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Benzimidazole resistance in a field population of *Haemonchus contortus* from sheep in Kenya

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ABSTRACT

A controlled anthelmintic efficacy test was used to confirm results of faecal egg count reduction test obtained earlier from a field survey where field strains of *Haemonchus contortus* were suspected to be resistant to thiabendazole (TBZ), fenbendazole (FBZ) and levamisole (LEV). Worm-free sheep (32) were experimentally infected with 2 strains of *H. contortus* (M1-3) and 3 anthelmintics were tested.

There was 85 and 43% reduction, as compared with controls, in mean worm burdens (MWB) of sheep infected with M1 strain and killed 5 days after treatment with TBZ and FBZ, confirming their resistance to these anthelmintics. TBZ had an efficacy of 98% in sheep infected with M2 strain but FBZ reduced MWB of the same strain in 70% indicating a moderate level of resistance to this anthelmintic. There was a 96% reduction in MWB of sheep infected with the suspected LEV-resistant M3 strain and then treated with LEV, indicating that this strain was not resistant to LEV.

There is a need to determine the anthelmintic efficacies of a gastrointestinal nematode population in any investigation on farms where chemotherapeutic control measures against helminthiasis appear to have failed.

The methods in use in the diagnosis of anthelmintic resistance (Hall 1982) are the faecal egg count reduction (FECR) test and the *in vitro* egg hatch technique (LeJambre 1976, Dobson *et al.* 1986). The FECR test was used to investigate the prevalence of anthelmintic resistance is sheep nematodes in 3 districts of Kenya. Five commonly used broad-spectrum anthelmintics were tested and an efficacy of less than 90% with thiabendazole and fenbendazole was obtained in 2 farms. Levamisole was less than 90% effective in 1 farm. To confirm these findings, a controlled anthelmintic efficacy test was conducted using 3 strains of *Haemonchus contortus* from these farms. This paper presents results on the efficacy of thiabendazole (TBZ), fenbendazole (FBZ) and levamisole (LEV) against these strains in experimentally infected sheep.

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MATERIALS AND METHODS

Female indigenous sheep (32) of mixed breeds and 6-12 months old were used. They were treated with ivermectin on arrival at the Faculty of Veterinary Medicine, Kabete, housed and treated again 3 weeks later. Faecal samples were collected per rectum and examined for helminth ova after treatment using a modified McMaster method (MAFF 1986).

The 3 *Haemonchus contortus* strains used, originated from Machakos Veterinary Farm, Machakos District (M 1), Machure farm, Kiambu District (M 2) and Managu farm, Nakuru District (M 3). All the farms kept sheep intensively and anthelmintic resistance was suspected (Waruiru *et al.* 1991). After isolation from the field, the strains were not exposed to anthelmintic selection and were

passaged only once in the laboratory before use.

Experimental sheep were divided into 3 groups, viz. 12 in group 1 were infected with M 1 strain, 12 in group 2 were infected with M 2 strain, and 8 in group 3 were infected with M 3 strain. In all the cases, the sheep were infected orally with 10 000 *H. contortus* infective larvae, 30 days before the proposed treatment day.

Anthelmintics used were proprietory preparations administered orally at the manufacturer's recommended dosage. Animals in specific groups, 30 days after infection were weighed, identified by ear-tags and randomly allocated to treatment groups on the basis of their worm egg counts (Hagarth-Scott *et al.* 1976). Sheep infected with M 1 strain were divided into 3 groups of 4 animals in each. Group 1 was treated with TBZ (62 mg/kg) and group 2 with FBZ (5 mg/kg); group 3 animals were untreated controls. The same treatment regimen was carried out for sheep infected with the M 2 strain. Sheep infected with M 3 strain were assigned to 2 groups of 4 sheep in each. Sheep in one group were treated with 7.5 mg of LEV/kg body weight and the remainder served as untreated controls.

The sheep were slaughtered 5 days after treatment (day 35 post-infection) and total worm counts were conducted using standard parasitological technique (MAFF 1986). The abomasal contents of each animal was washed into a separate bucket and 10% aliquots were collected and cleaned by washing through 210 µm aperture sieve. The abomasal mucosa was digested at 42°C in 300 ml of a 1% pepsin and 2% hydrochloric acid mixture for 4 hr. Worms were collected on a 40 µm sieve and a 10% aliquot retained. Worms in this aliquot were transferred to several petri-dishes and counted at 10% magnification under a dissecting microscope (LeJambre and Royal 1976).

