Onderstepoort J. vet. Res., 47, 137–142 (1980)

A FIELD STRAIN OF *HAEMONCHUS CONTORTUS* SHOWING SLIGHT RESISTANCE TO RAFOXANIDE

J. A. VAN WYK and H. M. GERBER, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

VAN WYK, J. A. & GERBER, H. M., 1980. A field strain of *Haemonchus contortus* showing slight resistance to rafoxanide. *Onderstepoort Journal of Veterinary Research*, 47, 137–142 (1980).

A field strain of *H. contortus*, already resistant to benzimidazole anthelmintics, was also found to be slightly resistant to rafoxanide. This is apparently the first report of resistance to rafoxanide in a field strain of *H. contortus*.

Résumé

*UNE SOUCHE SAUVAGE D'*HAEMONCHUS CONTORTUS *REVELANT UNE RÉSISTANCE LÉGÈRE AU RAFOXANIDE*

*Une souche sauvage d'*H. contortus *déja résistante aux anthelmintiques de benzimidazole, a également été trouvée être légèrement résistante au rafoxanide. Ceci est apparemment le premier rapport signalant la résistance d'une souche sauvage de* Haemonchus contortus *champêtre au rafoxanide.*

INTRODUCTION

The development of resistance of some strains of *Haemonchus contortus* to anthelmintics to which the species was previously susceptible is well established and is gaining in importance in various parts of the world (Le Jambre, 1978).

In South Africa, a strain of *H. contortus* resistant to thiabendazole was isolated at Onderstepoort (Van Wyk, unpublished data, 1974), and Berger (1975) described a field strain of the same species relatively resistant to thiabendazole, mebendazole and parbendazole. Another strain, isolated from Kaalplaas, an experimental farm adjacent to Onderstepoort, was found to be resistant to thiabendazole, mebendazole and fenbendazole (Van Wyk, unpublished data, 1976). Very little work has hitherto been done on the prevalence of resistant strains in South Africa and it is probable that the problem is more common than appears from these limited reports.

During investigations on the strain from Kaalplaas (designated the OP-M strain) it was discovered by chance that it was also slightly resistant to rafoxanide. This paper describes preliminary investigations with this strain as well as a trial to assess the efficacy of rafoxanide by the non-parametric (NPM) method of Groeneveld & Reinecke (1969), as modified by Clark (cited by Reinecke, 1973), to test the efficacy of rafoxanide.

I. PRELIMINARY INVESTIGATIONS

Method

Upon arrival at Onderstepoort, 11 goats from Kaalplaas, an experimental farm adjacent to Onderstepoort, were treated unsuccessfully for haemonchosis with mebendazole.

Two worm-free Dorper sheep (1 & 2) were each infested with 5 000 infective larvae (L3) of *H. contortus*, isolated from 4 of the 11 goats mentioned above.

From 21–41 days after infestation egg counts were carried out regularly on the faeces of the 2 sheep by a modified McMaster method (Reinecke, 1973); thereafter the egg counts were continued for only one of them (Sheep 1).

On Day 28 after infestation both sheep were treated with mebendazole* (15 mg/kg) and on Day 34 they were dosed with fenbendazole** (5 mg/kg). On Day 41 after infestation, Sheep 1 was dosed *per os* with rafoxanide*, while the other was retained, untreated, as a donor of the OP-M strain of *H. contortus*.

Subsequently, 2 additional Dorper sheep (3 & 4) were each infested with 5 000 L3 of *H. contortus* isolated from the donor (Sheep 2) which had not been exposed to rafoxanide. Sheep 3 and 4 were treated with rafoxanide 38 days after infestation, and were necropsied for worm recovery 17 days later.

Moving 3-point averages, interrupted at the points of treatment, were used for plotting Fig. 1 & 2.

Results

The results are summarized in Fig. 1 & 2.

On the 4 days before the mebendazole treatment, the mean faecal egg count of Sheep 1 and 2 was 8 100 eggs per g (e.p.g.) of faeces (range 4 300–14 400). Between the mebendazole and fenbendazole treatments the mean egg count was 5 492 (range 2 700–11 900) and for the 6 days after fenbendazole treatment, the mean count was 5 100 e.p.g. (range 300–8 100). After the rafoxanide treatment, the mean count of Sheep 1 was 2 400 e.p.g. (range 1 300–4 400).

