A FIELD STRAIN OF *TRICHOSTRONGYLUS COLUBRIFORMIS* RESISTANT TO LEVAMISOLE AND MORANTEL IN SOUTH AFRICA

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ABSTRACT

VAN WYK, J. A., BATH, G. F., GERBER, H. M. & ALVES, REGINA M. R., 1990. A field strain of *Trichostrongylus colubriformis* resistant to levamisole and morantel in South Africa. *Onderstepoort Journal of Veterinary Research*, 57, 199–122 (1990).

A strain of *Trichostrongylus colubriformis* from Nottingham Road, in Natal, was found to be solidly resistant to levamisole and morantel at the recommended dose levels. Untreated control sheep in fact harboured fewer worms at slaughter than either of the 2 treated groups. In contrast, the benzimidazoles and ivermectin were more than 99,9 % effective against this worm strain.

The possible implications of escalating resistance to anthelmintics in the gastrointestinal nematodes of sheep are discussed.

INTRODUCTION

Arundel (1985) placed the available anthelmintics for use in sheep and cattle according to their modes of action into 5 separate groups that are pharmacologically unrelated. According to this classification, levamisole and morantel belong to the same pharmacological group.

Anthelmintic resistance in *Trichostrongylus* spp. has previously been recorded in Australia and New Zealand (Waller, 1985), but not in South Africa. In addition, no instances of resistance had been recorded against the levamisole/morantel group of anthelmintics in South Africa, until Malan, Gruss, Roper, Ashburner & Du Plessis (1988) described resistance to levamisole in a strain of *Libyostrongylus douglassi* in ostriches, and Van Wyk, Van Schalkwyk, Gerber, Visser, Alves & Van Schalkwyk (1989) found 2 strains of *Haemonchus contortus* which showed resistance to both levamisole and morantel.

This paper deals with a strain of *Trichostrongylus colubriformis,* showing resistance to the levamisole/morantel group of anthelmintics.

HISTORY

During 1987 Bath (unpublished observations, 1987) recorded a strain of *T. colubriformis* (from Nottingham Road, Natal) that appeared from a preliminary faecal worm egg count reduction test to be insensitive to levamisole at a dosage of 7,5 mg kg^{-1} All the sheep had been drenched 27 days previously with levamisole[1].

The drenching history, which is incomplete as regards the various drugs and dates of drenching, records that broad spectrum drugs were alternated, and in addition some narrow spectrum compounds were used for controlling *Oestrus ovis* infection. Levamisole and niclosamide were apparently not administered in accordance with the manufacturers' recommendations, but were mixed and diluted with water before drenching.

MATERIALS AND METHODS

The strain of *T. colubriformis* used in the anthelmintic efficacy trial was isolated from naturally infected sheep at Nottingham Road, Natal. It was passaged twice in sheep maintained since birth under conditions of minimal exposure to worms at the Veterinary Research Institute, Onderstepoort. During maintenance in the laboratory the strain was not exposed to anthelmintics.

The infective larvae used in the investigation were stored for 5 days in the dark in tap-water at ambient temperature before being administered to the experimental animals.

The methods used for faecal worm egg counts, faecal cultures and infection of animals were according to Reinecke (1973).

Thirty-four 8-month old Merino rams, transferred to the laboratory from the field, were housed under conditions of minimal exposure to worms on concrete-floored pens that were swept twice weekly. As the sheep were not raised worm-free they were dewormed with a mixture of fenbendazole and trichlorphon (at dosages of 10 mg kg^{-1} and 124,8 mg kg^{-1} respectively) 10 days before the trial commenced. Subsequent faecal examinations of every sheep by total flotation of 5 g of faeces (Whitlock, 1959) 6 days later failed to reveal any worm eggs.

Particulars of the anthelmintics used in the experiment are shown in Table 1. Although they were off-the-shelf commercial formulations, these anthelmintics were assayed by the various manufacturers to confirm the precise concentrations of the active ingredients for accurate dosage determination.

TABLE 1 Particulars of the anthelmintics used in these investigations

Active ingredient	Trade name	Manufacturer	Dosage* (mg kg^{-1})
Ivermectin	Ivomec	Logos	0,2
Levamisole	Ripercol	Janssen	7,5
Morantel	Banminth II	Pfizer	12,5
Oxfendazole	Synanthic	Logos	5,0

* Registered dosage rate for sheep in South Africa

Commencing 28 days before treatment (Day −28), the 34 sheep were each infected daily for 3 days with about 1 620 infective larvae of the Nottingham Road strain of *T. colubriformis* (Table 2). On the day of treatment (Day 0), all the sheep were mass-measured, ranked according to the mean faecal worm egg counts, and allocated to the various trial groups with the aid of tables of random numbers. As the

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[1] Tramisol (Coopers)

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groups were of unequal size, the method of allocation was as described by Van Wyk & Gerber (1980).

