Anthelmintic Drugs Resistance of Gastrointestinal Nematodes of Naturally Infected Goats in Haramaya, Ethiopia

Anteneh Wondimu (anteneha7@gmail.com)
Haramaya University https://orcid.org/0000-0002-4850-0181
Yehualashet Bayu
Haramaya University

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Abstract

Background: The occurrence of anthelmintic resistance to commonly used drugs is becoming worldwide constrain in livestock production. Considering the narrow range of available drugs and slow rate of new drug development, anthelmintic resistance presents an alarming global threat demanding vigilant monitoring and management. It is likely that anthelmintic resistance of gastrointestinal goats present in Ethiopia, but little information regarding the prevalence and species of nematode resistance to drugs is available. Current study done with objective to assess anthelmintic resistance of gastrointestinal nematodes of goats to commonly used anthelmintic drugs.

Results: The result of the FECRT % and lower 95% confidence limit reported the presence of anthelmintic resistance for tested drugs; albendazole (41.5%, 36.9), tetraclozan (69.5%, 66.1), tetramisole (91.1%, 87.4) and ivermectin (43%, 38.2) respectively. Trichostrongylus spp, Teladorsagia spp and Haemonchus spp showed anthelmintic resistance for all tested drugs. Coproculture from different treated group revealed of Trichostrongylus spp (69.2% in ivermectin and 59.6% in albendazole) was the predominant nematode followed by Teladorsagia spp (21.9% in albendazole and 14.7% in ivermectin). In tetraclozan treatment group, Trichostrongylus spp (42%) and Teladorsagia spp (41.3%) were comparable, followed by Haemonchus spp (13%). In group treated with tetramisole, Teladorsagia spp (54.3%) was the major nematode detected followed by Trichostrongylus spp (25.7%) and Haemonchus spp (11.4%).

Conclusions: The study demonstrated the presence of multi-drug resistant nematodes mainly Trichostrongylus spp, Teladorsagia spp and Haemonchus spp. Control strategies including management practices of pasture rotation, supplementary feeding and encouraging traditional nematode control methods. Further studies covering wider areas of Ethiopia and mechanism of nematode resistance need to be studied in future.

Background

Gastrointestinal (GI) nematodes are worldwide problem which reduces production of livestock in many countries. The impact is more greater in sub-Saharan Africa including in Ethiopia due to the availability of a wide range of agro-ecological factors suitable for diversified host and parasite species (1, 2). The severity of helminthes parasites vary considerably depending on prevalence, genera, species involved and local environmental, such as humidity, temperature and rainfall (3).

Several scholars confirmed a widespread prevalence of small ruminant nematodes in different parts of Ethiopia. For example, 69.01% of small ruminants harbor one or more genera of nematodes (4). Study in eastern part of Ethiopia stated the prevalence of

nematodes in sheep and goats with *Haemonchus contortus* being the most prevalent followed by *Trichostrongylus* spp (5). Other study in south west Ethiopia Kaffa reported that 54.1 % of small ruminants were positive for GI parasites eggs (6).

Resistance of GI parasites to currently available anthelmintics has been occurred worldwide. Different researchers confirmed the occurrence of anthelmintic resistance (AR) to commonly used drugs and the problem associated with development of anthelmintic resistant parasites is becoming a major worldwide constrain in livestock production and hence need to detect and monitor resistance nematodes (7-9).

In Ethiopia, anthelmintic drugs commonly, used for management of livestock GI nematodes, fall under three families, including; Bezimidazoles (albendazole and triclabendazole), imidazothiazoles (tetramisole and levamisole) and macrocyclic lactones (ivermectin). Misuse of these anthelmintic drugs has failed to decrease livestock GI parasite infestation; but instead, led to the development of anthelmintic resistant nematodes in different agro-ecological zones of Ethiopia (10-12). Failure to identify anthelmintic resistant parasite will also incur severe production consequences due to the impact of parasitic gastroenteritis (13).

Evidence of anthelmintic failure indicates the likelihood of sub-optimal worm control and hence it is important to understand the geographic spread and severity of resistance for appropriate nematode control. Moreover considering the narrow range of available drugs and slow rate of new drug development, AR presents an alarming global threat demanding vigilant monitoring and management. It is therefore considered likely that AR of GI nematodes of goats present in Ethiopia, but little information regarding the prevalence and species of nematode resistance to drugs is available. Therefore the objective of this paper was to assess anthelmintic resistance of GI nematodes of goats to commonly used anthelmintic drugs.