The mean egg count of Sheep 3 was 1 200 e.p.g. (range 200–2 300) before treatment with rafoxanide and 200** (range 0–500) after treatment; for Sheep 4 the respective figures were 1 600 (range 500–2 500) and 1 200** (range 500–1 800).

At necropsy 668 adult *H. contortus* were recovered from Sheep 1, 106 from Sheep 3 and 464 from Sheep 4.

Comment

While, from the faecal egg counts the 2 benzimidazole remedies appeared (Fig. 1) to have had little effect upon this strain of *H. contortus* in 2 sheep, rafoxanide reduced the egg count considerably in 1 sheep and somewhat less in 2 other sheep.

II. NPM TRIAL

Because it appeared from the preliminary trials that this OP-M strain of *H. contortus* was resistant not only to benzimidazole remedies but was also relatively resistant to rafoxanide, a controlled trial was carried out to determine the efficacy of rafoxanide against it.

* Multispec (Ethnor)
** Panacur (Hoechst)

Received 18 April 1980—Editor

* Ranide (MSD)
** These figures exclude the high egg counts a day after treatment, since rafoxanide is known to act slowly (Snijders, Horak & Louw, 1973, Table 5: Sheep treated on 7th June and e.p.g. considerably lowered only on 9th June)

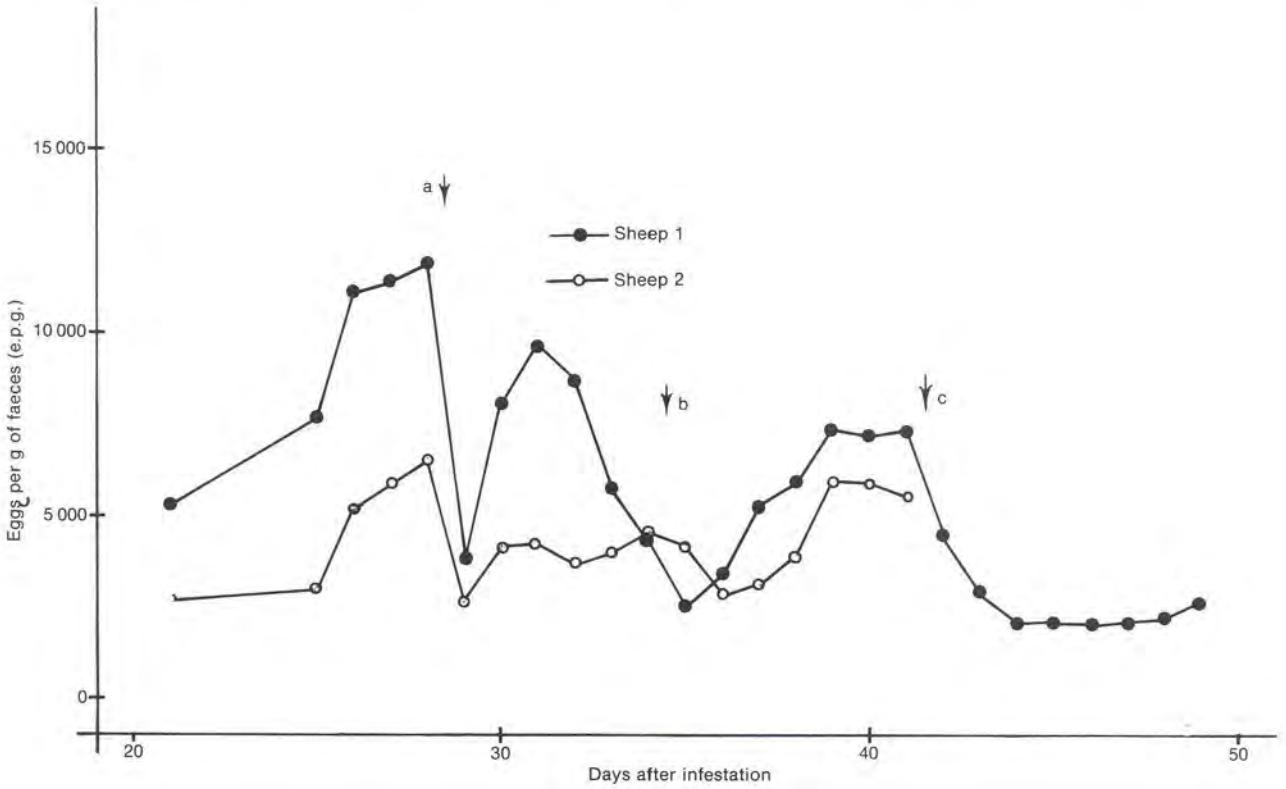

FIG. 1 Moving 3-point averages of the faecal egg counts of Sheep 1 and 2. The arrows indicate treatment: a mebendazole; b fenbendazole; c rafoxanide (only Sheep 1)

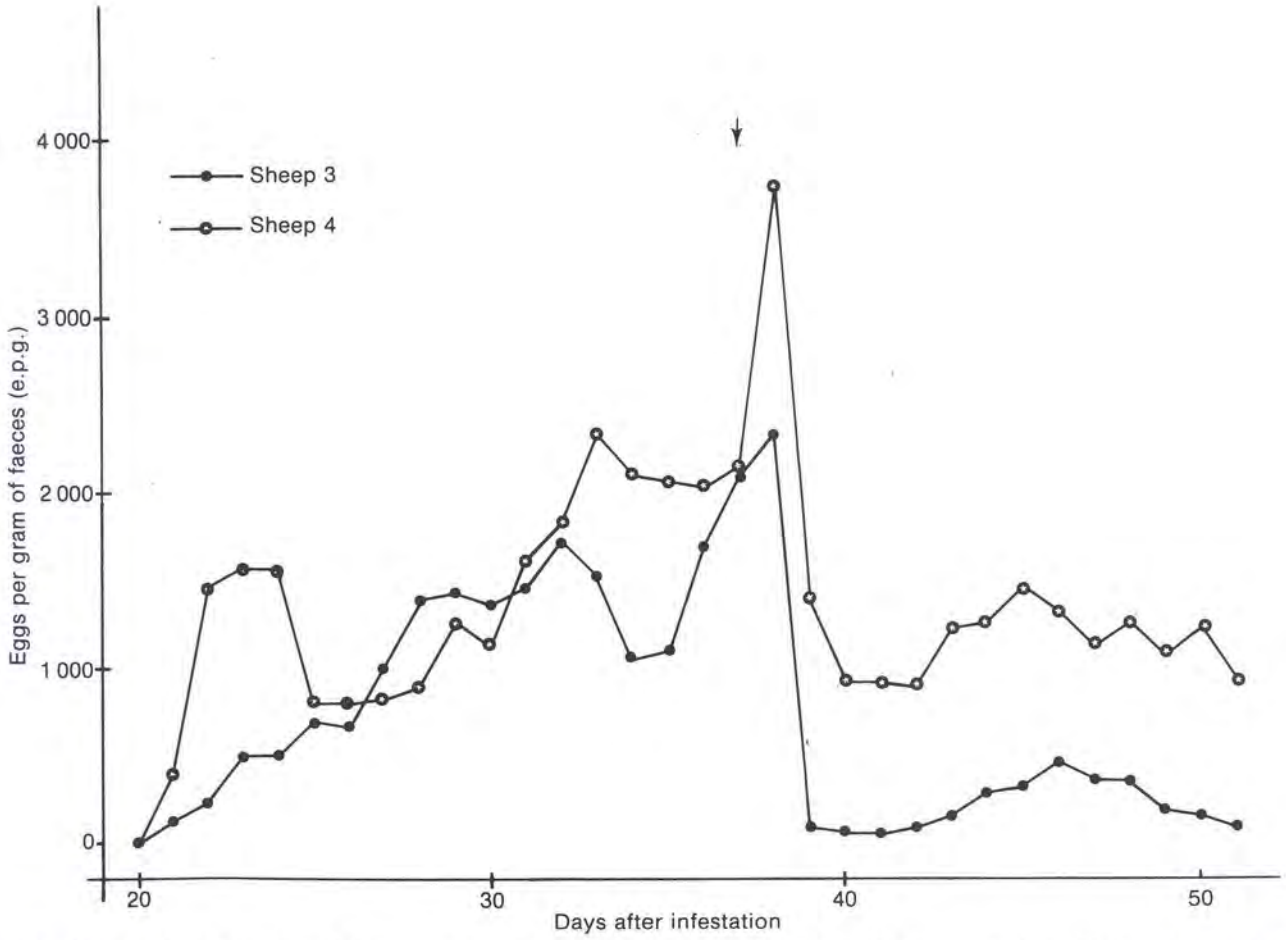

FIG. 2 Moving 3-point averages of the faecal egg counts of Sheep 3 and 4. The arrow indicates treatment of both sheep with rafoxanide

