them were tested both for albendazole and levamisole. The fecal egg count reduction percentages tested according to Coles *et al.* (1992) and (Presidente (1985) in sheep treated with albendazole were {77%, 46} and {65%, 66%} at Chacha and Sheno in north Shewa, and {67%, 65%} at Mojo in east Shewa respectively (Table 2). Albendazole resistance was suspected on one of the smallholder farms at Shashemene in east Shewa where the FECR% were {96%, 90%} (Table 2). Similarly, levamisole resistance was detected in goats on one of the smallholder farms at Zewai in east Shewa, where the FECR% was {95%, 72%} (Table 2).

Resistance in a flock of sheep at Debre-Birhan government sheep farm showed a 94 % reduction in the faecal egg count and the lower 95 % confidence interval was less than 90 (Table 3). Therefore, the results obtained at this farm suggest that there was a low resistance or resistance can be suspected. Resistance to both benzimidazole and levamisole groups of anthelmintics occurred in all the goat herds on the Hawasa University goat farm. The level of resistance ranged between 45 % & 65 % in the FECRT obtained in the Benzimidazole/ albendazole and levamisole tests both at institutional and at peasant farms levels (Tables 3-6).

Table 2. Results of FECRT of local breeds of sheep and goats on smallholder farms. Numbers in brackets are the 95% confidence intervals

Number	Survey zone	Localities	Species	n	Drug	Coles et al. (1992)	Presidente (1985)
1	East Shewa	Shashemene	Goat	35	Albendazole	96 (88-99) s	90 (91-99)
2		Meki	Sheep	26	Albendazole	98 (93-100)	91 (90-98)
3		Meki	Goat	26	Levamisole	98 (90-100)	92 (91-99)
4		Zewai	Sheep	30	Albendazole	98 (93-100)	91 (92-99)
5		Zewai	Goat	30	Levamisole	95 (84-98) r	72 (65-90) r
6		Dugdabura	Goat	23	Albendazole	99 (90-99)	92 (92-98)
8		Wolenchiti	Sheep	22	Albendazole	98 (91-99)	93 (91-99)
9		Fentale	Goat	20	Levamisole	99 (95-100)	90 (92-100)
10		Alemgena	Goat	30	Albendazole	98 (93-100)	90 (92-99)
11		Arsi Negele	Sheep	25	Albendazole	98 (93-98)	93 (91-100)
12		Arsi Negele	Goat	35	Levamisole	98 (93-100)	91 (92-99)
13		Boset	Goat	22	Albendazole	98 (91-100)	92 (94-100)
14		Chabi	Sheep	30	Albendazole	99 (95-100)	88 (58-98)
15		Chabi	Goat	35	Levamisole	98 (90-99)	92 (91-99)
16		Mojo	Sheep	35	Albendazole	67 (25-86) r	65 (32-88) r
17		Mojo	Goat	35	Levamisole	98 (93-100)	91 (90-98)
18	North Shewa	Karafino	Sheep	27	Albendazole	98 (90-100)	92 (91-99)
19		Yato	Sheep	37	Albendazole	98 (95-100)	93 (92-99)
20		Chacha	Sheep	25	Albendazole	77 (50-89) r	46 (25-68) r
21		Faji	Sheep	30	Albendazole	97 (90-99)	94 (93-99)
22		Sheno	Sheep	45	Albendazole	65 (15-89) r	66 (25-79) r

s = low resistance (resistance suspected)
r = resistance
- = missing data

Table 3. Results of FECRT on institutional farms

Localities	Species	Breed	N	Drug	Coles et al. 1992	Presidente 1985
DSBIC	Sheep	MC	15	Albendazole	97 (88-99) s	86(72-98)
"	Sheep	"	15	Levamisole	95 (93-98)	84 (89-98)
HU	Goat	rv	15	Albendazole	86 (61-95) r	31(25-80) r
"	"	"	15	Levamisole	89 (77-94) r	55 (27-72) r
"	"	Rvc	10	Albendazole	88 (71-95) r	24 (12-26) r
"	"	"	10	Levamisole	93 (80-98) r	35 (2-65) r
"	"	Toggenburg	7	Albendazole	92 (77-98) r	50 (1-20) r
"	"	trc	10	Albendazole	71(28-88) r	12 (1-42) r
"	"	Trc	10	Levamisole	96 (82-99) r	32 (15-68) r

DSBIC =Debre-Birhan sheep breeding & improvement center, HU=Hawasa University
s = resistance suspected (low resistance), r= resistance, MC=Menz sheep crossed with Awasi rv = local goat breed (HU goat farm refer them as rift valley goats)
trc = crossed with Togenburg

The results of pre- and post-treatment larval recovery in the anthelmintic resistance test were determined using the faecal egg/ worm count reduction test analysis using RESO a simple software method of calculation (1990). In the pre-treatment larval cultures, *Haemonchus* was the predominant nematode (75-89 %), while *Trichostrongylus* and *Oesophagostomum* were present in small numbers (1-11 %). Post-treatment faecal cultures indicated that *Haemonchus* was the only parasite recovered at the highest level and hence, resistant to albendazole. Detailed results of the calculations on FECR are presented in Tables 4-6.

