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SOUTH AFRICAN FIELD STRAINS OF *HAEMONCHUS CONTORTUS* RESISTANT TO THE LEVAMISOLE/MORANTEL GROUP OF ANTHELMINTICS

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ABSTRACT

VAN WYK, J. A., VAN SCHALKWYK, P. C., GERBER, H. M., VISSER, EVELYN L., ALVES, REGINA M.R. & VAN SCHALKWYK, L., 1989 South African field strains of *Haemonchus contortus* resistant to the levamisole/morantel group of anthelmintics. *Onderstepoort Journal of Veterinary Research*, 56, 257–262 (1989)

A strain of *Haemonchus contortus* from the Pietermaritzburg district of Natal was found to be resistant to levamisole (geometric mean efficacy 76,5 %), morantel (41,9 %), the benzimidazoles (oxfendazole: 33,7 %) and rafoxanide (82,0 %), but apparently fully susceptible to closantel and disophenol. In the case of ivermectin, a mean of 5,2 % of the *H. contortus* was not removed at a dosage of 200 µg kg^{-1} live mass.

A second strain of *H. contortus*, from Amsterdam in the south-eastern Transvaal, showed reduced susceptibility to levamisole (80,8 %) and morantel (46,2 %), the only 2 drugs tested.

This is apparently the first report of resistance to the levamisole/morantel group of anthelmintics in sheep in South Africa.

INTRODUCTION

During the recent past resistance of worms to the available anthelmintics has been recorded at an increasing rate. In South Africa a series of papers has appeared describing resistance of gastrointestinal nematodes of ruminants to the benzimidazoles and the salicylanilides (Van Wyk & Gerber, 1980; Van Wyk, Gerber & Alves, 1982; Van Schalkwyk, Geyser & Rezin, 1983; Van Schalkwyk, 1984). Thereafter, Van Wyk & Malan (1988) and Van Wyk, Malan, Gerber & Alves (1989) described 4 strains of *H. contortus* that showed resistance to ivermectin. One of the 4 strains was simultaneously resistant to 3 anthelmintic groups.

At the time of the most recent of these papers only the levamisole/morantel and organophosphate groups of anthelmintic were not affected by resistance.

This paper concerns 2 field strains of *H. contortus* that have developed resistance to both levamisole and morantel.

GENERAL MATERIALS AND METHODS

Unless otherwise stated the methods used for faecal egg counts (epg), making faecal cultures and for infection of animals are those described by Reinecke (1973).

Particulars of the anthelmintics used in the investigations are listed in Table 1.

In each of the 3 trials the sheep were mass-measured on the day of treatment (Day 0), ranked according to mean worm egg counts, and allocated to the various trial groups with the aid of tables of random numbers. In the case of groups of unequal size the method of allocation is that described by Van Wyk & Gerber (1980).

Worm recovery: The abomasal ingesta of the sheep in Trials 2 and 3 were concentrated by sieving through sieves with apertures of 150 µm onto sieves with apertures of 37 µm, whereafter the residues in both were retained for worm recovery.

Worm totals not marked with an asterisk in the various tables are the sum of macroscopic[1] examination of 10 % of the abomasal ingesta and total microscopic examination of the digested abomasal mucosa of each animal. Counts with an asterisk were derived from total macroscopic and microscopic examination of abomasal ingesta and mucosal digests, respectively. These accurate total counts are essential for analysis of the data by the non-parametric method (NPM) of Groeneveld & Reinecke (1969), as modified by Clark (cited by Reinecke, 1973).

For calculation of the geometric mean epg or worm burden a value of 1 was substituted for zero if the faecal worm egg counts were negative (Trial 1), or if no worms were recovered from the sheep that were slaughtered.