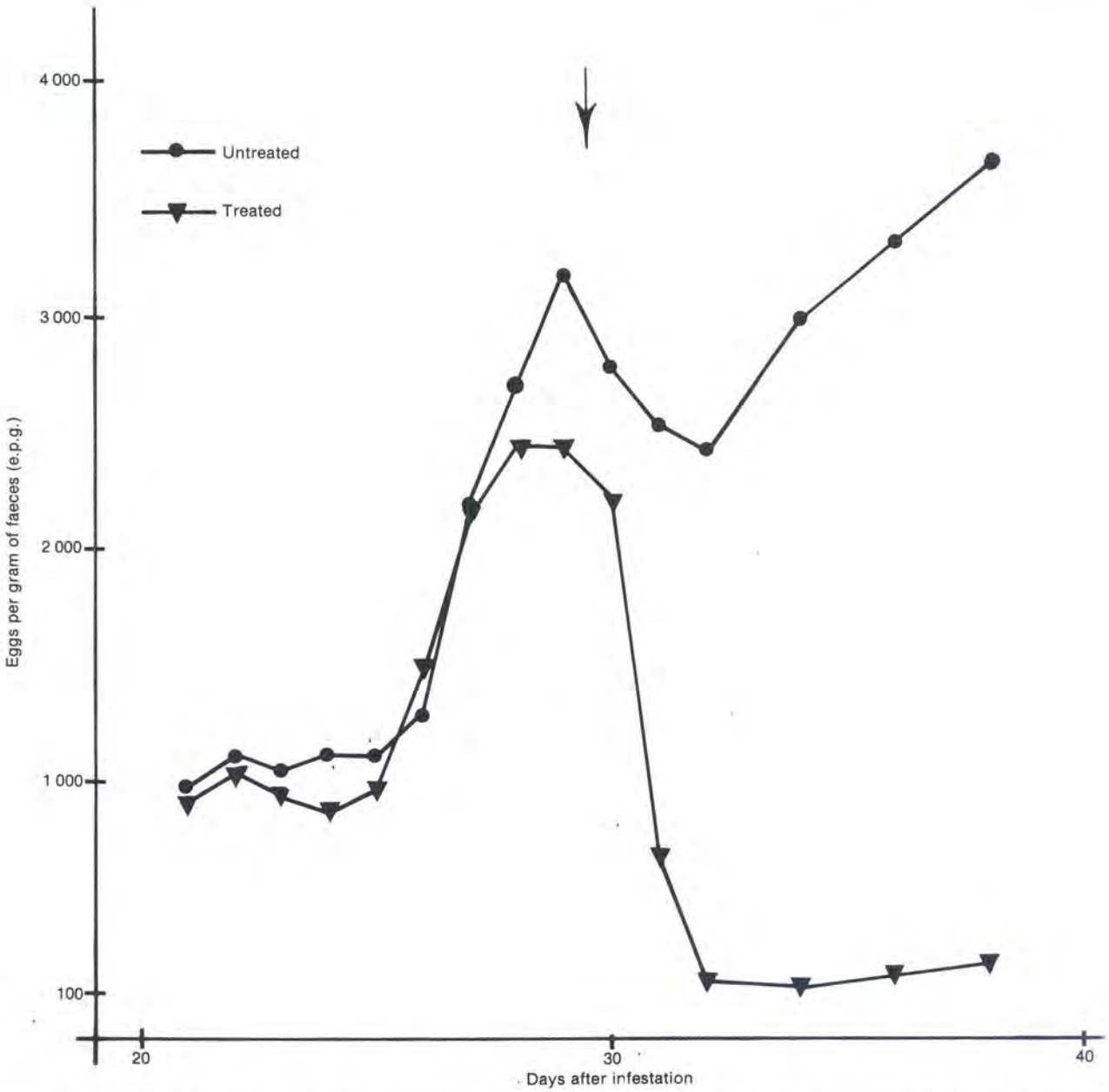

FIG. 3 Mean daily faecal egg counts of the 2 groups of sheep in the NPM trial. The arrow indicates treatment of Group B with rafoxanide; the other group remained as untreated controls (—●— Group A; —▼— Group B)

