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Comparative anthelmintic efficacy of commonly used anthelmintic brands against naturally infected Red Maasai-crosses at KALRO-VRI Muguga, Kenya

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ABSTRACT

A comparative efficacy test for three broad-spectrum chemical anthelmintics and three plant extracts was carried out using naturally infected Red Maasai cross sheep at the Veterinary Science Research Institute in Muguga in October 2015. A group of 42 sheep was randomly assigned to seven groups of six sheep each, based on faecal egg counts and age category. Each of the first three groups was treated with a Benzimidazole, Levamisole and Ivermectin injection according to the manufacturer's recommendation while the other three groups were treated with three herbal extracts according to recommendations by the practitioner. The efficacy was based on the faecal egg count reduction on Day 14 post-treatment.The efficacy of the broad-spectrum anthelmintics was 75%, 81% and 97% for the benzimidazole, ivermectin and Levamisole respectively. All the herbal extracts had low efficacies of 25%, 34% and 37%. It was concluded that levamisole showed a higher efficacy compared to other anthelmintics and plant extracts. Thus levamisole can be useful for controlling gastrointestinal nematodes in Muguga.

Keywords: Anthelmintic resistance, gastrointestinal nematodes, Helminths, Sheep

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INTRODUCTION

Helminth infections amongst sheep and goats are among the leading causes of mortality and morbidity in many production systems. The problem presents as a parasitic gastro-enteric syndrome caused by a mixture of different nematode helminthes (Fox, 2018). *Haemonchus contortus* in combination with other nematodes, chiefly *Trichostrongylus* spp, *Nematodirus* spp, *Cooperia,* spp. *Oesophagostomum* spp and *Trichuris* are usually implicated in this condition (Nginyi et al., 2001; Fox, 2018). The characteristic clinical signs of this infection manifest in a variety of signs, including anaemia, diarrhea, poor body condition, bottle jaw, and even death, particularly in young stock.

The main method of management of this condition is dependent on the use of anthelmintics, whose regime of application is varied from farm to farm and region to region (Maingi et al., 1997; Kinoti et al., 1994; Mbaria et al., 1995). This has come with challenges of cost, especially with farmers with huge flocks of small ruminants. Additionally, with continued use of these anthelmintics, development of anthelmintic resistance has set in for some farms and regions in Kenya (Wanyangu et al., 1996; Gakuya et al., 2000) and in the rest of the sheep and goat rearing regions of the world. To overcome some of these challenges, it's important to monitor the continued efficacy of the commonly marked anthelmintics to take remedial measures when some are found to perform below the acceptable levels. Recently, as alternative medicine, many extracts of plants have been screened and evaluated for their efficacy. Some of

Table 1: The treatments and dosages given to different groups on Day 0 of the trial.

Group No	Description	Treatment	Dosage
1	6 sheep	Macrocyclic lactones	0.2 milligrams/kilogram body weight
2	6 sheep	Benzimidazole	7.5 milligrams kilogram body weight
3	6 sheep	Levamisole HCl	7.5 milligrams/ kilogram body weight
4	6 sheep	H1 (50E)	20mls/adult sheep
5	6 sheep	H2 (NGEKOP)	40mls/adult sheep
6	6 sheep	H3 (3ROS + ATG30)	50mls/adult sheep
7	6 sheep	Control	No treatment

The dose rates for the commercial anthelmintics were those recommended by respective manufacturers while the herbal practitioner gave those for the herbal extracts.

these plants and herbs have been used by practitioners and animal keepers, but not screened and evaluated for efficacy (Gakuya, 2004).

This study was designed to test the efficacy of three commonly used anthelmintics as well as screen the three herbal preparations (with claims of efficacy by the practitioner) against naturally acquired gastrointestinal infections of sheep.

MATERIALS AND METHODS

This study was carried out at the Veterinary Science Research Institute, Muguga North Centre and used a flock of permanently grazed Red Maasai crosses. The animals were assigned into uniform treatment groups of six sheep based on age category and faecal egg counts. The faecal samples were tested individually with a modified McMaster method (Coles et al., 1992) based on 3 grams of faeces in 42 ml of water and with a detection level of 50 eggs per gram.