Methods

Study Area

The study was conducted in Haramaya district, which is located approximately 510 km east of Addis Ababa capital of Ethiopia. The estimated animal population is about 63,723 cattle, 79,950 sheep, and 120,350 goats. Topographically, it is situated at altitude of 1600 to 2100 m above sea level with the mean annual temperature and relative humidity of 18°C and 65%, respectively and the surrounding farming areas are semi-arid. Haramaya, it is located 041º 59' 58" N latitude and 09º 24' 10"S longitudes.

Study Animals

Apparently health goats maintained in Haramaya University (HU) goat farm were used to identify the GI nematodes of goats and anthelmintic resistance (AR) to commonly used anthelmintic drugs. HU goat flock of approximately 150 heads of different breeds; Abergel, Somale, Cross breed and Hararghe highlander, either sexes, different age, and weight groups was used. Each goat was individually marked with ear tag before the start of experiment. The goats allowed grazing pasture at Haramaya University pasture during the day time. In addition supplement hay and concentrate provided during the dry season. Approximately 20 to 30 goats are bedding in group for night shade and house cleaning is done every day during the morning after goats released for grazing. Sick goats and goats with newborn separated for especial treatments. Since experimental parameters don’t affect the health and production parameters of goats the study goats after experimental work rejoined the remaining groups of goats and continues to use for production at Haramaya University. All considered factors/variable evaluated based on criteria of ARRIVE guideline.

Determination of EPG

Around 15g of faecal samples were collected directly from the rectum of each goat, early morning before sent out for grazing; before and after treatment of anthelmintic drug. Samples were placed in labeled polythene bags and immediately transported to the Haramaya University Animal Science Parasitology Laboratory for examination. Faecal samples were examined for helminth eggs using modified McMaster technique with

saturated sodium chloride solution as the floating medium (14, 15). In each case, 3 g of faeces were mixed in 42 ml of saturated salt solution, and the number of nematode eggs per gram of faces (EPG) was obtained by multiplying the number of nematode eggs counted in two squares of the McMaster slide by a dilution factor of 50. The parasitic burden of the animals was evaluated based on EPG and goats with EPG count greater than 150 counts were included in the research then grouped randomly in to five treatment groups then four group received drug treatments while one group left untreated control in the same day of laboratory analysis. At ten day intervals faecal samples was collected from the goats to determine the concentrations of the parasite eggs (EPG) as described above and anthelmintic resistance was evaluated based on the standard guideline.

Faecal Culture and Larval Recovery

The unexploited faecal materials after EPG determination was incubated at room temperature (approximately 22–27°C) for 10 days, subjected to Baermann technique. Centrifuge tubes were filled to two thirds and spin at 1500rpm for 5 minutes in an electrically powered centrifuge to concentrate the larvae to the bottom (15). The larvae was recovered and pooled together after decantation of the supernatant fluid. A drop of the lugol's iodine added to kill recovered larvae, then a drop of sample placed in microscope slide and cover-slip applied, then the nematode larvae were identified to genera level and quantified according. Where possible, 100 larvae were identified and counted for each experimental group of animals (16).

Experimental Design

The study goats were divided into five treatment groups; four treated and one untreated control groups; ideally 20 goats were included in each group and naturally exposed to GI nematodes. Each goat's weight measured, in order to administer the correct dose of the anthelmintic drug and the drug given to animals based on the manufacturer recommendation. On day 0, fecal samples collected from each goat enrolled in the study,

and then the goats are either treated with an anthelmintic or left untreated. Subsequently, faecal samples collected 10days after treatment. Use of larval cultures in pre and post treatment samples helps to determine specific nematode genera involved, in order to identify AR within specific parasitic genera. AR was assessed based on FECRT% explained by Coles *et al.* (17) in which AR is occur when FECRT% is less than 95% and the lower 95% confidence level is lower than 90%. If only one of these fulfilled there was suspected AR. The sources of anthelmintic drugs were Chongoing Fangton Animal Pharmaceutical Co.LTD China (albendazole) from, Ashish Life Science Pvt Limited India (tetramisole, and tetraclozan) and Shenyang sunvictor pharmaceutical co.ltd./China (ivermectin).

