[illegible] EFFICACY OF OXFENDAZOLE AGAINST A STRAIN OF HAEMONCHUS CONTORTUS [illegible]TANT TO FENBENDAZOLE AND THIOPHANATE

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SUMMARY

A critical controlled test was conducted to determine the efficacy of oxfendazole against a strain of *Haemonchus contortus* isolated at Sokoine University of Agriculture (SUA) and known to be resistant to fenbendazole and thiophanate. A susceptible strain for comparison was obtained from Kimamba in Kilosa district. Based on post-mortem worm count, the efficacy of oxfendazole at the recommended dose of 5mg/kg body weight was 99.8% against Kimamba strain but only 15% against SUA-strain. The results show that SUA-strain of *H. contortus* is highly resistant to oxfendazole at the recommended dose.

INTRODUCTION

Haemonchosis caused by *Haemonchus contortus* is an important disease of domestic ruminants in tropical and subtropical countries resulting in heavy losses due to poor production and deaths. Routine use of anthelmintics in the control of gastrointestinal nematode infection has resulted in the development of strains of nematodes resistant to the anthelmintics that have been in use over long periods (Kelly and Hall, 1979). Much interest is now directed in developing nematode control methods that do not rely on anthelmintic treatment such as selective breeding for resistant hosts, development of effective vaccines and biological control of free living stages of helminths.

At Sokoine University of Agriculture (SUA) Morogoro several anthelmintics have been used over the years for controlling gastrointestinal parasitism (Table, 1). Frequent use of anthelmintics may have resulted in resistance due to selection over a period of time. Drug failure due to resistance leads to reappearance of clinical signs of internal parasitism and deaths even after anthelmintic treatment.

In previous studies at SUA a strain of *H. contortus* was found to be resistant to thiabendazole and fenbendazole (Kassuku and Tibaijuka, 1987) and to thiophanate (Ngomuo *et al.*, 1990). Currently, oxfendazole is being used at SUA for the control of gastrointestinal parasitism in small ruminants. The objective of this study was therefore to determine the efficacy of oxfendazole to the SUA strain of *Haemonchus contortus* in view of the fact that side resistance may have developed to some anthelmintics of the benzimidazole group (Kassuku and Tibaijuka, 1987).

MATERIALS AND METHODS

The test strain of *H. contortus* was isolated from the Faculty of Veterinary Medicine goat flock grazing SUA pastures. A goat excreting 8,000 epg was taken from the flock, killed and adult *H. contortus* females recovered from the abomasum. These worms were ground in a mortar and pestle to release the eggs and then cultured for L3. Gastrointestinal nematode control consisted of anthelmintic treatment of the goats at about 4 – 6 weeks interval.

Kimamba strain of *H. contortus* which was used as a standard, was isolated

from a flock of goats grazing natural communal pastures at Kimamba area. A goat with an epg of 10,000 was selected, killed and adult *H. contortus* females recovered from the abomasum. These were ground and cultured for L3. Anthelmintic treatment as a control measure against gastrointestinal nematodes in the flock and other flocks in the area had never been practiced on a routine basis.

Experimental animals

Twenty two goats raised under conditions of minimal exposure to worms (concrete floored pens, swept daily to remove accumulating manure) were used. The goats were fed on parasite free lucerne hay, concentrates and fodder. Water was provided *ad libitum*. Prior to the commencement of the trial, faecal worm egg counts were determined and found to be zero. Nevertheless, as a further precaution, the goats were treated with levamisole at a dose of 7.5mg/kg live body weight, 7 days before experimental infection.

Third Stage larvae of the two *Haemonchus contortus* strains

Two parasite free goats were used to amplify the number of nematode L3 of each strain of *H. contortus*. One goat was infected with 10,000 L3 of the SUA strain. The other goat was infected with 10,000 L3 of the Kimamba strain. Each goat was then placed in a metabolic cage and fed on the same parasite free lucerne hay, concentrates and fodder.

Three weeks after infection, faeces were collected every morning and cultured for L3 of SUA and Kimamba strains separately as follows: The faeces were mixed with vermiculite and water to make a consistency of horse faeces. The mixture was placed in plastic cups and covered with muslin cloth and incubated at 26 °C for 7 days.

L3 were then recovered on the 7th day of incubation by a modified Baermann technique using conical flasks. The L3 were recovered in the sediment and placed in tissue culture flasks and stored at 10° C until required for experimental infection.

