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Efficacy of two anthelmintics against gastrointestinal nematodes in naturally infected goats in a pastoral Karamoja region, Uganda

C. Byaruhanga[1], G. Egayu[1] and S. Olinga[1]

[1]Nabuin Zonal Agricultural Research and Development Institute, National Agricultural Research Organization, P. O. Box 132 Moroto, Uganda
Author for correspondence: cbyaruhanga27@yahoo.com

Abstract

A study was conducted to determine the efficacy of albendazole (ABZ) and ivermectin (IVM) against gastrointestinal nematodes (GIN) in naturally infected goats in the pastoral region of Karamoja, Uganda. Fifty four (54) small East African goats (female = 36, male = 18), of 4-6 months and from 18 flocks, were allocated to treatment groups (albendazole, ivermectin and untreated control), by a randomized complete block design. Each group included 18 goats and treatments were administered according to weight of each goat, with 5 Mg/Mg bw albendazole and 0.2Mg/Kg bw ivermectin. Fecal egg counts (FEC), expressed as eggs per gram and larval cultures were done on day zero before treatment and on day 13 post-treatment. Anthelmintic efficacy was determined by the Fecal Egg Count Reduction Test (FECRT). ABZ and IVM reduced FEC by 81.03 and 96.55%, respectively. The results indicated that nematode resistance was present in the ABZ group. Coproculture results following albendazole treatment showed that only *Haemonchus* spp. survived treatment. Information from 60 pastoralists indicated that reduced efficacy of the drugs could be due to prolonged and frequent use of the same anthelmintic and under dosing. Steps should be taken to limit the spread of resistant nematodes and to restore anthelmintic efficacy.

Key words: Anthelmintic resistance, albendazole, goats, ivermectin, pastoralists

Introduction

Gastrointestinal nematodes are a major factor that affects goat production and productivity (Lapenga and Rubaire-Akiiki, 2009). Anthelmintic treatment is the most common way of controlling nematode infections in ruminants in many countries (Waller, 2006; Domke *et al.*, 2011).

However, anthelmintic resistance has been reported worldwide, representing a limitation for sustainable small ruminant production (Kumsa *et al.*, 2010; Domke *et al.*, 2012; Rialch *et al.*, 2013). In Africa, anthelmintic resistance has been reported in a number of countries, including Kenya (Waruiru *et al.*, 2003), Ethiopia (Kumsa *et al.*, 2010) and South Africa (Tsotetsi *et al.*, 2013).

In Uganda, anthelmintic resistance was reported in goats at a Research Institute (Byaruhanga and Okwee-Acai, 2013), due to prolonged and frequent use of the same anthelmintic, administration of anthelmintics to all goats irrespective of their infection levels and grazing goats on the same traditional pastures for a long time. Sustainable helminth control

practices should rely on factors including prevention of resistance and preservation of anthelmintic effectiveness.

In sub-Saharan African, most rangelands are inhabited by pastoralists and agro-pastoralists that live on the edge of disaster and are always amidst of poverty (Teer, 1986). Because they live in climatically marginalized environments, mobile pastoralists depend on livestock as the only economic activity to sustain livelihoods (Lengoiboni *et al.*, 2010; Kipronoh *et al.*, 2011). In such areas, veterinary services are scarce; as a result, the pastoralists more often than not, control diseases using inappropriate methods (Anderson and Robinson, 2009). Information on the state of efficacy of anthelmintics in transhumant pastoral regions of Africa is scanty. Information from such areas would be useful to design and implement appropriate control strategies, thereby preventing the further development and spread of resistant worms (Kaplan, 2006).

The present study was, therefore, conducted to establish the efficacy of albendazole and ivermectin in naturally infected goats in the semi-arid pastoral region of Karamoja, Uganda. It also aimed to obtain information on the current practices by pastoralists to control helminths in goats.

Materials and methods

Study area

The current study was conducted in Nakapiripirit district in the semi-arid Karamoja sub-region, located in northeastern Uganda, from July to October 2012. The study area is located at 1,458 m, 2p 02' N and 34p 34' E. The rainy season is from March to September with an annual average of 500-800 mm. It is typical for a short period of dryness to occur during the rainy season, especially in the months of June and July (Ondoga *et al.*, 2010). Living conditions and quality of life of the people in the study area are low, due to factors including, harsh weather and insecurity and marginalization. People in the region depend highly on livestock for their livelihoods (Anderson and Robinson, 2009).

