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Anthelmintic efficacy of Albendazole, Levamisole and Ivermectin against gastrointestinal nematode (GIN) infections in goats on natural pastures in Gomba District, Uganda

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Abstract

Efficacy of albendazole (ABZ), levamisole (LVM) and ivermectin (IVM) against gastrointestinal nematodes (GIN) was evaluated in commercial goat farms in Gomba District, Uganda. On the farms, goats were randomly assigned to four groups (18-30 goats each). The first group served as the untreated control, the second was treated with ABZ (5 mg kg^{-1} BW), the third with LVM (7.5 mg kg^{-1} BW) and the fourth with IVM (0.2 mg kg^{-1} BW) at doses recommended by respective drug manufacturers. Fecal egg counts, expressed as eggs per gram and larval cultures were done on day zero before treatment and every 7 days after treatment. Anthelmintic efficacy was determined by the Fecal Egg Count Reduction (FECR) test. The observed FECR were 77.3%, 85% and 83%, for ABZ, LVM and IVM, respectively. Though FECR in all treated animals were significantly ($P<0.05$) lower than controls, there was no FECR of 95% or more. Coprocultures showed larvae of haemonchus species in albendazole treated goats. Continued shading (FECR <95%) of fecal eggs in all treated goats is indicative of anthelmintic resistance in the goat farming sector in Uganda. Further studies are needed to clarify the state of efficacy of commonly used anthelmintics in the different farming systems in Uganda.

Key words: Anthelmintic resistance, fecal egg count reduction (FECR), goat farms, Uganda

Introduction

Gastrointestinal nematode (GIN) infections pose the greatest challenge to commercial goat production worldwide. In the tropics, GIN infections are the principal cause of mortality, retarded growth and general production losses in goats (Baker *et al.*, 2001). On commercial farms, the control of GIN infections is heavily reliant on use of anthelmintics (Magona *et al.*, 2000). However, overzealous use of anthelmintics is widely blamed for development of parasite resistance worldwide (Sutherland and Leathwick, 2011). Currently, anthelmintic resistance is becoming widespread in nematodes of major livestockspecies (Silvestre *et al.*, 2002; Jabbar *et al.*, 2008; Sutherland; Leathwick, 2011). Widespread resistance against common anthelmintic groups: benzimidazole, levamisole and ivermectin have been reported. A more worrisome situation is that, parasites with multiple resistances against all the three drug groups have been identified (Jabbar *et al.*, 2008).

To avoid development of widespread anthelmintic resistance, the efficacy of commonly used anthelmintics in worm control should be constantly monitored (Ward *et al.*, 2000). This enables early detection of reduced efficacy or resistance against a given anthelminthic (Ward *et al.*, 2000). An agent with significantly reduced efficacy may be withdrawn by state regulatory authorities while farmers are advised to switch to more effective alternatives.

In Uganda, although anthelmintics are widely used in commercial goat farming, routine monitoring of the efficacyof commonly used drugs is not done. There is hence, a dearth of current informationabout efficacy to inform judicious veterinary use of anthelmintics in Uganda. We hence, evaluated the efficacy of anthelmintics: albendazole (ABZ), levamisole (LVM) and ivermectin (IVM)purchased form registered veterinary pharmacies in Uganda. Efficacy was evaluated in goats with natural GIN infections on selected farms in Gomba District, Uganda using the standard Fecal Egg-Count Reduction (FECR) tests. It is hoped that our results will inform prudent use and/or development of sustainable strategies for effective control of GIN infections in Uganda.

Materials and methods

The study animals

Naturally infected goats in 3 commercial farms in Gomba District, Central Uganda were studied. Both males and female goats aged between 3-12 months old were studied. Aging of the goats was based on farmers' records coupled with dental examination (Oltenacu, 1999). The goats were mainly indigenous Ugandan goats (the Small East African) or their crosses with the South African Boar goat grazed on natural and non-paddocked pastures in predominantly woodland vegetation with shrubs for browsing. The average flock size in the 3 farms was 300 goats.

Experimental design and treatment of study goats

Fecal egg counts expressed as eggs per gram (epg) of feces were done on samples from all goats within the age bracket of 3-12 months. Goats with counts above 200 epg were selected for experimental treatments. On this basis, 93 goats were selected for efficacy studies on the 3 farms. Selected goats were identified using ear tags and allocated to four groups using a randomised complete block design (Gomez and Gomez, 1984) as shown in in Table 1. All goatsin each farm were subjected to prophylactic anthelmintic treatments but samples for efficacy studies were collected from the tagged goats (with epg >200) only. Commercially available anthelmintic drugs from registered pharmacies were used. However, for purpose of confidentiality and legal concerns, the brand labels are not indicated in this study. Dosing and dosage routes followed brand recommendations.