Infection and treatment

The remaining 20 parasite free goats were randomly divided into four groups, each of five goats. Group 1 and 2 goats were infected with the Kimamba strain of *H. contortus* using a single dose of 10,000 L3 given intraruminally to each goat. After patency, Group 1 was treated with oxfendazole at 5 mg/kg orally, while Group 2 served as untreated control. Goats in groups 3 and 4 were infected with the SUA strain of *H. contortus* using the same dose and route of administration as group 1 and 2. After patency, Group 3 was treated with oxfendazole at 5 mg/kg orally, while group 4 served as untreated control.

Pre- and post-treatment epg values were determined for each goat in the four groups. Seven days post-treatment all experimental animals were killed and *H. contortus* worms recovered from the abomasal mucosa and contents by washing through sieves of 150 µm and 37 µm aperture size, respectively. The total worm count was recorded for each goat.

Statistical analysis

Mean faecal worm egg count and mean worm count for groups 1, 2 and 3, 4, respectively, were tested using "t"-test at 5% level of significance.

RESULTS

Three weeks after infection 18 goats out of 20 excreted strongyle eggs in the faeces and by the fourth week post-infection all the 20 goats passed strongyle eggs in their faeces.

Table 2 shows pre- and post-treatment faecal worm egg counts in all the groups.

The pre-treatment mean epg was 6100 while post-treatment means of 80 epg and 0 were obtained on the 4th and 6th day post-treatment with oxfendazole in Group 1. The corresponding mean epg values for the untreated Group 2 were 4180, 3900 and 4800, respectively. The efficacy of oxfendazole in Group 1 based on faecal worm egg count depression (FECD) was 98.6% and 100% on the 4th day and 6th day post-treatment, respectively.

Table 1: Anthelmintics used from 1981 to 1989 at SUA.

Time	Generic name	Trade name
1981	Fenbendazole	Panacur
	Levamisole HCL	Nilverm
1982	Fenbendazole	Panacur
	Thiophanate	Nemafax
1983	Thiophanate	Nemafax
	Albendazole	Valbazen
	Thiabendazole+Rafoxanide	Ranizole
1984	Albendazole	Valbazen
	Thiabendazole+Rafoxanide	Ranizole
1985	Ivermectin	Ivomec
	Thiabendazole+Rafoxanide	Ranizole
	Levamisole HCL	Nilverm
1986	Fenbendazole	Panacur
	Thiophanate	Nemafax
	Albendazole	Valbazen
1987	Thiophanate	Nemafax
1988	Tetramisole	Ripercol
	Oxfendazole	Synanthic/ Systamex
1989	Tetramisole	Ripercol
	Oxfendazole	Synanthic

Group 3 had pre-treatment mean epg of 800 and post-treatment epg mean values of 360 and 340 on the 4th and 6th day post-treatment, respectively. In Group 4 the mean epg values were 340, 2240 and 2300, respectively. The efficacy of oxfendazole in Group 3 based on FECD was 57.5% on the 6th day post-treatment.

Table 3 shows postmortem worm counts in the four groups. In Group 1 only one *H. contortus* worm was recovered from the five abomasi after slaughter.

Table 2: Faecal worm egg counts after a single oral dose of oxfendazole (5mg/kg body weight) against SUA strain (Group 3 and 4) and Kimamba strain (Group 1 and 2) of *Haemonchus contortus*

	DAYS POST-TREATMENT			
	0	4	6	7
	No. eggs/ gram of faecesX10^3 (mean ± SEM)			
G.1	6.10±2.23	0.08±0.06	0	0
G.2	4.18±0.93	3.90±0.88	4.80±1.40	3.08±0.74
G.3	0.80±0.29	0.36±0.14	0.34±0.16	0.56±0.27
G.4	0.34±0.10	2.24±0.10	2.30±0.19	3.20±0.30

Group 2 and 4 were controls

Table 3: Total worm counts in abomasi of goats infected with Kimamba strain (Group 1 and 2)* and SUA strain (Group 3 and4)** treated with a single dose of oxfendazole (5 mg/kg body weight)

		Goat Number							
		1	2	3	4	5	Mean	±	SEM
Group	1	1	0	0	0	0	0.2	±	0.2
	2	131	144	221	38	174	162	±	16.5
	3	2	14	8	16	14	11	±	2.5
	4	2	16	21	13	13	13	±	3.0

Group 2 and 4 were controls

* Efficacy = 161.6 − 0.2 X 100 = 99.8%

** Efficacy = 13−11 X 100 = 15.3%

Unlike in the untreated Group 2 which had a mean worm count of 162.

There was a statistically significant difference between the mean total worm count of the treated Group 1 and that of the untreated control

Group 2. The efficacy of oxfendazole, based on total worm count was shown to be 99.8%. Oxfendazole was therefore effective against the Kimamba strain of *H. contortus*. However, in the SUA strain of *H. contortus* mean total worm count of 11 was obtained in the treated Group 3, while in the untreated control Group 4 a mean of 13 was obtained. These means do not differ significantly. The efficacy of oxfendazole against the SUA strain was only 15.3%.