Method

Twenty Dorper sheep, 6–12 months old, not raised worm-free, were introduced for the trial. On arrival the sheep were dewormed with levamisole* at 7,5 mg/kg, and at 15 mg/kg 8 weeks later. The second treatment given 10 days before the commencement of the trial was merely a double precaution, since faeces collected immediately before this treatment were negative for worm eggs.

Throughout the trial the sheep were fed sterilized lucerne hay and were kept under conditions which precluded unintentional exposure to worms.

The L3 used in this trial were isolated from Sheep 2 which had not been exposed to rafoxanide.

The experimental design is summarized in Table 1. On the day of treatment (Day 0), the 20 sheep were mass-measured, ranked according to the e.p.g. and allocated to the 2 treatment groups, using tables of random numbers. Because the 2 groups were unequal, 2 numbers were drawn first, while the remaining 18 were allocated to 2 groups of 9 sheep and the groups then allocated to the 2 treatments, again using tables

TABLE 1 Experimental design (NPM trial)

Day	Treatment
−29	Dosed 1 108 L3 *H. contortus* to 20 sheep
−28	Dosed 1 108 L3 *H. contortus* to 20 sheep
−27	Dosed 1 108 L3 *H. contortus* to 20 sheep Total L3—3 324
0	Weighed 20 sheep and, while 9 sheep (Group A) remained as untreated controls, 11 sheep (Group B) were treated with rafoxanide at 7,5 mg/kg
+13	Killed 5 sheep from Group B for worm recovery
+14	Killed 6 sheep from Group B for worm recovery
+15	Killed Group A for worm recovery

* Ripercol (Ethnor)

TABLE 2 Faecal egg counts (modified NPM trial)

Group	Sheep	Days after infestation														
		Before treatment of Group B									After treatment of Group B					
		21	22	23	24	25	26	27	28	29	30	31	32	34	36	38
A (controls)	5	500	400	600	200	600	800	900	1 800	900	1 500	1 100	900	700	3 300	1 300
	6	1 100	200	1 200	700	900	700	900	2 000	3 500	2 100	2 500	1 600	2 800	2 600	2 000
	7	200	800	0	300	200	1 400	1 800	3 100	1 000	2 100	1 300	1 600	2 100	2 600	3 000
	8	700	600	1 900	900	900	500	600	2 700	600	1 300	1 400	400	900	1 600	700
	9	1 000	900	1 100	500	600	300	1 400	3 200	3 500	3 400	2 600	2 100	2 200	4 400	2 900
	10	900	800	1 400	900	1 400	1 400	1 400	4 400	4 200	4 700	3 700	2 200	3 900	3 200	3 000
	11	1 200	700	1 500	1 500	1 500	2 600	1 700	6 200	3 300	2 700	1 800	2 100	3 800	4 600	3 700
	12	1 500	800	2 600	1 700	1 900	2 100	3 000	4 400	6 000	5 400	3 700	3 800	5 600	6 300	5 900
	13	2 900	2 600	2 100	1 500	1 600	2 100	2 700	5 100	2 500	4 200	3 900	4 400	2 200	9 000	5 500
	Mean	1 111,1	866,7	1 377,8	911,1	1 066,7	1 322,2	1 600,0	3 655,6	2 833,3	3 044,4	2 444,4	2 122,2	2 688,9	4 177,8	3 111,1
	S.D.	773,7	687,4	783,9	548,7	561,3	809,0	812,4	1 469,8	1 771,8	1 461,3	1 113,7	1 275,5	1 547,9	2 273,1	1 740,3
B (treated)	14	600	1 000	1 100	800	600	1 100	900	1 100	700	1 200	100	0	0	0	0
	15	300	400	500	600	1 300	900	1 200	2 100	1 700	500	0	0	0	200	100
	16	500	900	1 400	400	400	400	1 300	2 500	1 100	1 400	400	300	200	400	500
	17	500	500	1 200	700	600	900	1 200	2 900	1 400	3 800	200	0	0	100	100
	18	1 000	1 000	1 100	400	700	1 500	2 100	1 900	2 100	1 800	0	100	0	0	0
	19	400	500	800	500	500	1 700	2 700	2 800	2 000	1 900	800	200	500	200	800
	20	1 800	1 100	2 500	600	1 300	2 800	3 700	3 300	5 200	3 500	200	0	100	100	100
	21	1 800	2 500	3 000	1 000	500	1 600	2 500	4 500	2 900	4 000	0	0	0	200	400
	22	1 400	1 100	400	800	1 100	3 300	6 000	4 200	4 000	1 600	400	0	200	700	500
	23	600	1 000	1 100	500	400	1 400	1 300	2 200	2 400	3 100	200	200	200	200	100
	24	1 000	700	1 000	400	1 100	1 300	1 100	3 100	2 900	800	600	0	0	0	0
	Mean	900,0	972,7	1 281,8	609,1	772,7	1 536,4	2 181,8	2 781,8	2 400,0	2 145,5	263,6	72,7	109,1	190,9	236,4
	S.D.	547,7	567,6	791,0	197,3	355,2	842,9	1 534,8	991,8	1 312,3	1 239,7	261,8	110,4	157,8	207,2	269,3