Experimental animals and study design

The study animals were selected from the settlement areas known as 'Manyattas'. In a 'Manyatta,' there are variable numbers of households. 'Manyattas' selected for the study were those that were easily accessible. One household was selected randomly in each 'Manyatta', with the help of the local extension workers and community leaders. A total of 54 small East African goats (female = 36, male = 18), from 18 'Manyattas' were selected for the study. From each household, four goats of same sex, aged 4-6 months and of uniform size and weight were selected by systematic random sampling. The age of the goats was determined from owners' memory. The goats were communally grazed on rangelands, with other animals-cattle, sheep and donkeys. Unlike cattle and donkeys, which were seasonally moved to far locations in such of pastures and water, the small ruminants were grazed in the vicinity of 'Manyattas', throughout the year.

Randomized complete block design (Gomez and Gomez, 1984) was employed for this field experimental study. Goats were blocked by household, and, from each block, the animals were randomly allocated to three groups, before treatment

on day zero (0). Each treatment group included 18 goats. Each goat was then ear tagged for identification.

The three groups were as follows: group 1, untreated control; group 2, albendazole liquid suspension (Wormita® -Cosmos Limited, Nairobi, Kenya; 5 Mg/ Kg body weight); group 3, ivermectin injectable (Kelamectin®-KELA N.V., 0.2 Mg/Kg body weight). Treatments were administered according to the weight of each goat with doses recommended by the manufacturers. The weight of each goat was determined by means of a girth tape. None of the goats received any anthelmintic treatment at least one month before the start of the experiment. Albendazole was administered orally using calibrated syringes whereas ivermectin was administered via the subcutaneous injection route with calibrated syringes and needles. A pre-treatment fasting of 12 hours was instituted to promote effectiveness of the anthelmintics administered. Fecal egg counts (FEC) expressed as eggs per gram (epg) were done on day 0 before treatment and then 13 days after treatment with anthelmintics. Pooled fecal samples were also cultured for respective groups for larvae identification before and after treatment.

Fecal collection and examination

Rectal fecal samples were collected from each goat in labeled plastic bags, on day zero before treatment and again on day 13 after treatments. The bag was tightened as close to the feces as possible to keep off air. The samples were kept in a cool box containing ice packs and examined in the laboratory of Veterinary Parasitology at Makerere University within 12 h of collection. The samples were examined for GIN eggs using saturated salt solution with a 1.2 specific gravity as flotation solution. Identification of nematode eggs was done as described by Soulsby (1982).

A FEC of strongyle type nematodes, expressed as eggs per gram (epg) of feces, was performed for each sample using the modified McMaster technique (MAFF, 1971). The detection level of the McMaster method used was 50 epg.

The degree of infection by strongyle type nematodes of the study goats was categorized as light (50-800), moderate (801-1200) and heavy (>1200) based on the epg record from pre-treatment fecal samples, as described by Hansen and Perry (1994).

Fecal egg count reduction test (FECRT)

Arithmetic means of pre and post-treatment FECs were used to calculate the percentage efficacy of each anthelmintic using a formula as described by Coles *et al.* (1992): FECR% =100 (1-T_2/C_2) where T_2 and C_2 are arithmetic mean epg in the treated and untreated groups, respectively at day 13 post treatment. Efficacy of each anthelmintic was tested and interpreted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for efficacy evaluations of anthelmintics (Coles *et al.*, 1992). Reduction in efficacy and presence of anthelmintic resistance is considered to exist if the FECRT percentage of an anthelmintic treatment is < 95% and the lower confidence limit for the reductions is <90% (Coles *et al.*, 1992). If only one of the two criteria is met, reduction in efficacy is suspected.

Third stage larvae identification

For specific identification of nematode genera, about four grams of rectal fecal

samples from each goat were pooled for each group. The pooled samples were incubated at 27p C for seven days before and after treatment. The infective larvae (L3) were isolated from the feces with a Baermann apparatus (Hansen and Perry, 1994), differentiated to the generic level, and counted under a compound microscope (at magnification x 250) using morphological keys given by MAFF, (1971) and Wyk *et al.* (2004).

Questionnaire survey

A total of 60 pastoralists, who owned 40-100 goats, were interviewed using a structured questionnaire with a combination of qualitative and quantitative, open-ended questions. Information on grazing system, housing, widely used anthelmintics, treatment methods, selection criteria for anthelmintics, application interval, methods of dosage administration, rotation of anthelmintic family and stocking dynamics was collected and analyzed. The study was conducted by competent teams; at last one member kept a record of the discussions, while the second asked most of the questions and facilitated the discussion. The third member, who was identified from the community acted as the interpreter.