Haemonchus Strain I

History

A group of 64 sheep was received at the Veterinary Research Institute, Onderstepoort from an agricultural experimental farm near Pietermaritzburg in Natal. They were born in the South-western Cape, but had been moved to the experimental farm during August 1986, where they grazed with resident sheep on dry-land kikuyu *(Pennisetum clandestinum)* pastures in an intensive system until February 1987. The mean annual rainfall for the region is 800–850 mm, received principally in the summer.

Worm control on the farm relies exclusively on the use of anthelmintics drenched at approximately monthly intervals. Anthelmintic groups are alternated annually, and during 1986 and the beginning of 1987 only levamisole was used for control of gastrointestinal nematodes.

On arrival at the laboratory the sheep were housed under conditions of minimal worm transmission (concrete floors that are swept twice weekly) and were drenched with levamisole[2] at a dosage of approximately 7,5 mg kg^{-1} live mass. Eleven days later some of the sheep presented with what appeared to be severe clinical haemonchosis, including

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[1] Approximately a tenth of each 10 % aliquot was, however, examined stereoscopically in order to ensure that the worms were in the adult stage and thus easily seen macroscopically

[2] Ripercol (Janssen)

TABLE 1 Particulars of the anthelmintics used in the efficacy studies

Active ingredient	Trade name	Manufacturer	Dosage* (mg kg^{-1})
Albendazole	Valbazen	SmithKline	3,8
Closantel	Flukiver	Janssen	5,0
Disophenol	Trimintic LA	Cyanamid	10,0 s/c
Fenbendazole	Panther	Hoechst	5,0
+ Trichlorphon			+ 62,4
Ivermectin	Ivomec	MSD	0,2
Levamisole	Ripercol–1	Janssen	7,5
Morantel	Banminth II	Pfizers	12,5
Nitroxynil	Trodax	MayBaker	10,0 s/c
Oxfendazole	Synanthic	MSD	5,0
Rafoxanide	Ranide	MSD	7,5

* Recommended dosage rate for sheep in South Africa; s/c denotes subcutaneous injection, all the other being drenched orally. Although Flukiver is recommended at 10 mg kg^{-1}, we drenched it at 5 mg kg^{-1}, because no Seponver (which is recommended at 5 mg kg^{-1}) was available to us at the time of the trial

FIG. 1 Sheep showing prominent anasarca of the facial region and the submandibular space

anasarca of not only the submandibular space, but also of the entire facial region (Fig. 1).

Strain 1: Preliminary investigation

Materials and Methods

In order to investigate whether haemonchosis was the cause of the clinical syndrome observed in the group of sheep from Natal, epg counts and larval identifications were done for the 3 most severely affected animals before and daily for 4 days after treatment with levamisole at a dosage of 7,5 mg kg^{-1}. No untreated controls were included in this preliminary investigation.

Other than at the first treatment on arrival at the laboratory, the levamisole used in the subsequent investigations was from a container, the contents of which had been assayed to confirm the concentration of levamisole listed on the label (Table 1).

Results

While the pretreatment faecal egg counts of the 3 sheep were 1 100, 6 470 and 18 300 (geometric mean: 5 069), those after treatment were 500, 1 200 and 2 275 respectively (geometric mean: 1 109), giving a mean reduction of 78,1 %. Faecal cultures yielded a pure culture of *H. contortus*.

Comment

The epg counts of 2 of the 3 sheep were lower than could be expected from their clinical condition. Furthermore, the counts were further reduced to such an extent by treatment with levamisole, that it seemed incredible that the treatment on introduction to Onderstepoort had failed to alleviate the condition.

It is possible that the 3 experimental sheep harboured few worms at the time of treatment (the rest having been removed by the drench on arrival at Onderstepoort), but that they had been affected to such an extent by the worm infection they had contracted in the field, that the relatively few remaining worms were sufficient to cause the development of clinical signs. On the other hand the worm egg counts may not have been an accurate reflection of the worm burdens of these animals.

It was decided to test the susceptibility of this strain of *H. contortus* to various anthelmintics more fully.