Table 1. Experimental treatments

Group	Treatment	Dose (mg kg^{-1} of body weight)	Dosage route	Number of goats
1	Control	Untreated	-	24
2	Albendazole (10%)	5	Oral	21
3	Levamisole (1.5%)	15	Oral	30
4	Ivermectin (1%)	0.2	Sub-cutis	18
Total				93

Collection and handling of fecal samples

Rectal fecal samples were collected on day zero before treatment and then days 7, 14 and 21 after treatment. Using gloved fingers, about 10 grams of feces were obtained from each goat by digital rectal extraction and placed immediately in a plastic bag. The bag was tightened as close to the feces as possible to keep off air. For identification, each sample was labeled with the details of the individual goat and farm before being put in a cold box containing ice packs. The samples were transported for laboratory analysis at the National Livestock Resources Research Institute (NaLIRRI), Tororo, Uganda within 12 hours. All samples were collected between 9-10 am just before the goats were released for grazing each morning.

Detection of nematode eggs and estimation of fecal egg counts (FEC)

The simple test tube flotation method (Hansen and Perry, 1994) was used in the detection of the nematode eggs. Parasitological nematode eggs identification was on morphologic features as described by Soulsby (1982). Using a modified McMaster technique, FEC was determined as number of eggs per gram (epg) for each sample (Kaplan and Miller, 2006). The detection level of the McMaster method used was 100 epg.

Fecal egg count reduction (FECR) tests

To estimate anthelmintic efficacy, the fecal egg count reduction (FECR) test was performed in accordance with Dash *et al*., 1988. Arithmetic means of pre-and post-treatment fecal egg counts were used to calculate the percentage efficacy of each anthelmintic using the following formula: Percentage FECR = {1" [(T2/T1) × (C1/C2)]} ×100, where T1 and T2 are pre- and post-treatment arithmetic means of the epg in treated groups, and C1 and C2 are pre- and post-treatment arithmetic means of the epg in the control group.

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). Reduced efficacy and development of anthelmintic resistance is considered to exist if percentage FECR of an anthelmintic is <95% (Coles *et al.,* 1992).

Identification of third stage nematode larvae

For specific identification of nematode genera, about three grams of rectal fecal samples from each goat were pooled for each group. Pooled samples were then incubated at 27^{0}C for seven days. Using the Baermann technique, third stage larvae (L_3) were recovered in accordance with Foreyt, 2001. The larvae were identified using key morphological features and counted under a compound microscope.

Statistical Analysis

Descriptive statistics on the data were generated using the frequency functions of Excel. T-tests were used to compare mean values for fecal egg counts or egg count reductions using GENSTAT (GENSTAT®, Version 13). P-values < 0.05 were considered significant.

Results

Egg counts in untreated (control) goats

The mean fecal egg counts amongst the untreated (control) goats ranged between 381-532 epg over the 21 day study period. There was however variation in egg counts amongst the untreated goats during the period (Fig.1). This variation was however not statistically significant (R^2 = 0.003, $P > 0.05$).

Fecal egg counts amongst the treated goats

Compared to the control group, there was significant decline ($P < 0.05$) in epg in all treated goats from time of treatment (day 0) up to the 14th day after treatment. Though the mean epg rose steadily from the 14th day in all treatment groups, it still remained significantly lower ($p<0.05$) compared to the control group (Fig. 2). The mean epg values in the different treatment groups are summarised in Table 2.

Percentage efficacy of anthelmintic agents

Percentage efficacy of Albendazole, levamisole and ivermectin is shown in Table 3. The efficacy of albendazole was 82, 80 and 61% at days 7, 14 and 21 days post treatment, respectively. The efficacy of levamisole on the other hand was 95, 91 and 69% on the same respective days. That of ivermectin was 85, 84 and 81% over the same treatment days, respectively. Generally, efficacy was highest for levamisole (85%), followed by ivermectin