Dosage was given as per manufacturer recommendations and considering the heaviest weight of goat in each group. Albendazole tetramisole and tetraclozan given at the dosage rate of 7.5 mg/kg body weight orally while ivermectin injected subcutaneously at the dosage rate of 200 µg/kg body weight. All considered variable meet criteria of ARRIVE guideline.

Fecal Egg Count Reduction Test (FECRT)

FECRT was done based on reduction of the concentration of eggs per gram of faeces (EPG) by more than 95 percent; measured ten days after treatment, in comparison with the EPG measured at the time of treatment and failure to do so was indicative of resistance. The EPGs count of goats in the pre-treatment group exceed 150 to 200 included in the research (17). The distribution of parasites within the experimental groups before administration of drugs done by determination of the mean level of egg excretion across animals (mean pre-drug administration (preDA) FEC) then after the administration of the drug (postDA FEC) as described above for the preDA FEC. Subsequently, the FECRT was calculated using calculation as described by Coles *et al.* (18) method.

FECRT (%) = 100 (1-[T_2/ C_2]).

Where

T2 arithmetic mean FEC after treatment,

C_2 arithmetic mean FEC control group at day 10.

Approximate 95 % confidence interval for R 100 = [(1-(Xt/Xc)) exp (± 2.1√v)]

Variance of reduction (on log scale v=[(s2 t /(ntX2t))]+[(s2 c /(ncX2c))])

Upper confidence limit 100[1-(Xt/Xc)exp(-2.1√v)]

Lower confidence limit 100[1-(Xt/Xc)exp(+2.1√v)]

Where Xt is the mean egg count of the treated group at 10days and Xc is that of the control group at 10days.

Based on Coles *et al.,* (18) method resistance to an anthelmintic was considered to be present if the percentage reduction in egg count was less than 95%, and the lower 95% confidence limit is less than 90. But as most natural infections include a mixture of species, therefore third stage larvae cultured from pretreatment and post treatments groups was separately estimated to identify resistant nematode (19).

Data Analysis

The efficacy of anthelmintics was evaluated based on the reduction in faecal egg count and percentage of larvae found in the cultures. Calculation of the arithmetic mean, percentage of reduction and 95% upper and lower confidence limits were conducted according to the procedures described by Coles *et al*. (18). Resistance was declared when the percentage of reduction was less than 95% and the 95% lower confidence limit was less than 90%. If only one of the two criteria was met, resistance was suspected.

Results

The result of EPG count showed the minimum and maximum number of EPG count in pretreatment 200 to 4600. Mean FEC before treatment for different drugs was from lower 690.5 to higher 1295.5 and the mean FEC post treatment ranges from 36.4 to 240.6. The result revealed FECRT% (41.5%, 69.5%, 91.1%, and 43%) and lower 95% confidence limit (36.9, 66.1, 87.4, and 38.2) for albendazole, tetraclozan, tetramisole and ivermectin respectively. This figure showed FECRT% less than 95% and the lower 95% confidence level lower than 90% which demonstrated the presence of anthelmintic resistance (Table 1).

The dominant nematodes identified in pre-treatment groups showed 66.2%, 57.6%, 56.4%, and 39.4% of *Trichostrongylus* spp in ivermectin, albendazole, tetramisole and tetraclozan treatment group respectively (Table 2). The result after treatment showed *Trichostrongylus* spp (69.2% in ivermectin and 59.6% in albendazole) as the predominant nematode followed by *Teladorsagia* spp (21.9% in albendazole and 14.7% in ivermectin). In tetraclozan treated group 42% of *Trichostrongylus* spp and 41.3% of *Teladorsagia* spp were comparable followed by 13% of *Haemonchus* spp. Though FECRT revealed AR was recorded to tetramisole, relatively low number of parasitic count was seen than other drugs. Predominant nematode identified were *Teladorsagia* spp 54.3% and *Trichostrongylus* spp 25.7% (Table 3).

