Detection of Anthelmintic Resistance in Gastrointestinal Nematodes of Small Ruminants in Haramaya University Farms

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Abstract: The present study evaluated the status of anthelmintic resistance of gastrointestinal (GI) parasites of small ruminants. The study was conducted from December 2014 to January 2015 in Haramaya University sheep and goat farms. A fecal egg count reduction test (FECRT) was performed in naturally infected sheep and goats. A total of 30 black head Ogaden sheep and 30 Hararghie highland goats of age form 6-18 months not treated in the previous 8 weeks and with a fecal egg counts (FECs) greater than 150 eggs per gram of faeces were selected for the test. Both sheep and goats were grouped into two treatments and one control group (albendazole, ivermectin, and the control). In sheep, the percentage reductions in FECs and the 95% (lower and upper) confidence limit (CL) for albendazole was 82% (95%, CL 60-92), and for ivermectin 68% (95%, CL 0-90). In goats, the percentage reductions in FECs for albendazole was 63% (95%, CL 28-81), and for ivermectin 41% (95%, CL 0-72). The result show that albendazole and ivermectin resistance was detected in nematode parasites of sheep and goats. To overcome the problem, the farm should use anthelmintics only when necessary, employ rotation of anthelmintic every two or three years, use the correct dose of anthelmintics, reduce dependence on anthelmintics and use other management options such as rotational grazing, and adopt strategies to preserve susceptible worms.

Keywords: *Anthelmintic resistance; Sheep and goat; Albendazole; ivermectin, Fecal egg count reduction*

Introduction

Ethiopia has 25,489,204 sheep and 24,060,792 goats' populations (CSA, 2015). The proportion of total annual meat production in Ethiopia from cattle, sheep, and goats was 63%, 25%, and 12%, respectively. At the national level, sheep and goats account for about 90% of the live animal and 92% of skin and hide export trade value (FAO, 2004). In Ethiopia, small ruminant production and productivity is affected by diseases, inadequate nutrition, and poor management system (Addis, 2015). Several studies in different parts of Ethiopia revealed that gastro intestinal (GI) parasites are one of the major problems causing morbidity, production loss, and mortality (Shimelis *et al*., 2011; Sabkeber *et al.,* 2014; Sisay *et al*., 2007). The treatment and control of parasitic helminthes largely depends on the use of few classes of anthelmintics drugs. Consequently, anthelmintic resistance is becoming a serious problem worldwide, especially in developing countries where there is no rational use and variety of anthelmintic drugs (Urquhart *et al*., 1996).

Anthelmintic resistance is a heritable change in the ability of individual parasites to survive the recommended therapeutic dose of anthelmintics (Taylor *et al*., 2002). According to World Association for the Advancement of Veterinary Parasitology (WAAVP), resistance is present if the percentage reduction in egg count is less than 95% and the lower limits of 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected (Coles *et al*., 1992; Coles *et al*., 2006). Anthelmintic resistance in GI nematodes of small ruminant has been reported in different parts of the world, which made it a seriously increasing problem (Wolstenholme *et al*., 2004). Resistance to the major classes of anthelmintics has been recorded in Europe (Coles *et al*., 2004), Asia (Gills, 1993), North America (Uhlinger *et al*., 1992) and Latin America (Echevarria *et al*., 1996). In Ethiopia, anthelmentic resistance in small ruminants has been reported by many researchers from different part of the country (Ayalew *et al.,* 2014; Desie *et al*., 2013; Getachew *et al*., 2013, Sisay *et al*., 2006a; Sisay *et al*., 2006b).

A study conducted by Sissay (2007) on small ruminant helminth parasites in eastern Ethiopia identified more than nine genera of nematode parasites and the use of anthelmintics has been practiced for a long time and constitutes a considerable share of the costs spent by the country in the control of helminthosis (Demelash *et al*., 2006). However, the control of GI nematode parasites of livestock in smallholder farmer and pastoralist communities is done with limited anthelmintic drug and is performed mainly during the rainy seasons (Sisay *et al*., 2007). Drugs are relatively expensive and are often not easily accessible to smallholder farmers and stock owners in pastoralist communities, while frequent and indiscriminate use of different classes of anthelmintics

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has been reported in institutional and large commercial farms (Sissay *et al*., 2006a). Despite the frequent use of anthelmintic drugs in Haramaya University sheep and goat farms, the prevalence of GI nematodes is extremely high (Sabkeber *et al*., 2014). The higher prevalence might be due to anthelmintics drug resistance or problem in the quality of the drugs. Therefore, the objective of this study was to assess if there is anthelmintics drug resistance in the GI nematodes of sheep and goats in Haramaya University farm.