Trial 1 (Strain 1): Controlled trial based on reduction of faecal worm egg counts

Materials and methods

Those sheep of the remaining 61 that passed 100 or more eggs per gram of faeces were ranked according to epg counts. They were randomised into 9 groups of 5 sheep each, which were treated as indicated in Table 1 and 2, and a tenth group of 6 sheep which served as untreated controls.

Faecal worm egg counts were conducted on all the animals 3 days before treatment, and on 3 occasions, 4, 7 and 12 days after treatment.

The anthelmintic efficacy was estimated by the following method:

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

where T and C are the geometric means for the treated and control groups and the subscripts 1 and 2 designate the mean epg counts on dates before and after the date of treatment, respectively (Presidente, 1985).

Results

The results are summarized in Table 2.

Albendazole, closantel, disophenol, ivermectin, nitroxynil and a mixture of trichlorphon/fenbendazole caused more than a 96 % reduction in the geometric mean egg count. In contrast, rafoxanide caused an 89,5 % reduction and both levamisole and morantel failed to reduce the epg.

Comment

The levamisole/morantel group of anthelmintics

TABLE 2 Trial 1 (Strain 1): Anthelmintic efficacy (epg results)

Group*	Treatment	Faecal egg count†		Efficacy (%)††
		Before treatment	After treatment	
1	Control	1 329	2 418	—
2	Levamisole	637	1 428	0,0
3	Morantel	1 359	2 124	0,0
4	Albendazole	1 300	84	96,4
5	Rafoxanide	1 289	247	89,5
6	Ivermectin	643	12	99,0
7	Closantel	1 163	2	99,9
8	Disophenol	1 087	4	99,7
9	Nitroxynil	1 028	1	99,9
10	Trichlorphon + Fenbendazole	825	1	99,9

* Five sheep per group, excepting for Group 2 (6 sheep)
† Geometric mean faecal egg count
†† Reduction on geometric mean

(Arundel, 1985) was apparently ineffective when judged by faecal egg count reduction. However, it must be remembered that the strain had already been selected with levamisole when all the sheep were drenched with this drug on introduction to the Institute. It was then decided to re-isolate the strain of *H. contortus* from the field for a controlled efficiency test, based on the reduction of the worm counts of treated compared with control groups of sheep.

Trial 2 (Strain 1): Controlled trial based on reduction of worm burdens

Materials and methods

Strain 1 of *H. contortus* was re-isolated from the field during June 1986 by culturing sheep faeces obtained from the farm of origin, and infecting worm-free donor sheep in the laboratory at Onderstepoort. The infective larvae (L3) used in Trial 2 were collected from these donor sheep, and were kept in water at room temperature in flat medicine bottles for about a week before the trial animals were infected. Thus the strain was passaged only once in the laboratory and, in contrast with Trial 1, was not selected with anthelmintics during this time.

The trial animals, consisting of 33, 6–12 month-old Merino rams and 25 similarly aged wethers were not raised worm-free, but were obtained from the field. On introduction to the laboratory these animals were housed on concrete, as previously described and were dewormed with both morantel and a mixture of fenbendazole and trichlorphon at double the therapeutic dosages shown in Table 1. Subsequent faecal examinations of each sheep by total flotation of 5 g of faeces (Whitlock, 1959) failed to reveal any worm eggs. During the course of the trial the sheep were kept under conditions that precluded unintentional exposure to gastrointestinal nematodes.

Although the anthelmintics used in this trial were off-the-shelf commercial formulations, they were assayed by the manufacturers to confirm the concentration of active ingredient in each, for accurately determining the various dosages.

The trial design is summarized in Table 3.

After infection with a total of approximately 3 100 L3 of *H. contortus,* between Days −33 to −31 the sheep were ranked according to faecal egg counts on Day 0 and were either treated with an anthelmintic, or kept as untreated controls (Table 3). One sheep allocated to the disophenol group was excluded from the trial as a portion of the intended dose was lost during injection.