A total of 42 animals was used for this study, three anthelmintics used and herbal extracts screened (Table 1). On Day 0, the animals in treatment groups 1, 2 and 3 were dewormed with the three broad-spectrum anthelmintic drugs according to the manufacturers' recommended dosages while those in group 4, 5 and 6 were treated with the herbal extracts according to the dosage given by the herbal practitioner. The identity of the plants from which the extracts were derived was not disclosed by the herbal practitioner. Those in group 7 which serve as the control group remained.

The animals were monitored for any side effects throughout the trial while faecal egg counts, differential larval counts (as described by Bairden et al., 1985) and faecal egg count reduction assay were carried out on Day 7, Day 10, Day 14 and Day 21. The faecal egg count reduction test was carried out as recommended by Wood et al. (1995) with the Day 14 results used to determine the efficacy of the different treatments. This is the day when the effect of treatment is expected to be evident, based on faecal egg counts.

All the trial animals were grazed together during the day and housed at night. During housing, they were provided with Rhodes grass hay and water provided *ad libitum*.

The description of the different anthelmintics used in the trial are:

Ivermin injection 1% w/v: This is a clear liquid injectable anthelmintic formulation containing 1% Ivermctin. Manufactured by Herbel pharmaceutical co. Ltd and marketed by Murphy. It is injected subcutaneously for the control of nematode infections (and ecto-parasites) at 0.2 milligrams per kilogramme body weight.

Valbazen 10%: This is a light green liquid oral drench, composed of 10% ablbendazole BP, recommended for nematodes, cestodes and trematodes in ruminant livestock. It is manufactured by Ultravetis East Africa Limited. It's recommended at 7.5 milligrams per kilogramme body weight when treating for nematode infections.

Wormicid liquid: This is a light yellow liquid oral drench, compost of 1.5% levamisole BP, manufactured by Cosmos Limited and recommended for treatment of nematode infections at a dose rate of 7.5 milligrams per kilogramme body weight.

Herbal preparations H1, H2 and H3: These were oral herbal extracts (coded by the practitioner as 50E, NGEKOP and 3ROS + ATG30 respectively) provided by the practitioner and which he claimed to have anthelmintic activity according to his observations on treated animals.

Data on faecal egg counts were entered in MS Excel and arithmetic means at Day 14 used to determine efficacy.

The approval for this study was granted by the Veterinary Research Institute, Institutional Animal Care and Use Committee (IACCUC), with approval reference CMMTE/45(27) dated 4th October 2015.

RESULTS

All the trial animals exhibited no unusual behavior or signs immediately following treatment and the subsequent days, except two animals in group 5 that died on Day 8. Postmortem findings indicated that haemonchosis could have led to their death in view of the

Table 2: The percentage efficacy of the three broad-spectrum anthelmintics and three herbal extracts on Day 14 post-treatment.

Group no	Treatment	n (on Day 14)	Mean EPG	% Efficacy
1	Benzimidazole	6	1200	75
2	Levamisole	6	160	97
3	Macrocyclic lactones	6	917	81
4	50E	6	2983	37
5	NGEKOP	4	3125	34
6	3ROS & ATG30	6	3588	25
7	Control	6	4800	

Table 3: Differential larval counts (L_3) from pooled samples for different nematode general on Day 0 and Day 14 post-treatment for the different treatment groups.

Day and treatment	Percentage of L_3 counts for the different treatments on Day 0 and Day 14 post-treatment				
	Haemonchus	*Trichostrongylus*	*Oesophagostomum*	*Strongyloides*	*Cooperia*
Day 0	45.7	28.3	8.7	10.8	6.5
Day 14					
Macrocyclic lactones	62.5	26.5	11	0	0
Benzimidazole	50	25	16.7	8.3	0
Levamisole HCl	60	40	0	0	0
H1 (50E)	64.3	25	3.7	0	0
H2 (NGEKOP)	70	20	5	5	0
H3 (3ROS + ATG30)	64	20	8	8	0
Control group	66.7	26.7	3.3	3.3	0

heavy parasite load found in the abomasa at post-mortem.

The efficacy of the six different treatments based on Day 14 faecal egg count reduction tests is indicated in Table 2. Out of the three broad-spectrum anthelmntics, only levamisole resulted in an acceptable efficacy of 97%. The macrocyclic lactone drug used was next in efficacy (81%) followed by the benzimidazole drug (75%). The mean faecal egg counts for the same drugs on Day 14 were 160, 917 and 1200 eggs per gram (EPG) respectively.