Table 4. Results of FECRT calculated using RESO according to Coles *et al*., 1992 in goats and sheep on smallholder farms in East Shewa

	Zeway				Mojo			
	Goats control	Goats Treated	Sheep control	Sheep Treated	Goats Control	Goats Treated	Sheep control	Sheep Treated
Drench number	20	20	10	20	15	20	15	20
Arith.mean epg (pre)	1013	1135	860	805	973	1220	933	1055
Arith.mean epg (post)	“	80	“	45	“	40	933-	305
Variance (FEC)	411238	376079	347100	9974	482095	6737	463810	223658
% Reduction		92		95		96		67
Variance (Reduction)		0.25		0.29		0.24		0.16
Upper 95% c.l.		97		98		99		86
Lower 95% c.l.		78		84		88		25
Interpretation		R		R		LR		R
Post treatment L3								
H. contortus	87	89	90	80	90	74	88	80
T. colubriformis	10	11	7	15	10	16	12	14
O. columbianum	3	0		5	0	10	0	6

Benzimidazole/ albendazole treatment: Pre-treatment $L_{3=}$ 89 *H. contortus*; 11 *T. colubriformis* and 5 *O. columbianum* larvae were recovered froma pooled samples

Table 5. Results of FECRT calculated using RESO according to Coles et al. 1992 in sheep on smallholders farm in north Shewa

	Sheep control 1	Sheep Treated1	Sheep control 2	Sheep treated 2	Sheep control3	Sheep treated3
Drench number	10	20	7	15	15	15
Arith.mean epg (pre)	855	805	757	640	650	1067
Arith.mean epg (post)	“	45	“	27	“	113
Variance (FEC)	347111	9974	469524	3524	116071	24095
% Reduction		95		96		85
Variance (Reduction)		0.29		0.35		0.24
Upper 95% c.l.		98		99		95
Lower 95% c.l.		84		86		58
Interpretation		R		LR		R

	Sheep control 1	Sheep Treated1	Sheep control 2	Sheep treated 2	Sheep control3	Sheep treated3
Post-treatment L3						
H. contortus	90	80		94	89	91
T. colubriformis	7	15		15	0	0
O. columbianum	0	5		0	0	0

Benzimidazole/albendazole treatment: [1] at Karafino, [2] at Faji, [3] at cha-cha, " = same mean epg count as above Pre-treatment L_3: 90 *H. contortus*, 10 *T. colubriformis* were recovered from pooled samples

Discussion

The results of the fecal egg count reduction percentage tests are in agreement with studies carried out in South Africa (Van Wyk *et al*. 1997a, b; 1999), in Kenya (Mwamachi *et al*., 1995), in Denmark (Maingi *et al.* 1996), in Malaysia (Pandey and Sivarja 1994) and in Peninsular Malaysia (Dorny *et al*.1994) where resistance of *Haemonchus* and *Trichostrongylus* spp. to benzimidazoles and levamisole was found.

The factors responsible for the occurrence of resistance on the study at small-holders (peasant farms) can be speculated. The mean numbers of anthelmintic treatments per year for goat and sheep as reported in the questionnaire survey (Desalegn, 2005) were between 2 and 4 during the wet seasons. The lower frequency of treatments recorded at peasant farm level implies lower selection pressure for resistance worms. However, other practices observed in the study areas may have contributed to the occurrence of resistance. Prolonged use of the same classes of anthelmintics, and the distribution and availability of drugs of dubious quality, might have contributed to the development of resistance in some flocks of sheep and goats belonging to smallholders. Under dosing was also very common on the study farms and veterinary clinics because of the probably under estimating of animals weight. Anthelmintic doses recommended for sheep were similarly used for the treatment of goats, which could have promoted selection for resistant worms (Waller, 1987; Coles and Roush, 1992). Reports on resistance of nematodes to anthelmintic are scanty from different regions of the country. Bersissa and Abebe (2007) reported a high efficacy of albendazole and tetramisole tested against *H. contortus* in experimentally infected lambs with the Ogaden isolates of the parasite, which indicate that in areas where farmers or pastoralists practice not frequent chemo-therapeutic treatments has no yet developed resistant worms to drugs.