On Day +7 the sheep were killed for worm recovery. At necropsy, the ingesta of the small intestines were concentrated by sieving through sieves with apertures of 150 μm onto sieves with apertures of 37 μm, the residues from both being retained for worm recovery. The mucosae of the small intestines were digested and examined for worms, as described by Reinecke (1973). With the exception of the worm burdens in Tables 3 and 4 that are marked with an asterisk and for which total worm counts were done, the numbers of worms in the intestinal ingesta were estimated by stereoscopic examination of a single 10 % aliquot.

TABLE 2 Experimental design

Day	Treatment
−28	34 sheep each dosed with 1 600* L3 *T. colubriformis*
−27	34 sheep each dosed with 1 600* L3 *T. colubriformis*
−26	34 sheep each dosed with 1 600* L3 *T. colubriformis*
0	12 sheep drenched with levamisole at 7,5 mg kg⁻¹
	6 sheep drenched with morantel at 12,5 mg kg⁻¹
	4 sheep drenched with ivermectin at 0,2 mg kg⁻¹
	4 sheep drenched with oxfendazole at 5,0 mg kg⁻¹
	8 sheep remained untreated as controls
+7	34 sheep killed for worm recovery

* Estimated from aliquots of larval suspension

Statistical analysis

Sufficient numbers of sheep were treated with levamisole and morantel for analysing the data by the non-parametric method of analysis (NPM) of Groeneveld & Reinecke (1969), as modified by Clark (Reinecke, 1973). In the groups treated with other anthelmintics the percentage efficacy was estimated by comparison of the geometric mean worm burdens of the treated and control groups of sheep, as outlined by Van Wyk, Malan, Gerber & Alves (1989). For the calculation of the geometric mean worm burdens, a value of 1 was substituted for zero if no worms were recovered from an animal.

RESULTS

The worm burdens are summarised in Tables 3 and 4.

The geometric mean worm burdens of the groups of sheep treated with levamisole and morantel were 247 and 781 larger, respectively, than the 1 873 of the untreated control group. Those for the sheep treated with ivermectin and oxfendazole were 3 and 2 worms, respectively. Thus the geometric mean efficacies were 0 % for both levamisole and morantel and 99,9 % for the other 2 remedies.

Too few sheep were treated for evaluting the efficacy of ivermectin and oxfendazole by the NPM, but both levamisole and morantel were ineffective (Class ×), as more than 5 of the treated sheep in each of these groups had worm burdens greater than the median of the controls × 0,5 (= 934 worms).

DISCUSSION

Unfortunately, the drenching history on the farm of origin of the Nottingham Road strain of *T. colubriformis* is apparently not sufficiently detailed or trustworthy to glean much useful information. The practice of mixing levamisole and niclosamide and

TABLE 3 *T. colubriformis* recovered from untreated controls and from sheep drenched with levamisole

Numbers of *T. colubriformis*	
Untreated (Control group)	Levamisole (7,5 mg kg⁻¹)
1223	469
1597*	1202
1651*	1781
1724*	1823
2009*	2220
2065	2235
2483	2518
2649	2610
	3034
	3064
	3186
	4658
Mean: # 1873	2120
Median: 1867×0,5 =934	NPM: 11/12 > 934

NPM efficacy: Class X (ineffective) Geometric mean efficacy: 0 %

* Total worm counts

\# Geometric mean

TABLE 4 *T. colubriformis* recovered from untreated controls and from sheep drenched with morantel, ivermectin and oxfendazole

Numbers of *T. colubriformis*	
Untreated (Control group)	Morantel (12,5 mg kg⁻¹)
1223	2111
1597	2462
1651	2567
1724	2577
2009	2919
2065	3481
2483	
2649	
Mean: # 1873	Mean: # 2654
Ivermectin (0,2 mg kg⁻¹)	Oxfendazole (5,0 mg kg⁻¹)
1	0
1	1
2	2
30	16
Mean: # 3	Mean: # 2
Geometric mean efficacy (%): # #	Morantel: 0,0
	Ivermectin: 99,9
	Oxfendazole: 99,9

\# Geometric mean

\#\# Based on the geometric mean values of the treated compared to the untreated control groups

diluting the mixture with water before drenching appears to be quite widely used in South Africa (P. C. van Schalkwyk, Personal communication, 1987), and one wonders what harm could be caused, as regards resistance, by use of such drenches if their efficacy is adversely affected.