Statistical analysis

The 95% confidence intervals of the fecal egg count reduction were analyzed using PASW Statistics 18, Release Version 18.0 (D3 SPSS, Inc., 2009, Chicago, IL). BootStreat, using a before/after treatment evaluation of the fecal egg counts and a re-sampling number of 2000 was used (Cabaret and Antoine, 2008).

Results

Gastrointestinal nematodes (GIN) in pre-treatment fecal samples of goats

Coproscopic examination for the presence of GIN eggs in pre-treatment fecal samples of the 54 goats revealed 100% strongyle and 19.4% *Strongyloides*. The greatest proportion of goats had light infection (61.1%), as compared to moderate (19.4%) and heavy (19.4%) infection.

Anthelmintic efficacy

The anthelmintic efficacies, based on fecal egg count reduction (FECR) of albendazole and ivermectin in goats, are shown in Table 1. Ivermectin was found to be more effective with FECR of 96.55%, while albendazole was less effective with FECR of 81.93 %.

Third stage larvae in pre and post-treatment cultures of experimental goats

Table 2 shows the proportions of third stage larvae (L3) identified from pre-treatment fecal cultures of goats. There was a predominance *Haemonchus* spp. followed by *Cooperia* spp. Other nematodes identified were *Strongyloides* spp. and *Trichostrongylus* spp. Post-treatment coproculture results showed that only *Haemonchus* survived albendazole treatment. No GINs were identified in ivermectin groups on day 13 post-treatment (Table 3).

Questionnaire survey

All the pastoralists interviewed, kept their goats in an extensive type of production system, on communal land utilized by goats, sheep, cattle and donkeys. With the

Table 1. Anthelmintic efficacy using arithmetic means and confidence intervals in goats

Groups	n	Arithmetic mean (mean ± SEM)		95 % CI for FECR		
		Day 0	Day 13	FECR %	LCL	UCL
ABZ	18	672.22 ± 86.99	61.11 ± 25.74	81.03	58.33	97.22
IVM	18	866.67 ± 110.26	11.11 ± 11.11	96.55	96.87	100
Control	18	1161.11 ± 187.72	322.22 ± 99.19	-	-	-

ABZ = albendazole; IVM = ivermectin; FECR, fecal egg count reduction; SEM, standard error of mean; LCL, lower confidence limit; UCL, upper confidence limit; CI, confidence interval; n, number of goats. Bootstrap results of 95% confidence intervals based on 2000 bootstrap samples

Table 2. Genera of nematodes identified in pre-treatment coprocultures of experimental goats

Groups	Proportion of nematode genera (%)			
	Haem	Coop	Stroi	Trich
ABZ	88.13	10.17	0.85	0.85
IVM	96.67	3.33	NL	NL
Control	79.91	9.4	4.27	6.41

Haem = Haemonchus; Coop = Cooperia; Stroi = Strongyloides; Trich = Trichostrongylus; NL = No larvae

Table 3. Genera of nematodes identified in post-treatment coprocultures of experimental goats

Groups	Proportion of nematode genera (%)				
	Haem	Coop	Stroi	Trich	Ost
ABZ	100	NL	NL	NL	NL
IVM	NL	NL	NL	NL	NL
Control	93.26	3.37	3.37	NL	NL

Haem = Haemonchus; Coop = Cooperia; Stroi = Strongyloides; Trich = Trichostrongylus; Ost = Ostertagia; NL = No larvae

exception of goats of up to 3 months of age, which were grazed around homesteads, other age categories were grazed together. At night, most pastoralists (68.3 %) kept their goats in unroofed enclosures and a few (31.7 %) kept their goats in houses with a ground floor. A big proportion of pastoralists (88.3 %) introduced new goats 12 months before the study, out of which 39.6% dewormed the goats before mixing with existing ones. Introduced goats were bought at weekly markets, supplied by farmers from within the same community as the present study.

Eighty one point seven (81.7 %) of pastoralists controlled worms using one anthelmintic family, 13.3 % two families, 1.7 % three families and 3.3 % did not use anthelmintics at all. Most pastoralists used benzimidazoles (96.7 %), while a few used macrocyclic lactones (10 %) and imidazothiazoles (6.7 %). For pastoralists that applied benzimidazoles, the drugs had been used more than 5 years (63.5 %) and 3-5 years (15.5 %) preceding the study.

Fifty three point three (53.3 %) of pastoralists dewormed goats in the flock every 1-4 weeks, 35% when goats were sick, 1.7 % once a year, 3.3 % each for 2-3 times/year and 4 times a year. Pastoralists dewormed goats by 51.7 % signs of disease (diarrhea, ruffled coat and decline in body condition and with a potbelly), 29.3 % all goats at same time and 5.2 % dewormed only adult goats. There was a category of farmers (13.8 %) who administered dewormers to goats that showed signs of disease and sometimes to all goats. In the present study, 50 % of the farmers used uncalibrated equipment for drenching goats.