Table 1. Anthelmintic resistance test based on percentages faecal egg count reduction test (FECRT%) according to Coles *et al.,* [18] methods

Drug	Ivermectin	Albendazole	Tetramisole	Tetraclozan	Control
No. of animals	20	20	20	20	20
Min & Max EPG before Rx	200-4000	200-2200	200-4600	300-2200	50-1900
Min & Max EPG after Rx	0-1050	0-1000	0-150	0-700	100-1000
MpreDA FEC(±SEM)	690.5 (±175.8)	781.2 (±172.7)	1295.5 (±492.9)	900 (±160.9)	850 (±343.4)
MpostDA FEC(±SEM)	230.9 (±130.6)	240.6 (±87.5)	36.4[a] (±15.2)	125[a] (±52.3)	800 (±89.5)
FECRT (%)	43	41.5	91.1	69.5	
95% CL	38.2-46.8	36.9-46.1	87.4-94.8	66.1-73.9	
Interpretation	Resistance	Resistance	Resistance	Resistance	

[a] Statistically different from EPG of control group day 10 ($p < 0.05$).
Mpre DA FEC: mean pre-drug administration FEC, MpostDA: mean post drug administration FEC, FEC: Fecal Egg Count, FECRT: fecal egg count reduction test, Min: minimum egg count, Max: maximum egg count, EPG: Egg per Gram of feces, CL: Confidence Level, SEM: standard error of mean

Table 2. The percentage of nematode infective larval identified based on the morphology of larval (L3) from coproculture before administration of anthelmintic drugs.

Drug	Nematode larval (L3)								
	Hae [a]	Tri [a]	Cha [a]	Oes [a]	Nem [a]	Str [a]	Tel [a]	Muc [a]	Coo [a]
Ivermectin	15.5	66.2	0	1.4	0.7	0	9.1	7	0
Albendazole	17.4	57.6	0	0	5.4	1	18.5	0	0
Tetramisole	8.3	56.4	0	2.5	1.9	2.5	18.6	6.4	3.2
Tetraclozan	17.8	39.4	2	2	0	0	24.6	14.4	0

[a] values in pre-treatments are percentages of nematode L3 larvae composition out of the 100 larvae counts.
Tri: *Trichostrongylus* spp Hae: *Haemonchus* spp, Cha: *Chabertia* spp, Oes: *Oesophagostomu* spp Nem:*Nematodirus* spp, Str: *Strongyloides* spp, Tel: *Teladorsagia* spp, Mc: *Muellerius capillaris*, Coo: *Cooperia*

Table. 3 The percentage of different nematode infective larval identified based on the morphology of larval (L3) from coproculture of goats after administration of anthelmintic drugs.

Drug	Nematode larval (L3)								
	Hae[a]	Tri[a]	Cha[a]	Oes[a]	Nem[a]	Str[a]	Tel[a]	Muc[a]	Coo[a]
Ivermectin	4.9	69.2	0	0	0	0	14.7	2.8	8.4
Albendazole	10.5	59.6	0	0	1.7	0	21.9	5.2	0.9
Tetramisole	11.4	25.7	0	2.9	0	0	54.3	2.9	2.9
Tetraclozan	13	42	0	0	0	0	41.3	3.6	0

[a] values in post-treatments are percentages of nematode L3 larvae composition out of the 100 larvae counts.
Tri: *Trichostrongylus* spp, Hae: *Haemonchus* spp, Cha: *Chabertia* spp, Oes: *Oesophagostomu* spp Nem: *Nematodirus* spp, Str: *Strongyloides* spp, Tel: *Teladorsagina* spp, Muc: *Muellerius capillaris*, Coo: *Cooperia* spp

Discussion

Anthelmintic resistance (AR) has been global issue in small ruminant industry during past few decades (20). Many parasites of veterinary importance have genetic feature that favor the development of AR. Resistance by different species of nematodes to all major groups of anthelmintic drugs has been reported worldwide (8, 9, 21).

The present study also revealed the presence of multi-drug resistance by different GI nematode spp predominantly by *Trichostrongylus, Teladorsagia* and *Haemonchus* spp. Comparable findings were reported in different parts of the world. In Uganda 58%, 52% and 38% AR prevalence in goat farms were detected for ivermectin, levamisole and albendazole respectively (22). According to study by Crook *et al.,* (23), *Haemonchus contortus, Trichostrongylus colubriformis* and *Teladorsagia cirumcincta* developed resistance against levamisole and oxfendazole. Multi drug resistance was also recorded by *Haemonchus contortus* in sheep and goat. In addition, AR of *Teladorsagia/Trichostrongylus* against Benzimidazole was found in Norway goats (24).