Materials and Methods

Study Area and Duration

The study was conducted in Haramaya University sheep and goat farms from Decmber 2014 to January 2015. The farm is situated at an altitude of 1600 to 2100 meter above sea level, with the mean annual temperature and relative humidity of 18°C and 65%, respectively (Sisay *et al*., 2006a). Geographically, it is located at latitude 09º 24' 10"N and longitude 041º 59' 58" E. There are four seasons in the area; a short rain season (from March to mid-May), a short dry season (from end of May to end of June), a long wet season (early July to mid-October) and a long dry season (end of October to end of February). Haramaya area receives an average annual rain fall of 900 mm, with a bimodal distribution pattern, picking in mid April and mid-August. The vegetation that constitutes the available pasture lands of the university is predominantly native grasses and legumes (HADB, 2009).

Study Animals

The sheep farm consist more than 134 (26 male and 108 female) sheep of different breeds. The flock consists of indigenous black head Ogaden breed (69), Hararghie highland (46), Washera (12), and exotic Dorper pure breed (7). The sheep breeds, except the Dorper, are kept in semi-intensive management system that is they graze the same field with Borena cattle breed and supplemented with concentrate at night. In addition to these, the farm has small paddocks where sheep graze for some time and are housed in separate housing units that accommodate 30-40 sheep per unit. The goat farm has 226 goats (32 male and 194 female) of different breeds and their crosses. Hararghie highland (56), Somali (52), Abargelle (45), Boer (10), Anglo-Nubian (1), and crosses of different breed (30) forms the goat population of the farm at the begging of the study. Hararghie highland, Somali and Abargelle goats are managed in semi-intensive system. All the remaining goat breeds are managed under intensive management. All the different breeds and cross breeds have separate pen. The separate grazing land for goat is also occasionally grazed by cattle, but sheep and goats are almost kept on separate grazing areas with rare cases of grazing the same area one after the other.

The farms have been using anthelmintics to control GI nematodes infection and the most frequently used anthelmintics were albendazole and ivermectin. The treatment frequency is at least twice per year and changed based on the condition of the animals. The conditions used as a justification for deworming were poor appetite, coughing and sneezing, diarrhea and loss of body condition. Sheep and goats that show these clinical signs are treated with the available anthelmintic without confirmatory diagnosis.

Experimental Design

Animals in both farms have identification number and based on their ear tag the animals used for the experiment were selected using simple random sampling technique. Before the actual experiment, equal number of black head Ogaden sheep (40) and Hararghie highland goat (40) were randomly selected considering the dropout during the experiment. Only animals that did not taken anthelmintics for the past 2 months were included in the study. Three gram of fecal materials were collected directly from the rectum by inserting two fingers and placed in universal bottle and labeled with necessary information. The fecal samples were screened in the Veterinary Parasitology laboratory of the University for the presence of nematode eggs within 2 hours after collection. Simple floatation and modified McMaster egg counting technique were used. Only sheep and goats with more than 150 egg per gram (EPG) of faeces were included in the study. The FECRT compares the treatment groups with untreated groups. The efficacies of albendazole and ivermectin were tested and interpreted according to the guideline provided by WAAVP recommendation (Coles *et al*., 1992).

Finally, 60 animals (30 for each species) were selected for the experiment based on the criteria set by WAAVP and assigned randomly to albendazole (10), ivermectin (10) and control (10) groups. Different age groups (6-18 months) and female sheep and goats were used for the study since there were no sufficient male goats in the farms. Individual animals were weighed and the two groups treated according to manufacturers recommended dose orally or subcutaneously depending on the type of drug, but the control groups were not. Albendazole 300mg bolus manufactured by Chengdu Qiankun veterinary pharmaceuticals Co. Ltd, China was used at a dose of 7.5mg/kg, orally. Ivermectin 1% (50ml injection) manufactured by Laboratorios Microsules, Uruguay, was used at a dose of 0.2mg/kg, subcutaneously.

The second rectal sample was taken after 10 days post treatment for albendazole and 14 days for ivermectin with their corresponding control group. The FECs were performed using Modified McMaster counting technique and the changes in the EPG count were recorded with a minimum detection limit of 50 EPG (Cole *et al*., 2006). Furthermore, the EPG was classified as light, moderate and heavy infection for a count of 50 to 799, 800 to 1200 and over 1200, respectively (Urquhart *et al.,* 1996).