TABLE 3 Trial 2 (Strain 1): Trial design

Day	Treatment
−33	58 sheep each dosed with 1 099* L3 *H. contortus*
−32	58 sheep each dosed with 1 021* L3 *H. contortus*
−31	58 sheep each dosed with 1 021* L3 *H. contortus*
0	13 sheep drenched with levamisole at 7,5 mg kg^{-1}
	12 sheep drenched with morantel at 12,5 mg kg^{-1}
	5 sheep drenched with closantel at 5,0 mg kg^{-1}
	4 sheep injected with disophenol at 10,0 mg kg^{-1}†
	5 sheep drenched with ivermectin at 0,2 mg kg^{-1}
	5 sheep drenched with oxfendazole at 5,0 mg kg^{-1}
	5 sheep drenched with rafoxanide at 7,5 mg kg^{-1}
	9 sheep remained untreated as controls
+7	25 sheep killed for worm recovery
+8	33 sheep killed for worm recovery

* Estimated from aliquots of larval suspension
† Administered subcutaneously

All the sheep were killed for worm recovery on Day +7 and Day +8, individuals being selected at random for slaughter, in such a way that similar proportions of each trial group were killed on each of the 2 days.

Statistical analysis: In the case of the levamisole and morantel groups sufficient sheep were used for analysis of the results by the non-parametric method (NPM) of Groeneveld & Reinecke (1969), as modified by Clark (cited by Reinecke, 1973). In the other groups the efficacy was calculated by comparison of the geometric mean worm burdens of the treated (T) and control (C) groups:

$$\% \text{ efficacy} = 100 - \frac{T\,100}{C}$$

Results

The results are summarized in Tables 4 and 5.

The efficacy of levamisole and morantel were Class B (60 % or more effective in at least 60 % of the treated flock) and Class X (ineffective) when analysed by the NPM and 76,5 % and 41,9 % respectively when based on reduction of the geometric mean worm burdens of the treated, compared to the untreated control animals.

In the other groups the geometric mean reductions were 33,7 %, 82,0 %, 94,8 %, 99,8 % and 99,9 % for oxfendazole, rafoxanide, ivermectin, closantel and disophenol respectively.

TABLE 4 Trial 2 (Strain 1): *H. contortus* recovered from untreated controls and from sheep drenched with levamisole or morantel

Numbers of *H. contortus*		
Untreated (control group)	Levamisole (7,5 mg kg^{-1})	Morantel (12,5 mg kg^{-1})
1 504	116	458
1 629	132	504
1 638	252	666
2 248*	297	1 202*
2 268*	632	1 205*
2 358*	655	1 301*
2 382	693	1 621
2 679	712*	1 693
3 174	729*	1 778
	740*	1 818
	957	2 225
	967	2 506
	1 166	
Mean:† 2 149	504	1 248
Median: 2 268 × 0,4 = 907	NPM: 3/13 > 907 (Class B)	NPM: 9/13 > 1 134 (Class X)
Geometric mean efficacy†† 76,5 %		

* Total worm counts
† Geometric mean
†† Reduction on geometric mean

TABLE 5 Trial 2 (Strain 1): *H. contortus* recovered from untreated controls and from sheep drenched with oxfendazole, rafoxanide, ivermectin, closantel or disophenol

Numbers of *H. contortus*	
Untreated (control group)	Oxfendazole (5,0 mg kg^{-1})
1 504	1 123
1 629	1 364
1 638	1 376
2 248*	1 573
2 268*	1 771
2 358*	
2 382	Mean:† 1 425
2 679	
3 174	
Mean:† 2 149	
Disophenol (10 mg kg^{-1})	Rafoxanide (7,5 mg kg^{-1})
0	96
0	281*
2	420*
3	615*
	1 234
Mean:† 2	Mean:† 386
Ivermectin (0,2 mg kg^{-1})	Closantel (5,0 mg kg^{-1})
62*	1
93*	1
122*	11
134*	11
177*	32
Mean:† 111	Mean:† 5
Geometric mean efficacy (%): ††	Oxfendazole: 33,7 Rafoxanide: 82,0 Ivermectin: 94,8 Closantel: 99,8 Disophenol: 99,9

* Total worm counts
† Geometric mean
†† Reduction on geometric mean

Comment

Only in the case of levamisole and morantel were sufficient animals used for analysis of the data by the NPM. However, most other groups consisted of 5 sheep each, which is sufficient for giving a good indication of the efficacy of the drugs tested.