Estimation of anthelmintic efficacy

Anthelmintic efficacy, before slaughter of the experimental animals, was calculated from the faecal egg count depression defined as the difference between the group mean eggs/g (epg) pre- and post-treated, and expressed as a percentage of the pre-treatment value. The percentage reduction (FECR) was corrected for change that occurred in the control group by equation.

$$(FECR)\% = \left(1 - \frac{T_2}{T_1} \times \frac{C_1}{C_2}\right) \times 100$$

where, T and C are the geometric means for the treated and control groups, and subscripts 1 and 2 designate the counts before and after treatment respectively (Campbell *et al.* 1978, President 1985). The anthelmintics on test which demonstrated efficacies of less than 90% were considered ineffective (Riffkin *et al.* 1984).

The efficacy of anthelmintics after slaughter was calculated from the counts of adult and immature stages of *H. contortus* using the following formula:-

$$\% \text{ Efficacy} = \frac{C - T}{C} \times 100$$

where, C and T are the geometric mean counts from untreated and treated groups respectively (Gibson 1964).

RESULTS AND DISCUSSION

The mean number of *H. contortus* eggs per gram of faeces (epg) for the experimentally infected sheep on day of treatment (day 30 post-infection) for strains M 1, M 2 and M 3 were 1 011, 2 371 and 3 313 respectively. Mean epg counts 5 days after treatment (day 35 post-infection) in the control and treated sheep are given in Table 1. FBZ depressed mean faecal epg counts of experimentally infected sheep with M1 and M2 strains of *H.contortus* by less than 90%. TBZ depressed group mean epg by less than 90% in sheep infected with M1 strain but had an efficacy of 94% in sheep infected with M 2 strain. LEV had an efficacy of 78% in sheep infected with M3 strain of *H. contortus*(Table 1).

Table 1. Mean epg of treated and untreated sheep after infection with larvae of three strains of *H. contortus*

Strain	Treatment group	Geometric mean	% efficacy
M1	Control	450	-
	TBZ	200	56%
	FBZ	500	*
M2	Control	4150	-
	TBZ	250	94%
	FBZ	1550	63%
M3	Control	3544	-
	LEV	775	78%

*Did not satisfy criteria for determining % efficacy.

Table 2. Results of slaughter trials conducted on the 3 strains (M1, M2 and M3) suspected as being resistant to test anthelmintics

Strain	Treatment group	Worm burden	
		Geometric mean (range)	Change compared with control (%)
M1	Control	1 767 (400-2 700)	-
	TBZ	267 (0-800)	85% decrease
	FBZ	1 000 (500-1 400)	43% decrease
M2	Control	4 700 (1 200-8 200)	-
	TBZ	100 (0-300)	98% decrease
	FBZ	1 400 (600-2 400)	70% decrease
M3	Control	1 133 (400-2 500)	
	LEV	50 (0-100)	96% decrease

The overall per cent reduction in group MWB following anthelmintic treatment is shown in Table 2 for each of the 3 strains of *H. contortus*.

In animals infected with M 1 strain, TBZ and FBZ reduced the MWB by less than 90%, confirming that the presence of TBZ reduced MWB by 85%, indicating a low level for resistance whereas FBZ reduced MBW by 43%, indicating a high level of resistance. TBZ reduced MWB by greater than 90% in sheep infected with M 2 strain, whereas FBW had an efficacy of 70%. Thus, M 2 strain was highly susceptible to TBZ but a moderate level of resistance to FBZ was observed.

The findings were unexpected because cross-resistance between benzimidazole or pro-benzimidazole would equally be reflected in the use of another anthelmintic having the same mode of action (Sutherland *et al.* 1988, Prichard 1990). The suspected resistance of LEV in the M 3 strain of *H. contortus* was not confirmed in the more exacting slaughter trials. There was 96% reduction in the worm burden of the group of sheep treated with LEV as compared with untreated controls, indicating that this strain was not resistant to LEV (Table 2).

The present study showed that resistance to benzimidazole (BZ) anthelmintics might be widespread in Kenya as reported by Nadrathi (1992). The use of these anthelmintics in regular drenching programme in the farms where M 1 and M 2 strains of *H. contortus* were isolated should be discontinued, and replaced with unrelated drugs like ivermectin or LEV.

It is highly probable that a similar pattern of resistance to BZ anthelmintics occurs within other sheep populations where climatic and management practices are generally similar to those of the present study. Thus, there is a need to monitor the distribution and extent of anthelmintic resistance in sheep and goat nematodes in large-scale farms in Kenya, and appropriate control and management measures instituted.

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