Factor like illegal marketing of drugs by non-animal health professional and purchasing of drugs by self-experience possibly allow inappropriate dosing or misuse that ends with survival of heterozygous resistant nematode (25, 26). In addition, species difference is also

associated with AR and goats have a higher metabolic rate and require higher dose rates of drugs than sheep; this may explain the fact that AR is of greater concern in goats than in sheep (18, 24, 27).

The current study reported the development of AR to all tested drugs (tetramisole, albendazole, tetraclozan and ivermectin). Likewise researchers from different parts of the world reported the occurrence of AR to this class of drugs. For example, study from India stated that most of the GI nematodes were found to have some degree of resistance against albendazole, levamisole and ivermectin used in goats (28). Another study in Italia revealed 40% and 20% of the goats flocks had resistant GI nematodes for benzimidazoles and ivermectin respectively (29). In addition, Adediran and Uwalaka, (30) reported that GI nematode of goat showed low resistance to ivermectin and levamisole but susceptibility to albendazole. Similar study in the same study area and species of animal conducted by Sissay *et al.* (19) reported contrary result i.e FECRT% of tetramisole, albendazole and ivermectin showed high level of efficacy. The difference may explain the development of drug resistant parasitic nematode through time.

In the present study *Trichostrongylus* spp were the dominant nematodes developed resistance for albendazole and ivermectin followed by *Teladorsagia* and *Haemonchus* spp*,* while *Teladorsagia* spp were the dominant nematode developed resistance for tetramisole followed by *Trichostrongylus* and *Haemonchus* spp; but in case of tetraclozan, *Trichostrongylus* and *Teladorsagia* spp showed almost equal percentage of resistance (Table 3). Comparable finding was recorded elsewhere in Ethiopia reported by Aga *et al.,* (31) who stated a suspected resistance against albendazole by *Haemonchus contortus* and *Trichostrongylus* spp but contrary to this study result albendazole and teramisole were found to possess a 100% efficacy against Ogaden isolate of *Haemonchus contortus* (32); tetraclozan and ivermectin also demonstrated high efficacy against all nematode genera isolated on the farms (31). Other study conducted by Sheferaw *et al.,* (12) indicated that among the drugs used for treatments of nematodes, resistance to albendazole was suspected. Bersissa *et al.,* (33) also recorded that albendazole and ivermectin was effective

treatment for *Trichuris* and *Strongyle* spp but teramisole showed low efficacy, which partly agreed with low efficacy of tetramisole but disagreed with 100% efficacy of albendazole with the current report.

FECRT of the four drugs showed the presence of AR to albendazole, tetraclozan. tetramisole and ivermectin. The development of variable degrees of resistance among different species of GI nematodes has been reported for all the major groups of anthelmintic drugs by different scholars (30, 34, 35) which calls the attention to AR and inculcates other possible nematode control methods which include management practices.

Conclusion

The current study demonstrated the presence of multi-drug resistant parasitic nematodes against albendazole, tetraclozan, ivermectin and tetramisole, of which *Trichostrongylus* spp, *Teladorsagia* spp and *Haemonchus* spp were commonly, identified genera on post-treatment culture. Therefore, an appropriate nematode control strategies including management practices of pasture rotation, supplementary feeding, treating goats at times of the year when the majority of the parasite population is in the host, treatment of the whole goats instead of only the animals with the highest parasite burden, and encouraging traditional nematode control methods. Moreover further studies covering wider areas of Ethiopia, selecting genetically resistant goats and on mechanism of nematode resistance need to be studded in future.

Declarations

ARRIVE guidelines

Methods section of the manuscript fulfill the ARRIVE guidelines particularly study animals, and experimental design part meets the criteria of the checklist for the reporting of animal experiments.

Abbreviations

AR (Anthelmintic Resistance), FEC (Fecal Egg Count), FECRT (Fecal Egg Count Reduction Test), EPG (Egg per Gram of Feces), GI (Gastro Intestine)

Ethics approval and consent to participate

Haramaya University don’t have ethical committee on animal related research due to this we can’t give you the ethical approval statement and committee’s reference number. But we followed all the relevant international and national guidelines for the care and use of animals.

Consent for publication

Not Applicable

Data availability and material

Data supporting the finding available from corresponding author upon reasonable request.

Competing interests

The authors agreed and don’t have any financial and non-financial competing interests.

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Authors' contributions

All authors have read and approved the manuscript for publication.

AW contributed data collection, laboratory analysis and paper write up. YB contributed laboratory analysis, data analysis and write up.

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