The rafoxanide group also consisted of only 5 sheep, which is ordinarily too few for classifying the results by the NPM. Nevertheless, it can be calculated that rafoxanide failed to qualify for a Class A efficacy, and can be rated at best as Class B. If 11 animals are treated, then for an "A"-efficacy claim only 1 animal in the treated group may harbour more worms than the reduced median worm count of the controls (Median × 0,25). As the median worm burden of the controls in this trial was 2 268, it follows that only 1 sheep in the rafoxanide group may have more than 567 worms at slaughter. It can be seen in Table 5 that 2 of 5 counts of the rafoxanide group exceeded 567, thus indicating an efficacy less than Class A, which is the registered claim for this drug in South Africa. In fact, as discussed by Van Wyk & Gerber (1980), rafoxanide was traditionally practically 100 % effective against adult *H. contortus* in South Africa, before the first instances of resistance were encountered.

Similarly, it can be calculated that oxfendazole failed to obtain a Class C efficacy claim (more than 50 % effective in more than 50 % of the treated flock), and was thus ineffective (Class X).

Trial 3 (Strain 2): Controlled trial based on reduction of worm burdens

History

During 1988 clinical observations and faecal egg counts of sheep on improved pasture on a farm near Amsterdam in the south-eastern Transvaal indicated that the strain of *H. contortus* present was possibly resistant to the levamisole/morantel group of anthelmintics.

The pastures, consisting of Midmar grass, which has to be re-established annually were first established in 1986. Thereafter outbreaks of coccidiosis were experienced in young lambs within the same season. Serious outbreaks of haemonchosis soon followed and this led to short interval dosing with morantel and levamisole in an irregularly alternating sequence, but apparently without controlling the infection effectively.

An anthelmintic efficacy trial was planned, involving animals that had acquired a natural infection on the farm.

Materials and metods

After the pasture had been re-established during the summer of 1987/1988, a group of 15 young weaner Merino lambs were thoroughly dewormed and placed in one of the paddocks towards the end of January 1988, before any other animals were introduced.

Approximately one month later the 14 surviving lambs were removed to the laboratory, at Kempton Park, where the efficacy trial was conducted. In the laboratory the lambs were housed on raised wooden slats, under conditions that precluded unintentional worm infection.

The lambs were ranked according to epg count, and randomized into an untreated control group of 4 animals, and 2 groups, each of 5 lambs, for testing the anthelmintic efficacy of morantel and levamisole respectively. Details concerning the drugs and dosages appear in Table 1.

Five days after treatment all the lambs were slaughtered and processed for worm recovery.

Results

At the end of the grazing period the lambs had high faecal epg counts, ranging from 4 700–24 400 (Table 6).

Morantel at 12,5 mg kg^{-1} and levamisole at 7,5 mg kg^{-1} removed a geometric mean of 46,2 % and 80,4 % of the *H. contortus,* respectively (Table 7). The corresponding arithmetic mean efficacies were 44,1 % and 57,3 %.