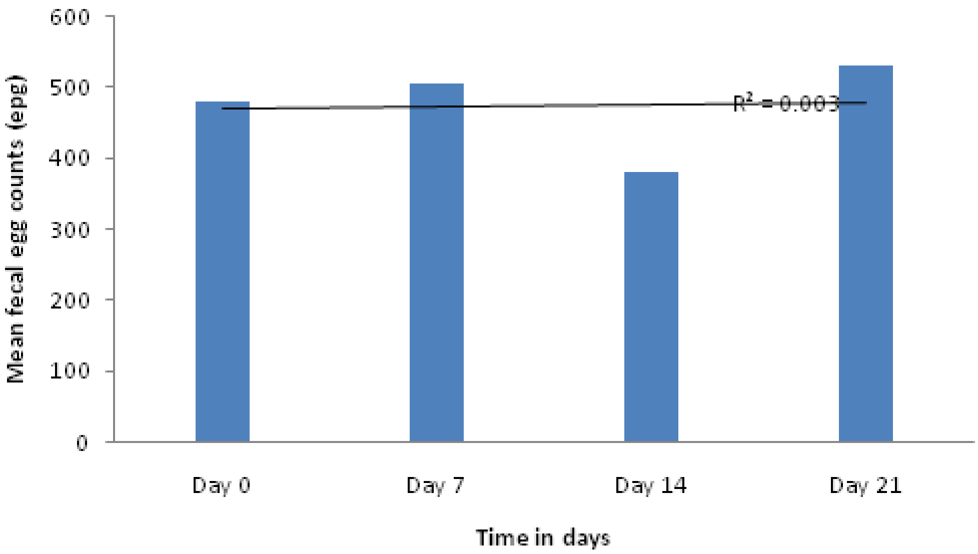

Figure 1. Changes in fecal egg counts amongst the untreated (control) goats.

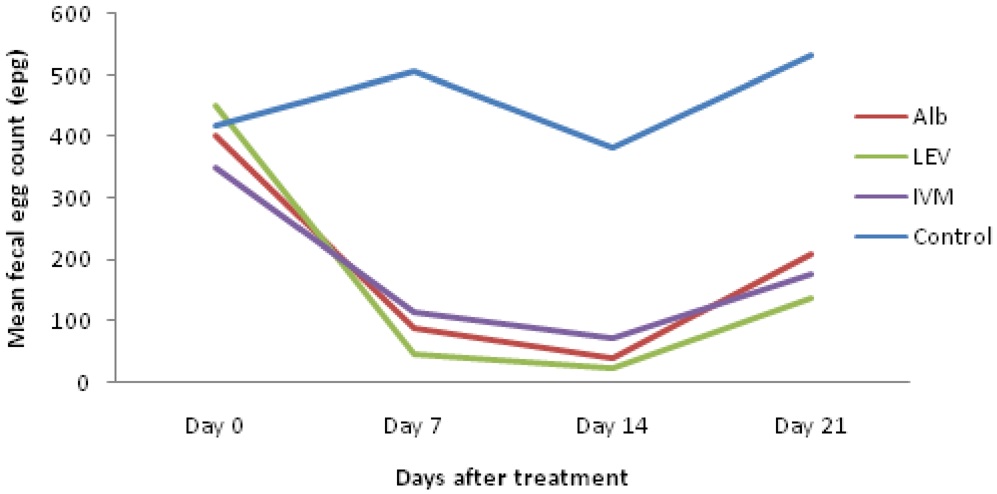

Figure 2. Changes in fecal counts amongst the treated goats.

Table 2. Summary of mean egg values amongst the treatment groups

Agent	Time after treatment	Mean egg counts (epg)	t-statistic	P-Value
Albendazole	Day 0	418		
	Day 0 Vs. Day 7	88	4.68	0.001
	Day 0 Vs. Day 14	40	3.83	0.003
	Day 0 Vs. Day 21	209	3.16	0.002
Levamisole	Day 0	452		
	Day 0 Vs. Day 7	45	6.30	0.001
	Day 0 Vs. Day 14	23	4.80	0.001
	Day 0 Vs. Day 21	136	3.90	0.002
Ivermectin	Day 0	350		
	Day 0 Vs. Day 7	116	3.95	0.002
	Day 0 Vs. Day 14	73	2.93	0.004
	Day 0 Vs. Day 21	176	3.20	0.002

(83%) while albendazole (77.3%) was the least efficacious over the entire treatment period. There was however, a shaper decline in efficacy of levamisole and albendazole between days 14 and 21 compared to ivermectin which remained relatively steady in efficacy throughout the 21 days (Fig. 3).