This is the first reported case in South Africa of resistance of *T. colubriformis* to any of the anthelmintics presently registered for its control. It is only the second South African report of resistance of nematodes of sheep to the levamisole/morantel group of anthelmitics.

The high level of resistance to levamisole and morantel reported in this paper is similar to that recorded by Van Schalkwyk, Geyser & Rezin (1983)

and Van Schalkwyk (1984) in 2 strains of *Ostertagia* spp. to the benzimidazoles. In contrast, most of the previous reports of resistance of *H. contortus* to various anthelmintics in South Africa indicate that this worm species seldom, if ever, becomes so markedly resistant (Van Wyk & Gerber, 1980; Van Wyk, Gerber & Alves, 1982; Van Schalkwyk *et al.*, 1983; Van Schalkwyk, 1984; Van Wyk, Malan, Gerber & Alves, 1987; Van Wyk & Malan, 1988). The present results also agree with those of McKenna & Seifert (1985), who recorded 0 % efficacy of morantel citrate at 8,8 mg kg^{-1} against a strain of *T. colubriformis* in the first report in New Zealand of resistance affecting this worm species.

In contrast to *H. contortus*, in which resistance to the levamisole/morantel group of anthelmintics is uncommon in sheep both here and in Australia (Van Wyk *et al.*, 1989; Waller, 1985), the present case of resistance involving *T. colubriformis* in South Africa concerns both drugs in this group. Waller (1985) states: "Quite unlike *H. contortus*, . . . early reports of resistance in both *Ostertagia* and *Trichostrongylus* spp. were just as frequent to levamisole and morantel as to the benzimidazoles." In Australia, resistance to levamisole is more common in worm infections in goats than in sheep, but this may be because levamisole is a less satisfactory anthelmintic in goats than in sheep (Waller, 1985).

The solid resistance of the Nottingham Road strain of *T. colubriformis* to levamisole lends support to the surmise that resistance to levamisole in *T. colubriformis* is caused by a single gene, which tends to favour the rapid development of resistance: ". . . under equal conditions of selection and fitness, resistance provided by a single gene will spread throughout the population at a faster rate than resistance depending on the presence of many genes." (Le Jambre, 1985).

The modern anthelmintics for which no resistance has been reported in South Africa, have thus been whittled down to a single group: the organophosphates. Recently, however, a strain of *H. contortus* was shown to be resistant to this group of anthelmintics (Malan & Van Wyk, personal observations, 1988).

That there is proven resistance of at least one worm strain to every one of the groups of anthelmintics is in itself possibly not as serious as the fact that certain strains of *H. contortus* in South Africa are already resistant to 3 different groups simultaneously, and that at least 1 of these strains has been disseminated by sale of the hosts when the farmer concerned abandoned sheep farming (Van Wyk, Malan, Gerber & Alves, 1989). The pessimism expressed by Van Wyk *et al.* (1987) regarding the future availability of effective anthelmintics in South Africa appears to be supported by the escalation of resistance in a growing number of worm strains. Recently, a strain of *H. contortus* acquired from the communal grazing of a cooperative organisation used for performance-testing of rams from numerous stud breeders in Natal, was shown to be simultaneously resistant to 3 anthelmintic groups (Van Wyk, Van Schalkwyk & Bath, unpublished observations, 1988). After the completion of each of the performance tests the best animals are sold at a public auction. In this way the resistance genes could be dispersed very widely, unless special precautions are taken.

Van Wyk *et al.* (1987) posed the question: What will happen if the stage is reached when worm strains develop resistance to all the available anthelmintics? It seems probable that progressively more resistant strains of gastrointestinal nematodes will be dispersed, as farmers realise that they are unable to control them, and sell their sheep. The chances of preventing this dispersal by regulatory measures appear remote (Van Wyk *et al.*, 1989).

It is probable that multiple resistance will develop in more and more strains of worms. At present it seems unlikely that sufficient new, effective anthelmintics will be developed to keep pace with developing resistance world-wide (Riffkin, 1988). The emergence of such widespread resistance (Waller, 1985; Van Wyk & Malan, 1989) lends urgency to the quest for efficient strategies, which do not rely solely on anthelmintics for controlling economic losses from helminth infection of domestic livestock.

ACKNOWLEDGEMENTS

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