TABLE 6 Trial 3 (Strain 2): Pretreatment faecal worm egg count (*H. contortus*)

Group	Sheep	Epg count
Untreated (control)	1	5 000
	2	7 500
	3	9 800
	4	24 400
		Geom. mean: 9 731
Morantel (12,5 mg kg^{-1})	5	4 700
	6	5 600
	7	8 800
	8	16 800
	9	19 800
		Geom. mean: 9 491
Levamisole (7,5 mg kg^{-1})	10	5 400
	11	7 000
	12	11 400
	13	11 400
	14	14 600
		Geom. mean: 9 357

TABLE 7 Trial 3 (Strain 2): *H. contortus* recovered from untreated controls and from sheep drenched with levamisole or morantel

Group	Numbers of *H. contortus*	Efficacy (%)
Untreated (control)	682	
	1 257	
	1 547	
	1 763	
Geom. mean	1 237	—
Arith. mean	1 312	—
Morantel (12,5 mg kg^{-1})	284	
	582	
	790	
	914	
	1 096	
Geom. mean	666	46,2
Arith. mean	733	44,1
Levamisole (7,5 mg kg^{-1})	10	
	165	
	476	
	810	
	1 340	
Geom. mean	243	80,4
Arith. mean	560	57,3

Comment

Previously, levamisole consistently achieved a geometric mean efficacy of more than 99 % in trials involving strains of *H. contortus* that were found to be resistant to various other anthelmintics in South Africa (Van Schalkwyk, 1984; Van Wyk & Malan, 1988; Van Wyk *et al.*, 1989). In fact, in most of these trials the drug was 100 % effective. In the present trial, however, the Amsterdam strain of *H. contortus* showed resistance to both levamisole and morantel.

The experimental lambs became quite heavily infected with *H. contortus* within 1 month on pasture that had not been grazed after re-establishment at the beginning of the summer of 1987/1988. Thus, the parasite had managed to survive on the Highveld of the Transvaal in large numbers through winter and the re-establishment of the pasture at the beginning of the subsequent summer.

This observation appears to be at variance with that of Horak (1980), who stated: "... the findings in Surveys 3 and 4, and 5 and 7 indicate that few or no larvae [of *Haemonchus* spp.] overwintered on pasture." Three of the 4 surveys listed by Horak (1980) were in sheep on the Highveld of the Transvaal, and the fourth (No. 7) in cattle in the northern Transvaal.

DISCUSSION

From the results of the various experiments it is obvious that both Strain 1 (Pietermaritzburg experimental station) and Strain 2 (Amsterdam) show marked resistance to the levamisole/morantel group of anthelmintics. Although resistance to this group of anthelmintics is quite common in *H. contortus* and *Trichostrongylus colubriformis* in sheep in Australia (Waller, 1985), these are apparently the first reported cases of resistance to these drugs in sheep in South Africa. However, the first case of resistance to levamisole in this country was recorded in *Libyostrongylus douglassi* of ostriches (Malan, Gruss, Roper, Ashburner & Du Plessis, 1988).

In the case of Strain 1 both oxfendazole and rafoxanide also failed to obtain their registered Class A efficacy claims against *H. contortus.* Thus Strain 1 is resistant simultaneously to 3 of the 5 anthelmintic groups, in common with the White River strain of *H. contortus,* as reported by Van Wyk & Malan (1988).

Van Wyk, Malan, Gerber & Alves (1987) stated that of the anthelmintics registered at that time for use against nematodes of sheep in South Africa, only disophenol, levamisole, morantel and trichlorphon had not yet been shown to be affected by nematode resistance in this country. Morantel and levamisole must now also be removed from the list of drugs not yet affected by resistance. Furthermore, disophenol is no longer registered for use in sheep and recently a strain of *H. contortus* was found to be resistant to trichlorphon (Malan & Van Wyk, unpublished observations, 1988).

Van Wyk *et al.* (1987) voiced the opinion that with escalating incidences of resistance a crisis could be reached in the treatment of *H. contortus* and that farmers might have to abandon sheep farming on those farms where all anthelmintic groups have failed. At the time this was regarded by some as alarmist, but at present such a possibility no longer seems improbable. At least 3 instances have already occurred where sheep farming was abandoned in South Africa after multiple resistance had developed (Van Wyk & Malan, 1988; Van Wyk *et al.*, 1989; M. D. Soll, Personal communication, 1987).

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