Third stage larvae identified in coprocultures of experimental goats

Based on morphologic features, nematodes of genera haemonchus (50%) and oesophagostomum (33%) were recovered from pre-treatment coprocultures (Table 4). In post-treatment coprocultures, haemonchus larvae were

Table 3. Percentage efficacy of anthelmintic agents

Agent	Percentage efficacy (FECR %)			Mean percentage efficacy over the study period
	Day 7	Day 14	Day 21	
Albendazole	82	80	61	77.30%
Levamisole	95	91	69	85.00%
Ivermectin	85	84	81	83.03%

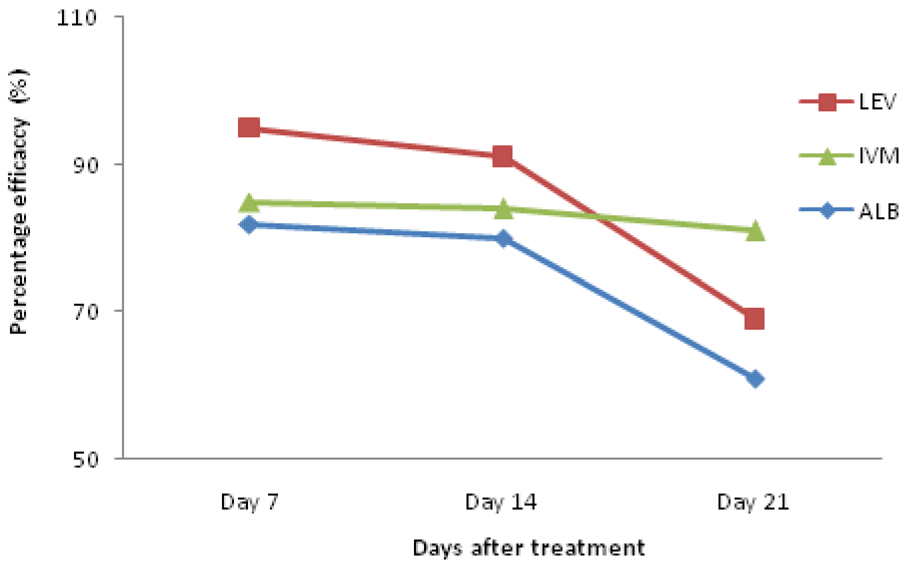

Figure 3. Changes in percentage efficacy with time after treatment.

recovered from 9.5% of albendazole treated goats.

Discussion

We recorded mean fecal egg values of between 350-481 epg amongst the untreated goats; much lower mean epg of 2350 reported by Nalule *et al*. (2011) in untreated Small East African goats in Kampala, Uganda. Waruiru (2002) also reported egg counts of above 1500 epg in Small East African goats in Kenya. A plausible explanation for the low egg counts for goats in the current study is that they were grazed on natural pastures with woody vegetation for browsing. Conversely, in the studies by Nalule *et al*. (2011) and Waruiru (2002), the goats were grazed strictly on grass pastures. It has long been postulated that browsing reduces GIN burdens in goats by limiting intake of infective larvae from the ground herbage (Kabasa *et al*., 2000). Browsed leaves also tend to contain significant amounts of tannins that are known to enhance nutrition, hence the ability to resist helminthes infections (Kabasa *et al*., 2000). It is therefore imperative that browse plants are promoted in goat farms as a strategy against helminthosis.

Table 4. Third stage larvae identified in coprocultures of experimental goats

Anthelmintic agent	Nematode species	Prevalence (%)			Mean Prevalence (%)
		Day 7	Day 14	Day 21	
Control (n = 24)	Haemonchus	18 (75%)	12(50%)	6(25%)	50.0%
	Oesophagostomun	10(42%)	14(58.3%)	0 (0%)	33.3%
Albendazole (n = 21)	Haemonchus	0	0	2(9.5%)	3.2%*
	Oesophagostomun	0	0	0	0.0%
Levamisole (n = 30)	Haemonchus	0	0	0	0.0%
	Oesophagostomun	0	0	0	0.0%
Ivermectin (n = 18)	Haemonchus	0	0	0	0.0%
	Oesophagostomun	0	0	0	0.0%