Pastoralists selected anthelmintics by 60.3 % veterinary advice, 36.2 % performance of drug, 1.7 % elders' advice and 1.7 % by dewomer packaging. All the interviewed respondents indicated that they do not have any idea about anthelmintic rotation. As a result they never rotated anthelmintic families.

Discussion

Benzimidazoles (BZ) were the most widely used anthelmintic family in the study area. This is in agreement with Kumsa *et al.* (2010) who reported that BZs were the most used anthelmintics in Ethiopia. In the present study, the efficacy of albendazole was less than 95 %, while efficacy of ivermectin was greater than 95 %. This indicated resistance of GINs to albendazole hence to the BZ family. These findings agree with those of Bakunzi (2003) and Tsotetsi *et al.* (2013) in South Africa, and Sissay (2007) in Ethiopia, who reported reduced efficacy and development of resistance to BZ drugs in nematodes of goats. Coproculture examination revealed that the predominant resistant nematodes were all *Haemonchus*. Results of the current study, in terms of *Haemonchus* being the encountered nematode, resistant against albendazole, agrees with a previous study in South Africa (Tsotetsi *et al.*, 2013)

The reason for reduced efficacy in albendazole was likely due to high selection pressure imposed by the pastoralists themselves: probably due to prolonged BZ use without rotation, frequent dosing by farmers and incorrect weight calculation which gives a risk of under-dosing. Underdosing, lack of anthelmintic class rotation and a high drench frequency, alone or in combination were reported to increase the risk of anthelmintic resistance in Ethiopia (Kumsa and Abebe, 2009), Uganda (Byaruhanga

and Okwee-Acai, 2013) and in South Africa (Tsotetsi *et al.*, 2013).

Resistance in BZ was also observed in goat flocks in India due to frequent treatment (Rialch *et al.*, 2013). More than half of the pastoralists reported that they dewormed their goats every 1-4 weeks. Such frequent use of anthelmintics increases the proportion of the exposed nematode population and thus concentrates the resistant alleles in the population (Ihler, 2010).

The other reason for reduced efficacy could be due to the fact that most farmers bought goats at auctions and introduced them into existing flocks. Animal introduction has been suggested as an important mechanism in the spread of resistance (Alvarez - Sanchez *et al.*, 2006). Introduced goats may have originated from flocks with resistant GINs, thereby disseminating resistant genes. Less than half of the farmers dewormed the newly introduced goats. Quarantine drenching against GIN in goats was reported to decrease the risk of spreading anthelmintic resistant nematodes (Domke *et al.*, 2012).

In addition, these animals grazed on pastures to which no livestock from other communities were introduced. Keeping closed flocks leads to increase in selected parasite populations which provide a pool of drug-resistant genes, with no chance of dilution by susceptible genes from outside flocks (Kaplan, 2006). This was reported to reduce refugia in pastures thus leading to development of resistance (Sissay, 2007).

The reason for good efficacy in ivermectin in the present study was probably due to very low selection pressure for the development of resistance. Very few pastoralists (10 %) used macrocylic lactones (ML), and the drugs were applied at low frequency. Therefore, majority of nematode parasite populations in goats remained unexposed to anthelmintic selection, thus remaining susceptible. This is in agreement with Domke *et al.* (2012) who reported that limited use of ivermectin seemed to have prevented the development of anthelmintic resistance in Norwegian goats. However, ML resistance in *Haemonchus* was reported in South Africa (Tsotetsi *et al.*, 2013) and Australia (Jabbar *et al.*, 2013) due to extensive and prolonged use of MLs.

Conclusion

We determined the efficacy of albendazole and ivermectin against GIN infections in naturally infected goats in a pastoral region of Karamoja, Uganda. The study revealed occurrence of BZ resistance in goat flocks, which is a serious threat to goat production. The BZ resistance observed in the present study suggests that regular monitoring for anthelmintic resistance in pastoral communities is essential to keep a track of their efficacy.

Steps should be taken to limit further spread of resistant nematodes, and to restore and maintain anthelmintic efficacy. Pastoralists should be educated on the importance of use of the most suitable drugs, correct dosage, possible combination of drugs, and treating selectively rather than the entire flocks. Other practices which reduce the risk of emergence to anthelmintic resistance such as: annual rotation of anthelmintic groups, avoiding high frequency of treatments and screening of new goats before introduction to flocks should be communicated to the pastoralists.

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