The efficacy of all the three herbal extracts was below the acceptable levels, as this ranged from 25% (3ROS & ATG30), 34% (NGEKOP) and 37% (50E). All the three groups had very high mean worm egg counts at the end of the trial as compared to those on conventional anthelmintic treatments. The corresponding mean faecal egg counts for these groups were 3588, 3125 and 2983 EPG.

Results of the differential larval counts at the beginning of the experiment indicated that *Haemonchus* was the most predominant nematode genera followed by *Trichostrongylus*, *Strongyloides*, *Oesophagostomun* and *Cooperia* respectively. The percentage of these genera on Day 0 pooled samples and Day 14 for the different treatments are shown in Table 2. The results showed that the majority of the surviving nematode genera on Day 14 were *Haemonchus*, whose proportion increased compared to the post-treatment period. The proportion (%) for *Trichostronylus* similarly decreased except for the levamisole treated group. The same was the case for *Oesophagostumum* except for the *Benzimidazole* treated group. *Cooperia*, which was recorded on Day 0, was not present in all the treatment groups on Day 14 (Table 3).

DISCUSSION

The use of broad-spectrum anthelmintics is the main method applied in the control of gastrointestinal nematodes in many parts of the world, including Kenya (Mbaria et al., 1995; Kinoti et al., 1993; Hoste and Torres-Acosta, 2011). Declining land sizes and urbanization limit the use of other methods like grazing management. These changes have resulted in overstocking and therefore subjecting grazing livestock to a high helminth infection pressure (Mbaria et al., 1995). The overreliance on anthelmintics to manage helminthoses has resulted in the development of anthelmintic resistance to these drugs. Earlier studies carried out in Kenya indicated that multiple resistance is quite common amongst many areas of the country covering all the agro-ecological zones (Wanyangu et al, 1996; Waruiru et al., 1994; Nginyi et al., 2014).

The paddocks at the Veterinary Research Institute at Muguga, Kenya have been grazed by sheep for many years and all the broad-spectrum anthelmintics have been used over the years. Given the high helminth infection challenge, the frequency of use has been high, especially when monthly monitoring indicated average worm egg counts of over 1,000 eggs per gram. This

made it necessary to monitor the efficacy of the commonly used broad-spectrum anthelmintics continuously. This is to ensure their continued suitability in the management of helminth infections in the sheep flock. In addition, three herbal extracts with anthelmintic activity claim and provided by a practicing herbalist were tested for anthelmintic efficacy.

It was evident from this trial that among the broad-spectrum anthelmintcs, only levamisole exhibited acceptable efficacy at 97%. The albendazole and ivermectin displayed efficacy below the acceptable level. The possibility that nematodes in the experimental sheep had developed resistance to the latter two products is a concern for the future management of sheep at the institute. Measures to manage the resistance need to be designed to sustainably continue keeping sheep and other ruminant livestock in the institute paddocks. The use of narrow-spectrum drugs can be alternated with levamisole particularly in this instance as the majority of the resistant nematodes were found to consist mainly of *Haemonchus*. Products containing molecules like closantel and rafoxanide could be used alongside some management practices like grazing of sheep paddocks by adult cattle while sheep are grazed on cattle paddocks ahead of the former.

Many authors have explored the possibility of using medicinal plants with anthelmintic activity (Gakuya et al., 2004; Githiori, 2004; Iqbal and Jabbar, 2010). However, the possible use of the herbal extracts as tested in this trial was excluded when they all displayed very low anthelmintic efficacy. As observed, there is probably need for more refinement to improve on the observed anthelmintic efficacy.

To manage the observed resistance or reduced efficacy of benzimidazole and ivermectin, it is recommended that strategies to manage this be adopted at the institute. These will include the use of levamisole-based anthelmintics or in combination with other broad-spectrum drugs.

The use of narrow-spectrum drugs like oxyclosanide or rafoxanide can be useful as these are effective against *Haemonchus*, the predominant resistant genera from post-treatment coprocultures. The management options available include the leader-and-follower strategy (Mahieu et al., 2015). The less susceptible animals (lambs, weaners) are grazed after the more resistant adult stock on ‘clean’ paddocks and are used to ‘mop’ infective larvae before the introduction of sheep into the paddocks.

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