*larvae were recovered from albendazole treated goats only

Egg counts amongst treated goats ranged between 23-209 epg throughout the 21 day study period. No group showed zero egg counts after treatment. Nalule *et al.* (2011) also observed a continued shading of nematode eggs at counts of 603, 23 and 11 epg on days 7, 14 and 21 respectively, after treatment with albendazole. Munyua *et al.* (2004) similarly reported continued egg shading in groups of goats treated with levamisole or albendazole with mean egg counts of 750 and 33 epg respectively, 14 days after treatment. They however observed that the goats treated with ivermectin had no counts (0 epg) by the 14th day after treatment. This was also the case in southern Ethiopia (Bersissa *et al.*, 2010). It may therefore be deduced that, as much as albendazole, levamisole and ivermectin do significantly reduce nematode egg counts, continued shading of eggs in feces following treatments is indicative of a possible parasite resistance against them. It should however, be noted that fecal egg counts were significantly ($P<0.05$) reduced in all treatment groups compared to the control goats, hence the 3 drugs may still be considered effective against common GIN infections in the study farms. Routine monitoring of fecal egg counts following deworming is important in monitoring success of treatments (Love and Hutchinson, 2003). In goats, a mean epg of 100-150 in 10-14 days after deworming suggests reduced effectiveness. Alternatively, it may mean the egg counts were very high prior to treatment or the pasture on which treated goats are grazed is heavily contaminated (Love and Hutchinson, 2003). In the present study, the worm burdens prior to treatments were comparatively low; hence continued egg shading after treatments is more likely to be due to parasite resistance against the three drugs. Mean percentage efficacies were: 85, 83 and 77.3% for levamisole, ivermectin and albendazole respectively. In Kenya, Keyyu *et al.*, (2002) reported efficacies of 97 and 59.4% for levamisole and albendazole respectively, against natural GIN infections in goats. Conversely, Bersissa *et al.* (2011) reported albendazole and ivermectin efficacies of 100% in a related study in southern Ethiopia. This is yet another pointer of a likely parasite resistance to these drugs. Most cases of reduced efficacies are often seen on commercial farms due to heavy reliance on chemotherapy. It is also worth noting that flock sizes matter in the development of reduced anthelminthic efficacy given that farmers with large flocks are more likely to be able to buy anthelmintic drugs (Wanyangu *et al*., 1996). In this study, the average flock size for each of the 3 farms was 300 goats, which may be considered large.

Albendazole was the least (77.3%) efficacious compared to levamisole (85%) and Ivermectin (83%). This is suggestive that albendazole is probably the most frequently or longest used anthelmintic in the study farms. Prolonged or too frequent use of a particular drug increases selection pressure for resistant individuals within a population. However, in the absence of genetic confirmation, one needs to be cautious in interpreting efficacy data involving commercially available drugs given that in poor countries, drug outlets often stock poor quality or even fake drugs (Wanyangu *et al.,* 1996). We however note that, at their current efficacies, judicious application of albendazole, levamisole and ivermectin in commercial farms in Uganda may still be considered sustainable. Farmers hence, need to be educated on rational use of these drugs so that their current efficacies are maintained while alternative approaches to complement chemotherapy are sought.

Amongst the untreated goats, larvae of nematodes of genera haemonchus and oesophagostomum were recovered from fecal samples in the proportions of 75 and 42% respectively. This strengthens previous findings which have reported that haemonchosis is the most prevalent and devastating GIN infection of goats in Eastern Africa (Okeyo *et al*., 1996; Bersissa *et al*., 2011). Therefore, a control strategy that specifically targets haemonchosis may be vital in improving goat production in the region. Such may include vaccination (Bakker *et al*., 2004) or selection for haemonchus tolerance (Okeyo *et al*., 1996).

Haemonchus larvae were recovered only from those treated with albendazole 21 days after treatment. This may be partly explained by the fact that albendazole was the least efficacious compared to levamisole and ivermectin. Ivermectin is also generally known to be more persistent in mammalian tissues than levamisole or albendazole, hence more long acting (Chiu *et al*., 1987). Besides, studies have shown that fecal samples from animals treated with ivermectin supports a significantly reduced invertebrate diversity (Iglesias *et al*., 2006). Additionally, better and sustained performance by levamisole may also be attributed to the fact that levamisole treatments tend to improve non-specific immunity of treated subject. Levamisole treated animals tend to be more resistant even to helminthes. It may therefore be implied that goats treated with albendazole resume pasture contamination with infective nematode larvae sooner than those treated with levamisole or ivermectin (Iglesias *et al.*, 2006).

We conclude that, much as albendazole, levamisole and ivermectin are effective against common GIN parasites in Gomba, Uganda, continued shading of eggs in feces (<9%% efficacy in all cases) after treatments is suggestive of a possible parasite resistance against the drugs. Farmer education is hence, recommended so as to ensure judicious use of these drugs in order to maintain current efficacies while alternative approaches to complement chemotherapy is sought and promoted.

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