Table 1. The degree of infestation of sheep and goats

Degree of Infestation	Number of Goat (%)	Number of Sheep (%)	Total (%)
Light	3 (10%)	10 (33.3%)	13 (21.7)
Moderate	3 (10%)	2 (10%)	5 (8.3)
Heavy	24 (80%)	18 (60%)	42 (70)

Analysis and Interpretation of Data

The effectiveness of albendazole and ivermectin was evaluated on the basis of the reduction in faecal egg count. Calculation of the arithmetic mean, percentage reduction and 95% upper and lower confidence limits was according to Coles *et al.* (1992). A computer program, RESO, Version 2 (Anonymous, 1990) was used for this calculation. Resistance is considered to be present if the percentage reduction in egg count is less than 95% and the 95% confidence level is less than 90%. If only one of the two criteria is met resistance is suspected (Coles *et al.*, 1992).

Results

The percentage reduction of faecal egg counts after treatment with albendazole and Ivermectin was 82% and 68%, respectively (Table 2). The lower confidence limit was within range of resistant parasites for both anthelmintics. The result revealed the development of resistance against albendazole and ivermectin by the GI nematodes of sheep in Haramaya University farm.

Table 2. Mean faecal egg counts and percentage reductions after treatment of sheep with Albendazole and Ivermectin

FECRT summary results	Treatment groups		
	Albendazole	Ivermectin	control
Number of animals (n)	10	10	10
Pre-treatment mean EPG	1640	1645	1370
Post-treatment mean EPG	180	465	1460
Reduction (%)	82	68	-
Upper 95% CI (%)	92	90	-
Lower 95% CI (%)	60	0	-
Interpretation	resistant	resistant	-

EPG= egg per gram; Mean EPG = arithmetic mean of faecal nematode egg counts; Control = Untreated group of animals, CI= confidence interval.

The percentage reduction of faecal egg counts after treatment with albendazole and Ivermectin was 63% and 41%, respectively (Table 3). The lower confidence limit was within range of resistant parasites for both anthelmintics. The result revealed the development of resistance against albendazole and ivermectin by the gastrointestinal nematodes of goats in Haramaya University farm.

Table 3. Mean faecal egg counts and percentage reductions after treatment of goats with Albendazole and Ivermectin

FECRT summary results	Treatment groups		
	Albendazole	Ivermectin	Control
Number of animals (n)	10	10	10
Pre-treatment mean EPG	3100	3455	1685
Post-treatment mean EPG	595	1220	2065
Reduction (%)	63	41	-
Upper 95% CI (%)	81	72	-
Lower 95% CI (%)	29	0	-
Interpretation	resistant	resistant	-

Mean EPG = arithmetic mean of faecal nematode egg counts; Control = Untreated group of animals, CI= confidence interval.

Discussion

The results obtained from the FECRT and 95% confidence limits indicated the presence of resistance to albendazole by the GI nematodes in Haramaya University sheep farm. The current finding was comparable with reports from Western Oromia for albendazole with 98% percentage reduction, 95% upper confidence limit (UCL) 100% and lower confidence limit (LCL) 86% (Aga *et al.,* 2013). Similar result was reported for albendazole against GI nematodes of sheep from Southern Ethiopia by Desie *et al.* (2013) with percentage reduction of 95%, 95%UCL (98.2%) and LCL (86.5%). However, the current finding of GI nematode resistance test against albendazole was contradictory with the reports from Wolaita Soddo by Desie and Amenu *et al.* (2010) with percentage reduction of 100%, Bersissa and Ajebu (2008) from Hawassa with percent reduction of 100%, Getachew *et al.* (2013) from Bedelle with percentage reduction of 95.6%, 95% UCL (97.6%) and LCL (93.6%). Similarly, Kassahun *et al.* (2005) from Southern Ethiopia reported with percentage reduction of 100% using albendazole against GI nematode in sheep, and Sissay *et al.* (2006b) in experiment done in eastern Ethiopia done in different peasant association with percentage reduction 95% and above, and 95% UCL (≥96%) and LCL (≥91%).

The result obtained from the FECRT and the 95% confidence limits showed the presence of resistance to Ivermectin by the GI nematodes in Haramaya University sheep farm. Ivermectin resistance is not common in GI nematodes of sheep in Ethiopia. However, in other countries, resistance againest GI nematodes of sheep were reported. A study conducted on 25 lambs in New Zealand showed a reduction of the FECs only by 18% which was an indication for the emergence of Ivermectin resistance by the GI nematodes of sheep (Leathwick *et al.*, 2001). However, many reports from Ethiopia showed the effectiveness of ivermectin against GI nematode of sheep. Sissay *et al.* (2006a) from Eastern Ethiopia reported a percentage reduction of (98%), 95% UCL (99%) and LCL (95%), Desie *et al.* (2013) from Southern Ethiopia, with percentage reduction of (96.7%), 95% UCL (100%) and LCL (91%), and Getachew *et al.* (2013) from western Oromia, with percentage reduction of (96.7%), 95% UCL (98.8%) and LCL (94.5%) confirmed the effectiveness of this drug.