Table 6. Results of FECRT calculated using RESO according to Coles *et al*., 1992 in goats at Hawasa Univesrity goat farm

Combined species	Control	Bz1	Lev1	Control	Bz2	Lev2	Control	Bz3	Lev3	Control	Bz4	Lev4
Drench number	10	10	10	10	10	10	15	15	15	10	10	10
Arith mean epg (pre)	1 010	1 290	2 390	720	820	720	850 (821)	1 607	1 007	2 696	1 490	1 560
Arith.mean epg (post)	"	800	420	"	90	50	"	120	93	"	790	80
Variance (FEC)	16 544	1 5111	510 667	315 111	7 667	5 000	491923	38 857	13 524	4 330 827	77 111	21 778
% Reduction		92	58		88	93		86	89		71	96
Variance (Reduction)		0.25	0.31		0.16	0.26		0.23	0.11		0.18	0.43
Upper 95% c.l.		97	87		95	98		95	94		88	99
Lower 95% c.l.		77	33		71	80		61	77		28	82
Interpretation		R	R		R	R		R	R		R	LR
Post-treatment L3												
H. contortus	87	89	90	79	90	74	88	80	91	75	86	72
T. colubriformis	10	11	7	15	10	16	12	14	5	15	14	18
O. columbianum	3	0		6	0	10	0	6	4	10	0	10

The superscript 1, 2, 3 and 4 designate the albendazole and levamisole tests on mixed goat breed, rift valley crossed, rift valley local, crossed Toggenburg breeds respectively. R- resistance, LR-low resistance. " = the same mean epg count as above
Pre-treatment L_3: 85 *H. contortus*, 15 *T. colubriformis*, 10 *O. colombianum* were recovered

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The mean number of anthelmintic treatments at Debre-Birhan sheep breeding and improvement center and at Hawasa University goat farm (6 or 8 times per year) was much higher than treatments carried out at peasant farms level. The use of the same groups of anthelmintics more frequently and for a number of years was very common in most of the government as well as private veterinary clinics. In the questionnaire survey that was carried out in the area, the majority of animal health workers (87-90 %) used benzimidazole groups of anthelmintics for four or more consecutive years before changing to a different group, like levamisole or for short periods with ivermectins (Desalegn, 2005).

Unless control measures are designed and uniformly applied, anthelmintic resistance has the potential to spread rapidly and widely. This is likely to further aggravate the "ill thrift" or nutritional inadequacies, poor helminth control and husbandry practices in small ruminant production in the area, and may further enhance selection for resistance. Possible introduction of worms to sheep and goat rearing farms or areas from within and outside the country which may be resistant to anthelmintics can also play the role in spreading the problem of resistance. For example, goats imported from New Zealand to Slovakia had multiple resistances to anthelmintics (Varady *et al*. 1993 cited in Maingi *et al*. 1996). Newly introduced animals to any farm should be treated with effective anthelmintics and held in a confined area for up to 48 hours before they are allowed to graze freely. This gives ample time to kill the worms inside the host animals.

Currently the responsibility of supplying and distributing of anthelmintics and other veterinary drugs to regional, zonal or district level veterinary clinics has been taken over by the zonal or regional bureau of agriculture of each of the regional states in the country. This should have brought about changes in the use of anthelmintics in most of the veterinary clinics. Conversely, because of the Government's free market economic policy, several private companies import veterinary drugs in bulk from various manufacturers in Africa, Europe and Asia and distribute these products in Ethiopia. This has contributed towards reducing the shortages of drugs. Anthelmintics of which the sources are not known are often available in the open market (guilt), and peasant farmers purchase them because of their trade names and affordable prices (Desalegn, 2005). However, no attempt has been done so far to study the quality and therapeutic values of anthelmintics that are being sold in most of the open markets through out the country. Waller (1997) stated that adulterated and/or anthelmintics of dubious quality obviously lead to development of resistance. Problems that require serious attention from the drug regulatory authority include the marketing of same group of anthelmintics under different trade names, formulations and presentation of the drugs.

Using the arithmetic mean, for calculating the percentage reduction in fecal egg count (Coles *et al*., 1992) is universally accepted because it provides a better estimate of the worm egg output and it is a more conservative measure of anthelmintic efficacy. In the current study both the arithmetic and geometric mean methods of calculating the percentage reduction in fecal egg count confirmed resistance (Tables 2 and 3) and Tables (4-6) in the flocks of sheep and goats that were examined.

The number of farms examined in both north and east Shewa were too few to represent the small holder farms in the country to declare the development and occurrence of anthelmintic resistance. However, conclusions can be made on the prevalence of anthelmintic resistance among the sheep and goat flocks in the smallholders of the two zones. The anthelmintic resistance tests that were carried out at Hawasa University using the local and cross-bred goats showed the occurrence of resistance mainly by *Haemonchus contortus*.

In order to reach a better understanding and positive decision on the current anthelmintic resistance situation in the country, a broader and extensive survey, involving more farms in other regions and at a national level, is crucial. Resistance to albendazole and levamisole should also be confirmed by other tests and observations, such as a controlled study or in vitro testing. This study, however, reports the anthelmintic resistance in nematode parasites of sheep and goats that occurs in selected areas of the country and will be an emerging problem to which attention should be paid.

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