DISCUSSION

Oxfendazole, being one of the new broad spectrum anthelmintics in the benzimidazole group has been claimed by several workers to possess high efficacy against gastrointestinal nematodes of most domestic animals. Michael *et al.* (1979) obtained 100% clearance of mature and immature *H.contortus* at a dose of 4.5mg/kg oxfendazole in experimentally infected goats. Webb *et al* (1979) reported that oxfendazole at 4.65mg/kg cleared all eggs from faeces and adult *Haemonchus* worms which had been found to be resistant to thiabendazole and parbendazole. In contrast to other benzimidazoles few workers have reported the presence of anthelmintic resistance to oxfendazole.

In the present study, oxfendazole at 5 mg/kg against SUA strain of *H. contortus* removed only 15% of the worm burden indicating low efficacy. On the other hand, oxfendazole at the same dose was able to remove 99.8% of worm burden in those goats infected with the Kimamba strain of *H.contortus* indicating high susceptibility of the strain to oxfendazole. Although the worm establishment rate for SUA strain was low, oxfendazole at 5 mg/kg body weight was unable to clear the worm burden in Group 3. Assuming that both strains were equally susceptible, then oxfendazole at the same dose should have been more effective in clearing a low worm burden, SUA strain, than a higher worm burden of Kimamba strain. During the 1987 outbreak of haemonchosis in sheep and goats at SUA, a single treatment with levamisole at 7.5mg/ kg body weight proved to be very effective except in animals with high epg of 27000 and above in which treatment had to be repeated after 7 days in order to clear the worm burden. In this study the low worm burden of SUA strain, could not be cleared by a similar dose of oxfendazole which was able to clear a comparatively higher worm burden of Kimamba strain. This seems to indicate that there is a strain difference in susceptibility to oxfendazole. It is thus likely that SUA strain of *H.contortus* is also resistant to oxfendazole at 5mg/kg body weight.

Hall *et al* (1978) suggested that the level of activity of each anthelmintic can be related to its chronological time of release for commercial use and that the longer and more frequently and intensively used is a compound, the more likely it is for the worms to develop anthelmintic resistance. However, for oxfendazole, this seems not to be the case since oxfendazole is one of the latest benzimidazoles to be released, for commercial use (Bjorn 1983) and at SUA, according to Ngomuo *et al* (1989) there has been no history of use of the drug before 1988 (Table 1). Kassuku and Tibaijuka (1987) reported resistance of SUA strain of *H.contortus*

up to three times there commended doses of the benzimidazole fenbendazole and a probenzimidazole thiophanate. The resistance of SUA strain of *H.contortus* to thiophanate was confirmed a year later by Ngomuo *et al* (1990) in a critical controlled test. It appears therefore that the SUA strain of *H.contortus* has developed side resistance to oxfendazole after selection with fenbendazole, thiophanate and thiabendazole. Webb and McCully (1979) observed that in an area where resistance of H. *contortus* to benzimidazoles other than oxfendazole was known to be widespread in many farms, resistance to oxfendazole on one farm was confirmed, and similar work done by Hall *et al* (1978) showed that side – resistance existed among all benzimidazoles tested including oxfendazole and that levels of resistance were shown to albendazole and oxfendazole although these drugs had not been used commercially at the time of the study. These findings agree with the present study on oxfendazole which has shown low efficacy just after its introduction for use at SUA.

Levamisole and tetramisole were found to have an efficacy of 100% against the SUA strain of *H.contortus* (Kassuku and Tibaijuka 1987) and was thus recommended to be used instead of the benzimidazoles in the control of *H. contortus* infection in the University farm. Forsyth and Gibbon (1980) also reported high efficacy of more than 95% when levamisole was administered either orally or subcutaneously to experimentally infected lambs with benzimidazole resistant *H.contortus* larvae. Similarly, Coles *et al* (1979) obtained an efficacy of 99% when levamisole was given to lambs experimentally infected with both benzimidazole resistant and susceptible strain of *H.contortus* with no apparent difference between susceptible and resistant worms.

However, it is just a matter of time before *H.contortus* strain at SUA develops resistance to levamisole and tetramisole if these anthelmintics are to be routinely used in the control of haemonchosis. The increasing prevalence of resistance to anthelmintics coupled with the increasing costs of development and registration of new anthelmintics means that this method of nematodes control may not be sustainable for much longer than the useful life of currently available anthelmintics.

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