of random numbers. Subsequently, the 2 sheep that were drawn first were included in the group to be treated with rafoxanide.

Faecal egg counts of all sheep were done on 9 occasions during the period prior to the treatment of Group A, and subsequently on 6 occasions.

At necropsy all abomasa were collected as described by Reinecke (1973) and total worm counts were done with the aid of a stereoscopic microscope.

The results of the trial (worm counts) were analysed by the NPM of Groeneveld & Reinecke (1969), as modified by Clark (cited by Reinecke, 1973). Moving 3-point averages, interrupted at the point of treatment, were used for plotting Fig. 3.

Results

The results are summarized in Tables 2 & 3 and in Fig. 3.

TABLE 3 Numbers of worms recovered in the modified NPM trial

Group	Sheep	Number of *H. contortus*		
		Adults	L4	Total
A (Controls)	12	**612**	0	612
	9	**540**	9	549
	11	**534**	0	534
	13	**476**	0	476
	7	**445**	0	445
	10	**432**	24	456
	6	**411**	0	411
	5	**385**	0	385
	8	**285**	0	285
	Mean	**457,8**	—**	—
	S.D.	**96,7**	—	—
	Median	**445**	—	—
B (Treated: rafoxanide at 7,5 mg/kg)	15	**139**	11	150
	19	**130**	0	130
	22	**102**	0	102
	16	**97**	4	101
	21	**93**	0	93
	23	**54**	0	54
	20	**54**	0	54
	24	**35**	0	35
	17	**23**	6	29
	18	**21**	1	22
	14	**12**	1	13
	Mean	**69,1**	—	—
	S.D.	**45,1**	—	—

NPM classification (adult worms only)
445×0,25=111,3 (2 exceed this total)
445×0,4 =178,0 (0 exceed this total)
Therefore *Class B**
Arithmetic mean efficacy: 84,9%

* >60% effective in >60% of the treated animals
** not applicable

Before the treatment of Group B, the mean e.p.g. of Group A (controls) was 1 638 and that of individual animals 867–3 656; after the treatment of Group B, the mean e.p.g. of Group A rose to 2 932, with a range in individual animals of 2 122–4 178. The mean e.p.g. in Group B before treatment was 1 493 and that of individual animals 609–2 782, and after treatment the figures were respectively, 175* and 73–264.

* This figure excludes the high e.p.g. on the day after treatment since rafoxanide is known to act slowly (Snijders, Horak & Louw, 1973, Table 5: Sheep treated on 7th June and e.p.g. considerably lowered only on 9th June)

The mean number of adult worms recovered from Group A was 457,8±96,7 (median burden: 445) and in Group B 69,1±45,1.