The result obtained from the FECRT and the 95% confidence limits showed the presence of resistance to Albendazole by the GI nematodes in Haramaya University goat farm. Similar findings were reported by Sissay *et al.* (2006a) from the same farm (Haramaya University goat farm) before ten years with percentage reduction of (57%), 95% UCL (63%) and LCL (49%) which confirmed the persistence and heritability of resistant genes in nematodes both in the host and in the refugia population for more than a decade. Desie *et al.* (2013) from Southern Ethiopia reported suspected resistance to albendazole against GI nematodes of goats with percentage reduction of (99.6%), 95% UCL (99%) and LCL (88.3%). On the other hand, Sissay *et al.* (2006b) from Eastern Ethiopia (Haramaya district small holder's goat flock) reported the effectiveness of albendazole with percentage reduction of (95%), 95% UCL (97%) and LCL (94%). This might be due to difference in the study area (different district), both the parasite population in the host and the refugia are different.

The present finding obtained from the FECRT and 95% confidence limits indicated the presence of resistance to Ivermectin by the GIT nematodes in Haramaya University goat farm. Similar result was reported by Sissay *et al.* (2006a) from the same farm before a decade with percentage reduction of 67%, 95% UCL (73%) and 95% LCL (60%). This might indicate the transfer of resistance genes vertically within generations for several years and its persistence after development in an area. On the other hand, many research findings were contradictory to the current finding of GI nematode resistance against ivermectin in Haramaya University goat farm. A study conducted by Sissay *et al.* (2006b) on goats in Eastern Ethiopia against GI nematodes indicated a percentage reduction of 96%, 95% UCL (97%) and 95% LCL (93%). Similarly, Desie *et al.* (2013) from southern Ethiopia reported a percentage reduction of 97.1%, 95% UCL (99.1%) and 95% LCL (99.0%).

The present finding confirmed the development of resistance for albendazole and ivermectin by GI nematodes of sheep and goats in Haramaya University farms. Different factors might contribute for the development of anthelmintics resistance in the farm such as the treatment frequency, inaccurate dosage determination, and indiscriminate use of anthelmintic, treatment without confirmatory diagnosis and management system (Suarez and Cristel, 2014). Besides these, the substandard drug quality might be the other probable reason for the ineffectiveness of the drug which needs to be investigated in the future in order to be sure about the cause for the development of resistance in both species.

In the majority of cases treatment frequencies in Haramaya University farms were influenced by clinical conditions of animals such as poor body conditions, coughing, sneezing, diarrhoea, emaciation and poor appetite. Indiscriminate use of anthelmintic will favor the selection pressure in which the susceptible population will extinct in the area whereas the resistant population will dominate both in the host and refugia.

The other contributing factor for the development of resistance in the farms were animal weight estimation by guessing to calculate the dosage which might accounted for error such as under or overdosing. Under dosing has been repeatedly blamed for the buildup of resistance which permits the survival of resistant heterozygous individuals and increases their chances of producing highly resistant parasites (Vadlejch *et al.*, 2014). Another factor that could explain the high levels of resistance in the farms were the use of the same drugs for many years in the same area. Frequent and longstanding use of the same anthelmintic is among the means to efficiently select one sub population of worms that presents the capability to survive for a particular type of drug. On the contrary, alternate use of the anthelmintic family reduces the effect of the selection pressure exerted by each type of drug and resistance development (Vadlejch *et al.*, 2014).

The management system in the farms is generally poor, the same grazing land was used before and after anthelmintic treatment which predisposed animals for the re-infection and contamination of the environment with surviving resistant strains. The gaps of the current study were inability to perform copro-culture before and after treatment, and lack to identify which species of nematodes developed resistance.

Moreover, the efficacy of the starting materials (drugs) is not known, hence it is not possible to directly declare that there is resistance; in previous reports, there was an evidence that dosage regimen for goats should be higher than sheep and manufacturers recommended doses usually are said not to be working. Hence different dose regimens should have been used in goats.

Conclusion and Recommendations

The growing resistance of GI nematodes of sheep and goats, to available veterinary anthelmintics, is a serious problem and a global issue. Our study findings indicated that GI nematodes of sheep and goats, in Haramaya University farm, might be resistant to albendazole and ivermectin. Based on literature and observations during the study period, it can be recommended that the farm should refrain from using frequent and unnecessary treatments, implement alternative use of anthelmintics such as a change of drugs every two/three years, reduce dependence on anthelmintics by employing management practices such as rotational grazing, improve the management of the farms and implement correct dosing of anthelmintics. Future researches should focus on the identification of resistant species by copro-culture before and after treatment, and perform *in vitro* egg hatchability assay using known standard drugs.

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Conflict of Interests

The authors declare that they have no competing interests.

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