The modified NPM classification for adult worms was Class B (>60% effective in >60% of the treated flock), since the worm burden of more than one of the treated sheep exceeded the median worm burden of the control sheep multiplied by 0,25 and no worm burden in the treated sheep exceeded the median burden of Group A multiplied by 0,4 (Groeneveld & Reinecke, 1969; Reinecke, 1973).

The arithmetic mean efficacy of the remedy was 84,9%.

Discussion and Conclusions

This appears to be the first report of resistance of a field strain of *H. contortus* to rafoxanide.

Horak, Snijders & Louw (1972), working with a South African strain of *H. contortus*, showed that rafoxanide at 7,5 mg/kg was 99,3% effective against adult worms, while Colglazier, Kates & Enzie (1971) recorded 99% efficacy at 5 mg/kg against 2 isolates of *H. contortus* resistant to thiabendazole. More recently Campbell, Hall, Kelly & Martin (1978) recorded 100% efficacy of rafoxanide at 7,5 mg/kg against a benzimidazole-resistant strain of *H. contortus*. In South Africa, for the purposes of registration for sale to the lay public in terms of Act 36 of 1947, rafoxanide was given "A" efficacy classification (i.e.>80% effective in >80% of the treated flock). This classification entailed analysis by the modified NPM test (Reinecke, 1973).

While it is obvious from the NPM trial in this paper that the level of resistance of this (OP-M) field strain of *H. contortus* to rafoxanide is not as high as appeared from the limited preliminary trials, there was certainly some resistance. The remedy failed, for the first time in South Africa, to qualify for the top NPM classification, "A", and a considerably larger mean percentage of adult worms survived than was previously encountered. In another trial with the OP-M strain of *H. contortus*, Schröder (personal communication, 1977) obtained similar results to ours, but in his case rafoxanide qualified for the NPM "A" classification by a narrow margin, while our results just failed to qualify.

Acknowledgements

The authors wish to thank Drs I. H. Carmichael and Anna Verster and Mr A. J. Morren for much help with the manuscript, and Messrs MSD (Pty) Ltd for conducting a search of the literature for references on resistance to rafoxanide.

References

BERGER, J., 1975. The resistance of a field strain of *Haemonchus contortus* to five benzimidazole anthelmintics in current use. *Journal of the South African Veterinary Association*, 46, 369–372.

CAMPBELL, N. J., HALL, C. A., KELLY, J. D. & MARTIN, I. C. A., 1978. The anthelmintic efficacy of non-benzimidazole anthelmintics against benzimidazole resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. *Australian Veterinary Journal*, 54, 23–25.

COLGLAZIER, M. L., KATES, K. C. & ENZIE, F. D., 1971. Activity of levamisole, pyrantel tartrate, and rafoxanide against two thiabendazole-tolerant isolates of *Haemonchus contortus*, and two species of *Trichostrongylus*, in sheep. *Proceedings of the Helminthological Society of Washington*, 38, 203–205.

GROENEVELD, H. T. & REINECKE, R. K., 1969. A statistical method for comparing worm burdens in two groups of sheep. *Onderstepoort Journal of Veterinary Research*, 36, 285–298.

HORAK, I. G., SNIJDERS, A. J. & LOUW, J. P., 1972. Trials with rafoxanide. 5. Efficacy studies against *Fasciola hepatica, Fasciola gigantica, Paramphistomum microbotrium* and various nematodes in sheep. *Journal of the South African Veterinary Association*, 43, 397–403.

LE JAMBRE, L. F., 1978. Anthelmintic resistance in gastrointestinal nematodes of sheep. *In*: Donald, E. D., Southcott, W. H. & Dineen, J. K. (Eds). The epidemiology and control of gastrointestinal parasites of sheep in Australia. Mt. Waverley: Shiels Printing, pp. 109–120.

REINECKE, R. K., 1973. The larval anthelmintic test in ruminants. *Technical Communication No.* 106, *Department of Agricultural Technical Services, Republic of South Africa.* iii+20 pp.

SNIJDERS, A. J., HORAK, I. G. & LOUW, J. P., 1973. Trials with rafoxanide. 6. The effect of repeated and single treatments with rafoxanide against *Haemonchus contortus* and *Oestrus ovis* in sheep. *Journal of the South African Veterinary Association*, 44, 251–263.

Printed by and obtainable from the Government Printer, Private Bag X85, Pretoria, 0001